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Research Article

Waterlogging Response of Lentil Cultivars Grown in Greenhouse Throughout The Early Vegetative and Recovery Period

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Abstract: Under conditions of global climate change, the frequency of climate anomalies is predicted to increase. One of these issues is the problem of waterlogging in agricultural areas as a direct result of the unexpected and severe rainfall that has occurred over the last decades. In this study, the morphological responses to waterlogging stress and the recovery capacity of the lentil cultivars were investigated. A waterlogging stress study was conducted in small water pools with four different lentil varieties (Çağıl, Fırat 87, Kafkas and Kayı). Lentil cultivars were exposed to waterlogging stress for 7 and 14 days in the same greenhouse conditions. Measurements were taken at the end of 7 and 14 days of waterlogging (W-7 and W-14) and during the recovery period after flowering (R-7 and R-14). Lentil cultivars and plant traits were negatively affected by waterlogging stress applications (W-7 and W-14). According to the study, 14-day waterlogging had a greater impact on lentil cultivars than 7-day waterlogging. Total biomass measured after flowering at R-7 and R-14 waterlogging decreased by about 31.5% and 49.3%, respectively. Çağıl cultivar had a tolerance to waterlogging stress, but Kafkas cultivar was sensitive to waterlogging stress.

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1. Introduction

Legume crops are a cheap source of protein, minerals, and carbs. They also contain a wealth of secondary metabolites, or "bioactive substances," which have a positive impact on health by influencing cellular and physiological processes (Zeroual et al., 2022). Although the origin of the cultivated lentil (*Lens culinaris* Medik. subsp. *culinaris*) is in the southeastern region of Türkiye, it has a widespread area along the Mediterranean, from the north to Western Europe and from the south to Egypt and from the Nile to Ethiopia. This has led to the development of many lentil species that are adapted to different climates and soil conditions (Alo et al., 2011; Biçer et al., 2018). Canada is the world's leading producer of lentils, with 2.9 million tons, followed by India (1.2 million tons), Australia (0.5 million tons), and Türkiye (0.3 million tons) (FAO, 2022). The increase in various biotic and abiotic stresses in the expanding grain legumes cultivation areas in recent years creates fluctuations in yield and this brings along the problem of food security that threatens food safety. Lentil is a crop that suffers from abiotic stress factors such as drought, cold, frost, logging, salinity, and high temperature. These stress factors affect the crop's physiological, biochemical, and molecular growth and development processes (Wiraguna et al., 2017). Food security is a major issue brought on by global climate change, but the nutritious and cheap lentil grain may serve an alternate role in addressing this issue.

Like animals, plants are what are known as obligatory aerobic beings, meaning they can't survive without oxygen. Some commercial cultivars cannot survive in waterlogged conditions due to the absence of aerenchyma tissue or adventitious roots in their roots. When water molecules replace air pores in soil cavities, waterlogging stress occurs in soils (Pan et al., 2021). Waterlogging is one of the most common causes of root zone oxygen deficiency. It causes more damage where there is poor drainage.

The waterlogging problem, which is commonly seen nowadays due to the changing global climate, deprives plant roots of oxygen, reducing or killing plant yields and threatening world food security. Increased porosity with the development of aerenchyma tissue in root systems facilitates oxygen movement from shoot to root by diffusion, increasing tolerance to waterlogging (Colmer, 2003). The plant’s ability to survive and recover after waterlogging depends on the development stage, the duration of the waterlogging, number of days exposed to waterlogging, and the genotypic variance in waterlogging tolerance (Setter and Waters, 2003). Flooding problem is survived not only just along the coast where spring wheat crops are grown but also in other places where winter wheat is grown, because of sudden heavy rain and excessive irrigation in Türkiye (Ozseven and Genctan, 2018). Worldwide, approximately 10% of irrigated areas or approximately 22 million hectares, are exposed to waterlogging (Bowonder et al., 1987).

