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Original article

Comparison of Drought Tolerance of Some Wheat Varieties Grown in Turkey

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Abstract

The effect of drought stress on 21 wheat (*Triticum aestivum* L.) varieties were screened at the germination stage for drought tolerance. Four levels of osmotic stress was assessed by applying different polyethylene glycol (PEG-6000; 0%, 10%, 15% and 20% (w/v)) concentrations. Germination rate, root-shoot length, total chlorophyll amount (SPAD), specific leaf area (SLA), relative water content (RWC), superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) accumulation were determined on the 8th day after sowing. The increased osmotic stress, significantly reduced germination rate, root-shoot length, SLA, chlorophyll amount and RWC in KateA-1, Gelibolu and Sultan-95 varieties. Pehlivan, Karahan-99 and Tekirdağ varieties were less affected by osmotic stress and these varieties were determined as drought tolerant varieties than other varieties.

Keywords: Drought Tolerance, Wheat, Germination, PEG, Osmotic Stress.

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INTRODUCTION

Drought is one of the most common environmental stress factors that affect the growth and development of plants. Drought stress is affecting the growth, development, and productivity of plants (Diab et al., 2004), causes many metabolic, mechanical, and oxidative changes in plants (Asseng et al., 2015). One of the earliest signals of many abiotic stresses, such as drought, is the formation of reactive oxygen species (ROS), which affects enzyme activities (Laxa et al., 2019). Plants can reveal the accumulation of ROS, such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$), and hydroxyl radical ($^{\cdot}OH$) under drought stress (Apel and Hirt, 2004).Wheat (*Triticum aestivum* L.) is a strategic cereal crop for the world's population. Drought conditions have inhibitory effects on wheat growth, impairing photosynthetic processes, leaf water content, and subsequent developmental processes. Additionally, the decline in photosynthetic pigments can be associated with an insufficient water supply, which reduces the leaf water content (Faroog et al., 2009).

Water deficit reduces crop growth, leaf expansion, shoot elongation, and biomass production. The effect of water deficit may vary according to the plant's variety and severity and duration of water stress as well as the growth stage. Under all stress conditions, including drought stress, excessive reactive oxygen species (ROS) accumulation due to insufficient antioxidant defense capacity causes oxidative stress in plants (Apel and Hirt, 2004; Abid et al., 2018; Hasanuzzaman et al., 2018). Physiological and morphological characteristics such as water content and area in leaf, root, and shoot length provide important data to understand the drought tolerance state of wheat during the seedling stage. Reduces leaf relative water content (RWC) which ultimately leads to reducing growth and biomass production. RWC is a plant water status indicator and was used successfully to identify drought-resistant varieties of wheat (Sharma et al., 2022). SLA is the ratio of leaf biomass to leaf area and is a measure of plant growth versus environmental factors (Wilson et al., 1999). Reduced chlorophyll causes chlorosis, leading to a reduction in photosynthesis. For this purpose, in this study, germination percentage, root shoot length, total chlorophyll content (SPAD), specific leaf area (SLA), relative water content (RWC), superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) accumulation were evaluated under drought stress.

The aim of this research is to determine the short-term drought tolerance of 21 wheat varieties grown in Turkey based on physiological and biochemical parameters. Accordingly, germination percentage, root shoot length, total chlorophyll content, SLA, RWC, $O_2^{\cdot-}$ and H_2O_2 accumulation were determined in 8-day-old wheat seedlings.

MATERIALS and METHODS

Plant Materials and Growth Conditions

In the study, 21 local bread wheat (*T. aestivum* L.) varieties were used as plant material. Tosunbey, Hamitbey, Bayraktar-2000, Demir-2000, Harmankaya-99, Nacibey, Sultan-95, Tekirdağ, Kate A-1, Pehlivan, Aldane, Gelibolu, Konya-2002, Sehzade, İkonya, Ekiz, Taner, Dağdaş-94, Selçuklu, Bozkır, Karahan-99 wheat varieties were obtained from different institutes (Table 1). The influence of polyethylene glycol (PEG 6000) induced drought stress on the physiological, and biochemical characteristics of wheat varieties were investigated. Seeds from different wheat varieties were germinated in petri dishes at 22 ±2^{0}C under a 16/8 h light and dark cycle. The experiment consisted of four treatments; each treatment contained 21 varieties with three replicates. A control treatment with only distilled water and stress treatments with 10%, 15% and 20% PEG 6000 were performed (Michel and Kaufmann, 1973).

