



## Interactive Effects of Water Salinity and Water-Deficit on Biochemical Content and Photosynthetic Activity of Melon (*Cucumis melo* L.) Seedlings

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### HIGHLIGHTS

- Salinity and water deficit are important stress factors during the melon seedling period.
- During the growth period of melon, high salinity of 4000 µmhos negatively affects.
- 25% water restriction can be applied in regions where irrigation water is limited.

### Abstract

The deleterious effects of abiotic stress factors, which have emerged in the context of global climate change, have a detrimental impact on agricultural production. Irregularity in seasonal rainfall and high temperatures make it difficult to meet the water needs of the plant. In addition, intensive fertilization, monoculture and use of poor-quality water in irrigation in vegetable fields cause salinity problems. It has been observed that both stress factors cause yield and quality losses in vegetable farming. For this purpose, in the present study, five different salt (NaCl-S) levels {control S<sub>1</sub> (main water=500 µmhos), S<sub>2</sub> (2000 µmhos), S<sub>3</sub> (4000 µmhos), S<sub>4</sub> (6000 µmhos) and S<sub>5</sub> (8000 µmhos)} and three different irrigation water levels (full irrigation-I<sub>100</sub>, 75% irrigation-I<sub>75</sub> and 50% irrigation-I<sub>50</sub>) were applied to melon seedlings in the study and their effects on photosynthetic activity and biochemical changes were tried to be determined. The study revealed that elevated levels of both stress factors resulted in a reduction in the growth of melon seedlings. Conversely, the findings indicated that superoxide dismutase (SOD) enzyme activity served as a significant indicator under both stress factor conditions. Furthermore, an increase in SOD activity was observed as stress levels escalated. In addition, it was observed that saline waters with a conductivity higher than 4000 µmhos would have a toxic effect on melon seedlings. It is important for the sustainability of melon farming that 25% water restriction can be applied in regions where irrigation water is limited.

**Keywords:** Abiotic stress; chlorophyll; *Cucumis melo* L.; leaf pigments; photosynthesis.

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## 1. Introduction

Climate change resulting from global warming negatively affects many sectors. The agricultural sector is among the sectors most affected. Climate change causes greenhouse gases: changes in temperatures, irregular precipitation regime, an increase or decrease in sea level, and many environmental events. The agricultural sector alone accounts for 20% of annual greenhouse gas production. A significant portion of this rate is attributed to the use of animal manure and excessive fertilizers. The negative effects resulting from this situation can be listed as geographical displacement, decreasing water resources for irrigation, and land loss due to rising sea levels. In addition, there is a serious increase in temperatures, and precipitation regimes are seen to become more irregular. The results of climate change vary across different regions of the world. Especially in tropical regions, climate change significantly affects agricultural productivity (Cumhur and Malcolm 2008).

Abiotic stress factors are increasing their effects due to the effects of climate change. Examples of abiotic stress factors are excessive light, exposure to UV rays, high and low temperatures, freezing, drought, salinity, presence of heavy metals in the soil, oxygen deficiency, air pollution, radiation, deficiency or excess of nutrients, flooding, and waterlogging (Hirayama and Shinozaki 2010). When we consider agricultural lands in the world, the areas affected by drought have a share of 26% among abiotic stress conditions (Blum 1985). Drought is the inadequacy of water needed by the plant due to transpiration and evaporation. Drought is a serious problem that increases its effect day by day and spreads to large areas, causing yield and quality losses (Çelik et al. 2024).

Drought stress is responded to by plants in two different ways: reversible and irreversible. Drought is divided into two groups: physical and physiological. Physical drought occurs when a negative water potential occurs in the protoplasm due to water scarcity in the plant's environment. The other is physiological drought, which is the situation where the plant cannot get the water it needs from the soil, even though there is enough water in the soil. Stomatal closure caused by drought conditions reduces gas exchange and increases leaf temperature, which in turn increases water loss through transpiration (Kuşvuran 2012; Ansari et al. 2019). Water deficiency and desiccation are two different terms related to drought that need to be distinguished. Water deficiency is a moderate water deficiency that causes stomata to close and gas exchange to be restricted. As a result of stomata closure, carbon dioxide uptake is restricted. Desiccation is defined as excessive water loss that causes complete disruption of metabolism and cell functions and failure to secrete enzyme activities (Smirnov 1993; Kalefetoğlu and Ekmekçi 2005). As plants begin to dry, signs of wilting begin to appear on the leaves. After desiccation occurs, the damage caused by dehydration to the plant cannot be recovered, even if the necessary water is given, and death occurs (Kaçar 2015). When the plant cannot provide the water it needs for photosynthesis from the root area, stress begins to appear in the plant, and when stress begins to show its effect, plants either reduce water consumption or try to take more water from the soil (Bray 1997). This causes a decrease in the photosynthesis rate in plants and, accordingly, a slowdown in vegetative growth (Sağlam 2004). Restriction of photosynthesis, reduction in stem diameter and length, cessation of stem and root development, also reduces leaf area, leaf number, and leaf life, causing the leaf to yellow and wither (Anjum et al. 2011; Öztürk 2015).

Similar to drought conditions, salinity stress represents a critical abiotic stressor that constrains vegetative development within agricultural environments. Salinity is characterized as the concentration of salts present in soil or aqueous mediums and is observed to exert a considerable influence on plant development (Fileccia et al. 2017; Ibrahim et al. 2019; Omid et al. 2022). It is seen that high salinity levels cause osmotic stress, and the available water that the plant will consume in respiration will decrease, and physiological drought will begin (Ibrahim et al. 2019). Similar to drought, salinity inhibits photosynthesis in plants and negatively affects nutrient uptake. It manifests itself in morphological changes, including the decrease in leaf number and leaf area, which are directly related to the decrease in photosynthesis. (Abdel-Farid et al. 2020; Omid et al. 2022). It has been noted that elevated salinity levels perturb ion homeostasis, resulting in the accumulation of deleterious ions, including sodium ( $\text{Na}^+$ ) and chloride ( $\text{Cl}^-$ ), within plant tissues. (Fileccia et al. 2017; Ibrahim et al. 2019; Ali et al. 2022). This ionic imbalance damages cellular structures, impairs enzyme functions and

inhibits metabolic processes. In a study, it was reported that salinity stress leads to a decrease in chlorophyll content and gas exchange, which are indispensable for photosynthesis (Alam et al. 2020; Hameed et al. 2021). It has been revealed that the presence of salinity in the environment increases the production of reactive oxygen species (ROS) and causes more damage to plant cells (Akram et al. 2019; Hameed et al. 2021).

