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Potential of Silicon and Melatonin Amendments to Enhance the Tolerance of Lettuce Plants to Water Deficit Stress

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HIGHLIGHTS

- Silicon treatments alleviated the impact of drought in lettuce.
- Melatonin induced significant improvement in lettuce under full irrigation.
- Silicon and melatonin together increased POD content in lettuce.

Abstract

Drought is one of the most significant problems nowadays, and it will be mandatory to cultivate crops with limited irrigation in the upcoming days. Some treatments are used to reduce the devastating effects of drought. Silicon (Si) and melatonin are popular treatments, and their efficacy was tested in lettuce under drought conditions for the purpose of this study. Thus, two doses of silicon (4 mM/8 mM), one dose of melatonin (150 µM), and their co-treatments were conducted in lettuce under two full irrigations (I100)/water-deficient (I25) irrigation regimes. The physiological, photosynthetic, and basic growth parameters were measured in lettuce. Based on these data, 8 mM silicon treatment significantly mitigates the severity of drought stress. It was found that the 8mM silicon treatment resulted in leaf dry weights similar to those of non-drought-treated plants, and leaf fresh weight was also slightly affected by drought. 8 mM silicon treatment also significantly improved the values of chlorophyll a, chlorophyll b, total chlorophyll, protein, and chlorophyll fluorescence in lettuce under drought stress. Additionally, melatonin treatments (150 µM) resulted in significant increases in the examined parameters of the non-drought-treated lettuce groups. Consequently, it was concluded that silicon fertilization alleviated the adverse effects of drought or limited irrigation conditions, whereas, under full irrigation conditions, silicon and melatonin treatments contributed significantly to lettuce cultivation.

Keywords: Silicon; Melatonin; Lettuce; Drought; Physiology

1. Introduction

The lettuce (*Lactuca sativa* L.) is a member of the Compositae family of leafy vegetables widely grown in cool seasons in Türkiye as well as in the world. It can be cultivated and accessed freshly throughout the year in Türkiye, and Türkiye is a significant lettuce producer (561.990 tons in 2022) due to its low calorie and high mineral and bioactive properties (Shi et al. 2022; TÜİK 2023). Lettuce groups commonly grown in Türkiye consist of leaf, Romaine (Cos), and Crisphead (iceberg). The common properties of lettuce that attract the attention of consumers are color, shape, and bioactive contents (Mampholo et al. 2016). While Michelon et al.

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(2020) reported an increase in yield and water use efficiencies in lettuce soilless cultivation. Casey et al. (2022) stated that the functioning of hydroponic systems requires a significant amount of energy and produces high environmental footprints. Hereby, the classical lettuce cultivation is more convenient and environmentally friendly for today (Casey et al. 2022). In any case, lettuce production with limited irrigation has come to the forefront in recent times. Especially, it is even more critical for producers located in a semi-arid climate zone such as Türkiye, where both classical (72.28%) and soilless (27.72%) lettuce cultivation is widely located in most production areas (TÜİK 2023).

The ever-increasing human population brings with it an enhanced demand for agricultural production. One of the most significant problems unveiled by the increasing population is the limited water resources used in agricultural irrigation due to climate change. These water limitations, known as drought, determine the fate of the ultimate usage of arable lands and the productivity of crops, together with salinization stress (Niu et al. 2014; Zhang et al. 2022). Some physiological impairments caused by drought stress, such as membrane damage, restricted cell enlargement, imbalanced redox homeostasis, cell respiration, and photosynthesis, lower yield and its components (Ahmad et al. 2018; Khan et al. 2021; Wahab et al. 2022). Particularly, vegetables containing 90% or more water and vegetables in the critical development period (fruit setting, flowering, etc.) were severely devastated by drought levels and durations (Khalid et al. 2023). To cope with the devastating effects of drought stress, two fundamental concepts are proposed by researchers: the development and usage of drought-resistance cultivars/genotypes (Li et al. 2023) and the usage of a mitigating agent approach (Tufail et al. 2023; Sun et al. 2023; Urbutis et al. 2023).

In the mitigating approach, some phytohormones, microelements, and amine/indoleamine derivatives applications have caused physiological and biochemical recoveries in plants under drought stress. Silicon is a highly abundant element in soil structure, available for plants in the form of monosilicic acid and stored as SiO2 in the leaves of plants. Melatonin, or N-acetyl-5-methoxytryptamine, is an indoleamine compound and is a multi-role stress-protective bioactive molecule. It could activate various responsive mechanisms within the plant cell during drought conditions, including regulating ascorbate-glutathione, nitrogen assimilation, protein biosynthesis, recovering chlorophyll content, and photosynthesis (Tiwari et al. 2021; Ren et al. 2021; Altaf et al. 2022). Common responsive mechanisms against drought stress triggered by the application of Si (metalloid) and melatonin (bioactive molecule) are to decrease the Reactive Oxygen Species (ROS) accumulation via enhancing the activity of antioxidant enzymes such as SOD, POD, and CAT; non-enzymatic antioxidant; reduce the transpiration rates, regulate the water balance of plants; and regulate light energy during photosynthesis (Zhang et al. 2018; Mavrič Čermelj et al. 2021; Altaf et al. 2022; Urbitus et al. 2023).

New and practicable strategies need to be adopted in lettuce production to address the drought stress worsened by increasing climate change. Previous studies have revealed that exogenous melatonin and silicon treatment separately can improve photosynthetic processes, promote antioxidant enzyme activity, and consequently contribute to drought tolerance in various species (Korkmaz et al. 2022; Seymen et al. 2024). On the other hand, no previously published study has investigated the effects of Si and melatonin on lettuce in response to drought stress. The primary objective of this experiment is to evaluate the growth patterns of lettuce plants treated with Si and melatonin under drought stress. Therefore, two practically applicable stress mitigants were selected and used to test on lettuce against drought under greenhouse conditions.

2. Materials and Methods

2.1. The Experimental Conditions and Design

The trial was conducted in the greenhouses of Selcuk University, Faculty of Agriculture. The temperature in the greenhouse was monitored between 9.2 and 23.4°C throughout the experiment, with an average temperature of 16.5°C. During the experiment, the relative humidity (RH) in the greenhouse ranged between 22.3% and 60.5% with an average relative humidity of 40.4% (Figure 1). In the study, the lettuce (*Lactuca sativa* L.) cultivar Yörünge was used as plant material and was purchased from Yüksel Tohum Company. The pots with a 19 cm bottom, 29 cm upper diameter, and 24 cm height were used in the experiment. First, the pots were filled with 10.4 kg of soil, and then 3-week-old lettuce seedlings were planted in all plastic pots by the experimental plan. Lettuce seedlings with a uniform and healthy structure were planted in pots. The lettuce seedlings were planted in April and harvested in May after treatments, and the measurements that were recorded. Fertilizers were supplied with the following instructions suggested by Şalk et al. (2008) for lettuce at the rate of 15 kg da^{-1} nitrogen (N), 10 kg da^{-1} phosphorus (P) and 18 kg da^{-1} potassium (K) to the pot before planting lettuce seedlings.

