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Physio-biochemical responses of registered bread wheat (*Triticum aestivum* L.) genotypes to drought stress: Variations in antioxidant parameters and photosynthetic pigment amounts

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Tescilli ekmeklik buğday (*Triticum aestivum* L.) genotiplerinin kuraklık stresine fizyo-biyokimyasal yanıtları: Antioksidan parametreler ve fotosentetik pigment miktarlarındaki değişimler

Abstract: In this study, physio-biochemical parameters of 7 registered bread wheat (*Triticum aestivum* L.) genotypes (Gerek 79, Sultan 95, Haymana 79, Grk/Cty, T98-9, Pastor, PM ME1) were investigated under drought stress conditions. Polyphenol oxidase (PPO), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), photosynthetic pigment, total protein, hydrogen peroxide, lipid peroxidation (malonyldialdehyde-MDA) and proline levels were determined in this wheat genotypes exposed to different drought duration (3rd, 6th and 10th day). As a result of this study, among 7 different wheat genotypes, Gerek 79 and Haymana 79 genotypes were the most physiologically sensitive to drought. In comparison, Pastor and Sultan 95 genotypes were the most drought-tolerant varieties. In addition, in parallel with the prolongation of the drought period in wheat varieties in general, it was determined that the content of photosynthetic pigments decreased significantly due to oxidative damage, while proline and MDA content increased.

Key words: Antioxidant enzymes, drought stress, photosynthetic pigments, wheat

Özet: Bu çalışmada, 7 tescilli ekmeklik buğday (*Triticum aestivum* L.) genotipinin (Gerek 79, Sultan 95, Haymana 79, Grk/Cty, T98-9, Pastor, PM ME1) fizyo-biyokimyasal parametreleri kuraklık stresi koşulları altında incelenmiştir. Farklı kuraklık şiddetine (3., 6. ve 10. gün) maruz bırakılan bu buğday genotiplerinde polifenol oksidaz (PPO), peroksidaz (POD), askorbat peroksidaz (APX), katalaz (CAT), fotosentetik pigment, toplam protein, hidrojen peroksit, lipid peroksidasyonu (malonildialdehit-MDA) ve prolin seviyeleri belirlenmiştir. Bu çalışma sonucunda, 7 farklı buğday genotipi arasında Gerek 79 ve Haymana 79 genotipleri fizyolojik olarak kuraklığa en duyarlı genotipler olurken, Pastor ve Sultan 95 genotipleri kuraklığa en toleranslı çeşitler olmuştur. Ayrıca buğday çeşitlerinde genel olarak kuraklık süresinin uzamasına paralel olarak oksidatif hasara bağlı olarak fotosentetik pigment içeriğinin önemli ölçüde azaldığı, prolin ve MDA içeriğinin ise arttığı tespit edilmiştir.

Anahtar Kelimeler: Buğday, kuraklık stresi, antioksidan enzimler, fotosentetik pigmentler

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1. Introduction

Plants are exposed to many biotic (insects and pathogenic fungi, etc.), and abiotic (drought, salinity, UV rays, etc.) stress factors throughout their lives. Drought, one of the abiotic stress factors, causes yield and quality losses, negatively affecting morphological and physiological characteristics in plants (Pulvento et al., 2022). Drought stress due to global climate change seriously affects the physiological functions of plants and appears as a limiting factor, especially in terms of agricultural activities. With the increase in arid areas in recent years, a lower amount of plant production than planned here is a significant loss in terms of economy. (Simelton et al., 2009). The severity of drought appears equivalent to global vegetation loss. It has been the focus of many (ecological, morphological, physiological, etc.) research on the response and adaptation mechanisms of plants to the severity and duration of drought. In this context, growing varieties that are compatible with climatic changes and especially drought-resistant in studies has become essential.

Typically, when plants face drought stress, their initial reaction is to curtail shoot growth, prioritizing water conservation. Simultaneously, they ramp up the production of protective phytochemicals, aiding in osmotic regulation while reducing their overall metabolic demands. Consequently, under water stress conditions, plants undergo a series of transformations in various aspects, including the morphology of leaves, flowers, fruits, and root structures, as well as adjustments in processes like photosynthesis and the activation of antioxidant enzyme systems. Moreover, they activate stress response genes through intricate signal transduction networks, synthesising numerous functional proteins that bolster the plant's resilience against drought stress. This has been corroborated by recent studies (Wahab et al., 2022; Yang et al., 2021). Wheat (*Triticum aestivum* L.), which plays a major role in the nutrition of more than 35% of the world's population, is one of the most important grains affected by drought stress (Zaheer et al., 2019). Deficiency of water causes the detrimental effects at all growth stages of wheat; most prominent effect was observed at the reproductive

stage, particularly at the grain filling stage, which leads to less and reduced grain size in wheat (Yu et al., 2018). In addition, water scarcity reduces nutrient availability, uptake, transport and accumulation in wheat, disrupts nutrient relationships in plants and reduces the rate of photosynthesis, leading to leaf senescence, thus shortening its life cycle (Maghsoudi et al., 2019). Water stress is a growing problem around the world, as well as having a significant impact on grain yield and quality. Global climate changes are making this situation more severe day by day (HongBo et al., 2005). Moreover, all these negativities result in a decrease in wheat yield and quality. The causes of these crop losses include the reduction of net photosynthesis rates due to metabolic limitations, oxidative damage to chloroplasts, and closure of stomata (Bhargava & Sawant, 2013).

