International Journal of Agricultural and Natural Sciences
E-ISSN: 2651-3617 14(2):61-77, 2021
Research Article

IMPACTS OF WATER STRESS AND HARVEST TIME ON PHYSIO-BIOCHEMICAL CHARACTERISTICS OF LETTUCE (*Lactuca sativa* L.)

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(Received 3th June 2021; accepted 06th August 2021)

ABSTRACT. Water scarcity has emerged as a serious concern for the agricultural sector. There is an urgent need to conserve the existing water resources by using them economically. Moreover, it can prevent the excessive use of water in agriculture by determining the water requirement of plants during different periods. This study determined the changes in the growth and physiological and biochemical contents of lettuce grown under limited irrigation conditions during different developmental periods. The study was designed with three replications in a random trial pattern of 20 parcels. Five irrigation levels, namely I_{100}, I_{80}, I_{60}, I_{40}, and I_{20}, and four harvest times, were used according to the development period of lettuce. The principal component analysis revealed significant changes in physiological and biochemical parameters, such as leaf water content (LWC), membrane damage (MD), carotenoids (CT), protein (PT), proline (PL), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), under water stress conditions in lettuce. In addition, significant changes in growth parameters were observed during harvest periods. Water productivity (WP) values ranged from 6.81 to 22.83 g/L, and the highest WP value was obtained at I_{100} irrigation level and harvest-IV. The I_{100} irrigation level in the fourth harvest period yielded the best results. The results show that water could be saved with limited irrigation in arid and semi-arid regions, with water scarcity of 48 days, after planting the lettuce seedlings.

Keywords: *Deficit irrigation, Antioxidant enzyme, Lettuce, PCA*

INTRODUCTION

With the continuous increase in the population, global warming, and rapidly exhausting water resources due to their inefficient use, the probability of meeting the daily irrigation needs during the development periods of agricultural plants is rapidly declining worldwide [1]. Moreover, drought stress has been reported to exert negative effects on the development of plants in approximately 45% of the world's agricultural areas [2]. Water scarcity is a global concern that has affected one-third of the world population; the problem is on the rise due to the increasing demand for water to meet the needs of the growing population, urbanization, industries, and agriculture. If the problem is not addressed immediately, it is believed that two-thirds of the world population will suffer from acute water shortage by 2025 [3].

In agriculture, water has a decisive effect on the vegetative production patterns and yield per unit area. The common approach in irrigation water management is to use water resources economically under conditions of insufficient water supply and to obtain the highest quality and quantity of the product with the existing water conditions [4]. Therefore, several studies have been conducted recently to find drought-tolerant genotypes [5] and their effects on the growth and yield of different plant species under limited irrigation conditions [6, 7]. It is an important criterion in the evaluation of water productivity irrigation programs in limited irrigation studies [8]. It has been explained in previous studies that significant increases in water productivity were achieved with the increase in water stress in limited irrigation works [6, 9, 10, 11]. This situation shows that limited irrigation is an important strategy in terms of saving water in arid and semi-arid regions with limited water resources. Plants have developed certain mechanisms to reduce and prevent the impact of stress factors on their tissues and thus survive the conditions. Furthermore, they can activate the antioxidant defense systems by secreting soluble substances [12]. In addition, plants can produce reactive oxygen species (ROS) under stress that react with protein, lipids, and other important macromolecules, and denature their structures, and impair the functions [13]. The generation of ROS activates several enzymes, such as glutathione S-transferase (GST), SOD, CAT, and POD [14, 15]. These biochemical changes are reflected as morphological changes in plants, depending on the severity and duration of the stress.

Lettuce (*Lactuca sativa* L.) is an important vegetable species that is cultivated worldwide and consumed as both fresh leaves or as a prepared vegetable. Its total production amounts to 27.3 million tons worldwide, with China, USA, India, Spain, Italy, Japan, Iran, and Turkey, reporting the highest production in that order [16]. Due to its high-water content and superficial root system, its leaves require a high level of irrigation; therefore, it is a drought-sensitive species [17]. Therefore, limited irrigation practices in lettuce are an important issue for developing new irrigation strategies. However, it is sensitive to water constraints in limited irrigation conditions. Moreover, the water requirements of the plant during different vegetation periods vary, and thus water limitation can severely affect the yield. However, it is possible to save some of the irrigation water required in the early periods. For this purpose, in addition to determining the effects of different irrigation levels, with a comparative analysis, on the growth and physiological and biochemical contents during the harvest at different vegetation periods, important parameters were analyzed that revealed the effect of water deficit and harvest period.

MATERIALS AND METHODS

Experimental area

The present study was conducted between November 29, 2019, and January 27, 2020, in glass greenhouses at the Faculty of Agriculture, Selcuk University, Konya, Turkey (located at 38° 01’ 49’’ N and 32° 31’ 32’’ E). Climatic parameters, such as temperature and relative humidity, were recorded in the experimental area using the automatic weather station (Davis Vantage Pro2). The average daily maximum and minimum temperatures during the experiment were 23 °C and 9 °C, respectively. Relative humidity in the greenhouse was between 40 and 66% (Fig. 1). None of the climatic factors restricted the cultivation of lettuce during the study.