Severe drought (20%) suppressed seed germination and seedling growth. Therefore, it could not be used to measure necessary plant characteristics.

Table 1. Wheat varieties used as experimental materials in this study with sources (1: Bahri Dağdas International Agricultural Research Institute (Konya/ TURKEY) 2: Directorate of Trakya Agricultural Research Institute (Edirne / TURKEY) 3: Transitional Zone Agricultural Research Institute (Eskişehir / TURKEY) 4: Field Crops Central Research Institute (Ankara / TURKEY)).

1	2	3	4
Konya-2002	Tekirdağ	Tosunbey	Harmankaya-99
Şehzade	Kate A-1	Hamitbey	Nacibey
İkonya	Pehlivan	Bayraktar-2000	Sultan-95
Ekiz	Aldane	Demir-2000	
Taner	Gelibolu		
Dağdaş-94			
Selçuklu			
Bozkır			
Karahan-99			

Germination Rate (%)

Germination percentage is an estimate of germination percentage that was determined by calculating the ratio of germinated wheat seeds to the total number of seeds.

Germination Percent (%) = (number of germinated seeds / number of experimental seeds) x 100

Shoot and Root Lengths

Shoot and root lengths were measured from 15 plants of each variety. The lengths of the root and shoot parts of the plants were measured with a ruler. Average values were calculated for each variety.

Total Chlorophyll Content (SPAD)

Total chlorophyll amounts of leaf samples were measured with a chlorophyll meter (Minolta, SPAD502) (Peryea and Kammereck, 1997). For this purpose, randomly selected 10 plants from each treatment were used to measure the chlorophyll content.

Relative Water Content (RWC)

To measure RWC, fully expanded younger leaves from each treatment were collected. Leaves were removed and weighed immediately for measurement of fresh weight (FW). Turgid weight (TW) was determined after leaves were immersed in distilled water for 6 h, and dry weight (DW) was measured after leaves were dried at 70°C in an oven for 24 h. RWC was calculated by following the formula as described by Smart and Bingham (1974).

$$RWC\ (\%) = (FW - DW)/(TW - DW) \times 100$$

Specific Leaf Area (SLA)

Specific leaf area was calculated using the leaf photos of wheat seedlings in the Image J program. Then the samples are dried in an oven at 70°C for 24 h and weighed on a precision scale. SLA is calculated by the formula (Wilson et al., 1999).

$$SLA = Area\ (cm^{2)}\ /\ Dry\ weight\ (mg^{-1})$$

Histochemical Localization of $O_2^{\cdot}-$ and H_2O_2

Generation of $O_2^{\cdot}-$ and H_2O_2 was analyzed histochemically using nitro blue tetrazolium (NBT) and 3,3-diamino-benzidine (DAB), respectively. Leaves were immersed in solutions at 25°C for 12 h. The incubated leaves were decolorized by immersion in boiling ethanol (90%) for 15 min to visualize the reddish-brown spots of H_2O_2 and blue spots of $O_2^{\cdot}-$. Then stained leaves were photographed against a contrasting background for proper visual (Kumar et al., 2014).

Statistical Analysis

The data were made with the Tukey test using a one-way analysis of variance (ANOVA). SPSS (Statistical Package for the Social Sciences, version 21.0) program was used for statistical analysis. Significance levels were shown in graphs. Those comparisons with $P \leq 0.05$ were taken as significantly different (Table 2).

RESULTS and DISCUSSION

Germination Percentage (%)

Drought stress decreased germination in all varieties. It was found that the effect of drought stress on germination percentage in wheat varieties was the lowest in Gelibolu and Selçuklu varieties. Compared to the control, drought stress induced with 10%, 15% and 20% PEG reduced the germination of Gelibolu by 17%, 69%, and 100%, respectively, and Selçuklu by 27%, 37%, and 73%, respectively. According to our results, the varieties that are least affected by drought stress are Karahan-99 and Aldane. The germination of Karahan-99 variety decreased by 3%, 7% and 27%, respectively, with increasing PEG applications compared to the control, while it did not change with 10% PEG application in Aldane variety but decreased by 10% and 17% with 15% and 20% PEG (Figure 1 and 2).