Salinity and drought are well-documented stress factors that have been shown to have a detrimental effect on the growth, development and yield of melons, a vegetable species that is widely cultivated around the world. Different varieties of melons are tolerant to different levels of salt. In general, it is reported that the salinity tolerance threshold in melons is approximately 2.2 dS m<sup>-1</sup>, beyond which yield can decrease significantly (Sobhani and Mohammadzadeh 2017). Melons exposed to drought stress exhibit low germination rates, impaired seedling growth and changes in biochemical pathways, including the accumulation of osmoprotectants such as proline and citrulline, which help alleviate stress effects (Kuşvuran et al. 2013; Ansari et al. 2019).

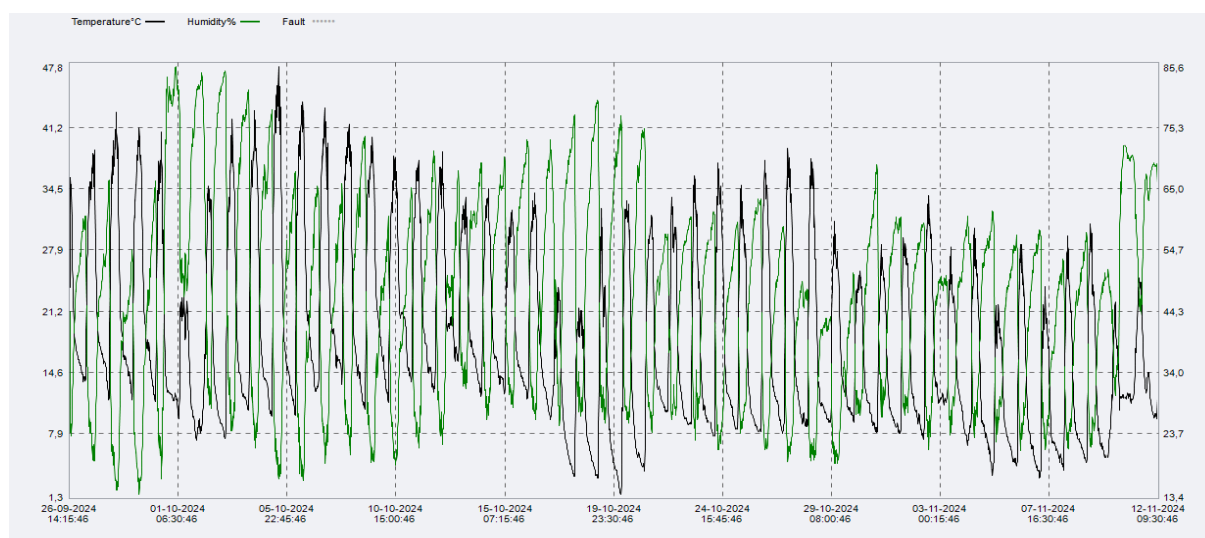
Melon (*Cucumis melo* L.) is a vegetable species that is eaten with pleasure by people and has a high production and consumption potential. Although previously believed to be native to Africa, recent research suggests that melon may be of Asian origin. There is a world production of melon of 29 541 294 tons. Turkey, in particular, is an important producer, ranking third after China and India with a production of 1 403 214 tons (FAO 2023). The regions where melon production is highest in Türkiye are the Mediterranean region, the Aegean region, and the Southeastern Anatolia region, respectively. The cities where production is highest are Adana, Denizli, Antalya, Mardin, Manisa, and Konya. (TÜİK 2023). Melon has a low protein and fat content in terms of nutritional content, while it contains plenty of carbohydrates and sugar. In addition, it is a vegetable species rich in vitamins A, B, C, calcium, potassium, iron and magnesium (Salunkhe and Kadam 1998). It is an economically important vegetable and is used in making fruit juice in addition to fresh consumption. In addition, some varieties are used as ornamental plants (Wien 1997).

The combination of salinity and drought stress causes more damage to yield, growth and development (Kuşvuran 2012; Chevilly et al. 2021). This interaction is especially important in semi-arid regions where both salinity and drought are common due to irregular rainfall patterns and inadequate irrigation practices (Pereira et al. 2017; Sousa et al. 2018). In such environments, effective irrigation management is mandatory to minimize salinity accumulation and ensure adequate water supply for melon cultivation. In studies on melon, salinity and drought stress have generally been evaluated separately. In our study, we tried to determine the separate and interactive effects of two important stress factors, such as drought and salinity, on photosynthetic activity and biochemical contents in melon seedlings.

## 2. Materials and Methods

### 2.1. Experimental area

The investigation was conducted in controlled pot environments within the greenhouses of the Faculty of Agriculture at Konya Selçuk University from September 26 to November 12, 2024. The geographical coordinates of the research site are situated at 38°02' north latitude and 32°30' east longitude, with an average elevation of 1 105 meters above sea level. Throughout the duration of the experimental period, climatic data within the greenhouse were systematically recorded, revealing an average temperature of 18.3°C and an average relative humidity of 45.7% (refer to Figure 1). Comprehensive physical and chemical analyses of the soil sample utilized in the experiment were conducted, indicating an organic matter content of 4.8% alongside a clayey-loamy texture. It was noted that the experimental soil exhibited favorable attributes concerning its nutrient composition, with a pH of 8.05, an electrical conductivity of 1 050 µS cm<sup>-1</sup>, and a calcium carbonate content of 13%. The field capacity and wilting point of the soil were ascertained to be 25.12% and 12.4%, respectively. Upon the evaluation of the collected results, it was determined that the climatic and soil characteristics did not induce any stressors in the cultivation of melons.



