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Determination of the Short-Term Drought Stress Tolerance of Three Barley Varieties Using Physiological and Biochemical Changes

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ABSTRACT

Barley (*Hordeum vulgare* L.) is an important agricultural plant that can adapt to stress conditions. Abiotic stress factors significantly reduce growth, photosynthetic efficiency and metabolic processes in barley. Drought stress increases reactive oxygen species (ROS) in plant cells, and the antioxidant defense system reduces damage caused by overproduction of ROS. The aim of this study was to determine the physiological and biochemical effects of short-term drought stress on some barley cultivars (Kalaycı-97, Harman and Yaprak) grown in Turkey. In 21-day-old seedlings, short-term drought stress decreased the total chlorophyll content. The amount of total protein reduced in the Harman variety, while it increased by 19% in Kalaycı-97 and 27% in Yaprak. The H_2O_2 content decreased in Yaprak while increasing by 76% in the Kalaycı-97 variety. It was demonstrated that TBARS levels increased by 62% in Kalaycı-97 and 26% in Yaprak. In other ways, while drought stress caused a 48% decrease in APX activity in Kalaycı-97, it caused a 42% and 20% increase in APX activity in Harman and Yaprak, respectively. However, in Kalaycı-97 and Yaprak, CAT increased by 48% and 69%, respectively. These results indicate that Yaprak genotype is tolerant, Kalaycı-97 sensitive and Harman moderately tolerant to short term drought stress.

Keywords: Barley, Drought stress, Relative water content, Catalase, Ascorbate peroxidase.

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Introduction

Abiotic stress conditions that affect plants include salinity, drought, excess or shortage of ions, extremes in temperature, and inadequate or excessive light [1]. Extended periods of stress cause physiological, biochemical, and molecular alterations in plants that hinder growth and development and reduce yield [2]. The degree and duration of the stress to which a plant is subjected directly correlate with its capacity to withstand stress. ROS production is a regularly observed cellular response in plants to stressors [3]. The accumulation of ROS results in the production of potentially harmful compounds for the cell, such as superoxide (O_2*) and hydrogen peroxide (H_2O_2) [4]. ROS concentrations increase in plant cells exposed to environmental stress. Overproduction of ROS has the potential to harm cells and ultimately cause cell death. Intracellular redox balance is preserved, nevertheless, because antioxidants scavenge these radical molecules and keep them at levels that are safe for plant cells. This is crucial for optimal plant growth and yield [5]. Antioxidant enzymes like catalase (CAT) and ascorbate peroxidase (APX) or a non-enzymatic antioxidants like vitamin C and E offer protection against ROS molecules [6]. Moreover, as markers of oxidative stress, changes in malondialdehyde (MDA), H_2O_2, and protein levels are crucial for assessing stress tolerance in plants subjected to abiotic stress [6].

Barley (*Hordeum vulgare* L.), a member of *Poaceae*, is an economically important cereal crop. Between 2018 and 2019, global barley production ranked third after maize and wheat. According to the barley crop report published by the Agricultural Economics and Policy Development Institute in January 2021, in terms of the area under barley, Turkey is the second largest producer after wheat [7, 8]. It has been reported that barley has a high tolerance to dry and saline soils. This makes it one of the most adaptable cereals to different climatic conditions [9]. However, with the increasing impacts of global climate change, barley production in Turkey has become one of the crops most affected by environmental stresses such as drought and salinity. According to the Intergovernmental Panel on Climate Change (IPCC), it is predicted that the severity and frequency of abiotic stresses will increase in the near future due to global climate change [10]. Increasing population, industrialization, soil degradation, and climate change are challenging agricultural water availability [9]. In this context, evaluating and identifying drought-tolerant genotypes is crucial for the future of agriculture and food production.