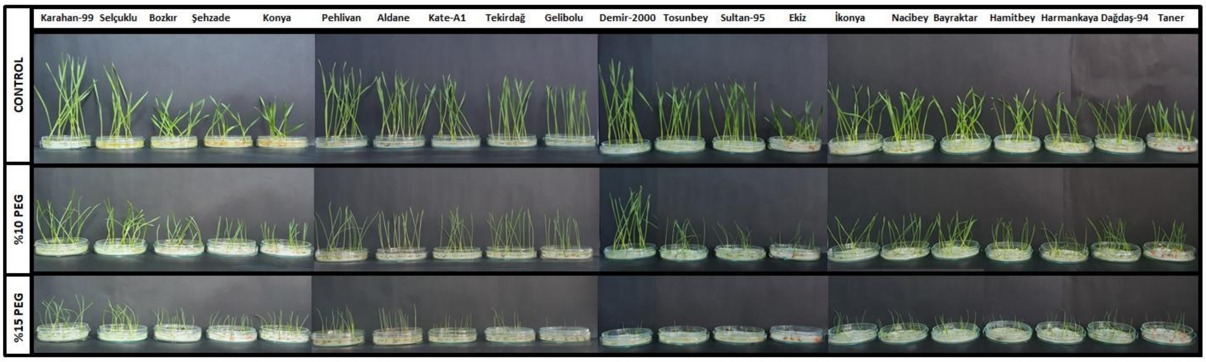

Figure 1. Effect of drought stress (0% (Control), 10% and15% PEG 6000) on seedling growth in different wheat varieties.

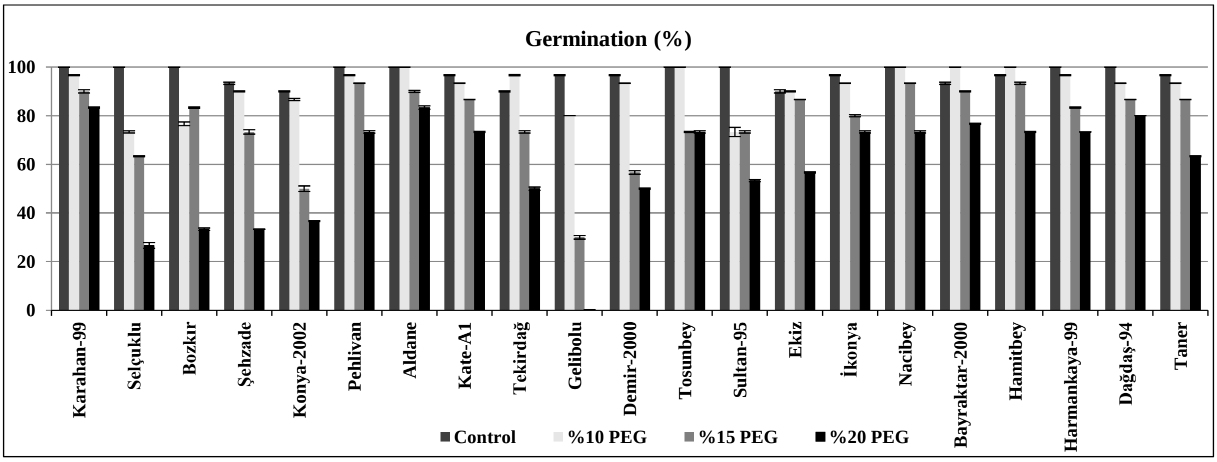

Figure 2. Effect of drought stress (Control (0%), 10%, 15% and 20% PEG 6000) on germination percentage in different wheat varieties.

Shoot and Root Lengths

Drought stress significantly reduced shoot length in all varieties compared to the control group. Shoot length was decreased by 10%, 15% and 20% PEG applications compared to the control group, the lowest decrease was observed in Şehzade and Bozkır varieties, and the highest decrease was determined in Kate A-1 and Sultan-95 varieties (Figure 1 and 3). Germination occurred at 20% PEG concentration in wheat varieties, but no shoot development was observed in all varieties.