The research was experimented on in (12 treatments x 6 replicates) 72 pots according to a completely randomized experimental design. Applications consist of the following categories: two foliar applications of possible protectants (S0, S4, S8, Mel0, and Mel150), and two irrigation levels (full irrigation = I100, deficient irrigation = I25). Considering the studies on melatonin in various species, including lettuce, it was observed that the optimum application dose varied between 100 and 200 µM (Can 2023; Kiremit et al. 2024). It was preferred to use a dose of 150 µM due to this perspective. AGRISILICA (26% soluble silicon) granule fertilizer was used as a silicon resource. Most of the studies on silicon have generally focused on concentrations of 2-4 mM Si (as SiO_2). In this study, considering the available research, 4 mM was selected as the optimum dose and 8 mM as the high Si dose. The lettuce seedlings were watered with tap water for the first eight days for adapting to the pots, and then drought stress was applied. Melatonin foliar and silicon application to pots were started 8 days after lettuce seedlings were planted together with drought stress. The silicon was applied once in the soil, and melatonin was applied once in the leaf throughout the experiment. Drought stress was applied to lettuce seedlings as described by Ünlükara et al. (2008). In this regard, the pots were weighed separately with a precision balance after being filled with soil, and then the pots were brought to field capacity and weighed again. Thus, the amount of water necessary to create drought stress was calculated using these weights. The lettuce seedlings were irrigated with 25% of the field capacity value to create drought stress while the control group was irrigated with full irrigation. In order to control the water loss due to evapotranspiration, the pots were checked daily with an electronic scale, and irrigation was provided for the deficient pots.

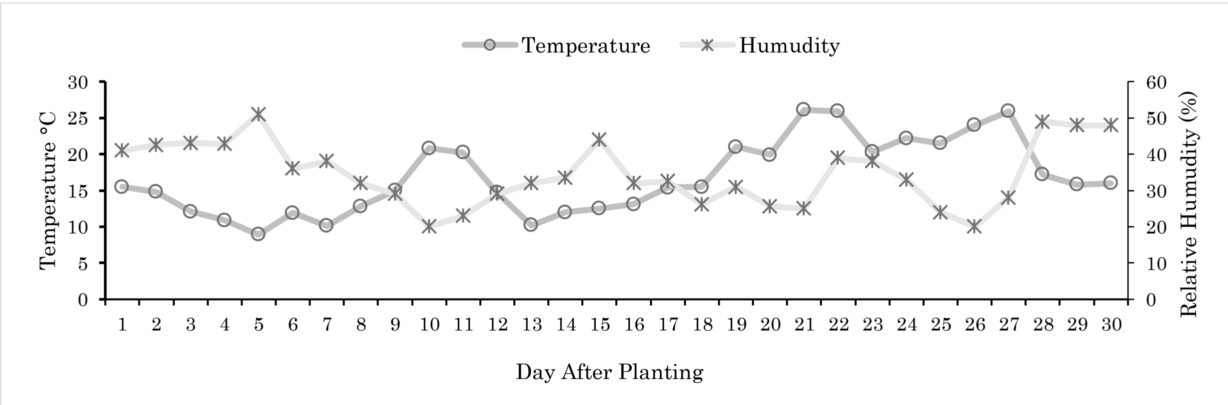

Figure 1. Average temperature and humidity records of experimental area

2.2. *Morphological Parameters*

During sampling, lettuce plants were carefully removed and washed with tap water and distilled water, respectively. After the wetness of the samples was dried, the fresh weights of lettuce leaves (LFW) and fresh weights of lettuce roots (RFW) were immediately weighed with a precision balance and expressed as grams (g). The lettuce roots and leaves were dried in a hot air oven at 70°C until they reached a constant weight, after which the leaf dry weight (LDW) and root dry weight (RDW) were weighed with a precision balance and expressed as grams (g).

2.3. *Chlorophyll Fluorescence Measurements*

Before harvesting, chlorophyll fluorescence was measured using a portable LI-600 fluorometer on fully expanded lettuce leaves between 9:00 and 11:00 under clear weather conditions. To measure photosynthetic activity, PhiPSII quantum yield, stomatal conductance, and leaf temperature were measured by a LI-600 fluorometer (Liang et al. 2019).

2.4. Physiological and Biochemical Analyses

The method proposed by Beyer Jr. and Fridovic (1987) was used to measure the SOD content in lettuce at the end of the experiment. Fresh leaves (0.5 g) were digested in liquid nitrogen and homogenized with 1.5 ml of extraction solution [100 mM K-PO_4 buffer (pH 7.0), 2% PVP (polyvinylpyrrolidone) and 1 mM Na_2 EDTA]. This mixture will be centrifuged at 14000 rpm for 20 minutes at +4°C and the supernatant will be mixed with 50 mM phosphate buffer (pH 7.8), 9.9 mM L-methionine, 57 μM NBT, and 1% Triton X-100, and 10 μL riboflavin will be added to start the reaction. Total SOD activity will be calculated in units mg protein^{-1}. The activity of one enzyme unit is defined as the amount of SOD required to produce a 50% inhibition of NBT reduction.

The method proposed by Chance and Maehly (1955) was used to measure the POD content in lettuce at the end of the experiment. The reaction mixture is 3 mL and contains 100 mM potassium phosphate buffer (pH 7.0), 20.1 mM guaiacol, 12.3 mM H_2O_2 and enzyme extract. The reaction will be triggered by the addition of the enzyme extract and monitored over a period of 10 min. Total POD enzyme activity will be calculated from the reaction initial rate (nmol H_2O_2 min^{-1} mg protein^{-1}) using the extinction coefficient of guaiacol (26.6 mM cm^{-1}, 470 nm).

The protein content in the lettuce seedlings was determined by the Bradford method using 0.5 grams of leaves (Bradford 1976). Leaf samples were ground in 0.05 M phosphate buffer (pH 6.5), and the samples were centrifuged at 15,000 rpm for 20 minutes to quantify the protein. After centrifugation, 0.2 mL of the supernatant was added to 3 mL of Bradford dye, and the samples were measured at 595 nm.