This research focuses on determining for the first time the drought tolerance of three barley varieties grown in Turkey (cv. Kalaycı-97, cv. Harman and cv. Yaprak) under short-term drought stress. The determination of drought tolerance of these varieties was investigated based on physiological [relative water content (RWC), leaf water loss (LWL), total chlorophyll content (SPAD), dry weight (DW)] and biochemical [total protein content, CAT

activity, APX activity, H_2O_2 level, histological localization of H_2O_2, MDA level] changes.

Materials and Methods

Plant Material and Growing Conditions

Barley (*H. vulgare*), a high-value cereal crop from the *Poaceae* family, was selected for this study. *H. vulgare* cv. Kalaycı-97 obtained from the Transitional Zone Agricultural Research Institute (Eskişehir/TÜRKİYE), while *H. vulgare* cv. Harman and *H. vulgare* cv. Yaprak were obtained from the Thrace Agricultural Research Institute (Edirne/TÜRKİYE).

The seeds were sterilized with 5% NaCl and placed in dishes with moist filter paper. Seedlings were grown in pots containing perlite and in a growth chamber (22-24°C, 65% relative humidity, 16/8h light/dark at 130-250 µmol m^{-2} s^{-1} light intensity) irrigated with Hoagland's solution (100%) [11]. The 21 days old seedlings (3 weeks old plants) were divided into two groups: Control and Drought. Each group contained 75 seedlings. In the drought group, 21 d old seedlings were subjected to drought stress through irrigation cessation. The control group continued with Hoagland (100%) irrigation. Physiological parameters such as RWC, LWL, DW, chlorophyll contents (SPAD), H_2O_2 levels, enzyme activities and histological localization of H_2O_2 were determined in leaf tissues of 31-day-old seedlings after 10 days of drought stress. Sampled leaf tissues were stored in a deep freezer (-20°C) until biochemical analyses [MDA, total protein content, CAT, APX]. The experimental design was a randomized complete block design with at least independent three replicates (each replicate is 1 pot x 20 seedlings).

Physiological Parameters

To determine LWL, the last fully developed leaf of each plant was sampled. The samples were kept in a cold chain (+4°C) to prevent water loss. After leaf fresh weight recording (0 min), leaves were left at room temperature (21°C) and leaf fresh weights were recorded at 10, 20 and 30 min. The LWL values were calculated as the percentage of weight loss from the fresh weight [12]. Five replicate samples were taken from each group.

Mature leaves of the seedlings were used to measure fresh weight. Leaves were then placed between filter papers in a plastic cuvette containing pure water for 4 hours and turgid weights recorded. Subsequently, the same leaves are dried at 70 °C for 24 h to determine their dry weight. The RWC was calculated according to the following formula (Equation 1) [13].

$$\mathrm{RWC} = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} \mathrm{X}100 \qquad (1)$$

(FW: Fresh weight, TW: Turgida weight, DW: Dry weight)

Total chlorophyll was measured using a chlorophyll meter (Minolta, SPAD-502, Osaka, Japan). [14]. Accordingly, the measurements were carried out on the fully developed mature leaves of the seedlings, with 15 replicates being taken from three different seedlings in each group, obtained from five pots.

Leaf samples from each group were dried at 70°C for 72 h and then used to determine the leaf DW of each plant. Sampling was carried out with at least 3 replicates in each group [15].

Determination of Antioxidant Enzyme Activities

The total protein content in leaf tissues was homogenized in 3 mL of 0.05 M sodium phosphate buffer (pH 7.8) containing 1 mM EDTA (Ethylenediaminetetraacetic acid). The homogenates were then centrifuged for 30 min at +4°C and 18895×g. The supernatant was used for protein analysis. All procedures were performed at +4°C. Protein concentration was determined against a blank at 595 nm using a spectrophotometer within 5-60 min after mixing 100 µL of supernatant with 5 mL of reagent [16]. Total protein content was expressed in mg/mL using bovine serum albumin as a standard.

Catalase activity (EC 1.11.1.6) was determined as the decrease in absorbance at 240 nm due to the amount of H_2O_2 consumed. The CAT activity is expressed in terms of µmol ml^{-1} of H_2O_2 consumed per minute [17].