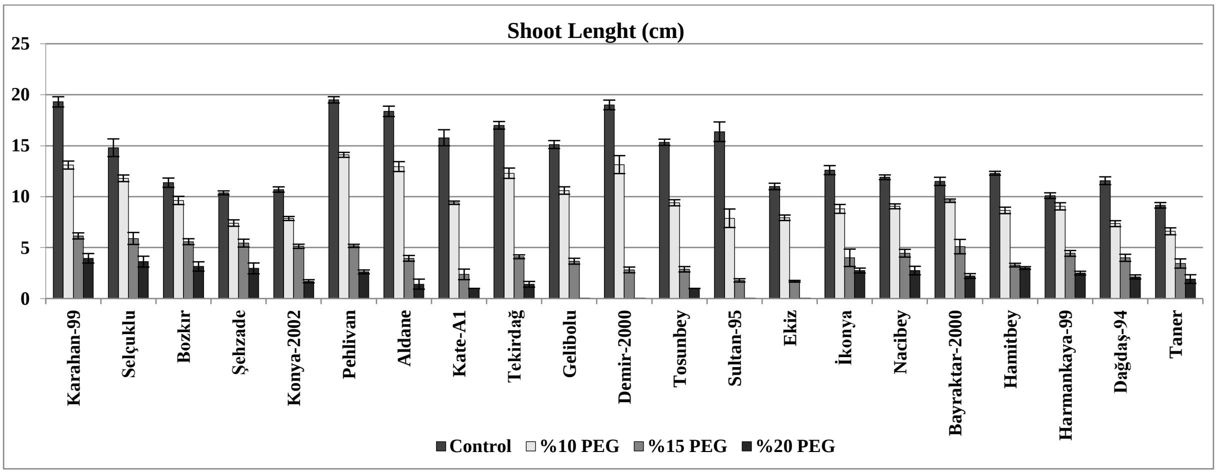

Figure 3. Effect of drought stress (Control (0%), 10%, 15% and 20% PEG 6000) on shoot length in different wheat varieties.

Increasing drought stress in wheat varieties significantly affected root length. Interestingly, the root length of Karahan-99 and Bozkır varieties increased by 110% and 51% with 10% PEG application compared to controls. Drought stress induced by 10%, 15% and 20% PEG reduced root length the most in Sultan-95 and Kate A-1 varieties. These decreases were 8%, 71% and 81% respectively in Sultan-95 variety and 28%, 63% and 79% in Kate A-1 variety compared to the control (Figure 4).

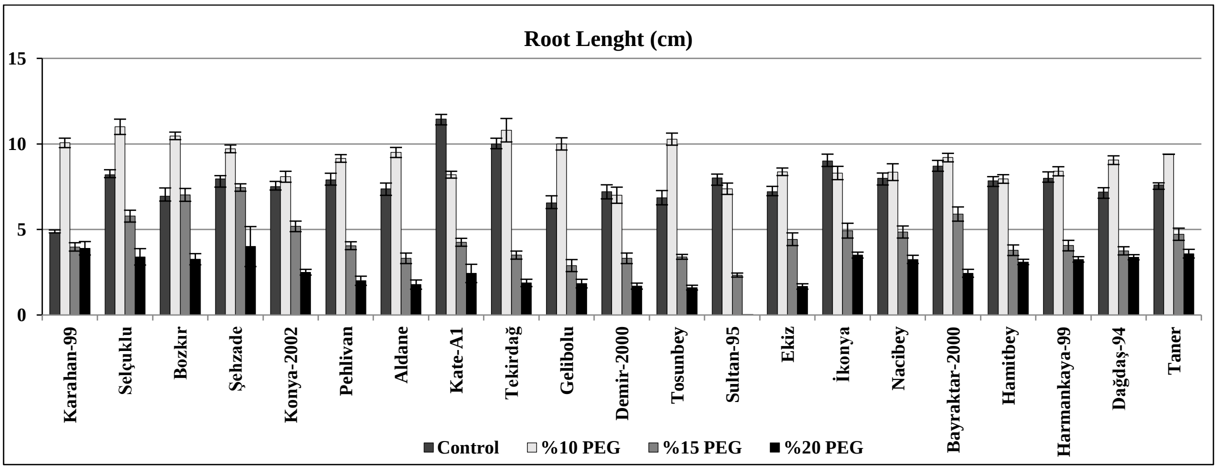

Figure 4. Effect of drought stress (Control (0%), 10%, 15% and 20% PEG 6000) on root length in different wheat varieties.

Total Chlorophyll Content (SPAD)

10% PEG application increased the total chlorophyll content by 11% and 7%, respectively, in Selçuklu and Konya-2002 varieties compared to the control group. It decreased by 35%, 35% and 31%

in Sultan-95, Kate A-1 and Hamitbey varieties, respectively. SPAD data could not be calculated because the leaves of wheat varieties are curled lamina at 15% and 20% PEG drought stress levels (Figure 5).