The most important reason why lentils cannot be grown on fertile lowlands in Türkiye is the stress of waterlogging or the accumulation of water in the soil. Because lentils are very susceptible to even short periods of waterlogging. Although excess water in lentil cultivation reduces the yield at any stage, the tolerance to waterlogging in the plant differs between and within species according to different growth stages. Poor lentil production is caused by the soils with poor drainage, such as fine textured with high clay and in subsoil compaction. The problem occurs seriously in long rainy weather conditions. It is impossible to achieve a high yield from lentils since they must be sown in stony, gravelly, and high-sloping lands that are lacking fertility (Malik et. al, 2015). Particularly waterlogging during germination can cause failed germination, late emergence, and prevention of root growth. In the flowering and pod-filling phase, a plant's ability to recover diminishes (Solaiman et al., 2007; Materne and Siddique, 2009; Wiraguna et al., 2017). In early flowering damage is most severe. In the damage, plants are stunted and turn yellow to red; wither, and eventually die. Root and collar portion rot are severe. In order to alleviate this problem, it is necessary to develop high-yielding, potential waterlogging resistant varieties and determine their genetic sources (Osman et al., 2013; Paudel et al., 2020).

The purpose of this research was to examine the impact that waterlogging caused on the development of different lentil cultivars when it was applied at varying times during the early stages of plant growth.

2. Material and Methods

This research was conducted under semi-controlled environments in the greenhouse of the Faculty of Agriculture, Dicle University.

The four lentil cultivars (Kayı, Kafkas,Fırat, and Çağıl) were used. Waterlogging treatments were given two times (7 days duration: W-7 and 14 days duration: W-14) in the begining of the 32nd day after seed emergence. The control plants were irrigated at the field capacity levels throughout the experiment.

The potting soil is clayey (79%), with low salt level (ds m^{-1} 0.85), slightly alkaline (pH 7.61), medium calcareous (6.43%), medium organic matter (2.88%), available phosphor (178.79 kg da^{-1}) and potassium (498.56 kg da^{-1}). Plastic pots were filled 1.76 kg with the soil. Eight seeds in each pot were sown to ensure good emergence on April 8, 2020, and after emergence, they were adjusted to four seeds per pot. Fertilization was applied 5.0 kg/da nitrogen and 9.0 kg/da phosphor by diluting with 30 ml/da water per pot before 5 days the waterlogging stress. The experiment arranged the randomized complete plots design in split plots in waterlogging treatments as the main factor and cultivars as subfactors with four replications.

Waterlogging treatments were initiated on the 32nd day of seed emergence. Plants were exposed to waterlogging during 7 (W-7) and 14 (W-14) days. The control plants were irrigated at the field capacity level throughout the experiment. Waterlogging stress was tested in a concrete brick-built pool that was filled to the pot's surface with water. It was covered with a thick plastic cover to prevent water leakage, and equipped with an electric air motor to assure the flow of oxygen.

The lentil plants to be subjected to waterlogging stress were cultivated under normal cultivation conditions for 32 days in pots. The pool was filled with water up to the surface of the pots. After 32 days, pots were transferred into the pool. At the end of the 7th and 14th days, all pots were removed from the poll and continued to be grown under normal conditions. After this stage, the plants were watered until the harvest. Plants were harvested 15 days after flowering dates.

Measurements were taken on W7 and W14 days of waterlogging and the fifteenth day (R7 and R14) of flowering. Plant height, root length, number of leaves per plant, plant diameter, above ground biomass, leaf weight, stem weight, fresh and dry root weight were measured. The dry weights of plant parts were determined after drying in an oven at 70 °C for 48 hours.

Cultivars and waterlogging treatments were tested using ANOVA. Data analyzed in the JMP statistical program (JMP Pro-13, SAS Institute).

3. Results and Discussion

The analysis of variance in two different waterlogging treatments for lentil cultivars is given in Table 1.