**Figure 1.** The distribution of temperature and relative humidity inside the greenhouse was measured and recorded throughout the study

## 2.2. Plant Materials, Planting, Irrigation and Salinity Procedures

The material used in the study was a standard melon variety, Kırkağaç 631. In the experiment, plastic pots with a height of 24 cm, a base diameter of 19 cm and an upper diameter of 29 cm were used. Each pot to be used in the experiment was filled with dry soil and weighed to 10 kg. The study was set up with three replications and 6 to 9 seeds were planted in the pots to form a triangle. After the seeds germinated, thinning was done so that three plants remained in each pot. In this study, five different salt (NaCl) levels {control S<sub>1</sub> (main water = 500 µmhos), S<sub>2</sub> (2000 µmhos), S<sub>3</sub> (4000 µmhos), S<sub>4</sub> (6000 µmhos) and S<sub>5</sub> (8000 µmhos)} were used. At the same time, three different irrigation water levels (full irrigation-I<sub>100</sub>, 75% irrigation-I<sub>75</sub> and 50% irrigation-I<sub>50</sub>) were used. After the seedlings reached two or three true leaves, the soil was brought to field capacity. The pots in S<sub>1</sub>I<sub>100</sub> subjects were weighed regularly and the decreasing water was applied to the pots. As of October 16, 2024, the calculated salt and irrigation amounts were started to be applied to the pots. A total of 6 water and salt stress applications were made and the seedlings were harvested on November 12, 2024. Cultural procedures were carried out regularly in the study.

## 2.3. Physiological and Biochemical Analyses

Following the demonstration of the impact of stress, measurements of g<sub>s</sub> (stomatal conductance), PSII (actual photosynthetic efficiency), and T Leaf (leaf temperature) were conducted on melon foliage. Illuminance assessments were executed utilizing a portable LI-600 fluorimeter device (LI-COR Inc. USA) during the time frame of 9:00 a.m. to 11:00 a.m. (Liang et al. 2019).

Spectrophotometric analyses (Shimadzu-UV-6300PC) were performed on samples procured from melon foliage. The protocol delineated by Lichtenthaler (1987) was employed to quantify total chlorophyll and carotenoid concentrations within the leaves of melon specimens. In order to ascertain the levels of malondialdehyde (MDA) and H<sub>2</sub>O<sub>2</sub> in the leaf tissues, the spectrophotometric technique articulated by Esterbauer and Cheeseman (1990) was taken into account. The Bradford method was utilized for the quantification of proline and protein concentrations within the leaf (Bradford, 1976). The activities of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) were evaluated according to the methodologies established by Havir and McHale (1987), Beyer and Fridovich (1987), and Bergmeyer et al. (1983), respectively.

## 2.4. Data Analysis

Data obtained from melons tested under all applied stress conditions were analyzed using JMP-17 Pro software at 5% and 1% significance levels. All data were then subjected to principal component analysis (PCA) using the same software to determine significant parameters and their relationships.

### 3. Results and Discussion

#### 3.1. Effects of Salinity and Irrigation Deficit on the Stomatal Conductance, Actual Photosynthetic Efficiency, and Leaf Temperature in Melon

An analysis of the data revealed that the application of different irrigation levels and salt applications to melon seedlings exhibited statistically significant effects on gs, PSII and T leaf (Table 1). Upon examination of the table, it was observed that irrigation water salinity and varying irrigation water levels did not demonstrate any statistically significant change in gs. When the measured PSII values were analyzed, salt applications and irrigation water amounts did not generate a significant difference; however, when both stress factors were combined, significant changes were observed. The study observed that under control conditions, melon seedlings exhibited a leaf temperature of 19.97°C, which decreased significantly with increasing salinity stress. When water stress applications are examined, the highest leaf temperature was observed to be 19.21 °C in the I 100 application. According to the findings of the study, leaf temperature decreases compared to the control with increasing water stress.