Ascorbate peroxidase activity (EC 1.11.1.11) was determined by homogenizing leaves in 1ml of 50mM Na-P buffer (pH 7.8) with 2mM ascorbate and 1mM EDTA. The supernatants were used for analysis of APX activity (extinction coefficient 2.8 mM^{-1} cm^{-1}). The amount of enzyme is the amount of 1 µmol ml^{-1} ascorbate oxidized per minute. [18].

For H_2O_2 determination, 0.1 g fresh sample was homogenized in a buffer containing H_2SO_4 and cold acetone (100%). The samples were subsequently centrifuged at 4000xg for 5 min. The supernatants were then mixed with e-FOX reading buffer and allowed to stand for 30 min. The results were read at 550-800 nm against a blank (water) in a polystyrene cuvette [19].

For the H_2O_2 histochemical localization leaves were incubated in a solution containing 1 mg ml^{-1} 3',3'-diaminobenzidine (DAB) at 25°C for 12 h. The incubated leaves were then immersed in 90% ethanol in a hot water bath for 15 min to remove color. Subsequently, stained leaves were photographed against a contrasting background for visualization [20].

The degree of lipid peroxidation (nmol g wet $weight^{-1}$) was determined by measuring the level of MDA, the product of lipid peroxidation (€=155 $mM^{-1}cm^{-1}$) [21].

Statistical Analysis

Data were subjected to multiple comparisons using one-way analysis of variance (ANOVA) followed by the Tukey test. Statistical analysis was performed using SPSS V21.0 (Statistical Package for the Social Sciences, Version 21.0) software. The significance levels are shown in the graphs, and comparisons with a significance level of $P \leq 0.05$ were considered significantly different. Table 2 presents the correlation analysis between the variables.

Results

Physiological Parameters

At the end of the experiment, the total LWL in the control group of Kalaycı-97 was 10%, while it was found to be 13% in the drought group. The best stomal conductance started after 20 min in the control group and 10 min in the drought group (Figure 1A). In the Harman variety, the total LWL within half an hour was 4% in the control group and 5% in the drought group. The best stomal conductance occurred after 10 min in the drought group (Figure 1B). At the end of the experiment, the total LWL in the Yaprak cultivar was 11% in both groups. In this variety, the best stomatal conductance started after 10 min in both groups (Figure 1C).

The relative water content decreased in all cultivars compared to the controls. However, only Kalaycı-97 showed a statistically significant decrease of 8% (Figure 1D).

Total chlorophyll content decreased by 14-28% in all varieties under short-term drought stress compared to the control (Figure 1E).

Dry weight decreased in all three cultivars due to drought stress. Indeed, Kalaycı-97 was found to have the greatest decrease in dry weight (17%) among the three varieties (Figure 1F).