Water stress induces oxidative damage due to the overproduction of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and superoxide anion (O_2^-), and that can damage biological membranes through biochemical reactions (Hasanuzzaman et al., 2020). ROS accumulation in plants during stress depends to a large extent on the relationship between ROS production and ROS cleaning system. The main reason for the increase in ROS during stress in plants is the limitation of carbon dioxide fixation in chloroplasts, and in addition, the over-reduction of the electron transport chain (Miller et al., 2010). H_2O_2, one of the free radicals, acts as a signalling molecule that is vital for plants such as photosynthesis processes, growth and development, response to biotic and abiotic stresses, as well as the cell cycle. However, excessive H_2O_2 accumulation causes oxidative stress in cells, and as a result, cell death is inevitable. Antioxidants, which carry out the task of clearing ROS in cells, are divided into two groups non-enzymatic antioxidants (ascorbic acid -AA, vitamins, alkaloids, nonprotein amino acids, carotenoids, glutathione, phenolic compounds) and enzymatic antioxidants (superoxide dismutase-SOD, ascorbate peroxidase-APX, glutathione reductases-GR, glutathione peroxidase-GPX, guaicol peroxidase-GOPX, glutathione-S-GR, polyphenol oxidase-PPO, peroxidase-POD, mono-dehydroasorbate reductase-MSHAR and catalase-CAT). In addition, proline (osmolyte), one of the protective molecules involved in the clearance of ROS, increases in the cell during stress (drought, salinity etc.) and helps to protect the organism. Among these important functions are optimizing mitochondrial functions, influencing cell proliferation and acting as a signal to activate gene expression for plant survival and recovery in stress conditions (Szabados and Savouré, 2010).

In this study, an antioxidant defence system was investigated in 7 different wheat genotypes exposed to drought stress. Comparative analyses were performed by determining the levels of PPO (EC 1.10.3.1), POD (EC 1.11.1.7), APX (E.C. 1.11.1.11), CAT (E.C. 1.11.1.6), photosynthetic pigments, total protein, H_2O_2, lipid peroxidation (malonyldialdehyde-MDA) and proline levels.

2. Materials and Method

2.1. Experiment design

In the research, 7 registered bread wheat genotypes were used (Table 1). Wheat genotypes that were used in the study developed by Republic of Turkey Ministry of Agriculture and Forestry Transitional Zone Agricultural Research Institute are drought tolerant genotypes related physiological tests results highlights.

Table 1. Bread wheat genotypes and abbreviation used in research.

No	Type Name	Abbreviation
1	GEREK 79	G_{79}
2	SULTAN 95	S_{95}
3	HAYMANA79/ALTAY2000	H_{79}
4	GRK/CTY//MESA/3/RL6043/4*NAC/4 MNCH	GRK
5	T 98-9//VORONA/HD2402	$T_{98\text{-}9}$
6	PASTOR/DEMIR2000//MUFITBEY	Pastor
7	PM ME1 IRR_S-32//TMP64/YY305/3/MUFITBEY	PM ME_1

Seeds of selected wheat genotypes were kept in 70% ethanol for 1 min, then put into 5% NaClO and mixed for 10 min. After this process, the seeds were rinsed 4 times with sterile distilled water. After germination of seeds in petri dishes, 7-10 days old seedlings were taken into pots and grown in pots under the same conditions in the greenhouse environment for 40 days and irrigation was left on the 40th day (Fig. 1). Then plant samples were collected from the application groups on days 3rd, 6th and 10th and stored at -80 °C for analysis (Chakraborty and Pradhan, 2012).

2.2. Determination of total soluble protein content

The total protein amount has been determined according to Bradford's (1976) method. 0.25 g leaf tissue was homogenized in porcelain mortar in 2.5 mL 50 mM KH_2PO_4 (pH:7) buffer with liquid nitrogen and the homogenate was transferred to microcentrifuge tubes. Then, the homogenate was centrifuged at 15.000 g for 20 min at +4 °C. 20 µl of supernatant was taken and 2.5 mL of Coomassie Brilliant Blue G-250 was added and vortexed. After 10 min of incubation, the absorbance of the samples at 595 nm was recorded and the amount of total protein in the leaves was determined by means of a standard curve of bovine serum albumin (BSA) (Öztürk & Demir, 2003).