Chlorophyll-a, chlorophyll-b, total chlorophyll, and total carotenoid contents in lettuce leaves were determined by the method described by Lichtenthaler and Buschmann (2001) and Witham et al. (1971), respectively. An amount of 0.2 g of leaf material was extracted in the dark place with 3 mL of 100% acetone solution in a pre-cooled mortar and pestle. After extraction, the homogenate was filtered, and the volume of the homogenate was made up to 5 mL using cold acetone. The absorbance values of the homogenate at 470, 645, and 662 nm were measured using a Shimadzu-UV1900i spectrophotometer. Lichtenthaler and Buschmann (2001) equations were used to calculate the amount of these photosynthetic pigments;

$$\text{Chl a} = 11.75\, A662 - 2.350\, A645$$

$$\text{Chl b} = 18.61\, A645 - 3.960\, A662$$

$$\text{Carotenoid} = 1000\, A470 - 2.270\, Ca - 81.4\, Cb/230$$

2.5. Statistical Analysis

Statistical analysis of the data generated within the scope of the study was performed with the Minitab 19 software (Mathews 2004). The data were first subjected to ANOVA analysis. Then the results were tested at a 5% significance level according to Fisher's exact test to determine statistically significant differences between the means of different groups. The same variables were subjected to Principal Component Analysis (PCA) and Pearson correlation coefficient using R Statistical Software, and the parameters found to be significant for melatonin and silicon treatments under drought stress in lettuce seedlings were discussed in detail.

3. Results and Discussion

The results in Figure 1 and Figure 2 indicate that vegetative growth (root-leaf fresh weight) and dry matter (root-leaf dry weight) were significantly changed ($p \leq 0.05$) by different doses of silicon and melatonin treatment under drought stress. Considering Figure 1, 8 mM silicon (I100+S8+M0) and 4 mM silicone+150 mM melatonin (I100+S4+M150) treatments resulted in a significant increase in leaf fresh weight (LFW) of non-drought treated groups compared to the control group (I100+S0+M). In terms of drought stress and treatments, all treatments significantly increased the lettuce leaf fresh weight under drought stress compared to the untreated group (I25+S0+M0), and this increase was highest in the I25+S8+M0 group (relatively higher than I25+S0+M0).

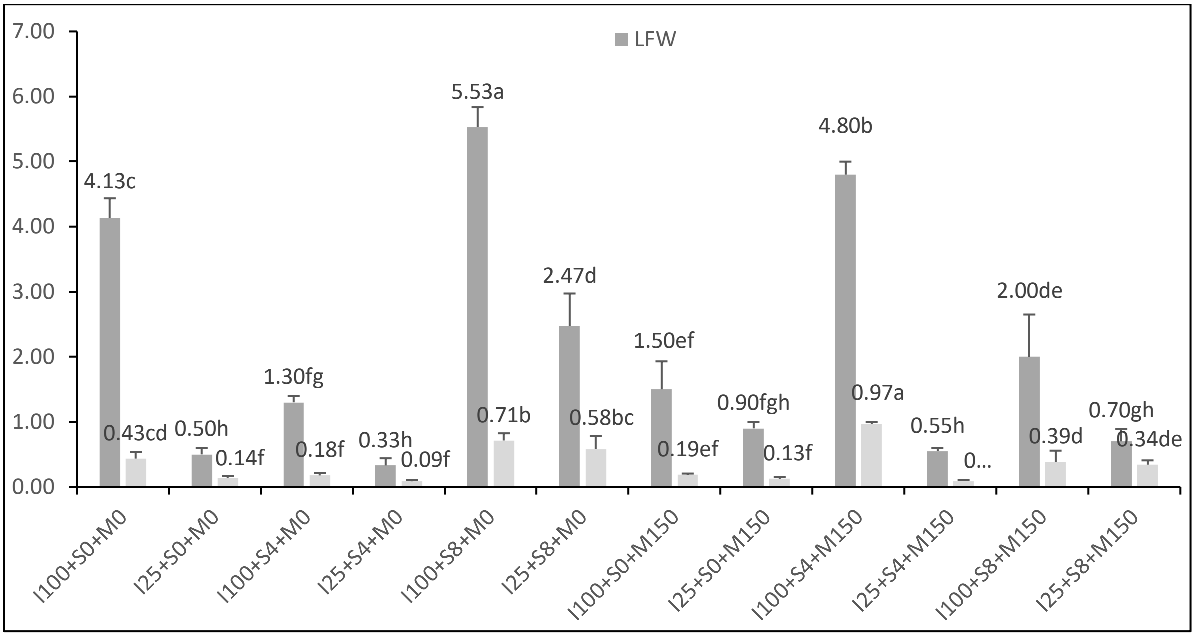

Figure 2. Leaf fresh and dry weight of lettuce (g), different letters represent statistical significance ($p<0.05$). I100: full irrigation, I25: drought, S0: non-silicon, S4: 4 mM silicon, S8: 8 mM silicon, M0: non -melatonin, M150: 150 mM melatonin.

It is even noticed that the LFW of the I25+S8+M0 group is higher than that of the I100+S0+M150 and I100+S8+M150 groups respectively. In other words, the 8 mM silicon application resulted in about 5-fold higher fresh weight compared to the I25+S0+M0 group, which not only performed the best among the drought treatments but also competed with some of the full irrigation groups (I100+S0+M150 and I100+S8+M150). In the I25+S8+M0 group, the LDW of lettuce was significantly higher than the control group (I100+S0+M0) and other drought-treated groups (I25+S0+M0, I25+S4+M0, I25+S4+M150, I25+S0+M150, I25+S4+M150, I25+S8+M150).

In some studies, the application of silicon alone without stress caused decreases in plant fresh weights. The general trend is that silicon application together with stress reduces the decrease rate of shoot fresh weights (Pei et al. 2009; Haghighi and Pessarakli, 2013). From this perspective, it is seen that Si, which was applied with drought stress in this study, contributed to the dry and fresh weights at different rates and reduced the effect of drought.