**Table 1.** The effects of salinity and irrigation deficit on the gs, PSII, T leaf, and pigment contents in melon

Treatments	gs (mol m <sup>-2</sup> s <sup>-1</sup> )	PSII	Tleaf (C°)	Chl a (mg g <sup>-1</sup> )	Chl b (mg g <sup>-1</sup> )	CT (mg g <sup>-1</sup> )
<u>Salinity (S)</u>						
S <sub>1</sub> (control)	0.03	0.71	19.97 <sup>A</sup>	17.94 <sup>B</sup>	5.13 <sup>B</sup>	4.42 <sup>C</sup>
S <sub>2</sub>	0.03	0.73	19.17 <sup>B</sup>	19.84 <sup>A</sup>	5.69 <sup>A</sup>	4.89 <sup>B</sup>
S <sub>3</sub>	0.03	0.72	18.93 <sup>B</sup>	19.98 <sup>A</sup>	5.61 <sup>A</sup>	5.42 <sup>A</sup>
S <sub>4</sub>	0.03	0.73	18.37 <sup>C</sup>	15.67 <sup>C</sup>	4.43 <sup>C</sup>	4.23 <sup>D</sup>
S <sub>5</sub>	0.05	0.71	18.19 <sup>C</sup>	14.83 <sup>D</sup>	4.27 <sup>C</sup>	4.06 <sup>E</sup>
<u>Irrigation (I)</u>						
I <sub>100</sub>	0.03	0.72	19.21 <sup>a</sup>	19.42 <sup>a</sup>	5.72 <sup>a</sup>	5.04 <sup>a</sup>
I <sub>75</sub>	0.03	0.72	18.69 <sup>b</sup>	17.22 <sup>b</sup>	4.87 <sup>b</sup>	4.51 <sup>b</sup>
I <sub>50</sub>	0.04	0.73	18.89 <sup>b</sup>	16.32 <sup>c</sup>	4.49 <sup>c</sup>	4.26 <sup>c</sup>
<u>S X I (Interactions)</u>						
S <sub>1</sub>	I <sub>100</sub>	0.04	0.69 <sup>bc</sup>	21.11 <sup>a</sup>	19.44 <sup>c</sup>	5.47 <sup>de</sup>
	I <sub>75</sub>	0.03	0.70 <sup>abc</sup>	19.52 <sup>b</sup>	20.99 <sup>b</sup>	6.37 <sup>b</sup>
	I <sub>50</sub>	0.02	0.74 <sup>a</sup>	19.29 <sup>bc</sup>	13.39 <sup>f</sup>	3.56 <sup>h</sup>
S <sub>2</sub>	I <sub>100</sub>	0.03	0.73 <sup>ab</sup>	19.20 <sup>bcd</sup>	16.77 <sup>d</sup>	4.88 <sup>f</sup>
	I <sub>75</sub>	0.03	0.72 <sup>abc</sup>	19.04 <sup>bcd</sup>	21.00 <sup>b</sup>	5.79 <sup>cd</sup>
	I <sub>50</sub>	0.02	0.75 <sup>a</sup>	19.28 <sup>bc</sup>	21.76 <sup>b</sup>	6.39 <sup>ab</sup>
S <sub>3</sub>	I <sub>100</sub>	0.02	0.71 <sup>abc</sup>	18.72 <sup>bef</sup>	23.15 <sup>a</sup>	6.75 <sup>a</sup>
	I <sub>75</sub>	0.03	0.72 <sup>ab</sup>	18.84 <sup>cde</sup>	17.37 <sup>d</sup>	4.82 <sup>f</sup>
	I <sub>50</sub>	0.03	0.74 <sup>ab</sup>	19.24 <sup>bcd</sup>	19.43 <sup>c</sup>	5.26 <sup>e</sup>
S <sub>4</sub>	I <sub>100</sub>	0.02	0.71 <sup>abc</sup>	18.80 <sup>cde</sup>	18.85 <sup>c</sup>	5.39 <sup>e</sup>
	I <sub>75</sub>	0.04	0.73 <sup>ab</sup>	17.89 <sup>g</sup>	12.96 <sup>f</sup>	3.69 <sup>h</sup>
	I <sub>50</sub>	0.02	0.74 <sup>ab</sup>	18.40 <sup>efg</sup>	15.20 <sup>e</sup>	4.21 <sup>g</sup>
S <sub>5</sub>	I <sub>100</sub>	0.03	0.73 <sup>ab</sup>	18.19 <sup>fg</sup>	18.92 <sup>c</sup>	6.12 <sup>bc</sup>
	I <sub>75</sub>	0.03	0.74 <sup>a</sup>	18.14 <sup>g</sup>	13.78 <sup>f</sup>	3.69 <sup>h</sup>
	I <sub>50</sub>	0.11	0.67 <sup>c</sup>	18.24 <sup>fg</sup>	11.80 <sup>g</sup>	3.01 <sup>i</sup>
<u>Significance</u>						
S	ns	ns	**	**	**	**
I	ns	ns	**	**	**	**
S X I	ns	*	**	**	**	**

\*Statistically significant according to P<0.05 and 0.001. ns not significant. gs (Stomatal conductance), PhiPSII (actual photosynthetic efficiency), T Leaf (Leaf temperature), Chl a (Chlorophyll a), Chl b (Chlorophyll b), CT (Carotenoid)

Plants try to prevent water loss from plant tissues by closing their stomata under water stress conditions or high temperature conditions. In this way, plants develop a defense mechanism against stress and photosynthesis is negatively affected. In a study conducted on lettuce, it was reported that gs and PSII were

not affected in applications where water stress was mild, while significant decreases occurred in applications where stress was intense (Yavuz et al. 2023). In a similar vein, it has been documented that water stress on cabbage growth has been shown to result in a significant reduction in gs and PSII values. (Seymen et al. 2023). In the present study, while the findings obtained from salt stress and water stress did not cause significant changes in PSII and gs when examined separately, both stress factors decreased leaf temperature compared to the control.

### 3.2. Effects of Salinity and Irrigation Deficit on The Biochemical Contents in Melon

The findings of the study demonstrated that the application of different irrigation and salt treatments to melon seedlings exhibited statistically significant ( $P < 0.01$ ) effects on pigment content (see Table 1). Upon examination of chlorophyll a content, it was observed that the S<sub>2</sub> and S<sub>3</sub> applications resulted in the highest levels of chlorophyll a, with concentrations of 19.84 and 19.98 mg g<sup>-1</sup>, respectively. However, when the impact of varying water levels was analyzed, the maximum chlorophyll a content was observed in the I<sub>100</sub> application, reaching 19.42 mg g<sup>-1</sup>. Conversely, as water stress levels increased, a decline in chlorophyll content was evident. When the interactive effect of both stress factors was examined, the maximum chlorophyll a content was attained by the S<sub>3</sub>I<sub>100</sub> application, reaching 23.15 mg g<sup>-1</sup>. The most significant decrease was observed in the S<sub>5</sub>I<sub>50</sub> application, which had the highest level of both stresses, with a value of 11.80 mg g<sup>-1</sup>. Chlorophyll b content was also found to be negatively impacted by salt stress. When water levels were analyzed, the maximum value for chlorophyll b content was obtained from the I<sub>100</sub> application, at 5.72 mg g<sup>-1</sup>, while significant decreases in chlorophyll b content were observed as water stress increased. In the interaction of salinity and water limitation, S<sub>3</sub>I<sub>100</sub> and S<sub>2</sub>I<sub>50</sub> applications exhibited the highest chlorophyll b values. Conversely, S<sub>5</sub>I<sub>50</sub> application, which had the maximum chlorophyll b content, had the lowest chlorophyll b content. In the examination of carotenoid content, it was observed that low-dose salt levels increased the carotenoid content compared to the control, while high-level salt applications caused significant decreases. The irrigation application of I<sub>100</sub> resulted in the highest carotenoid content of 5.04 mg g<sup>-1</sup>, while significant decreases in carotenoid content were observed as water stress increased. The interaction of stress factors revealed that the application of S<sub>3</sub>I<sub>100</sub> yielded the highest carotenoid content of 5.88 mg g<sup>-1</sup>.