2.3. Determination of proline content

In order to determine the amount of proline in the application groups, 0.4 g leaf tissue sample was homogenized in 4% $C_7H_6O_6S$ and filtered through filter paper. 0.5 mL of the filtrate 10 times diluted with distilled water and 1 mL of diluted sample, 1 mL of 96% glacial acetic acid and 1 mL of ninhydrin (2,2-dihydroxyindane-1,3-dione) were added and all tubes were incubated in a water bath for 60 min at 100 °C. After incubation, the tubes were simultaneously placed into an ice bath, held for 10 min, 2 mL of toluene were added to each tube and vortexed. Then incubated for a further 5 min and the absorbance of the pink phase formed at the top of each tube was recorded at 520 nm. The results were calculated as the amount of proline per fresh tissue g^{-1} using a standard graph prepared from proline (Öztürk & Demir, 2003).

2.4. Determination of lipid peroxidation

For the determination of lipid peroxidation (MDA); 0.5 g of leaf tissue was homogenized with 5 mL of 0.1% (w/v)

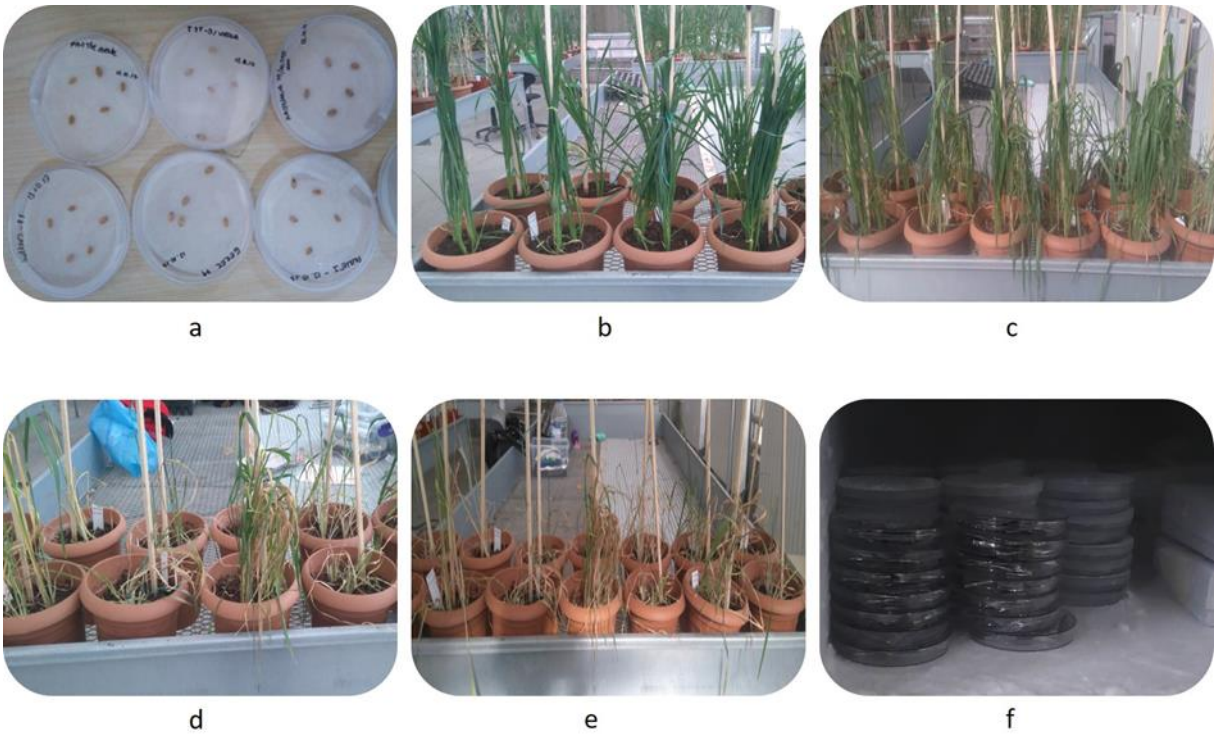

Figure 1. a. Wheat samples germinated in petri dishes **b**. Wheat plants, on the 40th day, grown under greenhouse conditions **c**. 3rd day of drought stress **d**. 6th day of drought stress **e**.10th day of drought stress **f**. Plant samples were stored for analysis at -80°C.

Trichloroacetic acid (TCA) and then centrifuged at 10.000 g for 20 min. On the 0.5 mL supernatant, 1 mL 0.5% (w/v) (Thiobarbituric acid) TBA prepared in 20% TCA was added. The mixture was allowed to stand at 95 °C in a water bath for 30 min and then quickly cooled in an ice bath. After centrifugation at 10.000 g for 5 min, the supernatant absorbance at 532 nm was recorded and corrected for nonspecific turbidity by subtracting the absorbance value at 600 nm wavelength. An extinction coefficient of 1.55×10^5 $M^{-1}cm^{-1}$ was used to quantify lipid peroxides and it was expressed as MDA µmol g^{-1} FW (fresh weight) (Sreenivasulu et al., 1999).

2.5. Determination of hydrogen peroxide (H_2O_2)

For the determination of H_2O_2, leaf tissues (0.5 g) were homogenized with 5 mL of 0.1% (w/v) TCA in an ice bath. The homogenate was centrifuged at 12.000 g for 15 min and 0.5 mL of the supernatant was added to 0.5 mL of 50 mM potassium phosphate (pH: 7) buffer and 1 mL of 1 M KI. Then, the absorbance of the mixture was recorded at 390 nm and the amount of H_2O_2 was determined using the standard curve (Velikova et al., 2000).