Considering Figure 3, the highest root fresh weight (RFW) values of lettuce in all irrigation treatments were recorded in the 8 mM silicon application of I100+S8+M0. Figure 3 also indicates that the 8 mM silicon treatment in drought lettuce (I25+S8+M0) significantly contributed to root growth, even exceeding the performance of the control groups (I100+S0+M0, I100+S4+M0, I100+S8+M0, I100+S0+M150, I100+S4+M150, I100+S8+M150). In lettuce, silicon application alone significantly increased RFW both under drought conditions (I25+S8+M0) and under full irrigation (I100+S8+M0) conditions. However, this was not observed in melatonin treatment alone (I25+S0+M150, I100+S0+M150). In parallel with the fresh weight, the highest lettuce root dry weight was also determined in the I25+S8+M0 and then in the I100+S8+M0 group.

Drought is a worldwide stress factor, causing severe biomass losses in crops, including lettuce. Drought affects the mobilization of stored nutrients for the embryo by affecting α-amylase, β-amylase, and proteases in seed germination, and during growth and development by reducing water content in leaves, closing stomata, and reducing cell growth (Hameed et al. 2021; Razi and Muneer 2021). In order to mitigate the adverse effect of drought on biomass production, various practices, including biochar, plant growth-promoting bacteria (PGPR), microelements, and some chemical treatments, are being employed for that purpose. Previous studies have reported that the application of silicon and melatonin alone under water-limited conditions mitigates the reduction in biomass (Hidalgo-Santiago et al. 2021; Kiremit et al. 2024). In the present study, an 86% reduction in LFW and a 51% reduction in RFW were detected under drought stress. Hidalgo-

Santiago et al. (2021)'s research reported significant biomass reduction in lettuce irrigated at 75% of field capacity, and this reduction was lowered when a silicon-containing fertilizer was applied. Shaffique et al. (2024) research reported that melatonin treatment significantly restored the shoot and root length of tomato seedlings under drought stress. In the present study, silicon application alone (I25+S8+M0) lowered the rate of reduction in lettuce LFW from 86% to 40% compared to the control group (I100+S0+M150), while lettuce RFW increased (I25+S8+M0) approximately twice as much as the control group (I100+S0+M150). Although melatonin and silicon applications caused biomass reductions at varying rates in parallel with previous studies, the greatest effect was observed in the 8 mM silicon application, and silicon application under drought conditions caused the RFW to increase above the control group, unlike other studies. The most probable explanation for this increase in lettuce RFW is an increase in the content of aquaporin in the root zone and an increase in osmotic regulation agents to restore water balance. The dry weight results in this study also support this explanation. When the dry weight data were compared (I100+S0+M0 and I25+S8+M0), it was observed that lettuce root and leaf showed related results (Figure 1 and Figure 2). Therefore, it is possible to conclude that this difference is due to the water contents of the lettuce leaf and root, and Si application improved root and leaf water contents under drought conditions. It is known that silicon-supplied studies in lettuces have low canopy temperature and high-water potential (Villa e Vila et al. 2024).

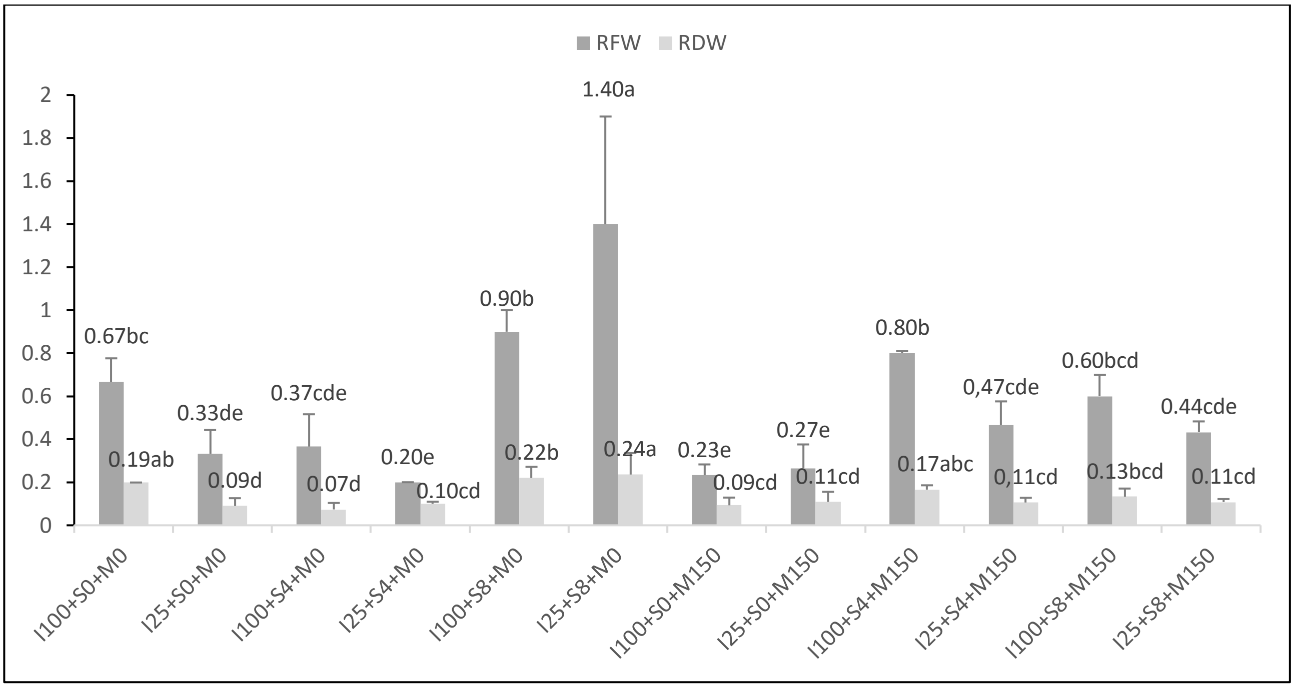

Figure 3. Root fresh and dry weight of lettuce (g), different letters represent statistical significance ($p<0.05$). I100: full irrigation, I25: drought, S0: non-silicon, S4: 4 mM silicon, S8: 8 mM silicon, M0: non-melatonin, M150: 150 mM melatonin.

In Figure 4, while the total chlorophyll content of lettuce leaves decreased at varying rates (in I25+S0+M0, in I25+S4+M0, I25+S0+M150 and I25+S4+M150, I25+S8+M150) in limited irrigation treatments compared to the control group (I100+S0+M0). In contrast to this decrease pattern, it was observed that chlorophyll content was significantly higher in the I25+S8+M0 group compared to the I100+S0+M0 group. The highest total chlorophyll content was observed in the I100+S4+M0 (28.58) and I100+S4+M150 (37.46) full irrigation groups compared to control group (I100+S0+M0). The results indicated that 4 mM silicon treatment increased leaf chlorophyll pigment in non-drought-treated lettuce leaves, whereas 8 mM silicon treatment contributed highly to lettuce leaf chlorophyll content under drought conditions. However, melatonin + silicon treatments did not show a significant change in lettuce leaf total chlorophyll content under limited irrigation pattern (I25+S0+M0, I25+S0+M150, I25+S4+M150 and I25+S8+M150).