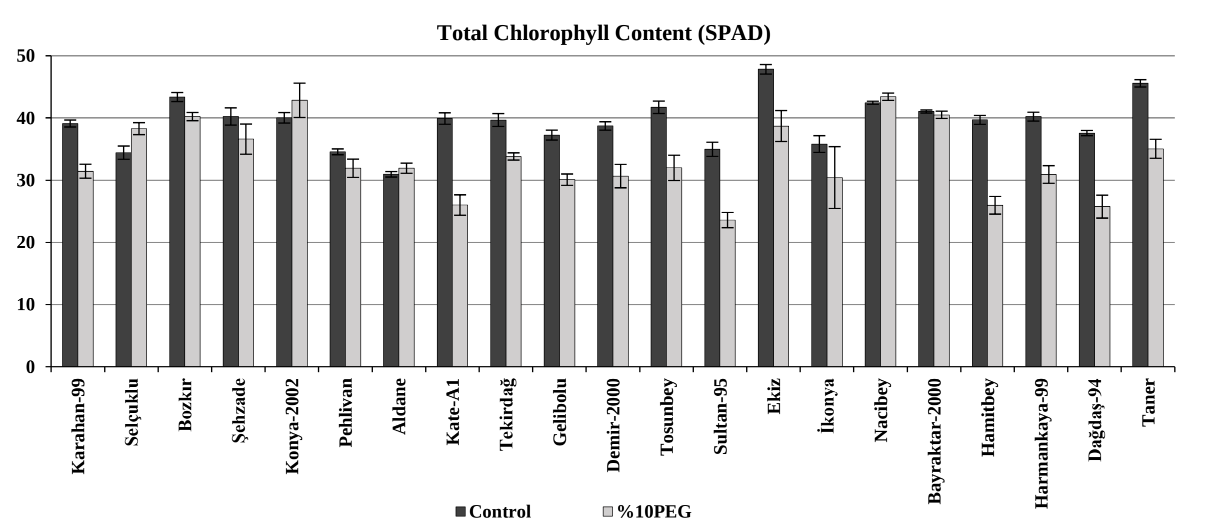

Figure 5. Effect of drought stress (Control (0%) and 10% PEG 6000) on total chlorophyll content in different wheat varieties.

Specific Leaf Area (SLA)

Drought stress decreased the SLA in all wheat varieties. The varieties whose SLA decreased the most under drought stress were Gelibolu and Demir-2000, while those with the least decreased were Karahan-99 and Pehlivan varieties. With 10% and 15% PEG, SLA decreased 32% and 55% in Karahan-99, 32% and 61% in Pehlivan, 56% and 91% in Gelibolu, and 45% and 91% in Demir-2000 (Figure 6).

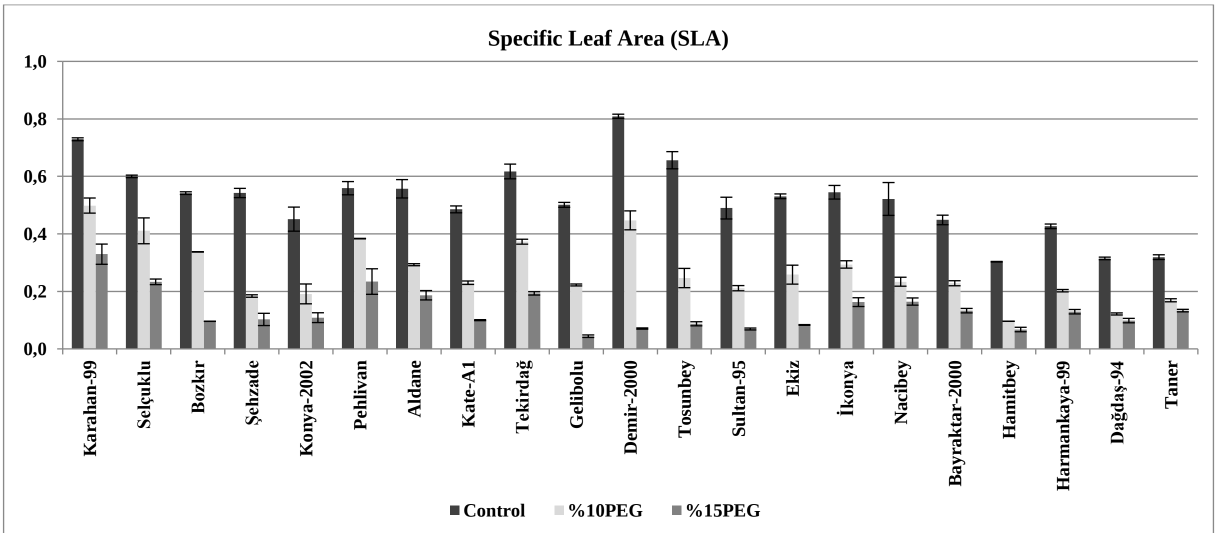

Figure 6. Effect of drought stress (Control (0%), 10% and 15% PEG 6000) on specific leaf areas in different wheat varieties.