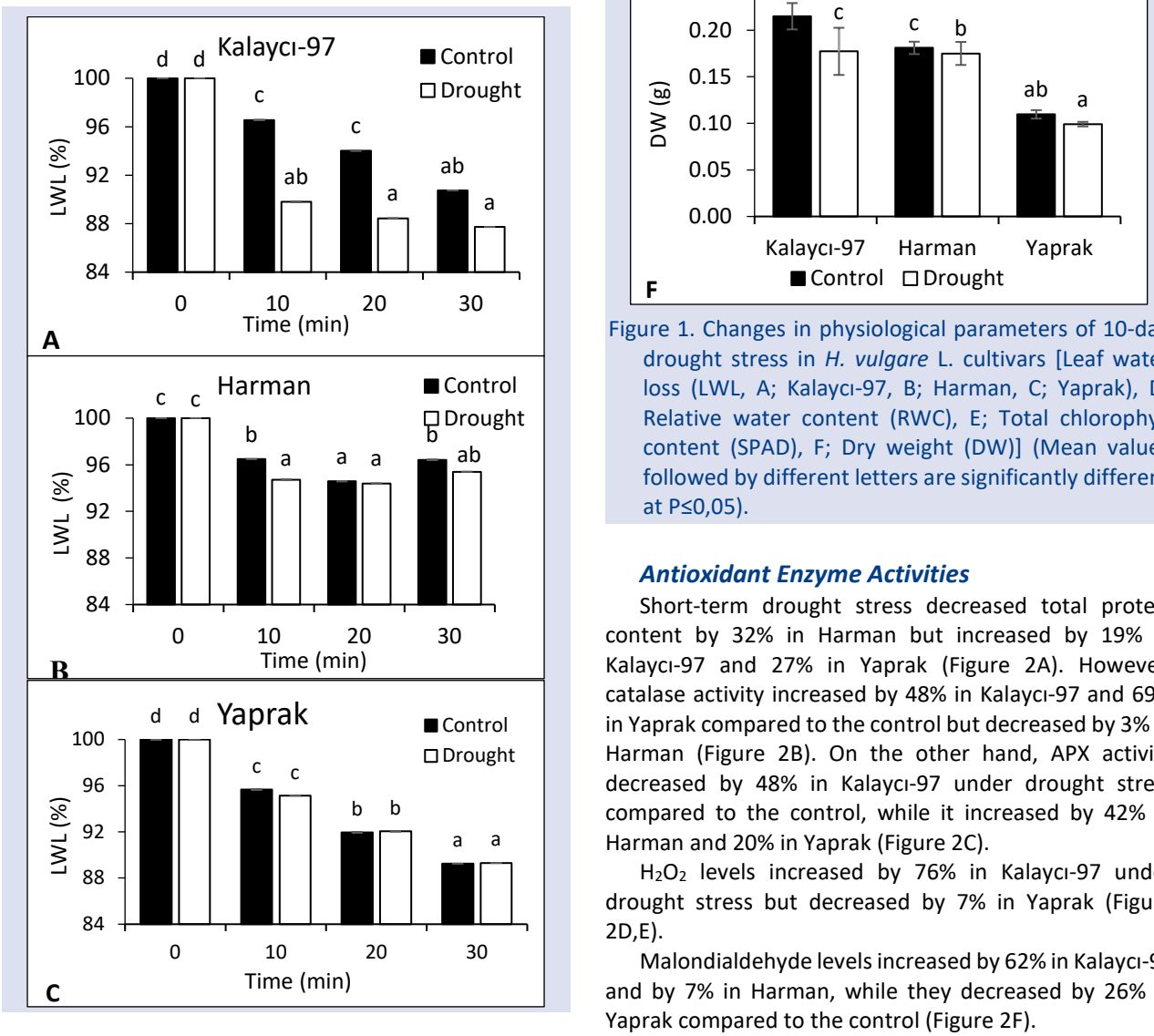


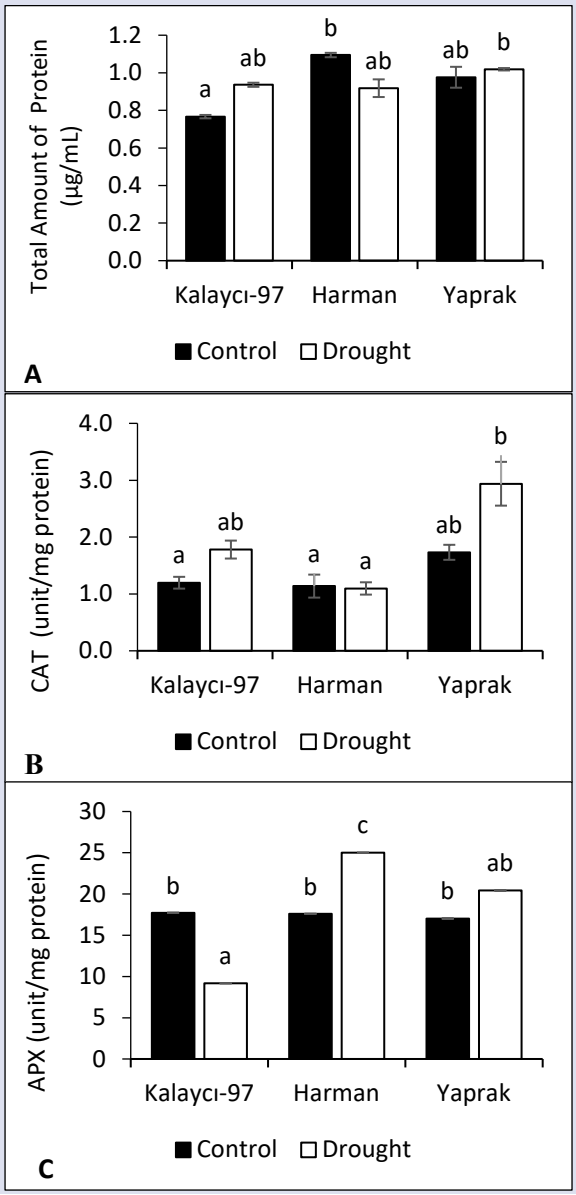
Figure 1. Changes in physiological parameters of 10-day drought stress in *H. vulgare* L. cultivars [Leaf water loss (LWL, A; Kalaycı-97, B; Harman, C; Yaprak), D; Relative water content (RWC), E; Total chlorophyll content (SPAD), F; Dry weight (DW)] (Mean values followed by different letters are significantly different at P≤0,05).

Antioxidant Enzyme Activities

Short-term drought stress decreased total protein content by 32% in Harman but increased by 19% in Kalaycı-97 and 27% in Yaprak (Figure 2A). However, catalase activity increased by 48% in Kalaycı-97 and 69% in Yaprak compared to the control but decreased by 3% in Harman (Figure 2B). On the other hand, APX activity decreased by 48% in Kalaycı-97 under drought stress compared to the control, while it increased by 42% in Harman and 20% in Yaprak (Figure 2C).