Pigments found in plant leaves play a pivotal role in the process of photosynthesis and the utilization of excess energy. A well-documented mechanism for mitigating ROS production under stress conditions involves enhanced tolerance through the dissipation of excess energy in chloroplasts via the xanthophyll cycle, facilitated by carotenoids (Latowski et al. 2011). Research findings have demonstrated significant alterations in pigment content under conditions of salinity and water stress (Seymen 2021; Yavuz et al. 2022; Kurtar et al. 2024). In a study conducted on spinach, it was reported that 50% water stress did not result in a statistically significant difference in chlorophyll a, b and carotenoid contents (Kayak et al. 2023). Conversely, a separate study revealed that salt and water stress significantly diminished chlorophyll a, chlorophyll b, and carotenoid contents in spinach (Yavuz et al. 2022). Conversely, a study on cabbage revealed significant alterations in pigment content under diverse irrigation water and salt stress conditions, accompanied by substantial declines in chlorophyll a, chlorophyll b, and carotenoid levels (Seymen et al. 2023). The findings of the present study are in alignment with these earlier research findings, underscoring the pivotal role of pigment content in mediating the impact of stress conditions.

It was observed that salt and water stress applied to melon had statistically significant effects on H<sub>2</sub>O<sub>2</sub>, MDA, protein and proline (Table 2). When we look at H<sub>2</sub>O<sub>2</sub> content, control (S<sub>1</sub>) application had the highest H<sub>2</sub>O<sub>2</sub> content with 15.46 µmol g<sup>-1</sup>. Other salt stress applications caused a decrease in H<sub>2</sub>O<sub>2</sub> content. While the highest H<sub>2</sub>O<sub>2</sub> content was obtained under full irrigation conditions, decreases in H<sub>2</sub>O<sub>2</sub> content were observed as water stress increased. In the interaction, S<sub>1</sub>I<sub>75</sub>, S<sub>1</sub>I<sub>50</sub> and S<sub>4</sub>I<sub>100</sub> applications were the applications with the highest H<sub>2</sub>O<sub>2</sub> contents and were statistically in the same group. When we look at MDA contents, S<sub>5</sub> (8000 µmhos) application, where the highest salt stress was applied, had the lowest MDA content with 0.55 nmol mL<sup>-1</sup>, while other salt applications were statistically in the same group. When different water level applications were examined, it was seen that water levels did not have a significant effect on MDA contents. Similarly, the interaction of both stresses was not found to be statistically significant. When protein contents were examined,

S<sub>2</sub> and control (S<sub>1</sub>) applications had the highest protein contents, while higher salt applications decreased the protein content. It was seen that different irrigation levels did not have a significant effect on protein content. When stress interactions were examined, the highest protein content was obtained from S<sub>4</sub>I<sub>50</sub> application. When proline was examined, control (S<sub>1</sub>) and S<sub>3</sub> applications gave the highest results with 17.30 and 15.43 µg g<sup>-1</sup> among different applications. At different irrigation levels, I<sub>75</sub> and I<sub>50</sub> applications had the highest proline contents with 15.06 and 16.38 µg g<sup>-1</sup>.

**Table 2.** The effects of salinity and irrigation deficit on the levels of H<sub>2</sub>O<sub>2</sub>, MDA, PT, and PL, as well as on the antioxidant enzyme activities in cabbage