2.5. Enzyme activity assays

Fresh leaf tissues (0.2 g) from control and drought treated plants were grounded with liquid nitrogen and homogenized in 3 mL of buffer containing 50 mM KH_2PO_4 buffer (pH 7.0), 0.1 mM EDTA, and 1% PVPP (w/v). The homogenates were centrifuged at 15.000 g for 15 min at 4°C and resulting supernatants were used for the determinations of CAT, POD, APX and PPO activities. Enzyme activity was expressed as enzyme unit (U) per g of fresh tissue.

Catalase (CAT, EC 1.11.1.6) catalyzes the breakdown of hydrogen peroxide (H_2O_2) into H_2O and O_2. Catalase activity measurement is based on the monitoring of the discolouration of H_2O_2 during the reaction of water and oxygen at 240 nm for 3 min by using a spectrophotometer (Schimadzu UV-1800, Japan). In the activity measurement, a 3 mL reaction mixture was prepared with 1450 µl 50 mM KH_2PO_4 buffer (pH: 7), 1500 µl 30% H_2O_2 and 50 µl homogenate. One unit of activity was defined as the amount of enzyme catalyzing the decomposition of 1 µmol H_2O_2 per min, calculated from the extinction coefficient (0.036 cm^2 μmol^{-1}) for H_2O_2 at 240 nm (Ulusu et al., 2017).

Peroxidase (POD, EC 1.11.1.7) activity was defined according to the oxidation of guaiacol previously described (Dursun, 2018). To determine the peroxidase activity spectrophotometrically, a 3 mL reaction mixture was prepared in 970 µl 50 mM KH_2PO_4 buffer (pH: 6), 30% 1000 µl H_2O_2, 1000 µl guaiacol and 30 µl enzyme extract. The reaction was started by finally adding enzyme solution to the activity mixture and the optical density was recorded for 3 min at 470 nm.

In order to determine ascorbate peroxidase (APX, EC 1.11.1.11) activity, a 3 mL reaction mixture was prepared with 1450 µl of 50 mM phosphate buffer (pH: 7), 750 µl of 30% H_2O_2, 750 µl of ascorbic acid and 50 µl of enzyme extract. The reaction was started by adding hydrogen peroxide to the activity mixture and the absorbance at 290 nm was recorded for 3 min. Enzyme activity was calculated using ascorbate Alan's extinction coefficient (2.8 $mM^{-1}cm^{-1}$) (Karabal et al., 2003).

Polyphenol oxidase (PPO, EC 1.10.3.1) activity was determined by measuring the increase in absorbance at 420 nm with a spectrophotometer (UV-1800 Shimadzu JAPAN). 50 µl of crude extract was added to a 3 mL substrate mixture containing 0.20 M sodium phosphate buffer (pH: 6.5), and 25 mM catechol. Enzyme activity was calculated from the linear portion of the curve. One unit of PPO activity was defined as the amount of enzyme that can

cause an increase in absorbance of 0.001/min (Flurkey, 1989).

2.7. Determination of photosynthetic pigment contents

Chlorophyll (Chlorophyll a-Chl a, chlorophyll b-Chl b, total chlorophyll) contents were determined by the methods of Arnon (1949). Leaf tissues (0.2 g) were homogenized with 80% (v/v) acetone and centrifuged at 3.000 g for 5 min. The absorbency of supernatant was measured by spectrophotometer at 450, 645 and 663 nm wavelengths. Photosynthetic pigment amounts were calculated according to the following equations:

Chlorophyll a (mg g^{-1} FW) = 12.7 A_{663} - 2.69 A_{645}

Chlorophyll b (mg g^{-1} FW) = 22.9 A_{645} – 4.68 A_{663}

Total chlorophyll (mg g^{-1} FW) = 20.2 A_{645} + 8.02 A_{663}

2.8. Statistical analysis

According to Duncan's multiple range test, differences in the application groups were analyzed with a significance value of $p \leq 0.05$. Four replicates were performed for each group (n = 4). Statistical analyses were performed with SPSS Standard Version package program and differences between control and application groups were analysed by one-way ANOVA.