Table 1. Two-way ANOVA for all traits

Measurements after 7 days waterlogging													
Source of variation	**Df**	**PH (cm)**	**RL (cm)**	**FAGB (mg)**	**RFW (mg)**	**LFW (mg)**	**NLP $^{-1}$**	**SFW (mg)**	**TFB (mg)**	**LDW (mg)**	**SDW (mg)**	**RDW (mg)**	**TDB (mg)**
Cultivar (C)	3	71.25**	103.36*	151.00**	77.60	51.60**	14.70	17.80**	295.00**	2.21**	1.90**	1.39**	11.10**
W-7	1	52.57**	140.36**	2.60	422.00**	3.33	6.54	0.72	490.00**	0.80**	0.03	7.05**	13.70**
C*W-7	3	0.30	65.57*	4.60	56.00	7.20	5.82	1.21	60.20	0.12	0.16	2.16**	2.87*
Error	17	61.62	113.45	67.10	258.40	14.62	32.70	8.51	251.20	0.93	0.50	1.24	4.24
CV(%)		11.50	11.20	17.61	19.12	13.48	14.85	15.27	12.20	14.90	16.98	10.50	9.20
Measurements after 14 days waterlogging													
	Df	**PH (cm)**	**RL (cm)**	**FAGB (mg)**	**RFW (mg)**	**LFW (mg)**	**NLP $^{-1}$**	**SFW (mg)**	**TFB (mg)**	**LDW (mg)**	**SDW (mg)**	**RDW (mg)**	**TDB (mg)**
Cultivar (C)	3	163.90**	163.15*	47.00**	234.00**	44.40**	65.40**	18.30**	433.00**	2.90**	1.39**	10.02**	34.90**
W-14	1	38.93**	470.19**	56.00**	1782.00**	59.70**	59.04**	3.10	2473.00**	4.27**	0.00	14.70**	34.40**
C*W-14	3	6.57	55.89	49.00**	333.00**	21.80**	27.73	8.24	409.00**	0.91*	0.32	10.40**	17.20**
Error	17	46.36	203.32	51.40	140.00	15.80	60.85	16.78	183.00	1.18	0.75	3.31	9.36
CV(%)		10.50	16.60	19.90	15.40	19.77	21.20	23.60	11.90	20.70	20.80	22.95	17.42
Recovery measurements after flowering at 7 and 14 days waterlogging treatments													
	Df	**PH (cm)**	**LDW (mg)**	**SDW (mg)**	**RDW (mg)**	**TDB (mg)**	**PD (cm)**						
Cultivar (C)	3	1573.70**	3.10**	33.60**	7.20**	68.00**	0.22						
R	2	1075.40**	22.80**	39.90**	26.00**	236.00**	0.12						
C*R	6	2195.50**	12.00**	32.30**	9.50**	89.00**	0.41						
Error	33	491.20	6.15	11.06	1.94	19.40	0.95						
CV(%)		9.70	17.70	15.77	13.10	9.60	14.90						

W-7: Waterlogging 7 days, SV: Source of variation, Df: Degrees of freedom, R: Recover days: (R-0, R-7, R-14), PH: Plant Height, RL: Root Length, FAGB: Fresh Above Gound Biomass, RFW: Root Fresh Weight, LFW: Leaf Fresh Weight, NLP-1:Number of Leaves Plant-1, SFW: Stem Fresh Weight, TFB: Total Fresh Biomass, LDW: Leaf Dry Weight, SDW: Stem Dry Weight, RDW: Root Dry Weight, TDB: Total Dry Biomass, PD: Plant Diameter, Significant: $*P < 0.05$; $**P < 0.01$.

In the plants exposed to waterlogging for seven days, the differences among cultivars in all parameters except the number of leaves per plant were significant. The waterlogging application was significant for all traits except for the number of plant leaves and fresh above ground biomass, stem fresh and dry weight, and number of leaves per plant. Cultivar x waterlogging application at the seventh day interaction was significant for root length, root dry weight, and total dry biomass.

In the plants exposed to waterlogging for 14 days, the differences among cultivars were significant for all parameters. The effect of waterlogging was significant on all properties except stem fresh and dry weight. Cultivar x waterlogging application at 14th day interaction was significant for all traits except for plant height, root length, number of leaves per plant, and stem fresh and dry weight.

The traits after the flowering time were evaluated as recovery in the control, 7th day and 14th day waterlogging treatments. The applications were called R0, R7, and R14, respectively. The analysis of variance for the recovery data reveals that cultivars, recovery, and cultivars x recovery interaction were significant for plant height, fresh and dry above ground biomass, stem, leaf and root dry weight (Table 1).

3.1. Measurements at 7 (W-7) and 14 days (W-14) waterlogging treatments

Plant height, root length, root fresh and dry weight, total fresh biomass, leaf dry weight, and total dry biomass decreased when lentil cultivars were subjected to seven days of waterlogging stress (W-7) in 32 days after sowing.