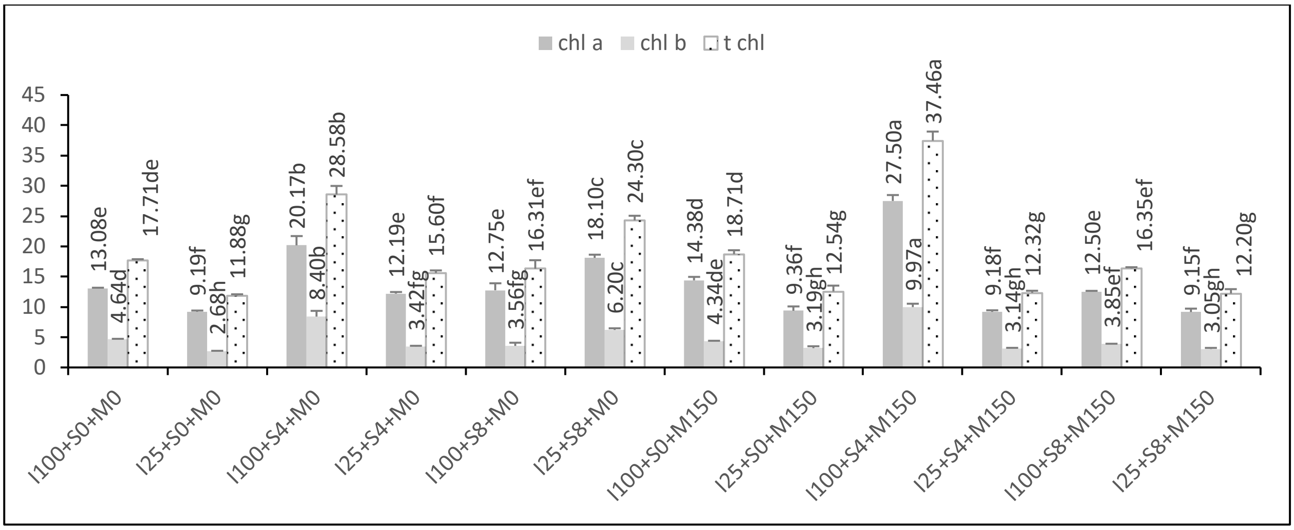

Figure 4. The pigments content (Units?) in lettuce, different letter represent statistical significance ($p<0.05$). chl a: chlorophyll a, chl b: chlorophyll b, t chl: total chlorophyll, I100: full irrigation, I25: drought, S0: non-silicon, S4: 4 mM silicon, S8: 8 mM silicon, M0: non-melatonin, M150: 150 mM melatonin. (Chlorophyll contents expressed as mg g^{-1})

The melatonin and silicon-treated fully irrigated lettuce leaves carotenoid contents were increased significantly compared to the I100+S0+M0 group. Regarding carotenoid content in drought-treated lettuce seedlings, only the 8 mM silicon treatment caused improvement. In contrast to the carotenoid content, all silicon and melatonin treatments except for I25+S4+M150 significantly increased the protein content of lettuce seedlings under drought stress compared to the I100+S0+M0 group (Figure 5). As in the carotenoid content, this increase was highest in the I25+S8+M0 and I100+S8+M150 groups. Oxidative stress and subsequent reduction in chlorophyll activity and protein breakdown are among the first responses of lettuce plants against drought stress (Shaffique et al. 2022). Shin et al. (2021) found that drought stress did not affect the chlorophyll content of lettuce on the 4th day of drought, while significant decreases in chlorophyll content were reported on the 8th day of drought. Damage to the chloroplast membrane/structure, chlorophyll photo-oxidation, high chlorophyllase activity, and inhibition of chlorophyll synthesis were reported to be the factors reducing chlorophyll content under drought conditions (Chakhchar et al. 2018; Kapoor et al. 2020). As a result, drought generates oxidative stress, which impairs the photosynthetic mechanism and damages the protein structure, thereby leading to cell expansion limitation, increasing lipid peroxidation, and hence inhibiting plant growth (Ors et al. 2021; Razi and Muneer 2021). Carotenoids are crucial for the maintenance of photosynthesis by protecting membrane lipid photo-destruction, acting as an antioxidant agent, and reducing the light pressure on chlorophyll by absorbing excess energy (Maslova et al. 2021). Plants increase some osmoprotectants and protein soluble content in order to maintain cellular turgor under drought stress (Ahmad et al. 2022). In Leitão et al. (2021)'s proteomic study, it was found that sulfurtransferase and aquaporin proteins accumulated in lettuce leaves as a result of abiotic stress. It is predicted that these proteins will provide resistance to drought stress by protecting photosynthesis through their antioxidative properties of sulfurtransferase and increasing water transport efficiency through their water channel properties. To reduce the common detrimental effect of drought, the successful results have been obtained in lettuce by using following applications such as selenium, silicon, organic acid, and melatonin (Kiremit et al. 2024; Kleiber et al. 2024; Tufail et al. 2023; Villa e Vila et al. 2024). In parallel with the mentioned results, in this study, a significant increase in chlorophyll, carotenoid, and protein content of lettuce was detected in the 8 mM silicon application under dry conditions. In particular, melatonin application resulted in increases in the studied parameters of lettuce under full irrigation conditions, while silicon applications resulted in significant improvements in lettuce under drought conditions.