3. Results

3.1. Total protein content

The total protein content of 7 different wheat genotypes exposed to drought in the study is shown in Table 2. A result of analysis of H_{79} and G_{79} genotypes showed a relative increase in total protein content, which can be considered statistically significant (Table 2). In H_{79}, a decrease was observed in total protein content in the first days of the drought, while an increase of 3 times was observed in the 10th day compared to the control group. Due to water stress, the total protein content initially decreased compared to the control. It increased again in parallel with the increases in the synthesis of antioxidant enzymes triggered by the continuation of these adverse conditions. In the G_{79} genotype, on the other hand, the total protein content increased periodically during the drought period. This increase may be due to the activities in the synthesis of antioxidant enzymes, which are one of the intracellular antioxidant defence systems. In addition, the increased viscosity due to water loss in the cell cytoplasm under prolonged drought stress can be considered the reason for the increase in protein per tissue compared to the early period. A decrease in total protein content of $T_{98\text{-}9}$, S_{95} and PM ME_1 genotypes was determined during drought. On the other hand, GRK resulted in a serious decrease on the 10th day, although this content increased on the 3rd and 6th days. In many plants, among several cellular responses, variations in protein expression, synthesis, and accumulation have been observed during water deficit, usually mediated by the hormone abscisic acid (Kuromori et al., 2018). Drought stress can induce both qualitative and quantitative changes in plant proteins. For example, Mohammadkhani and Heidari (2008) determined that in *Zea mays* L., in response to progressive drought conditions, about 50 proteins were expressed, while 23 proteins were suppressed and 10 proteins induced synthesis. On the other hand, while there was a significant decrease in the total protein content of the drought-sensitive (Bahar) wheat variety among the 2 different wheat cultivars tested for drought stress, this decrease was slight in the drought-resistant (Kavir) variety (Michaletti et al., 2018).

3.2. Proline content

In all wheat genotypes used in the study, proline content increased in parallel with the prolongation of drought ($p < 0.05$) (Table 2). In G_{79}, the genotype with the highest proline content, on the 3rd, 6th and 10th days of drought, the amount of proline in the plant increased by ~840%, 2089%, and 1988% compared to the control, respectively. All wheat genotypes were able to cope with drought stress in the first days (3rd) of drought, but proline levels increased significantly as the drought continued. Only the proline level of GRK from the investigated wheat genotypes decreased in the first days of drought stress, then increased 13-fold compared to the control.

Proline, which has an important role as an osmolyte, has important functions such as stabilization of membranes and protein conformation, and reduction of photodamage to thylakoid membranes by ROS scavenging when plants are exposed to stress (Ozturk et al., 2021). Similar to our study, it was noted that proline accumulation in pepper increased significantly with increasing drought stress (Kaya et al., 2019). In other experiments, drought has been reported to cause high proline content in peanut (Girija et al., 2002) and wheat genotypes (Keles & Öncel 2004; Sultan et al., 2012).

Proline is known to be able to regulate some drought-resistant genes (Per et al., 2017). Wang et al. (2015) identified the two proline synthesis enzymes pyrroline-5-carboxylate synthetase (P5CS) genes and the pyrroline dehydrogenase PDH gene in *Kosteletzkya virginica* L. It has been reported that although expression of the P5CS1 gene is upregulated in most plant organs during drought, the expression of the PDH1 gene is downregulated (Kiyosue et al., 1996; Strizhov et al., 1997). In this respect, proline accumulation induced by drought stress in plant tissues may occur by upregulating the P5CS gene (Liang et al., 2013).

3.3. Changes in H_2O_2 and MDA content

Toxic ROS production caused by water stress induces lipid peroxidation in plants. Determination of H_2O_2 and MDA content, two important oxidative stress indicators, are commonly used criteria. In general, H_2O_2 and MDA contents of wheat genotypes under drought stress showed a positive correlation with each other. After 10 days of drought, the highest increase in MDA content among genotypes was determined in Pastor with 14-fold, followed by H_{79} with a 9-fold increase. Especially in the G_{79}, H_{79}, GRK, PM ME_1 genotypes, MDA content which reached its highest level as of the 6th day was decreased on the 10th day of drought (Table 2).

In this case, it is thought that it is an indicator that especially membrane lipids begin to disappear due to stress and plant hemostasis is gradually disappearing. Lipid peroxidation in cell membranes is known to be one of the most challenging and most harmful effects on the membranes of all cells exposed to varying degrees of stress. Changes in the amount of malondialdehyde due to the oxidation of membrane lipids provide information about the degree of damage to the cell membranes of plants under stress conditions. Changes in MDA levels in plants vary according

Table 2. Effect of drought treatment on contents of total protein, proline, malondialdehyde and hydrogen peroxide in leaves of wheat types