Compared to W-7, the effects of stress on the plant traits were increased when it was exposed to waterlogging for 14 days (W-14).

Plant height, root length, root fresh and dry weight, total fresh and dry biomass, and leaf dry weight were also adversely affected at W-14 compared to control and W-7. When the waterlogging stress duration was raised from 7 days to 14 days, the effect of stress often doubled. Similarly, Lake et al. (2021) stated that increasing the duration of waterlogging stress generates an increase in stress in specific features of the plant, which in turn causes a decline in plant growth.

Root weight characteristics affected the plant parts the most as a result of W-7 and W-14 applications, and significant losses occurred in the root area depending on the applications.The decrease in root fresh and dry weights was about 34-35% in W-7 and 55-60% in W-14, respectively (Table 2). Similar results were also reported by Yavas et al. (2012). Several metabolic processes, including respiration, are suppressed and ATP synthesis in anaerobic respiration is reduced when roots are deprived of oxygen as a result of waterlogging stress. Although this scenario primarily affects root development, it also has a negative impact on vegetative growth by limiting water and nutrient uptake (Mustroph ve ark., 2006; Mustroph ve ark., 2013).

Stem fresh and dry weights were not affected by W-7 and W-14 applications. The results showed that the plant through the stress gave priority to stem construction in the first developmental stages.

The significant losses for total fresh and dry biomass results from applications were determined, and the reductions were about 25% in W-7 and 40-50% in W-14, respectively.

Table 2. Investigated traits of seedling plants of lentil under control and 7 and 14 day waterlogging treatments

Traits	Control for 7 day waterlogging	7 day waterlogging	Loss at 7 day waterlogging (%)	Control for 14 day waterlogging	14 day waterlogging	Loss at 14 day waterlogging (%)
Plant height (cm)	18.29 a	15.33 b	16.20	17.31 a	14.76 b	14.70
Root length (cm)	25.75 a	20.91 b	18.80	26.01 a	17.16 b	34.00
Fresh above gound biomass (mg)	357.00 a	336.00 a	5.90	333.00 a	236.00 b	29.10
Root fresh weight (mg)	793.00 a	528.00 b	33.40	901.00 a	356.00 b	60.50
Leaf fresh weight (mg)	226.00 a	202.00 a	10.60	212.00 a	112.00 b	47.20
Number of leaves plant $^{-1}$	9.92 a	8.87 a	10.60	10.60 a	7.50 b	29.20
Stem fresh weight (mg)	135.00 a	146.00 a	-8.10	148.00 a	125.00 a	15.50
Total fresh biomass (mg)	1150.40 a	864.60 b	24.80	1235.00 a	593.00 b	52.00
Leaf dry weight (mg)	55.30 a	43.70 b	21.00	55.50 a	28.80 b	48.10
Stem dry weight (mg)	33.00 a	30.90 a	6.40	31.50 a	31.10 a	1.30
Root dry weight (mg)	101.30 a	67.00 b	33.90	89.30 a	39.70 b	55.50
Total dry biomass (mg)	189.80 a	141.80 b	25.30	175.90 a	100.10 b	43.10

In lentil plants exposed to waterlogging stress, the leaves fall from the lower leaves upwards, and the number of leaves and leaf weight were reduced. Moreover, the leaf losses were high in W-14. The rate of decrease in the total number of leaves raised from 10.6% in W-7 to 29.1% in W-14. The leaf fresh and dry weights were more affected than the number of leaves by applications. This can be explained by the fact that the increase in the number of days the roots are without oxygen significantly affects the plant's growth potential. The effects of W-7 and W14 applications on cultivars were given in Fig. 1 and Fig 2. The Kafkas cultivar was negatively highly affected by W-7 for total fresh and dry weight. Kayı cultivar had the highest fresh and dry weight at W-7, W-14, and control conditions. However, Kayı cultivar lost unusually more dry and fresh weight in W-14 than the other cultivars, and it was unable to survive prolonged waterlogging.