H_2O_2 levels increased by 76% in Kalaycı-97 under drought stress but decreased by 7% in Yaprak (Figure 2D,E).

Malondialdehyde levels increased by 62% in Kalaycı-97 and by 7% in Harman, while they decreased by 26% in Yaprak compared to the control (Figure 2F).



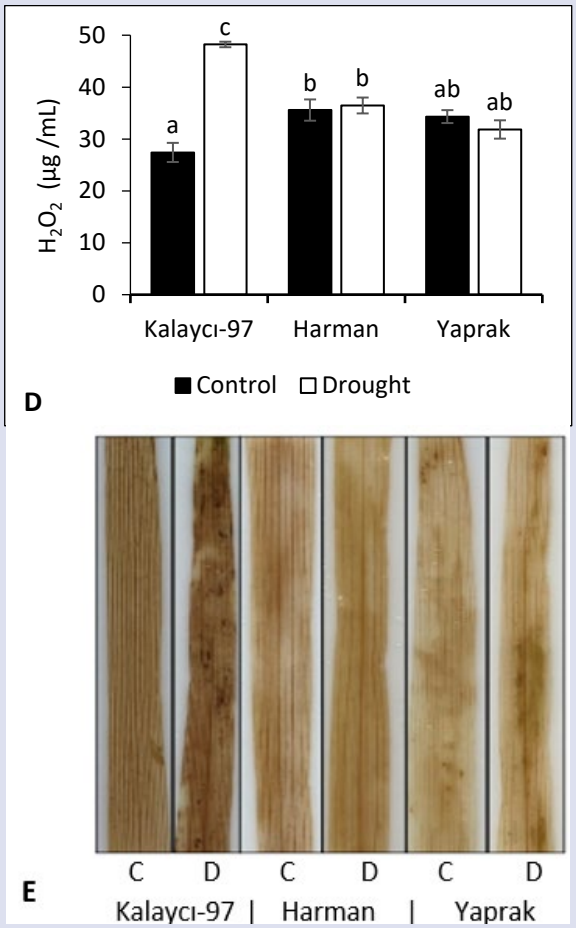


Figure 2. Changes in antioxidant enzyme activities caused by 10 days of drought stress in *H. vulgare* L. cultivars [A; Total protein amount, B; Catalase (CAT), C; Ascorbate peroxidase (APX), D; H_2O_2 amount, E; H_2O_2 Histochemical staining (C; Control, D; Drought), F; Malondialdehyde amount] (Mean values followed by different letters are significantly different at P≤0.05).