Type Name	Analysis	Day zero	3rd day	6th day	10th day
H_{79}	Total Protein (mg g^{-1}FW)	0.1595±0.0046c	0.0831±0.0186b	0.0351±0.0022a	0.3109±0.0156d
	Proline (µg g^{-1}FW)	0.9427±0.0245a	1.4244±0.2756a	1.1253±0.1019a	15.3258±0.212b
	MDA(mmol g^{-1}FW)	0.0165±0.0035a	0.051±0.0038b	0.0962±0.0112c	0.0934±0.0105c
	H_2O_2 (µmol g^{-1}FW)	1094.7±162.1b	1975.1±32.3c	185.6±56.9a	1094.7±162.1d
$T_{98\text{-}9}$	Total Protein (mg g^{-1}FW)	0.2168±0.0113c	0.3089±0.0105d	0.0644±0.0027a	0.0988±0.0005b
	Proline (µg g^{-1}FW)	0.9179±0.3656a	5.1177±0.6553b	14.7131±0.4619c	14.6212±0.7838c
	MDA(mmol g^{-1}FW)	0.0287±0.0013a	0.0402±0.0027a	0.0823±0.0082b	0.0991±0.0076b
	H_2O_2 (µmol FW^{-1})	327.6±57.6a	423.5±45.7ab	1072.9±233.9b	2081.6±343.8c
PM ME_1	Total Protein (mg g^{-1}FW)	0.1484±0.0167ab	0.0867±0.0029a	0.1815±0.0009b	0.0664±0.0024a
	Proline (µg g^{-1}FW)	0.7971±0.0475a	7.2482±0.1444b	13.4308±0.4160c	15.8096±0.019d
	MDA(mmol g^{-1}FW)	0.0147±0.0037a	0.0382±0.0076a	0.0657±0.0010b	0.0609±0.0057b
	H_2O_2 (µmol g^{-1}FW)	203.7±43.3a	700.5±45.6b	232.3±34.2a	870.5±50.2b
GRK	Total Protein (mg g^{-1}FW)	0.0632±0.0065a	0.1417±0.0004b	0.1862±0.0222b	0.26±0.01c
	Proline (µg g^{-1}FW)	0.7666±0.1173a	6.4449±1.2665b	15.881±0.0752c	15.8771±0.0728c
	MDA(mmol g^{-1}FW)	0.0518±0.0118a	0.085±0.0122ab	0.1403±0.0162b	0.0814±0.0196ab
	H_2O_2 (µmol g^{-1}FW)	350.5±83.8a	591.1±30.9b	272.2±16.1a	505.7±32.6b
S_{95}	Total Protein (mg g^{-1}FW)	0.2426±0.0063c	0.0965±0.0016a	0.1689±0.0218b	0.175±0.005b
	Proline (µg g^{-1}FW)	0.7854±0.1100a	9.6583±0.6363b	15.7275±0.0786c	15.7559±0.0280c
	MDA(mmol g^{-1}FW)	0.0335±0.0086a	0.1333±0.0060b	0.1709±0.0034b	0.1847±0.0245b
	H_2O_2 (µmol g^{-1}FW)	803.3±173.6a	2149.4±100.8b	495.1±17.4a	574.2±126.1a
Pastor	Total Protein (mg g^{-1}FW)	0.1948±0.0051b	0.0577±0.0028a	0.2457±0.0042c	0.071±0.0077a
	Proline (µg g^{-1}FW)	0.7365±0.09a	9.1423±1.8408c	4.8127±0.8533b	15.9259±0.064d
	MDA(mmol g^{-1}FW)	0.0122±0.0024a	0.0904±0.0215b	0.0585±0.0171b	0.1438±0.0134c
	H_2O_2 (µmol g^{-1}FW)	513.8±107.1a	833.2±71.8ab	318.2±57.9a	1302.6±63.9b
G_{79}	Total Protein (mg FW^{-1})	0.1635±0.0042b	0.2188±0.0019c	0.2227±0.0051c	0.0783±0.0102a
	Proline (µg FW^{-1})	0.9535±0.0652a	0.4588±0.0851a	0.1769±0.0571a	12.5004±1.061b
	MDA(mmol FW^{-1})	0.0032±0.0018a	0.0065±0.0024a	0.0371±0.0024b	0.0127±0.0037a
	H_2O_2 (µmol FW-^{1})	546.1±37.5a	1099.7±55.2b	389.8±15.9a	257.2±47.1a

to the degree of stress and cause an increase in MDA levels in general. If stress conditions cannot be eliminated, MDA levels will continue to increase and deterioration in the endomembrane system will increase, leading to cell damage, similar to our study. Similar to MDA levels, a drought-induced increase was observed in all wheat genotypes. While the levels of H_2O_2 reached their peak on the 3rd day of the drought in the H_{79}, G_{79} and S_{95} genotypes, it was observed that the peak levels were attained on the 10th day of the drought in $T_{98\text{-}9}$, pmme1 and pastor varieties. The results suggest that short-term, gradual temperature increases initially triggered H_2O_2 production in wheat cultivars. Reactive oxygen species (ROS), such as singlet oxygen, superoxide radical, hydrogen peroxide, and hydroxyl radical, responsible for cellular damage, come about due to water stress (Wahid et al., 2007). Damage to plants following exposure to water stress is recognised by impairment to the photosynthetic, mitochondrial and, specifically, cell membranes' fluidity (Gill and Tuteja, 2010; Choudhury et al, 2013). Membrane damage is a stress parameter used to measure the degree of lipid peroxidation (Demirci-Cekic et al., 2022). The elevation in lipid peroxidation and H_2O_2 during biotic and abiotic stress periods is the plant's response to these stress factors. In our study, different H_2O_2 and MDA levels observed in wheat genotypes during different drought periods offer critical information about the tolerance levels of genotypes against drought.