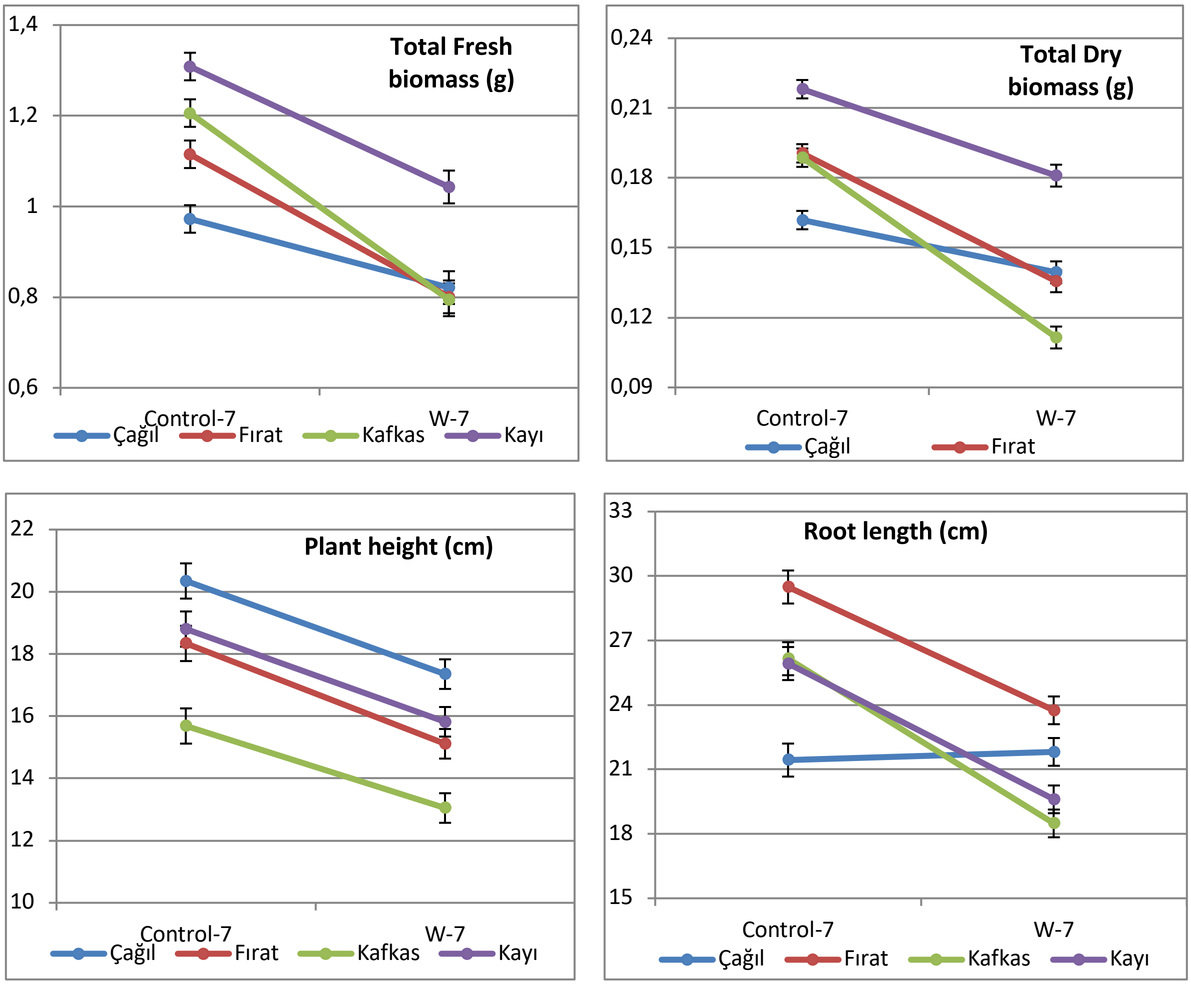

Figure 1. Total fresh biomass, total dry biomass, plant height, and root length in lentil genotypes at the end of 7 day waterlogging treatment. Vertical bars show ± SD of the mean.

Çağıl cultivar compared to other cultivars was low for total fresh and dry weight. However, it was proven to have a high tolerance, having the least amount of loss from waterlogging stress in W-7 and W-14. While plant height after W-14 stress was reduced by about the same amount in all lentil cultivars, plant height in the Çağıl cultivar was less affected (Figure 2). The root length loss of the Çağıl cultivar in W-7 and W-14 compared to other cultivars was low. It revealed that it may be a result of its overall tolerance.