Table 1. Correlations between physiological and biochemical parameters in all varieties.

		1	2	3	4	5	6	7	8	9
1	LWL	1								
2	RWC	,545**	1							
3	SPAD	,349*	,678**	1						
4	DW	0,163	0,326	0,284	1					
5	PR	-0,001	0,07	-0,046	-0,357	1				
6	CAT	-0,251	-0,343	-,450**	-,523*	0,155	1			
7	APX	-0,122	,440*	0,164	-0,148	-0,163	-,414*	1		
8	H_2O_2	-,345*	-,683**	-,466**	-0,328	0,138	-0,034	-0,196	1	
9	MDA	-0,175	-,499**	-0,308	0,366	-0,085	-0,144	-,424*	,535**	1

Note: LWL=Leaf water loss, RWC=Relative water content, SPAD=Total chlorophyll content, DW=Dry weight, PR= Total protein amount, CAT= Catalase activity, APX=Ascorbate peroxidase, H_2O_2 amount, MDA= Malondialdehyde amount.

**: Significant correlations P<0.01, and *: P<0.05

Discussion

Drought is a limiting factor for growth and yield in barley, as in most cereal crops. There is a need to understand the effects of global climate change induced drought stress on existing varieties. This data is important for potential farmers to use these varieties. This study focuses on determining the drought tolerance of three barley cultivars (cv. Kalaycı-97, cv. Harman and cv. Yaprak)

grown in Turkey based on physiological and biochemical parameters.

Relative water content reflects the water status in plant tissues [22], and drought stress negatively affects plant water content [23]. According to our results, short-term water deficit reduced RWC only in Kalaycı-97 among the three barley varieties. The LWL results indicated that Kalaycı-97 had the highest water loss of 3% compared to the control among the three varieties at the end of 30 min. On the other hand, there was no water loss in Yaprak variety, while it decreased by 1% in Harman. Accordingly, it was understood that drought stress decreased the water content most in Kalaycı-97. Similar studies have reported differences in RWC among genotypes with contrasting drought tolerance [24], and drought stress has been shown to reduce RWC in barley [22]. Furthermore, a significant relationship between RWC and drought tolerance was found in barley genotypes exposed to prolonged water shortage [25]. In addition, a positive phenotypic correlation between grain yield under water deficit conditions and RWC has been reported in barley [26]. Based on the RWC results, Kalaycı-97 seems to be less drought tolerant than other varieties.

Drought stress is known to reduce chlorophyll content in plants [3, 27, 28]. In this study, short-term drought stress resulted in reduced total chlorophyll content in all three cultivars. Similarly, severe drought stress [22, 29] and water scarcity [26] have been reported to reduce chlorophyll content in barley. Furthermore, changes in total chlorophyll contents in barley genotypes have been significantly associated with drought tolerance [30]. The decrease in the chlorophyll content of Kalaycı-97 under short-term drought stress suggests that it is much more sensitive to drought than other varieties.

Total protein content increased under drought stress in Kalaycı-97 and Yaprak, but surprisingly decreased in Harman. Drought stress has been reported to decrease protein content in barley [29]. Therefore, the increase in protein content in the cultivar Yaprak may indicate its drought tolerance.

Drought stress increases the concentration of ROS in plant cells. ROS include molecules with unpaired electrons, such as O_2^{*-}, OH^-, and H_2O_2 [31]. Increased levels of ROS can cause damage to lipids, proteins, and DNA. Therefore, the antioxidant defense system, consisting of enzymatic and non-enzymatic antioxidants, plays an important role in detoxifying ROS [32].