Treatments		H <sub>2</sub> O <sub>2</sub> (µmol g <sup>-1</sup> )	MDA (nmol mL <sup>-1</sup> )	Prot (µg g <sup>-1</sup> )	Prol (µg g <sup>-1</sup> )	SOD (EU/g <sup>-1</sup> )	POD (EU/g <sup>-1</sup> )	CAT (EU/g <sup>-1</sup> )
<u>Salinity (S)</u>								
S <sub>1</sub> (control)		15,46 <sup>A</sup>	0,81 <sup>A</sup>	104,70 <sup>AB</sup>	17,30 <sup>A</sup>	4509,36 <sup>C</sup>	3060,98 <sup>D</sup>	1572,44 <sup>A</sup>
S <sub>2</sub>		9,87 <sup>B</sup>	0,76 <sup>A</sup>	105,76 <sup>A</sup>	14,21 <sup>BC</sup>	4523,99 <sup>C</sup>	3253,69 <sup>D</sup>	1631,11 <sup>A</sup>
S <sub>3</sub>		9,84 <sup>B</sup>	0,74 <sup>A</sup>	103,67 <sup>BC</sup>	15,43 <sup>AB</sup>	4725,73 <sup>BC</sup>	4214,22 <sup>C</sup>	1700,22 <sup>A</sup>
S <sub>4</sub>		10,14 <sup>B</sup>	0,77 <sup>A</sup>	103,21 <sup>BC</sup>	14,32 <sup>BC</sup>	5044,53 <sup>B</sup>	4637,51 <sup>B</sup>	1453,78 <sup>A</sup>
S <sub>5</sub>		6,66 <sup>C</sup>	0,55 <sup>B</sup>	102,34 <sup>C</sup>	13,04 <sup>C</sup>	5443,15 <sup>A</sup>	5776,00 <sup>A</sup>	803,78 <sup>B</sup>
<u>Irrigation (I)</u>								
I <sub>100</sub>		11,54 <sup>a</sup>	0,72	103,35	13,14 <sup>b</sup>	4687,24	5581,76 <sup>a</sup>	1401,20 <sup>b</sup>
I <sub>75</sub>		9,99 <sup>b</sup>	0,69	103,99	15,06 <sup>a</sup>	4844,97	3871,04 <sup>b</sup>	1213,47 <sup>b</sup>
I <sub>50</sub>		9,65 <sup>b</sup>	0,77	104,47	16,38 <sup>a</sup>	5015,85	3112,64 <sup>c</sup>	1682,13 <sup>a</sup>
<u>S X I (Interactions)</u>								
S <sub>1</sub>	I <sub>100</sub>	12,60 <sup>d</sup>	0,81	106,48 <sup>c</sup>	16,74	4724,82	3161,07 <sup>hi</sup>	1556,00 <sup>b-e</sup>
	I <sub>75</sub>	16,89 <sup>e</sup>	0,79	107,13 <sup>bc</sup>	15,48	4358,71	3698,67 <sup>gh</sup>	1440,00 <sup>c-f</sup>
	I <sub>50</sub>	16,90 <sup>e</sup>	0,83	100,50 <sup>gh</sup>	19,69	4444,56	2323,20 <sup>jk</sup>	1721,33 <sup>bcd</sup>
S <sub>2</sub>	I <sub>100</sub>	14,51 <sup>bc</sup>	0,77	102,50 <sup>efg</sup>	13,23	4499,44	4387,73 <sup>ef</sup>	1830,67 <sup>abc</sup>
	I <sub>75</sub>	8,49 <sup>ef</sup>	0,69	109,51 <sup>b</sup>	15,07	4514,06	3845,87 <sup>fg</sup>	830,67 <sup>gh</sup>
	I <sub>50</sub>	6,62 <sup>g</sup>	0,83	105,28 <sup>cde</sup>	14,32	4558,45	1527,47 <sup>l</sup>	2232,00 <sup>a</sup>
S <sub>3</sub>	I <sub>100</sub>	6,90 <sup>fg</sup>	0,77	105,84 <sup>cb</sup>	12,35	4291,10	5355,20 <sup>c</sup>	1846,00 <sup>abc</sup>
	I <sub>75</sub>	9,57 <sup>e</sup>	0,66	102,07 <sup>gh</sup>	16,62	4800,69	5462,93 <sup>c</sup>	1298,67 <sup>def</sup>
	I <sub>50</sub>	13,05 <sup>cd</sup>	0,80	103,09 <sup>def</sup>	17,31	5085,42	1824,53 <sup>kl</sup>	1956,00 <sup>ab</sup>
S <sub>4</sub>	I <sub>100</sub>	16,02 <sup>ab</sup>	0,78	102,27 <sup>fg</sup>	11,04	4910,80	6642,13 <sup>b</sup>	1245,33 <sup>efg</sup>
	I <sub>75</sub>	7,68 <sup>fg</sup>	0,67	93,08 <sup>c</sup>	15,77	4853,16	2643,73 <sup>ij</sup>	1457,33 <sup>c-f</sup>
	I <sub>50</sub>	6,73 <sup>g</sup>	0,86	114,29 <sup>a</sup>	16,13	5369,63	4626,67 <sup>de</sup>	1658,67 <sup>b-e</sup>
S <sub>5</sub>	I <sub>100</sub>	7,71 <sup>fg</sup>	0,48	99,66 <sup>gh</sup>	12,31	5010,06	8362,67 <sup>a</sup>	528,00 <sup>h</sup>
	I <sub>75</sub>	7,32 <sup>fg</sup>	0,64	108,14 <sup>bc</sup>	12,35	5698,24	3704,00 <sup>gh</sup>	1040,67 <sup>fg</sup>
	I <sub>50</sub>	4,96 <sup>h</sup>	0,53	99,20 <sup>h</sup>	14,44	5621,16	5261,33 <sup>cd</sup>	842,67 <sup>gh</sup>
<u>Significance</u>								
S		**	**	**	**	**	**	**
I		**	ns	ns	**	ns	**	**
S X I		**	ns	**	ns	ns	**	**

\*Statistically significant according to P<0.05 and 0.001. ns not significant

H<sub>2</sub>O<sub>2</sub> (Hydrogen peroxidase), MDA (Malondialdehyde), Prot (Protein), Prol (Proline), SOD (Superoxide dismutase), POX (Peroxidase), CAT (Catalase).

MDA, which has been observed to increase under stress conditions, is an indicator of lipid peroxidation (Riasat et al. 2019). MDA is the end product of lipid peroxidation and its increase indicates that ROS accumulation increases as a result (Savvides et al. 2016). It has been explained that H<sub>2</sub>O<sub>2</sub> is a toxic substance that increases significantly in plant cells as a result of stress. Its high concentration has been observed to cause lipid peroxidation and membrane damage, with a consequent negative effect on the plant (Nayar and Kaushal 2002). MDA and H<sub>2</sub>O<sub>2</sub> have been observed to increase underwater stress conditions in many studies (Seymen et al. 2023; Kayak et al. 2024; Yavuz et al. 2024). In contrast, our study observed no such increase in melon under both stress conditions. The reduction of oxidative damage is achieved by proline and proteins through their synergy with enzymatic and non-enzymatic antioxidant defence systems under stress conditions (Hanif et al. 2021). Proline, a non-enzymatic antioxidant, has been shown to enhance the production of osmoprotectants (Khedr et al. 2003). It has been documented that proline and protein contents increase under both stress conditions and interactions (Yavuz et al. 2022; Seymen et al. 2023). In the present study, although proline content decreased under salinity stress conditions, it increased under water stress conditions.