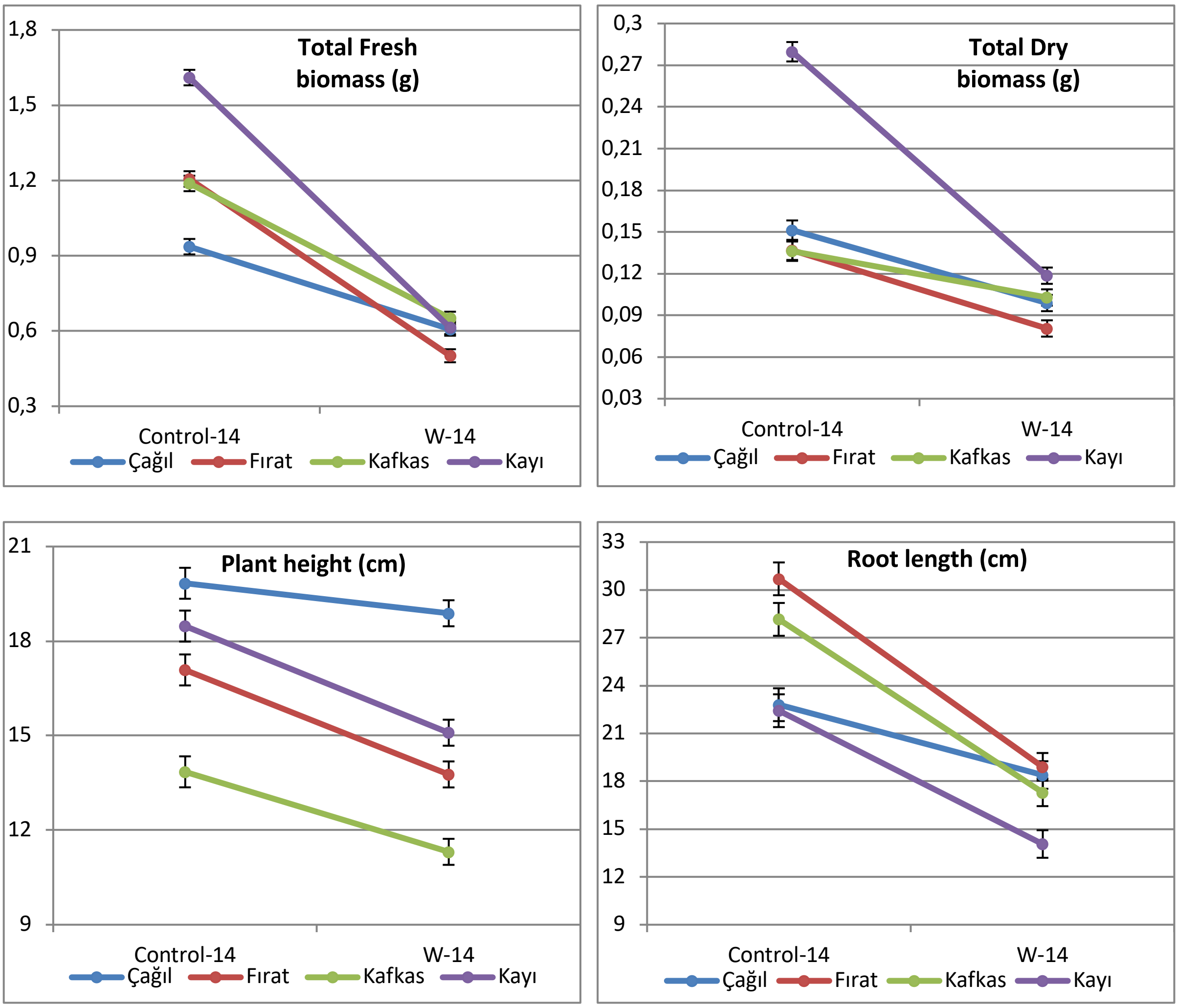

Figure 2. Total fresh biomass, total dry biomass, plant height, and root length in lentil genotypes at the end of 14 day waterlogging treatment. Vertical bars show ± standart deviation of mean