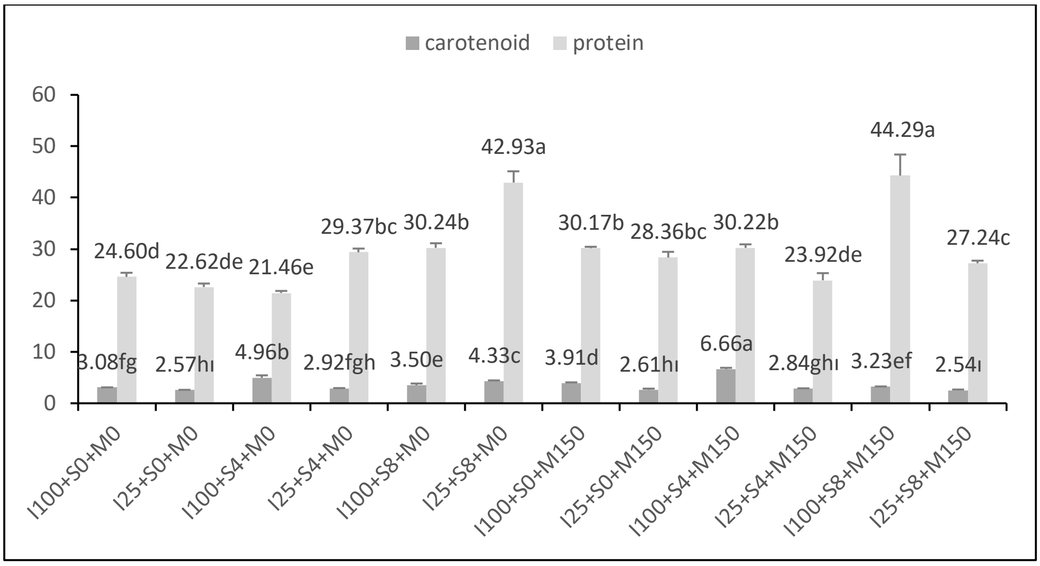

Figure 5. The carotenoid and protein content in lettuce, different letters represent statistical significance (carotenoid content expressed as mg g^{-1}, protein content expressed as μg g^{-1}) ($p<0.05$). I100: full irrigation, I25: drought, S0: non-silicon, S4: 4 mM silicon, S8: 8 mM silicon, M0: non-melatonin, M150: 150 mM melatonin.

Figure 6 summarizes the status of antioxidative enzymes in silicon- and melatonin-treated lettuces under drought conditions. Based on these data, SOD enzyme increases were recorded in the I25+S8+M150, I25+S4+M150, and I25+S0+M150 groups compared to the I100+S0+M0 group. According to the findings of the study, the highest activity of SOD and POD was observed in the I25+S4+M150 lettuce group. Regarding these findings, in the I25+S8+M150 group, silicon and melatonin treatments enhanced SOD activity and decreased POD activity compared to I25+S0+M0 group. One interesting detail was observed in POD enzyme activity, and while melatonin only slightly increased POD activity in drought-treated groups, silicon and melatonin co-treatment caused a significant increase in POD activity. It is known that the activity of antioxidant enzymes such as SOD, POD, and CAT increases under drought stress in different plant species, including lettuce (Ekinci et al. 2020; El-Bauome et al. 2024; Erol 2024).

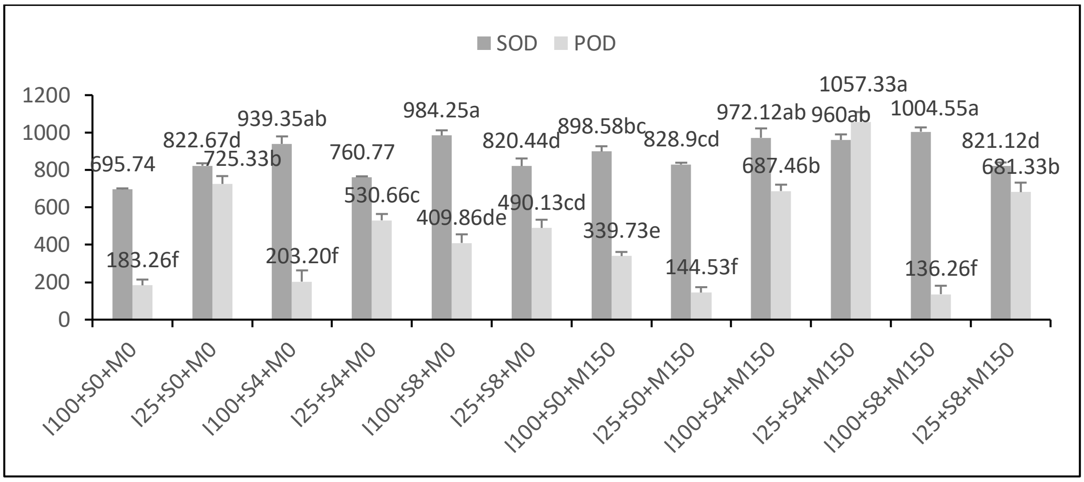

Figure 6. The SOD (μmol min^{-1} mg^{-1} FW) and POD (μmol min^{-1} mg^{-1} FW) content in lettuce, different letters represent statistical significance ($p<0.05$). I100: full irrigation, I25: drought, S0: non-silicon, S4: 4 mM silicon, S8: 8 mM silicon, M0: non-melatonin, M150: 150 mM melatonin

Silicon treatments have been reported to increase POD activity in drought, salinity, and heavy metal stress treatments in different plants (Akhtar et al. 2021; Basilio-Apolinar et al. 2021; Bhardwaj et al. 2024). Bhardwaj et al. (2024) reported that nitric oxide application together with Si significantly increased POD content in *Raphanus sativus* and also stated that Si+NO treatment improved the plant defense system during abiotic stress conditions by triggering antioxidant enzymes. Thus, while POD individually targets extracellular H_2O_2, SOD contributes to plant abiotic stress tolerance rate by targeting superoxide radical. In summary, silicon and melatonin decreased the pressure on energy production and photosynthetic mechanisms with the assistance of the antioxidant enzyme defense system in lettuce under drought stress, while silicon treatments had a positive effect on lettuce water balance.

Table 1 provides the parameters related to photosynthesis and chlorophyll fluorescence. There was a statistically significant difference between drought and whole irrigation groups of lettuce with respect to leaf temperature, stomatal conductance (GSW), and quantum productivity of photosynthesis (PhiPSII). In melatonin and silicon treatments, there was a statistical difference between drought and whole irrigation groups with respect to leaf temperature, stomatal conductance, and PhiPSII. Leaf temperature, stomatal conductance, and PhiPSII have a crucial role in the optimal functioning of the photosynthetic machinery. The higher chlorophyll a, carotenoid, and total chlorophyll content also led to better performance of PhiPSII in the drought-treated lettuce group (I25+S8+M0). The higher content of chlorophyll a, and carotenoid, and better functioning of PhiPSII were also confirmed in the study by Lemos Neto et al. (2021). There is another parameter that ensures the maintenance of photosynthesis: stomatal conductance, which ensures the supply of gas exchange. In the present study, silicon treatment under drought conditions contributed to photosynthesis and subsequently to the dry matter content of lettuce by increasing stomatal conductance and gas exchange (continuity). The present findings are also supported by the results of the study conducted by Hidalgo-Santiago et al. (2021). The stomatal conductance also resulted in a decrease in canopy temperature as a natural consequence of gas exchange through the leaf in the silicon-treated I25+S8+M0 group. The low canopy temperature studies with the aid of silicon treatment have been recorded in many species (Kiremit et al. 2024; Seymen et al. 2024b; Villa e Vila et al. 2024), and this study is in parallel with these studies.