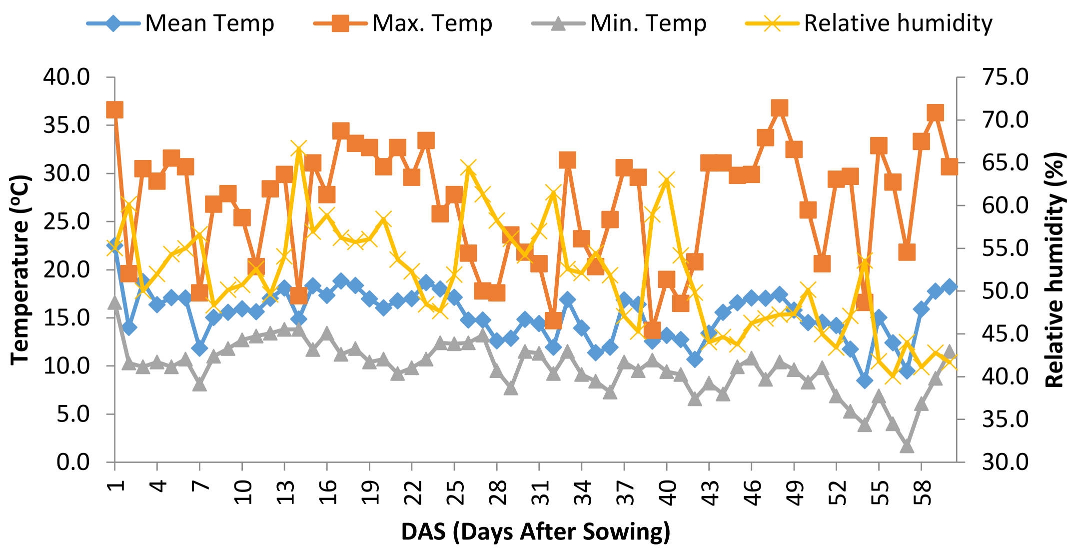

Fig. 1. *Temperature and humidity change graph at 59 days after sowing (DAS) under greenhouse conditions.*

Decomposed animal manure and soil mixture (1/3) were used in the experiment. An analysis of the soil revealed organic matter, pH, and EC to be 3.05, 7.98, and 1.28 dS/m, respectively. In terms of weight percentage (mass water content, %) of experiment soil, the field capacity and wilting point values were 27.4% and 14.8%, respectively. It was concluded that there were no factors that would restrict lettuce cultivation.

Planting and irrigation

The study was conducted in plastic pots with a bottom diameter of 19 cm, a top diameter of 29 cm, a height of 24 cm, and containing 13.3 kg of seedling mixture. "Presidential" lettuce cultivar, which is suitable for greenhouse cultivation and has a high-quality leaf structure, was used as the plant material. This study was based on a random parcel experimental design with five irrigation levels (I_{100}, I_{80}, I_{60}, I_{40}, and I_{20}) and four harvests times (26, 37, 48, and 59 days after transplanting). The experiment was performed in three replicates, with three pots in each parcel and two seedlings planted in each pot.

The volume of irrigation water applied to the pots was determined by the gravimetric soil moisture measurement method. For this, when the available water content in the control I_{100} subject decreased to 30 to 35%, the pots were irrigated, and soil moisture was completed to the field capacity each time. Immediately after planting, 1.52 L (approximately 32.3 mm) of irrigation water was applied to all pots, and the soil moisture was reached the FC level. Scheduled irrigation was started 13 days (Dec. 12, 2019) after the sowing of seeds; irrigation water was applied to trial subjects ten times. After the programmed irrigation was started, 20% (I_{80}), 40% (I_{60}), 60% (I_{40}), and 80% (I_{20}) water constraints were applied according to the I_{100} (full irrigation) subject. The volume of irrigation water applied to the subjects ranged from 62.8 mm (I_{20}) to 185.0 mm (I_{100}). The first harvest was done 14 days (December 26, 2019) after the start of scheduled irrigations; other harvests were done at 11-day intervals (on January 5, 16, and 27) four times.

Growth parameters

The number and area of leaves were determined before harvesting the plants during each harvest period. Next, the pots in each parcel were harvested separately, and the plants were removed together with their roots. They were cut over the root area, and aboveground fresh weight (g) and underground fresh weight (g) were determined per plant. These freshly weighed samples were subsequently dried at 72 °C in the oven until they reached a constant weight. They were finally weighed to determine the aboveground dry weight (g) and underground dry weight (g).

Physiological parameters

The discs obtained from the leaf samples were first wetted, subsequently saturated with water, and their weights in turgor status were determined. The samples were dried at 80 °C in the oven for 48 h, and the dry weights were determined again. Afterward, LWC (leaf water content) (%) was calculated according to the protocol described by Kaya et al. (2003) [18]. Similarly, the percentage of MD (membrane damage) was analyzed using the method described by Lutts et al. [19] using the leaf discs.

Biochemical parameters

To determine the content of CT (carotenoids) and that of a and b chlorophylls, the leaf samples were first ground in 10 mL acetone and centrifuged. The absorbance was read at 470, 652, and 663 nm using a spectrophotometer. The contents of chlorophylls a and b were determined according to the method described by Lichtenthaler and Buschmann [20] whereas the content of CT was determined according to the Jaspar formula [21]. Bradford's [22] method was used to determine PT content. The content of PL was determined from the absorbance at 520 nm, according to Bates et al. [23]. Hydrogen peroxide (H_2O_2) content was determined according to the method described by Pick and Mizel [24] considering absorbance at 390 nm. The MDA content was measured at 532 and 600 nm according to the method described by Cakmak and Horst [25].

To determine the antioxidant enzyme activity, the preparation of the antioxidant enzyme was made from fresh leaf samples of lettuce [26]. The samples, prepared according to the method described by Agarwal and Pandey [27] were read at 560 nm to determine the SOD content and the amount of enzyme causing the inhibition. The change in the absorbance was recorded at 240 nm for 3 min; 0.01 A_{240} unit per min was considered to be equivalent to 1 unit of CAT activity. The change in the absorbance was recorded every 30 s at 470 nm, and 1 unit of enzyme activity was considered to be equal to a change of 1.0 A_{470} units per min [28].