3. 2. Recovery measurements

The recovery capacity of lentil cultivars for all traits at the end of the treatment of waterlogging (W-7 and W-14) after the flowering period is given in Table 3. In the R-7 and R-14 recovery processes compared to the control were significantly reduced for all traits. In R-14, the recovery capacity of stem dry weight, root dry weight, and total dry biomass has deteriorated. Compared to the control, plant height was decreased by 18.9% in R-7 and 23.7% in R-14 (Table 3). Leaf dry weight decreased by 45.7% and 39.9%, respectively. Stem dry weight was not affected in the W-7 and W-14 applications compared to the control; however, it was reduced by 20.7% in R-7 and 46.9% in R-14. Root dry weight decreased in R-7 and R-14 applications, but it was as high as in W-7 and W-14 measurements. The root dry weight in R14 had the lowest recovery level of all parameters studied. The total dry biomass loss rate in the recovery period (R-7 and R-14) (31.5% and 49.3%, respectively) was higher than the loss rate in the W-7 and W-14 (25.3% and 43.1%, respectively). It showed that the healing process of the plant continued by getting worse (Prasanna and Rao, 2014). As a result, the plant metabolic activities and agronomic traits continue to deteriorate even after the waterlogging (W-7 and W-14) has ended.

Table 3. Investigated traits of lentil genotypes under control and 7 and 14 day duration waterlogging treatment followed by a recovery period until 15 days later of flowering

Traits	R-0	R-7	R-14	Loss at 7 day waterlogging (%)	Loss at 14 day waterlogging (%)
Plant height (cm)	46.20 a	37.50 b	35.80 b	18.90	23.70
Leaf Dry weight (mg)	107.50a	58.40 b	64.60 b	45.70	39.90
Stem Dry weight (mg)	150.20a	119.10 b	79.70 c	20.70	46.90
Root Dry weight (mg)	87.10a	58.75 b	30.56c	32.60	64.90
Total Dry biomass (mg)	344.90a	236.20 b	174.90 c	31.50	49.30
Plant Diameter (cm)	1.12	1.08	1.20	3.70	-7.20

R: Recover days: (R-0, R-7, R-14).

The recovery capacity of lentil cultivars at the end of the treatment of waterlogging (W-7 and W-14) after the flowering period were given in Figure 3.