Relative Water Content (RWC)

When exposed to 10% and 15% PEG-induced drought stresses, the RWC content decreased significantly in Gelibolu and Sultan-95 varieties compared to the control. These decreases were determined as 10% and 79% in Gelibolu and 43% and 51%, in Sultan-95 respectively. On the other hand, Bayraktar-2000 and Şehzade were the least affected varieties compared to other varieties (Figure 7).

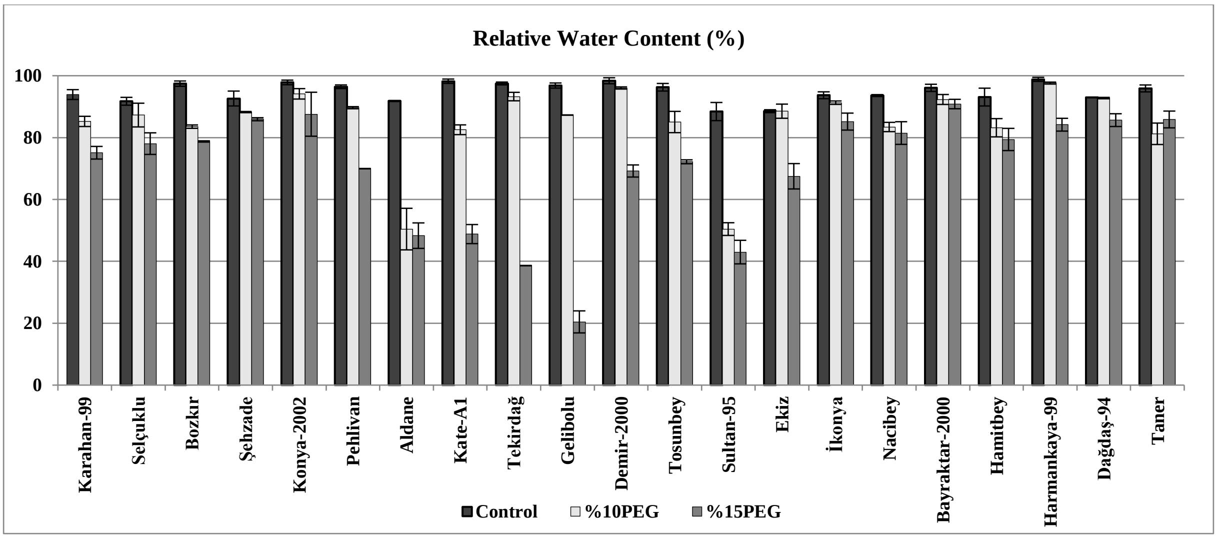

Figure 7. Effect of drought stress (Control (0%), 10% and 15% PEG 6000) on relative water contents in different wheat varieties.

Histochemical Localization of Superoxide Anion ($O_2^{\cdot-}$) and Hydrogen peroxide (H_2O_2)

The level of ROS species was determined by histochemical examination using two different dyes. Staining with NBT shows that dark blue spots contain $O_2^{\cdot-}$ (Figure 8), while staining with DAB shows that brown spots contain H_2O_2 (Figure 9). Accordingly, Pehlivan, Karahan-99 and Tekirdağ varieties were found to use antioxidant mechanism effectively. These varieties were evaluated to be drought-stress tolerant. On the contrary, it was determined that the antioxidant mechanism was not effective against drought stress in Kate A-1, Gelibolu and Hamitbey varieties, and thus these varieties were evaluated to be more sensitive to drought stress (Figure 8 and 9).