Table 1. Measurement of some photosynthetic parameters in lettuce under water deficit stress

Treatments		Photosynthesis		
		Tleaf (°C)	GSW (mol m^{-2} s^{-1})	PhiPSII
Irrigation	I100	25.43^{b}	0.521^{a}	0.671^{a}
	I25	26.11^{a}	0.391^{b}	0.593^{b}
Silicon	S0	25.80^{ns}	0.426^{b}	0.593^{b}
	S4	25.79^{ns}	0.443^{b}	0.630^{b}
	S8	25.71^{ns}	0.500^{a}	0.674^{a}
Melatonin	M0	25.77^{ns}	0.469^{a}	0.656^{a}
	M150	25.50^{ns}	0.443^{b}	0.608^{b}
Irrigation x Silicon x Melatonin Interactions				
	I100+S0+M0	25.16^{d}	0.508^{a}	0.617^{ab}
	I100+S4+M0	25.10^{d}	0.520^{a}	0.631^{ab}
	I100+S8+M0	25.10^{d}	0.514^{a}	0.733^{a}
	I100+S0+M150	25.64^{cd}	0.510^{a}	0.683^{ab}
	I100+S4+M150	25.54^{cd}	0.510^{a}	0.702^{ab}
	I100+S8+M150	26.02^{bc}	0.552^{a}	0.672^{ab}
	I25+S0+M0	27.02^{a}	0.318^{b}	0.413^{c}
	I25+S4+M0	26.95^{a}	0.378^{b}	0.586^{b}
	I25+S8+M0	25.26^{d}	0.578^{a}	0.672^{ab}
	I25+S0+M150	25.38^{cd}	0.356^{b}	0.658^{ab}
	I25+S4+M150	25.56^{cd}	0.364^{b}	0.631^{ab}
	I25+S8+M150	26.95^{ab}	0.356^{b}	0.631^{ab}

ns: non-significant, a,b,c Values within a row with different superscripts differ significantly at $P<0.05$, PhiPSII- quantum productivity of photosynthesis, GSW: Stomatal Conductance, Tleaf: leaf temperature.

The correlation matrix (Pearson) was generated using the features included in the study (Figure 7). In the current study, performed with silicon and melatonin treatments in limited irrigated lettuce, a negative moderate statistically significant relationship was found between Tleaf-PhiPSII, Tleaf-GSW, Tleaf-LFW, POD-PSII, and POD-GSW. The study also revealed a moderate positive statistically significant relationship between chlb-PhiPSII, chlb-GSW, chlb-LFW/LDW, Car-PhiPSII, Car-GSW, car-LFW, chl a-PhiPSII, chl a-LFW/LDW, chl a-GSW, t chl-PtiPSII, t chl-GSW, t chl-LFW/LDW, PhiPSII-LFW, GSW-protein, GSW-RFW, GSW-LFW/LDW, protein-RFW. A statistically significant and a very strong positive correlation was found between chl b-car, chl b-chl a, chl b-t chl, car-t chl and chl a-t chl. The final crucial finding extracted from Figure 7 is that there is a statistically significant and strong positive correlation between PhiPSII-GSW. According to the data, there is a positive correlation between stomatal conductance and protein content, root fresh weight, leaf fresh and dry weights of lettuce treated with silicon+melatonin under drought stress and stomatal opening-fresh/dry increase is confirmed by some other studies (Akhtar et al. 2022; Alotaibi et al. 2023; Ramadan et al. 2024). In the present study, it is clearly found that silicon treatment has a significant positive effect on stomatal conductance in lettuce. Arif et al. (2021) explain that silicon has a positive effect on stomatal conductance and gas exchange by stimulating genes related to ABA biosynthesis, and these positive effects are reflected in the photosynthetic properties of the plant. Evidence supporting this study from a distinct perspective comes from the study of Markovich et al. (2022), and they state that drought application in combination with low silicon fertilization reduces the stomatal conductance. However, the positive correlation between PSII of chlorophyll and carotenoid pigments, stomatal conductance, and leaf fresh-dry weights also indicates that the photosynthetic mechanism remained unaffected for a certain period of time under drought stress in lettuce treated with silicon (Bukhari et al. 2021; Fatemi et al. 2020; Hassan et al. 2021; Seymen et al. 2024a). Moreover, as a natural consequence of the strong correlation between PSII and GSW, an increase in fresh and dry weight was observed in silicon-treated lettuce under drought stress in the present study.

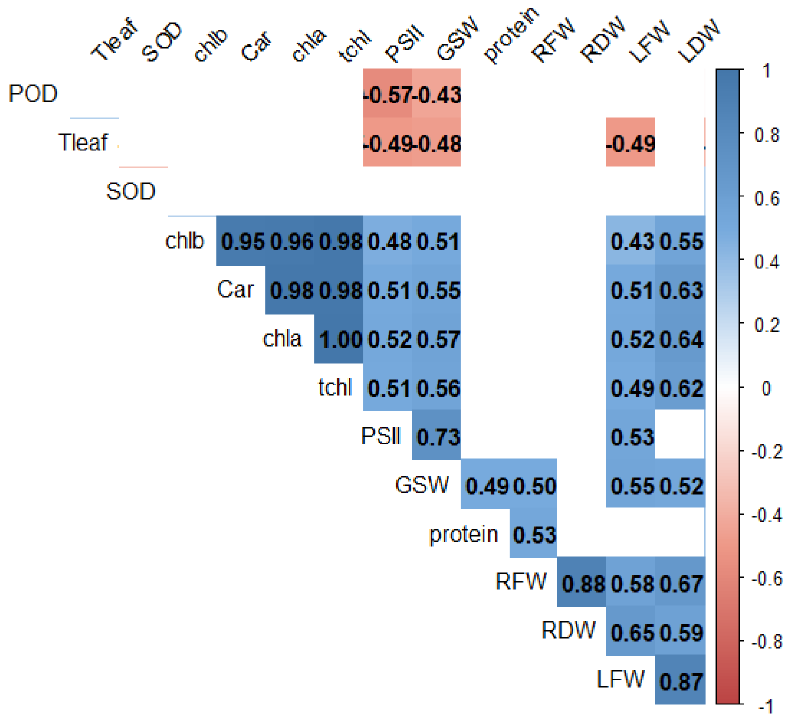

Figure 7. Pearson correlation matrix between the variables (blank boxes represent statistically insignificant data; the remaining-colored boxes are all statistically p<0.001)