Water productivity (WP)

The water use efficiency was determined by proportioning the aboveground wet lettuce weights (g) obtained from the experimental subjects to the plant water consumption amounts (liter) calculated for each experiment.

$$WP = \frac{E_y}{ET}$$

Eqn.1

WP=Water productivity (g/l); Ey=Above-ground wet lettuce weights (g); ET=Seasonal Evapotranspiration (liter)

Evaluation of data

The growth and changes in the physiological and biochemical contents in lettuce at different irrigation levels and harvest times were analyzed using the JMP–14 package program according to 1% and 5% levels. The differences between harvest periods were reflected in shape, so the effect of water constraint was subjected to statistical analysis. The principal component analysis (PCA) and correlation analyses were performed to determine the parameters that showed significant changes. The reciprocal relationship between harvest time and irrigation levels was demonstrated with PCA. The loading plot was drawn to show the relationship between parameters from PC1 and PC2. The score plot was drawn to determine the effects of harvest time and irrigation levels.

RESULTS AND DISCUSSION

Effect of water stress on growth parameters

The results of statistical analyses showed that water deficit had a significant effect on growth parameters. Irrigation deficit significantly affected the aboveground fresh weight in all harvest periods. The I_{100} level was distinguished from other applications with the best results (Fig. 2a).

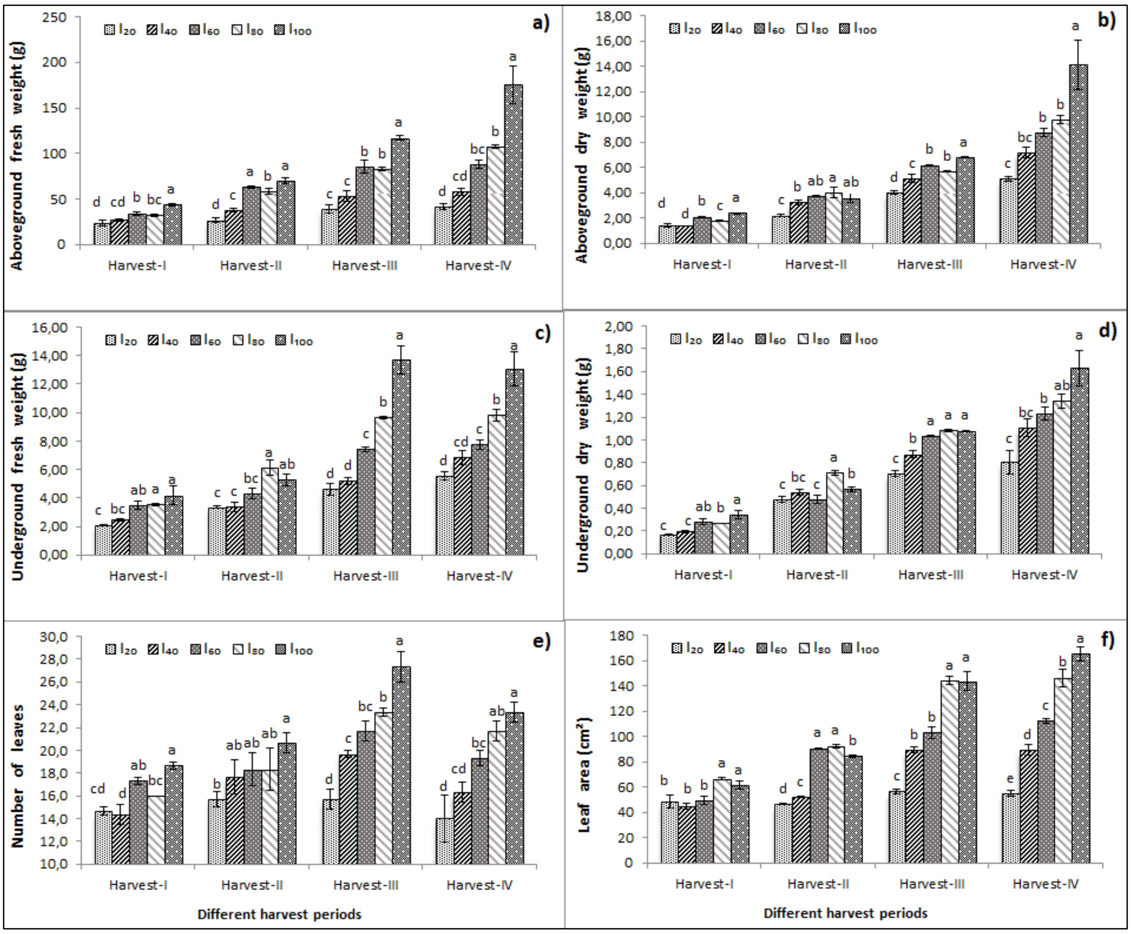

Fig. 2. *Growth characteristics in lettuce under water stress conditions and different harvest times (In deficit irrigation application statistically significant according to P<0.05)*

Similarly, the irrigation deficit exerted a significant effect on the aboveground dry weight; the highest dry weight was obtained from the I_{100} irrigation level (Fig. 2b). Oh et al. [29] reported that drought stress on lettuce adversely affected the aboveground fresh weight. Shawon et al. [30], in their study in Chinese cabbage, reported that drought stress reduced the aboveground fresh and dry weights during different vegetation periods. This result revealed that drought negatively affected cell division and growth. Considering the underground fresh and dry weights, the irrigation deficit caused significant reductions despite the increase in each harvest period. The highest values were obtained from the I_{100} irrigation level (Fig. 2c, 2d). Baslam and Goicoechea [31] reported that drought stress in lettuce decreased the underground fresh and dry weights. When the number of leaves was examined, it was observed that the irrigation deficit decreased the number of leaves (Fig. 2e). In the leaf area, although the results at the I_{80} irrigation level in the first three harvests were in the same statistical group as the I_{100} irrigation level, the I_{100} application in the last harvest was separated from other applications and had the highest leaf area (Fig. 2f). Oh et al. [29] reported that drought stress in lettuce reduced the leaf area. Shawon et al. [30] reported that stress in Chinese cabbage negatively affected the number of leaves and leaf area. Ors and Suares [9] reported a similar effect of drought on spinach. Thus, plants try to survive by consuming less water by restricting vegetative growth to escape the negative effect of drought. The most important factor in plant development is cell division. To accomplish cell division, certain chemical events occur in plants. Therefore, sufficient water must be taken from the soil and the decrease in the amount of water taken from the soil causes cell elongation and disruption of carbon assimilation and slows the growth of the plant.