Drought stress decreased APX activity in Kalaycı-97 but increased it in Harman and Yaprak. In addition, CAT activities increased in Kalaycı-97 and Yaprak, but remained unchanged in Harman. High concentrations of ROS can attack lipids in cells and lead to lipid peroxidation if not detoxified. As an indicator of this, MDA levels decreased in Yaprak under short-term drought stress compared to the control but increased by 62% in Kalaycı-97 and 7% in Harman. Short-term drought stress increased H_2O_2 levels in Kalaycı-97 but decreased them in Yaprak. The localization of H_2O_2 in barley leaf tissue is visualized by histochemical staining with 3',3'-diaminobenzidine (DAB) [20]. Our histochemical staining results support the findings on H_2O_2 levels. According to our results, the high H_2O_2 levels and low APX activity in Kalaycı-97 may indicate a weaker drought tolerance compared to the other varieties.

The significant increase in H_2O_2 and MDA levels under drought stress has been associated with drought susceptibility in barley [22]. On the other hand, severe drought stress [33] or decreasing soil moisture [34] has been reported to increase CAT activity in barley. In addition, drought stress induced by 10% PEG (-0.8 MPa) was shown to increase POX, SOD and APX isoenzyme activities in barley and was confirmed to be associated with growth parameters [35]. Furthermore, different barley genotypes have been shown to exhibit different molecular and biochemical responses under the same drought conditions [36]. Interestingly, in barley leaves under water stress, there was a sharp increase in APX, CAT, POX and SOD activities during flowering and stem elongation. However, the continued increase in MDA levels suggests that the increase in these enzymes may not be sufficient [29].

According to our research results, short-term drought stress decreased RWC and pigment content, increased H_2O_2 levels and increased lipid peroxidation in Kalaycı-97. Despite the increase in CAT activity, the decrease in APX activity in this genotype suggests that increased CAT activity alone may not be sufficient to reduce lipid peroxidation. Similar results have been reported in water-stressed barley [29]. Furthermore, the results for Kalaycı-97 suggest that this cultivar has a lower drought tolerance compared to other cultivars. On the other hand, in Yaprak genotype under short-term drought stress, RWC was preserved, less chlorosis was detected, the amount of H2O2 was lower, and lipid peroxidation was found in accordance with increased APX, and CAT activities compared to other genotypes. Moreover, high RWC, pigment and antioxidant activities have been shown to be associated with high drought tolerance [22, 35, 36]. This suggests that the Yaprak genotype is more tolerant to short-term drought stress compared to other genotypes. According to our results, the Harman genotype was found to have moderate tolerance to short-term drought stress compared to the Kalaycı-97 and Yaprak genotypes. The fact that increased APX and CAT activities in this genotype could not prevent the increase in lipid peroxidation is consistent with previously reported results in barley [29].

It has been reported that a 15% differential water deficit in barley leaves is associated with signaling and ABA metabolism, leading to appropriate defense activation [37]. Furthermore, it has been reported that high temperature stress, which is prominent in global climate change, in combination with drought, causes more severe damage to barley [4]. Our results indicate that the three barley genotypes used in this study, grown in Turkey and exposed to short-term drought stress, have different drought tolerances. Accordingly, it can be said that Yaprak is tolerant, Kalaycı-97 is sensitive, and Harman is moderately tolerant to short-term drought stress.

Conclusions

In this study, tolerance to short-term drought stress has been demonstrated physiologically and biochemically in three barley cultivars grown in Turkey. However, further research is needed to understand the effects of long-term drought tolerance and the tolerance mechanisms in these genotypes. In addition, studying the interactions of drought effects with major stressors such as temperature can provide valuable insights into potential scenarios related to global climate change.

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Conflict of interest

There are no conflicts of interest between the authors of the articles.

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