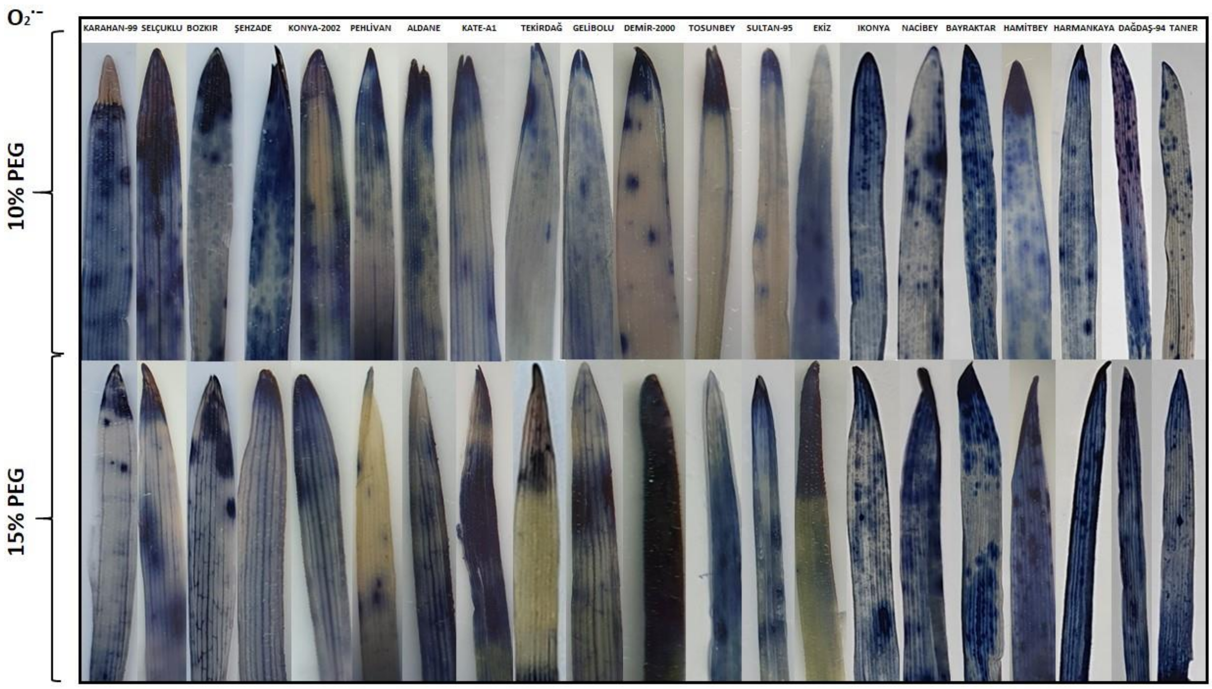

Figure 8. Detection of $O_2^{\cdot-}$ with NBT in leaves of wheat under drought stress (10% and 15% PEG 6000). The blue areas reflect $O_2^{\cdot-}$ accumulation.