Effect of water stress on physiological parameters

The irrigation levels have important effects on LWC and MD. The LWC values in the harvest periods from the first harvest to the last harvest decreased in all applications. Although the I_{100} level in harvest-I was statistically different from other irrigation levels, I_{100}, I_{80}, and I_{60} in harvest-II and harvest-III, and I_{100} and I_{80} in harvest-IV were in the same group (Fig. 3a). Researchers have reported that the drought stress reduced the LWC in lettuce [32] and spinach [33]. LWC is a widely used index to display water stress in plants [34]. If RWC is high in plants, there is more water nutrient intake from the soil, and in stress conditions, and water deficiency appears. When MD was examined, irrigation deficit did not effect on the harvest-I period, whereas significant MD was observed in the second harvest period and later in severe irrigation deficit (I_{20}) (Fig. 3b). Arıkan et al. [35] reported that abiotic stresses increased the MD in blackberry. In another study, Ipek et al. [36] found that deficit irrigation applications on blackberry had negative effects on LWC and MD. It is known that as a result of damage to cell membranes by stress, water-soluble substances in the cell flow into the intercellular spaces and increase tissue electrical conductivity. Therefore, the effect of drought stress in lettuce also caused MD to increase.

Effect of water stress on biochemical parameters

Deficit irrigation has a significant effect on biochemical parameters in lettuce. We found a difference in the value of chlorophyll a between the applications during three harvest periods, whereas we did not observe any significant difference between the deficit irrigation during the last harvest time (Fig. 3c).

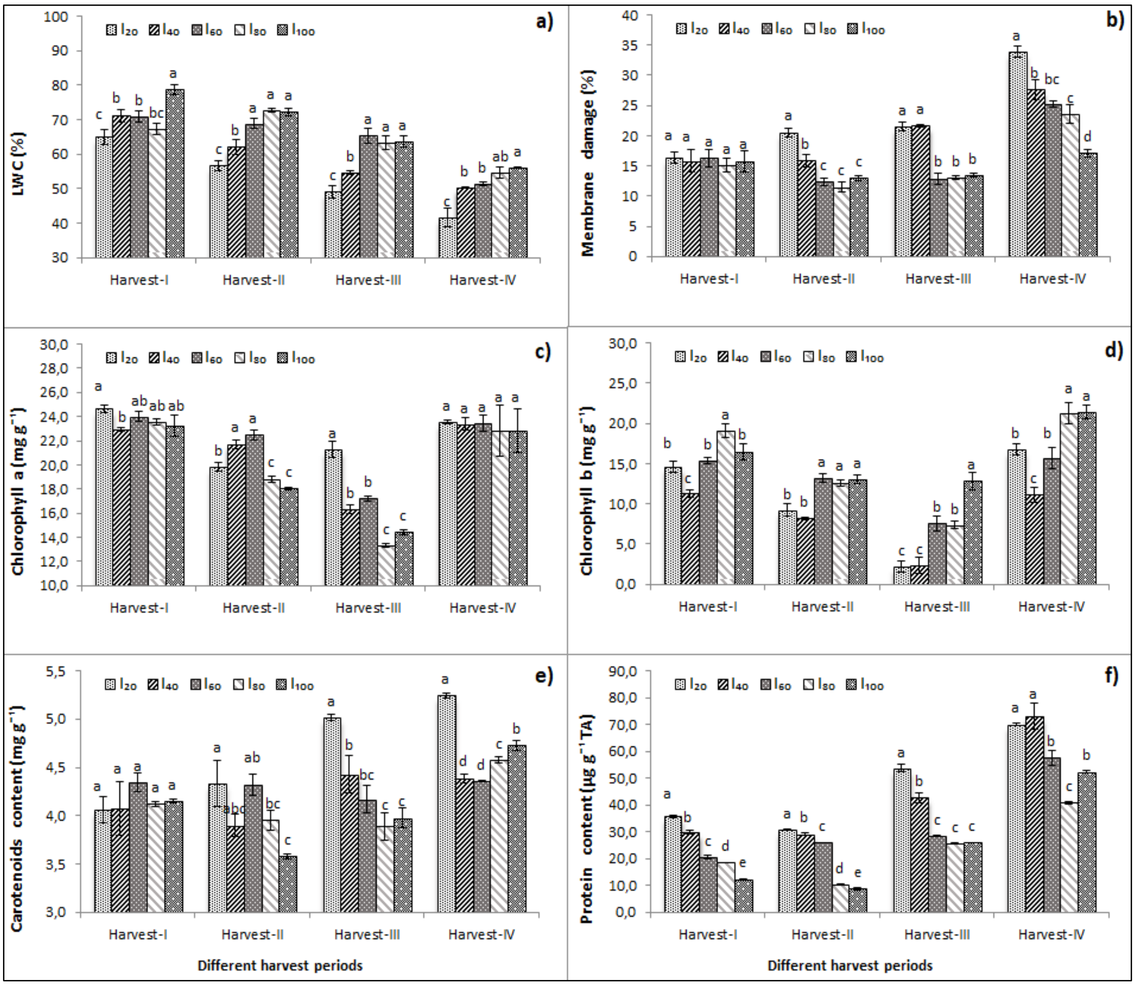

Fig. 3. *Physiological and some biochemical characteristics in lettuce under water stress conditions and different harvest times (In deficit irrigation application statistically significant according to P<0.05)*

The effect of deficit irrigation on chlorophyll b values revealed different results during different harvest times. However, when harvest-IV was examined, chlorophyll b values of I_{100} versus I_{80} irrigation levels were statistically significant in the same group, with the highest chlorophyll b content (Fig. 3d). It is reported that change in chlorophyll a and b content occurred due to inhibition of chlorophyll biosynthesis and enzymes, degradation of pigments caused by disruption of chloroplast membrane integrity, and interruption of other metabolic activities [37]. Basahi et al. [32] reported that drought stress did not effect on chlorophyll b content, while chlorophyll a content was increased. The deficit irrigation applications in the harvest-I did not affect the CT content, whereas a significant increase was observed in the CT content at the I_{20} irrigation level during the later harvest times (Fig. 3e). CT are molecules that are located together with the chlorophyll in the leaves, and work indirectly during photosynthesis and have antioxidant properties [38, 39]. Similarly, drought stress increased the CT content in spinach [33] and lettuce [32].