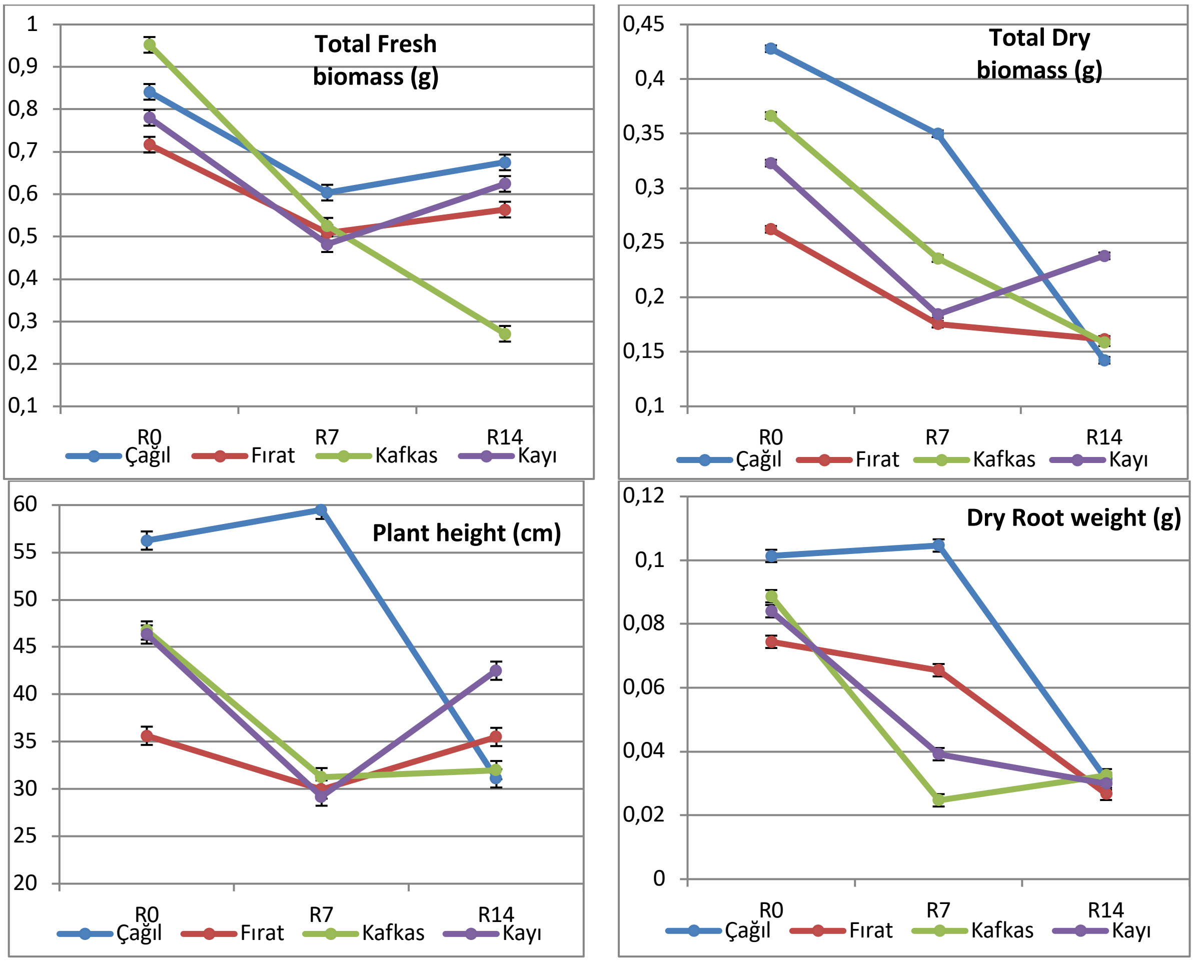

Figure 3. Total fresh biomass, total dry biomass, plant height, and dry root weight in lentil genotypes after waterlogging termination during recovery until 15 days after flowering, Vertical bars show ± standart deviation of mean.

Kafkas and Kayı cultivars for total fresh weight were more damaged under W-7 stress. In the W-14, the total fresh weight for all the cultivars except Kafkas was similar or slightly higher than in W-7. However, Kafkas cultivar was strongly negatively affected and lost more than 70% compared to the control (R-0). The total dry weight difference between cultivars was higher than the total fresh weight in R-0.

Kayı cultivar for all traits showed more loss in R-7 compared to other cultivars, but recovery in R-14 for the Kayı cultivar was high, unlike other cultivars.

Çağıl cultivar, which lost less in R-7 compared to other cultivars, lost excessive dry weight in R-14.

The waterlogging responses of lentil cultivars reveal that the Kafkas cultivar was sensitive to both waterlogging treatments. When compared to other cultivars, it might be possible to assert that Kayı had a higher tolerance to waterlogging conditions. In addition, the responses of cultivars to R-7 and R-14 treatments were different. For example, Çağıl was tolerating the R-7 application, but as the flooding continued, this situation changed in a decreasing direction. The tolerance or sensitivity to waterlogging of traits taken in the post-flowering stage (R-7 and R-14) were analyzed on the basis of cultivars. For this reason, it must be considered that the selection of resistant lentil cultivars in short-term flooding studies should be misleading, these results agree with Setter and Waters (2003).

Conclusion

To prevent major losses due to sudden flooding, which has grown increasingly common with global climate change, in lentil cultivation, it is vital to identify resistance mechanisms and resistant varieties. As the duration of waterlogging stress increases, the root zone remains oxygen-free for a longer time, preventing respiration and causing more damage to the plant. While the worst damage in lentils was particularly in roots and leaves, including all other vegetative parts. Recovery treatment in lentil increased the waterlogging damage as the stress duration (R-14) was extended. Lentil varieties respond differently to waterlogging stress and duration. The longer waterlogging duration caused more damage in lentil cultivars, and the cultivars that were able to resist short-term waterlogging weren't withstand long-term waterlogging. Also, it was not known that the responses of cultivars after waterlogging will not be the same as their responses in later development stages. This means that early detections might be misleading when trying to figure out which cultivars were tolerant or resistant. In this study, the Çağıl cultivar was generally tolerant to waterlogging, while the Kafkas cultivar was to be sensitive.

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