3.4. Antioxidant enzyme activities

Antioxidant enzymes such as CAT, POD, APX, and PPO provide plants with tolerance against stressful conditions and safeguard them from oxidative damage. Plants mitigate the detrimental impacts of reactive oxygen species by activating multiple antioxidant enzymatic systems during water stress exposure. The extent of antioxidant enzyme response in plants exposed to water stress primarily depends on their tolerance and stress levels. To better understand this process in wheat genotypes under gradually increasing drought, we analysed the activities of CAT, POD, APX, PPO antioxidant enzymes. Changes in the activities of four different antioxidant enzymes in wheat plants are shown in Table 3.

CAT activity decreased in all wheat cultivars examined in this study beginning from the initial days of drought. Notably, CAT activity significantly decreased on the sixth day of drought in the H_{79}, PM ME1, G_{79}, Pastor, and GRK genotypes. GRK exhibited the most pronounced decline in CAT (catalase) activity, with a 14-fold reduction compared to its initial levels on the first day of observation. This consistent drop in CAT activity throughout the drought periods suggests that wheat plants may have surpassed their

Table 3. Effect of drought treatment on activity of CAT, POD, APX and PPO enzymes in leaves of wheat types

Type Name	Enzyme Activity (EU FW-1)	Day zero	3rd day	6th day	10th day
H_{79}	CAT	90.375±8.3234b	119.0825±14.2b	22.685±3.8143a	36.5433±6.9284a
	POD	13.2688±0.4434b	12.3891±1.054b	13.4192±0.7466b	8.8891±2.3997a
	APX	2.5071±0.8905a	9.5786±0.7928b	9.45±0.2909b	21.3857±0.5142c
	PPO	2670±785.93a	2560±560a	3420±780a	5700±897.68b
T_{98-9}	CAT	124.6775±10.04b	56.65±5.7907a	82.94±15.2268ab	55.5025±10.63a
	POD	16.7011±0.7586a	18.0376±0.582a	17.0395±0.6328a	17.9605±1.5579a
	APX	3.5679±0.4620a	4.1571±0.3015a	9.5036±0.9604b	5.3893±0.7542a
	PPO	4200±668.13a	6080±335.46b	3080±1247.7a	3800±243.3a
PM ME_1	CAT	38.3756±4.3749b	50.4569±3.147b	5.7868±3.9593a	28.1726±3.961ab
	POD	13.7331±2.5225a	18.6485±0.71ab	16.3872±2.124ab	19.8289±1.3127b
	APX	2.6571±0.0726a	3.4286±0.2726a	2.2071±0.1026a	2.2179±0.2006a
	PPO	2640±202.7a	2520±240a	5100±220ab	5600±211.6b
GRK	CAT	112.0775±16.71c	82.99±15.6442b	14.025±1.505a	*
	POD	14.75±2.0833a	11.4474±1.571a	13.391±2.362a	*
	APX	4.1714±0.3377a	8.775±0.9679b	3.2286±0.3571a	*
	PPO	2640±382.2a	3990±527.5b	2160±600a	*
S_{95}	CAT	67.8173±5.0632b	40.2792±3.136a	44.0102±4.1691a	40.1015±5.9293a
	POD	9.0414±0.7088a	15.6071±1.268b	16.4643±1.762b	18.3008±0.1393b
	APX	2.3679±0.0649b	2.0786±0.0437b	1.7464±0.06671b	0.9571±0.05781a
	PPO	4840±520.7a	3750±453.6a	5910±317.7a	4200±212.9a
Pastor	CAT	96.2437±10.05b	71.4975±10.5ab	54.2132±11.865a	
	POD	10.1767±0.5946a	12.5132±0.156b	13.0226±0.9895b	10.7895±0.81ab
	APX	2.7±0.2693a	4.9071±0.492ab	7.0393±0.8071ab	7.9607±0.0543b
	PPO	4760±262.2b	2910±251.8a	4560±249.7b	4760±204.7b
G_{79}	CAT	112.3075±7.062c	46.5325±6.767b	8.5233±4.02a	26.9475±5.553ab
	POD	18.7782±0.0623a	19.3214±0.042b	19.2223±0.0323b	19.3647±0.0123b
	APX	4.5857±0.2486c	2.2821±0.0915a	2.6143±0.0524a	3.6107±0.5427b
	PPO	2960±693.9a	2220±60a	6720±1440b	2120±312.4a

threshold for drought tolerance, rendering them vulnerable to the adverse consequences of prolonged water scarcity. Furthermore, it's noteworthy that various cultivars display distinct profiles of antioxidant enzyme activities. This divergence underscores the diverse strategies different wheat cultivars employ to combat the effects of water stress. The process of plant adaptation is highly complex and needs fixed guidelines. The process of plant adaptation is complex and needs flexible guidelines. Based on our research, Illescas et al. (2021) also noted a significant decrease in catalase activity (CAT) in wheat plants as drought stress intensified. Similarly, *Festuca arundinacea* Schreb. demonstrated decreased CAT activity when exposed to increasing drought stress.