A lower PT rate was obtained at all harvest periods as compared with other irrigation levels in severe stress applications (I_{20}) (Fig. 3f). Considering the PL content, the water deficit did not significantly affect the PL content in the first harvest, whereas a significant increase was observed in severe water stress applications after the second harvest. At the harvest-IV time, the I_{40} irrigation level was significantly affected by stress and applications that were affected the most with the I_{20} water stress application during this period (Fig. 4a). It is known that plants synthesize PL when they encounter stress, and the PL content increases with the severity of the stress. It is reported that PL plays an important role in biological events, such as ensuring osmotic balance, eliminating radicals, maintaining membrane integrity, protecting PT and DNA structure, and accumulating usable nitrogen.[38] The PL content, after the second harvest of lettuce, indicated that significant stress occurred at the level of I_{20} irrigation. Rajabbeigi et al. [40] reported that stress significantly increased the content of PL in their drought stress in lettuce. Basahi et al. [32] obtained similar results for lettuce.

The highest H_2O_2 contents were obtained at the irrigation levels I_{20} and I_{40} during other harvest times, except the harvest-III time. However, the largest difference was obtained in the last harvest period (Fig. 4b). Basahi et al [32] reported that drought increased the H_2O_2 content. It has been reported that the content of H_2O_2 increases with increasing drought stress in spinach [41]. The MDA content was found to be high at I_{20} irrigation level in all harvests. However, when the last three harvests were examined, the I_{100}, $I_{80,}$ and I_{60} irrigation levels were statistically significant in the same group and were distinct from other applications (Fig. 4c).

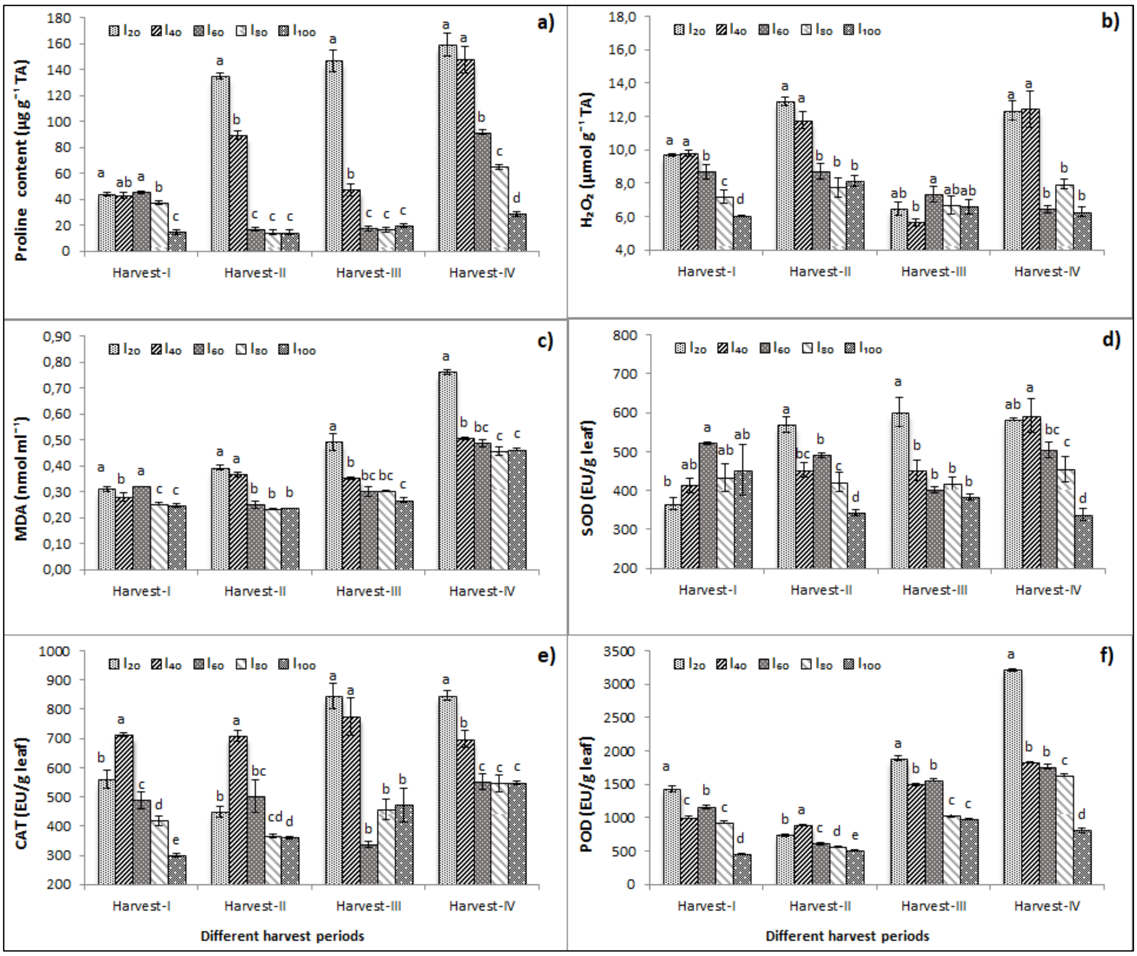

Fig. 4. *Biochemical characteristics in lettuce under water stress conditions and different harvest times (In deficit irrigation application statistically significant according to P < 0.05)*

A high amount of MDA content indicates an increase in lipid peroxidation due to stress. Similarly, Mesquita et al. [42] in soybean, Yadegari et al. [43] in tomato, and Hameed and Iqbal [44] in wheat reported that drought stress increased the MDA activity. We found that I_{20} and I_{40} water levels resulted in significant stress after 26 days of planting seedlings in lettuce.