The principal components graph provided in Figure 8 and Table 2 explains 63.57% (the first two components) of the total variation. 83% of the total variation is explained by 4 components. The first component explained 48% of the variance and was the most significant contributor. Except for the Tleaf and POD parameters, the other parameters contributed significantly to explain 48% of the variance of PC1 (Figure 8 and Table 2). On the other hand, the second component explained 14.8% of the variance, with Tleaf and POD contributing the highest to the variance. Explaining 60% or more variation in the first two components has been used in many studies as an acceptable threshold and has been used in data classification (Zhang et al. 2018; Martinez et al. 2020).

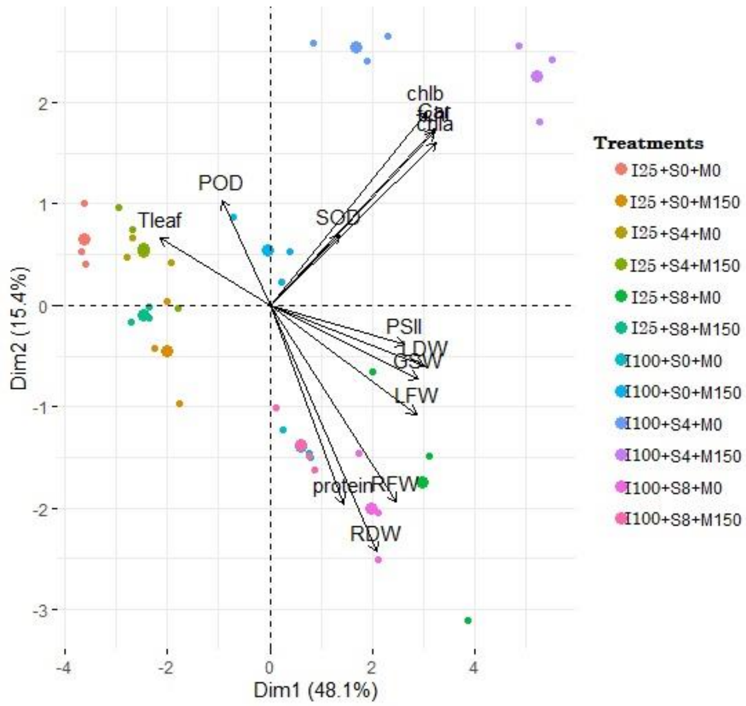

Figure 8. Principle Component analyses, Biplot graph visualized from PC1 and PC2 including variables

Table 2. Principle Component analyses results extracted from included traits

Items	PC1	PC2	PC3	PC4
Eigen value	6.738	2.162	1.596	1.065
Percentage of variance	48.12	15.45	11.40	7.60
Cumulative variance	48.12	63.57	74.97	82.57
Eigen vectors				
LFW	-0.296	0.196	-0.107	0.167
LDW	-0.313	0.109	-0.261	0.010
RFW	-0.256	0.354	-0.330	-0.020
RDW	-0.215	0.445	-0.282	0.179
Chl a	-0.336	-0.294	-0.046	0.079
Chl b	-0.316	-0.349	-0.019	0.117
Tchl	-0.333	-0.313	-0.039	0.091
Car	-0.333	-0.318	-0.066	-0.014
Protein	-0.149	0.358	0.097	-0.448
SOD	-0.140	-0.126	0.029	-0.781
POD	0.095	-0.188	-0.645	-0.273
Tleaf	0.222	-0.121	-0.144	-0.091
PSII	-0.272	0.070	0.446	-0.039
GSW	-0.300	0.133	0.275	-0.117

In the graph, all the points placed in rectangles indicate drought-treated lettuces with silicon and melatonin, while the points placed in the circle belong to the I25+S8+M0 group. The biplot plot indicates that the means of all treatments were placed at a different location from the origin and were grouped into three different treatments whose means differed from each other in general. In the study conducted by Sattar et al. (2023) similar to the present study, the treatments were grouped into 4 distinct categories: individual silicon-melatonin under drought, individual silicon-melatonin to control, combined silicon-melatonin drought and combined silicon-melatonin under drought. Except for I25+S8+M0, in the present study, the silicon, melatonin, and silicon+melatonin treated groups formed one cluster under drought conditions, while the silicon, melatonin, and silicon+melatonin treated groups formed two clusters under full irrigation conditions (first group: I100+S4+M0, I100+S8+M150, second group: I100+S0+M0, I100+S8+M0, I100+S8+M150 and I25+S8+M0). The I25+S8+M0 group was located in the area where the non-drought-treated groups were located, which is another indicator that silicon treatment significantly mitigated adverse effects of drought. It has been reported by Kal et al. (2023) that the principal component analysis method provides significant insights in stress studies. As evidenced in Figure 8, the LFW, LDW, RFW, RDW, GSW, protein, and PSII are the variables used in the non-drought-treated group allocation, and it validated with correlation analysis (Figure 7) that have a critical impact on biomass production. On the other hand, the variables Tleaf, POD, and partly SOD were also used as indicators for the allocation of drought stress-related groups. Faisal et al. (2024) used some of these variables effectively in the allocation of treatments to alleviate heavy metal stress in PCA analysis, and these results generated consistent results with this study.

4. Conclusions

In this study, drought stress in lettuce and melatonin/silicon as a stress mitigation agent were tested in greenhouse conditions. Out of all treatments, 8 mM silicon treatment reduced the severe effect of drought by improving various parameters such as RFW, RDW, LFW, LDR, PSII, GSW, and protein in lettuce. Based on the results of the present study, it was observed that silicon fertilization improves lettuce cultivation under limited irrigation conditions, which can be applied in practice. The study also revealed two more key points: (1) melatonin treatment of well-watered lettuce resulted an increase in biomass and some related parameters, and (2) co-treatment of silicon and melatonin resulted in an increase in POD values. Consequently, the physiological investigation of these two points will be a priority in the following periods, and silicon fertilization and melatonin treatments will contribute to lettuce cultivation at varying rates.

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