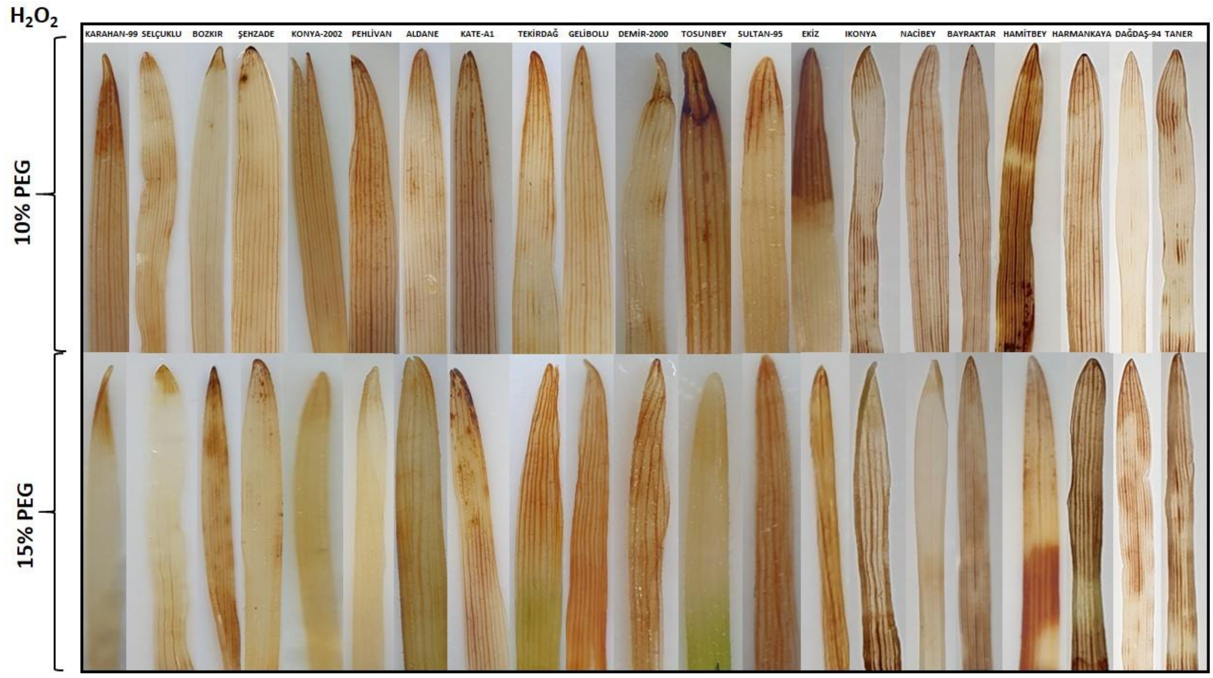

Figure 9. Detection of H_2O_2 using DAB staining in leaves of wheat under drought stress (10% and 15% PEG 6000). The brown areas reflect H_2O_2 accumulation.

Drought is one of the main abiotic stresses that seriously affect wheat production. The germination rate of wheat decreased significantly with the increase in drought stress. Our results supported with the studies of Sharma et al. (2022), which explained that the germination rate of wheat decreased significantly with the increase in drought stress. Under drought stress conditions, root growth occurs in search of water, but the growth of the shoot is reduced (Dhakal 2021; Li et al., 2021). Root length of Karahan-99, Tosunbey, Selçuklu and Bozkır varieties increased by 10% PEG treatment. In addition, it is consistent with the findings of Faisal et al. (2017) who reported that the seedling and root length decreased significantly due to the increase in drought stress in wheat.

Early wheat seedling's growth and physiology were adversely affected by the increase in the level of drought stress. Significant reduction in shoot and root length at higher concentrations of 20% PEG was observed as compared to the control group. Overall, SLA during the seedling stage had reduced significantly. Under drought stress conditions, the RWC is an important indicator of the water status in wheat (Bayomi et al., 2008). The reduction in RWC could be attributed to a decline in turgor and the reduction in cell expansion are known to contribute to a loss in leaf area (Alves et al., 2014).

In this study, it was determined that drought stress caused significant changes in germination rate, root-shoot length, SPAD, SLA and RWC parameters ($p<0.001$) (Arıcan and Demirbaş, 2022) (Table 2).

Table 2. The data were made with Tukey test using one-way analysis of variance (ANOVA). Significance levels were shown in table. Those comparisons with $P \leq 0.05$ were taken as significantly different.

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	16980.780	79	214.947	172.556	.000
Shoot	Within Groups	773.557	621	1.246		
	Total	17754.337	700			
	Between Groups	5603.036	82	68.330	75.361	.000
Root	Within Groups	602.050	664	.907		
	Total	6205.087	746			
	Between Groups	1580.405	83	19.041	11.848	.000
Germination	Within Groups	135.000	84	1.607		
	Total	1715.405	167			
Total	Between Groups	15945.036	41	388.903	19.041	.000
Chlorophyll	Within Groups	9885.647	484	20.425		
Content	Total	25830.683	525			
	Between Groups	866.052	62	13.969	63.463	.000
SLA	Within Groups	27.733	126	.220		
	Total	893.785	188			
	Between Groups	37789.667	58	651.546	6.984	.000
RWC	Within Groups	9235.805	99	93.291		
	Total	47025.471	157			

Conclusion

$O_2^{\cdot-}$ and H_2O_2 is one of the important indicators of oxidative stress. Histochemical localization of $O_2^{\cdot-}$ and H_2O_2, indicated an increased accumulation of these ROS in the leaves of plants (Tewari et. al., 2006). Drought stress has resulted in accumulation of $O_2^{\cdot-}$ and H_2O_2 the wheat varieties, tolerant varieties were able to maintain a significantly lower $O_2^{\cdot-}$ and H_2O_2 content as compared to the sensitive varieties (Figure 8 and 9).

According to root and shoot length, chlorophyll contents, germination, RWC, and SLA results, it can be attributed that Karahan-99, Pehlivan, and Tekirdağ varieties as drought tolerant; Kate A-1, Sultan-95, and Gelibolu were found to drought-sensitive varieties.

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