On the one hand, the SOD activity did not show a significant change in water levels in the first harvest, a significant increase in the I_{20} irrigation level in other harvest periods, and the I_{40} irrigation level in the last harvest was observed (Fig. 4d). Although the highest CAT activity was obtained from the I_{40} irrigation level in the first two harvests, the I_{20} and I_{40} irrigation levels had higher CAT activity than other applications in the last two harvest times (Fig. 4e). Moreover, although the irrigation levels had a significant effect on the POD activity in the first two harvests, a significant increase in POD was observed in the I_{20} irrigation level in the last two harvest times (Fig. 4f). Antioxidant enzymes are agents that get activated under stress conditions and are one of the best ways to determine the response to stress [45]. Naderi et al. [46], in their study in wheat, reported that drought increased the activity of antioxidant enzymes, and there was a positive correlation between them. The drought stress in lettuce [47], tomato [48], spinach [41], maize [49], radish [50], and myrobalan 29C rootstock [51] caused an increase in SOD, CAT, and POD activities. Plants have an antioxidant defense system with enzymatic and non-enzymatic antioxidants in cellular organelles, clearing different ROS to a certain level. If ROS production is higher than antioxidant scavenging ability, oxidative damage occurs [52]. Plants' major ROS scavenging mechanisms are SOD, APX and CAT [53]. In this study, the abundant rise in SOD, CAT, and POD activities induced by water deficit application provides additional evidence that drought regulates the degradation of O_2 to H_2O.

Principal component and correlation analysis

Growth, and physiological and biochemical contents were analyzed by PCA for the different parameters of different irrigation levels and harvest times (Table 1). The PCA revealed a high rate of 76.14% in two components. Some studies have reported that the first two components should explain more than 25% of the variance [54, 55, 5]. PCA should be able to support the usability of the analysis and the parameters being considered. PCA is a widely used method in defining and evaluating stress tolerance in drought studies [56, 57]. In the present study, the first component (PC1) explained 42.56% variance, with the LWC as a negative and the MD, CT, PT, PL, MDA, SOD, CAT, and POD as positive parameters. The second component (PC2) explained 33.58% variance, and growth parameters, such as AFW, ADW, UFW, UDW, NL, and LA, were the positive parameters. Pouri et al. [58] determined important parameters with the same method in their drought study in corn.

A loading plot was obtained using the PC1 and PC2 components to examine the interrelation between growth and physiological and biochemical contents (Fig. 5). An angle of <90° between the vectors in the figure indicates a positive relationship, whereas that of >90° reflects a negative relationship. If the angle between the vectors is 90°, there is no significant relationship [59]. When the graph was examined, a high positive correlation was observed between the physiological and biochemical parameters described in PC1. These parameters showed a high negative relationship with LWC.

Table 1. *PCA results regarding growth, antioxidative enzyme activities, and chlorophyll content in lettuce under water stress conditions and different harvest times*

Items	PC1	PC2
Eigenvalue	7.66	6.04
Percentage of variance	42.56	33.58
Cumulative variance	42.56	76.14
Eigenvectors		
AFW	–0.073	0.385
ADW	0.057	0.386
UFW	–0.047	0.389
UDW	0.064	0.389
NL	–0.191	0.318
LA	–0.095	0.382
LWC	–0.314	–0.160
MD	0.337	0.056
Ca	0.160	–0.136
Cb	–0.024	0.098
CT	0.294	0.094
PT	0.319	0.145
PL	0.334	–0.050
HO	0.181	–0.177
MDA	0.335	0.116
SOD	0.279	–0.105
CAT	0.286	–0.011
POD	0.306	0.065

Principle component (PC); aboveground fresh weight (AFW); aboveground dry weight (ADW); underground fresh weight (UFW); underground dry weight (UDW); number of leaves (NL); leaf area (LA); leaf water content (LWC); membrane damage (MD); chlorophyll a (Ca); chlorophyll b (Cb); carotenoids content (CT); protein (PT); proline (PL); H_2O_2 (HO); malondialdehyde (MDA); superoxide dismutase content (SOD); catalase (CAT); peroxidase (POD)

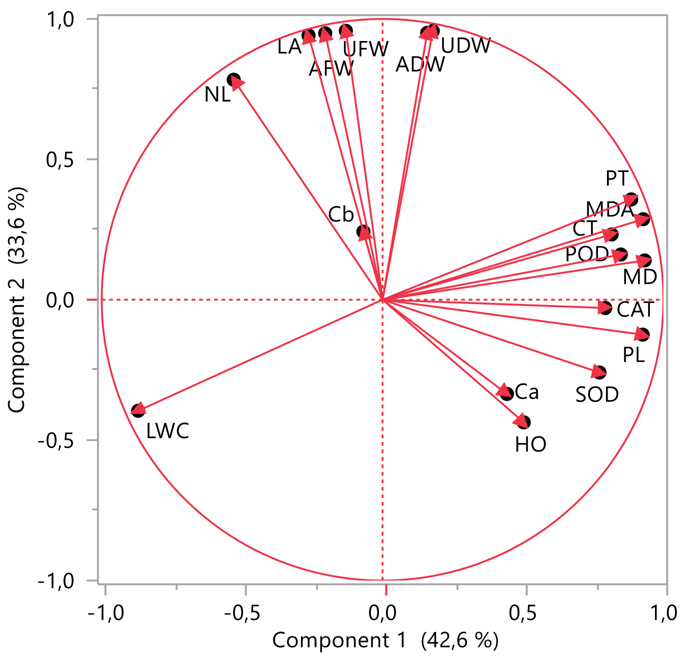

Fig. 5. *Loading plot based on PC1 and PC2 obtained from PCA using growth, antioxidative enzyme activities, and chlorophyll content in lettuce under water stress conditions and different harvest times.*