It was observed that the effects of salinity and water stress applications on antioxidant enzyme activities in melon were statistically significant (Table 2). When SOD enzyme activity was examined, it was seen that S<sub>5</sub> application had the highest SOD enzyme activity with 5443.15 EU/g<sup>-1</sup> and it was observed that SOD activity increased as stress increased. Different irrigation levels and both stress interactions did not have statistically significant effects on SOD enzyme activity. When POD activity was examined, the highest SOD value among different salinity applications was obtained from S<sub>5</sub> application with 5776 EU/g<sup>-1</sup>. Similarly, it was observed that POD activity increased as salinity level increased. In different irrigation conditions, I<sub>100</sub> application was the application giving the highest value with 5581.76 EU/g<sup>-1</sup> and POD activity decreased as water restriction increased. When the interaction of these two was examined, S<sub>5</sub>I<sub>100</sub> application had the highest POD value. When CAT enzyme activity was examined, S<sub>5</sub> application gave the lowest enzyme activity of 803.78 EU/g<sup>-1</sup> from different salt levels and other salt applications were statistically in the same group. When water restriction applications were examined, the highest CAT activity was obtained from I<sub>50</sub> application with 1682.13 EU/g<sup>-1</sup>. When the interaction was examined, the highest activity was obtained from S<sub>2</sub>I<sub>50</sub> and S<sub>3</sub>I<sub>50</sub> applications.

Although reactive oxygen species (ROS) formed under stress conditions restrict the development of plants, antioxidant enzymes secreted by plants (such as CAT, SOD, POD, etc.) are important mechanisms in scavenging ROS. SOD, for example, attempts to reduce the negative effects of stress by converting the formed O<sub>2</sub> anion into less reactive species O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> (Kireçci 2018). Numerous studies have documented the augmentation of SOD activity under abiotic stress conditions in diverse plant species (Gao et al. 2020; Seymen 2021; Yavuz et al. 2022). CAT, an enzyme that can directly modify H<sub>2</sub>O<sub>2</sub>, plays a pivotal role in the scavenging of ROS (Van Breusegem et al. 2001). POD has also been observed to increase stress conditions and contribute to the accumulation of secondary metabolites and osmolytes to scavenge ROS (Sattar et al. 2020). In many stress studies, it has been observed that CAT and POD enzyme activities increase (Yavuz et al. 2022; Seymen et al. 2023). In the present study, salinity stress in melon revealed significant increases in SOD and POD enzyme activities, like literature. A considerable increase in CAT enzyme activity was observed in the context of underwater stress conditions.

### 3.3. PCA

PCA was performed on photosynthetic activity and biochemical contents of different salt and irrigation water applications applied to melon (Table 3). The analysis revealed that four components explained 81.21% of the study. In the first component, with the highest explanation, Chl a, Chl b, Car, MDA, CAT and T Leaf were the most strongly explained parameters in the positive direction. Similarly, in the first component, SOD and gs were the most strongly explained parameters in the negative direction. The second component exhibited Chl a, Chl b, Car and POD as the most strongly explained parameters in the positive direction, whilst MDA, Prol and CAT were the most strongly explained parameters in the negative direction. The examination of the loading plot graph elucidated a strong positive correlation between the pigment contents. Numerous studies have demonstrated the presence of a strong correlation between the pigment contents under conditions of water and salinity stress (Yavuz et al., 2022; Kayak et al., 2023; Seymen et al., 2023). While a strong positive correlation was identified between T Leaf, H<sub>2</sub>O<sub>2</sub>, MDA, CAT, PSII and Prol, it was observed that these parameters exhibited a strong negative correlation with POD (Figure 2). In numerous studies, it was observed that the correlation relationship between biochemical and photosynthetic activities under water and salinity stress conditions was interpreted with a loading plot graph (Seymen 2021; Seymen et al. 2024; Yavuz et al. 2024). In order to interpret the significant differences between the applications, a scoria plot graph was drawn from the first two components (Figure 3). The examination of this graph revealed that the S<sub>5</sub>I<sub>50</sub> application, which was subjected to the highest levels of water and salt stress, was associated with elevated SOD activity. This finding indicated that SOD was the predominant enzyme responsible for both stresses. Furthermore, it was observed that the S<sub>3</sub>I<sub>100</sub>, S<sub>2</sub>I<sub>75</sub> and S<sub>1</sub>I<sub>75</sub> applications, which exhibited high levels of pigment content, were the least impacted by stress. The S<sub>1</sub>I<sub>100</sub> application was found to be high in terms of protein content, and it was revealed that the protein content was high in the absence of stress. Conversely, the first component is generally the component explaining salinity stress, while the second component is the component explaining water stress. According to these results, it has been observed that waters with salinity