It is observed that there was a high positive correlation between growth parameters that are important in PC2. In addition, to interpret similar relationships, the subjected correlation analysis was performed, which revealed significant correlations ($p < 0.01$) (Table 2). Moreover, the significant relationships concorded with the correlation table, with a high positive correlation between MD, CT, PT, PL, MDA, SOD, CAT, and POD (r = –849, r = –753, r = –910, r = –799, r = –903, r = –560, r = –716, and r = –805, respectively). Their LWC showed a high negative correlation. In addition, a high positive correlation was found between all growth parameters. Xiu et al. [57] reported correlation analysis as an important method for evaluating parameters. They also reported that antioxidant enzyme contents showed a high correlation with each other under drought stress. Mozafari et al. [55] determined the relationship between parameters with a similar method in the drought stress study conducted in strawberry and reported a positive correlation between H_2O_2 and MDA and a negative correlation in others. In addition, there was a high positive correlation between SOD, POD, PL, and growth parameters. Similarly, another study reported a high positive correlation between H_2O_2 and MDA in *Achillea* species [60]. Naderi et al. [46] reported a high positive correlation between antioxidant enzyme activities, PT, PL, and CT in wheat.

Using the PC1 and PC2 components, a score plot was created to explain the harvest periods and irrigation levels (Fig. 6). The parameters important in PC1 explained the stress, whereas those important in PC2 explained the harvest times. In this case, physiological and biochemical parameters, such as LWC, MD, CT, PT, PL, MDA, SOD, CAT, and POD, revealed significant changes in water stress conditions in lettuce. Growth parameters, such as AFW, ADW, UFW, UDW, NL, and LA, showed significant changes during harvest times. It has been revealed that physiological and biochemical parameters do not change significantly in different vegetative periods of the plant.

Table 2. *Correlation coefficients between growth, antioxidative enzyme activities, and chlorophyll content in lettuce under deficit irrigation conditions and different harvest times*

	AFW	ADW	UFW	UDW	NL	LA	LWC	MD	Ca	Cb	CT	PT	PL	HO	MDA	SOD	CAT	POD
AFW	1.00																	
ADW	0.91	1.00																
UFW	0.92	0.85	1.00															
UDW	0.86	0.95	0.87	1.00														
NL	0.81	0.60	0.85	0.65	1.00													
LA	0.93	0.85	0.93	0.86	0.86	1.00												
LWC	–0.16	–0.48	–0.25	–0.54	0.12	–0.15	1.00											
MD	–0.08	0.27	0.00	0.29	–0.37	–0.11	–0.84	1.00										
Ca	–0.27	–0.08	–0.44	–0.29	–0.65	–0.43	–0.10	0.39	1.00									
Cb	0.35	0.31	0.24	0.12	0.08	0.25	0.11	0.08	0.54	1.00								
CT	0.08	0.35	0.09	0.31	–0.28	–0.02	–0.75	0.74	0.39	0.08	1.00							
PT	0.16	0.50	0.21	0.50	–0.21	0.10	–0.91	0.86	0.31	–0.01	0.75	1.00						
PL	–0.31	0.02	–0.20	0.08	–0.54	–0.36	–0.79	0.83	0.35	–0.19	0.64	0.75	1.00					
HO	–0.46	–0.29	–0.41	–0.27	–0.58	–0.51	–0.26	0.39	0.34	–0.00	0.10	0.28	0.61	1.00				
MDA	0.09	0.42	0.15	0.43	–0.27	0.00	–0.90	0.92	0.34	0.10	0.82	0.88	0.81	0.38	1.00			
SOD	–0.43	–0.15	–0.30	–0.06	–0.53	–0.41	–0.56	0.67	0.31	–0.25	0.61	0.53	0.83	0.45	0.60	1.00		
CAT	–0.19	0.07	–0.14	0.08	–0.41	–0.23	–0.71	0.66	0.23	–0.32	0.62	0.73	0.68	0.31	0.68	0.49	1.00	
POD	–0.08	0.21	0.04	0.27	–0.29	–0.08	–0.80	0.83	0.21	–0.05	0.72	0.80	0.67	0.26	0.85	0.53	0.66	1.00

Aboveground fresh weight (AFW); aboveground dry weight (ADW); underground fresh weight (UFW); underground dry weight (UDW); number of leaves (NL); leaf area (LA); leaf water content (LWC); membrane damage (MD); chlorophyll a (Ca); chlorophyll b (Cb); carotenoids\ (CT); protein\ (PT); proline\ (PL); H_2O_2*(HO); malondialdehyde\(MDA); superoxide dismutase\ (SOD); catalase (CAT); peroxidase (POD)*

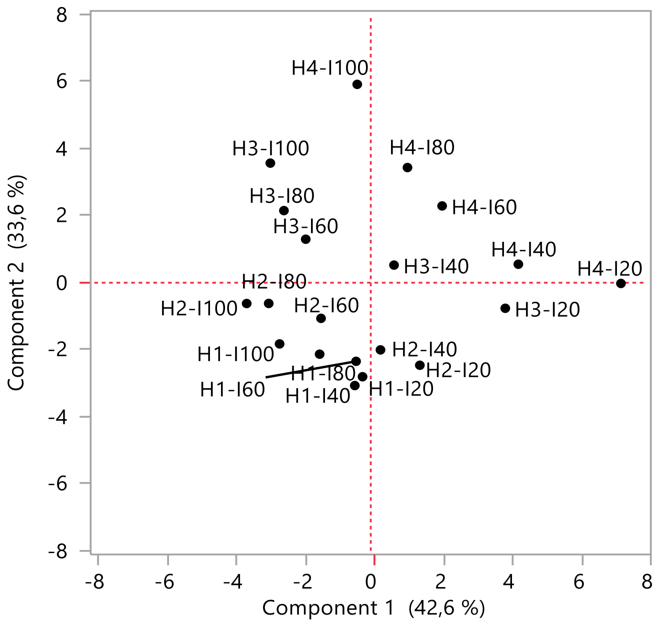

Fig. 6. *Score plot based on components PC1 and PC2 obtained from PCA using growth, antioxidative enzyme activities, and chlorophyll content in lettuce under water stress conditions and different harvest times.*

According to these results, the I_{100} irrigation level in the harvest-IV period was located in the positive region of PC2, separated from other applications. Moreover, it provided the best results in terms of growth parameters. On the contrary, I_{20} versus I_{40} irrigation levels in harvest-IV and I_{20} irrigation levels in harvest-III were the most affected applications by drought stress in the positive region of PC1.

Water productivity

The WP results, an important criterion in the evaluation of irrigation programs and expressed as the unit water utilization rate, are presented in Fig. 7. When different irrigation levels and different harvest times were evaluated together, the WP values ranged from 6.81 to 22.83 g/L, and the highest WP value was obtained at I_{100} irrigation level and harvest-IV. When irrigation levels were examined according to different harvest times; the highest WP values in irrigated I_{20}, I_{40}, I_{60}, and I_{80} irrigation levels were obtained in harvest-III. After this period, WP values decreased in other deficit irrigation subjects with moderate and excessive water stress, except for the I_{80} irrigation level, where light water stress was applied. WP values increased continuously from the first harvest time to the last harvest time at the I_{80} irrigation level, where light water stress was applied with a completely irrigated I_{100} irrigation level. WP values were found to be considered close between I_{60} and I_{80} irrigation levels, where mild and moderate water stress were applied during the last harvest, respectively. Similarly, there were no significant differences in WP between the I_{20} and I_{40} irrigation levels, where excessive water stress was applied. The WP values in the I_{100} irrigation level, which was not exposed to any water deficit condition during the complete development period of the lettuce, increased significantly during the last harvest, and it was positively separated from other applications. Based on these results, we conclude that harvesting lettuce in harvest-IV is important for high yield.

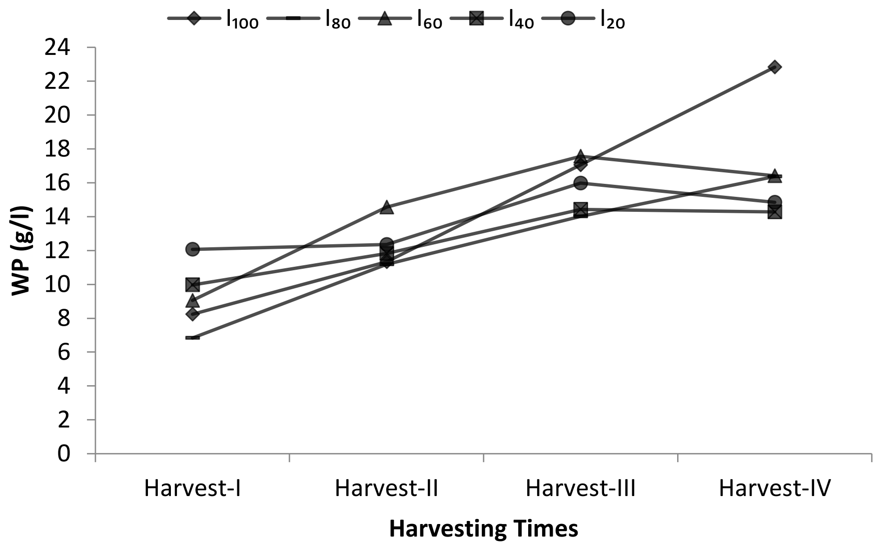

***Fig. 7.** Water productivity of lettuce under water stress conditions and different harvest times.*

However, to save irrigation water in the arid and semi-arid regions with limited water resources, it is important to have a sustainable irrigation management system to harvest the lettuce at the time of harvest-III and irrigation with the I_{60} irrigation level, where the highest WP was obtained during this period. In addition, the limited irrigation (I_{60}) of 48 days after planting the lettuce seedling will conserve water and reduce the pressure on water resources.

CONCLUSION

This study demonstrated that significant changes occur in the growth and physiological and biochemical contents under water deficiency during different harvest times of lettuce. While severe water deficit negatively affected the growth parameters at all harvest times, a decrease in LWC and a significant increase in MD, CT content, PT, PL, H_2O_2, MDA, and antioxidant enzyme activities were observed. The PCA revealed LWC, MD, CT, PT, PL, MDA, SOD, CAT, and POD as important parameters in explaining the stress and inducing significant changes in lettuce under water stress conditions. In addition, the study of growth parameters, such as AFW, ADW, UFW, UDW, NL, and LA, revealed significant changes in harvest times. The I_{100} irrigation level in the harvest-IV period provided the best results. On the contrary, I_{20} versus I_{40} irrigation levels in harvest-IV and I_{20} irrigation levels in harvest-III were the most affected applications under drought stress. Based on these results, complete irrigation should be performed during the last harvest period in lettuce. Deficit irrigation during this period greatly restricts plant growth. Thus, water resources can be conserved with limited irrigation in arid and semi-arid regions (water scarcity of 48 days) after planting lettuce seedlings.

Acknowledgement. I would also like to thank Prof. Dr. Ertan Sait Kurtar, Assoc. Prof. Dr. Muzaffer Ipek, and Assist. Prof. Dr. Şeyma Arıkan, who helped me with laboratory studies.

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