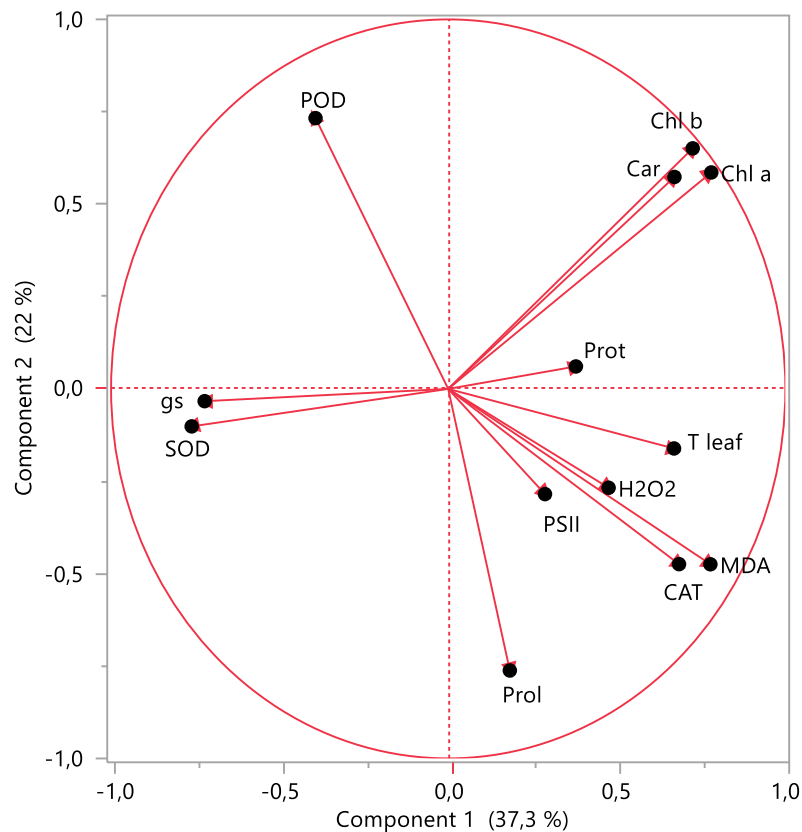


higher than 4000  $\mu\text{mhos}$  in melon irrigation water limit plant growth. Conversely, in regions where water resources are scarce, it has been demonstrated that imposing a 25% water restriction during the initial melon development stages can lead to substantial water savings.

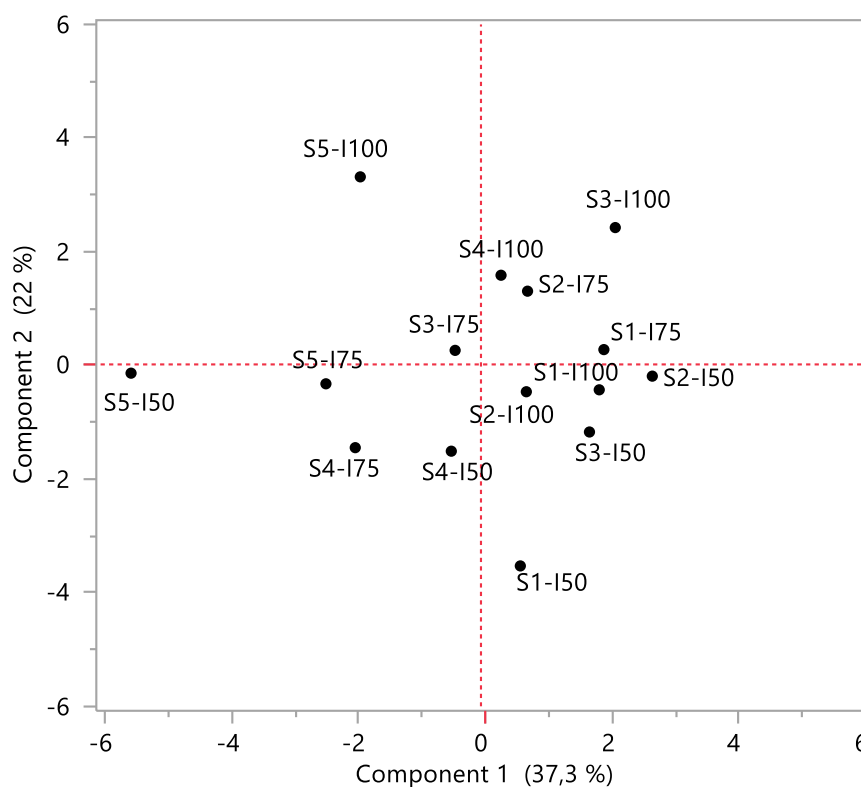
**Table 3.** Results of the principal component analysis (PCA) performed for biochemical contents, and photosynthetic activity in melon under salinity and irrigation deficit conditions

Items	PC1	PC2	PC3	PC4
Eigenvalue	4.87	2.86	1.69	1.16
Percentage of variance	37.30	21.98	13.03	8.91
Cumulative variance	37.30	59.27	72.30	81.21
Eigenvectors				
Chl a	0.354	0.346	0.030	0.096
Chl b	0.329	0.385	0.011	0.008
Car	0.304	0.339	0.026	-0.090
H <sub>2</sub> O <sub>2</sub>	0.216	-0.158	0.309	-0.313
MDA	0.353	-0.280	-0.052	0.183
Prot	0.172	0.035	-0.206	0.757
Prol	0.083	-0.450	0.194	-0.012
CAT	0.310	-0.280	-0.110	-0.011
SOD	-0.345	-0.060	-0.194	0.352
POD	-0.178	0.433	0.011	-0.080
T leaf	0.304	-0.095	0.442	0.225
gs	-0.328	-0.019	0.407	0.187
PSII	0.130	-0.168	-0.638	-0.245

\*Statistically significant according to  $P < 0.05$  and  $0.001$ . Chl a; Chlorophyll a, Chl b; Chlorophyll b, Car; Carotenoid, H<sub>2</sub>O<sub>2</sub>; Hydrogen peroxidase, MDA; Malondialdehyde, Prot; Protein, Prol; Proline, SOD; Superoxide dismutase, POX; Peroxidase, CAT; Catalase, gs; Stomatal conductance, PSII; actual photosynthetic efficiency, T Leaf; Leaf temperature



**Figure 2.** Loading plot for PC1 and PC2 as a result of PCA of the biochemical contents, and photosynthetic activity in melon under salinity and irrigation deficit conditions



**Figure 3.** Score plot for PC1 and PC2 as a result of PCA of the biochemical contents, and photosynthetic activity in melon under salinity and irrigation deficit conditions

#### 4. Conclusions

It was observed that saline irrigation water and water deficit applications applied to melon seedlings had significant effects on photosynthetic activity and biochemical contents. As a result of the research, it was revealed that there was a positive correlation between pigment contents in the plant and that they were important parameters in determining stress. It was observed that saline waters higher than 4000  $\mu\text{mhos}$  would limit plant development in the seedling period of melon. SOD enzyme activity was an important indicator of both stress factors and significant increases were experienced in SOD activity as stress increased. It was observed that 25% water restriction could be applied in the seedling period of melon in agricultural areas where irrigation water was limited, and that water saving would be achieved.

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