However, it should be noted that in certain studies, e.g. those investigating *Mentha pulegium* L. (Ulusu et al., 2022) and *Cicer arietinum* L. (Mafakheri et al., 2011) flora, CAT activity has been reported to rise during the initial phases of drought stress before declining over the subsequent days. The decrease in CAT activity is commonly observed as a broad response to various stressors (Abedi & Pakniyat, 2010; Gunes et al., 2008). This decline in CAT activity can be attributed to several factors, including the inhibition of enzyme synthesis, alterations in the composition of enzyme subunits, and potential enzyme degradation mediated by induced peroxisomal proteases, all occurring under stressful conditions.

POD enzymes affect plant growth, development, lignification, suberization, and cross-linking of cell wall compounds (Passardi et al., 2005). In addition, POD decomposes H_2O_2 into H_2O and effectively hinders the accumulation of H_2O_2 in cells, helping to reduce the toxic effect of water stress on plants by protecting them from oxidative damage. In current study, the peroxidase (POD) activity in wheat genotypes subjected to drought conditions was generally higher than in the control. However, only the H_{79} and G_{79} genotypes significantly reduced POD activity on the 10th and 3rd days of drought, respectively. Various research groups have studied the activity of peroxidase enzymes (POD). These studies found that the activity of POD increased in four cultivars of *Carthamus tinctorious* L. (Farooq et al., 2020), in *Brassica napus* L. (Shafiq et al., 2014), in two tomato cultivars (Ghorbanli et al., 2013), and in wheat (Malik & Ashraf, 2012) when subjected to drought stress compared to the controls. These results are in support of the present study.

APX utilises ascorbate as an electron donor to reduce H_2O_2, and its significance in detoxifying H_2O_2 is widely acknowledged (De Gara et al., 2003). The enzyme is activated in the water-water and glutathione-ascorbate cycles. In the Mehler reaction, APX eliminates ROS by reducing them to water (Sui, 2015; Weng et al., 2007). The study found that APX activities varied significantly among wheat genotypes under water stress. H_{79} and Pastor

genotypes showed a significant increase in APX activities, while S_{95} and GRK genotypes exhibited a decrease in APX activities during the later days of drought. PM ME_1 and T_{98-9} genotypes did not present any statistical difference in APX activity under water stress ($p< 0.05$). Another study on antioxidant enzyme activities in three different types of wheat subjected to water stress reported that three varieties showed the highest APX activity at -4 bar water potential level. However, this enzyme activity reduced as the drought level increased (Esfandiari et al., 2007). Enhanced APX activity in plants during water stress augments oxidative stress tolerance (As, 1993). Thus, it minimises the damage caused by drought in plants and protects the plant until a specific period.

PPO activities were directly proportional related to drought stress levels in all the genotypes of wheat studied in this study. In general, PPO activity remained stable in the samples analysed during early drought stress periods, while a relatively increase was observed in H_{79} and PM ME_1 genotypes under long-term drought stress conditions. The results showed that, in general, PPO enzyme activity did not show a clear relationship with stress. Especially in H_{79} and PM ME_1 genotypes, the enzyme activity was significantly lower than the control in the first days of drought, while an increasing trend was observed under severe stress. The diverse reactions of enzymatic antioxidant activities observed across all the tested wheat varieties in this study can be attributed to the genetic distinctions inherent to each of these cultivars (Malik & Ashraf, 2012).

3.5. Chlorophyll (Chl a, Chl b and total Chl) content

In this study, in which Chl a, Chl b, and total Chl analyses were performed to determine how pigment degradation, which reduces the photosynthetic efficiency, changes with drought, it was determined that drought stress significantly reduced these pigment contents (Table 4). Significant differences were identified between the analysed genotypes ($p <0.05$). Especially in the S_{95} on the 10th day of drought, Chl a, Chl b and total Chl contents decreased by 72.17%, 89.74% and 77.25%, respectively and it was the genotype most affected by drought in terms of chlorophyll content. Although Chl a and total Chl content increased on the 3rd and 6th days of drought in the G_{79} genotype, it was greatly affected by the continuation of drought and its vegetative tissues were completely damaged. Among the analyzed genotypes, T_{98-9} was the least affected wheat variety in terms of pigment content from drought. While photosynthetic pigment levels differed according to the genotype difference under stress conditions, the total amount of chlorophyll decreased in general among the cultivars. In Chl a, chlorosis, which occurs due to drought conditions, was less from Chl b. In this case, when Chl a is the dormant type in total Chl, the share of Chl b in the total Chl pool in plants has increased proportionally. These results showed that the water stress experienced had reduced the rate of photosynthesis due to the loss of chlorophyll in plants. Oxidative stress in plants causes chloroplast disruption and can decrease chlorophyll content (Arora et al., 2002). Therefore, the results obtained from the present study can be considered a distinctive oxidative stress symptom (Smirnoff, 1993). Similar to our results, the gradual increase in drought stress resulted in significantly decreased pigment contents in Lilium species (Zhang et al., 2011), *Olea europaea* L. (Khaleghi et al., 2012) and tomato (Zgallaï et al., 2006).

Table 4. Effect of drought treatment on contents of Chlorophyll a, Chlorophyll b, Total Chlorophyll in leaves of wheat types

Type Name	Analysis	Day zero	3rd day	6th day	10th day
H_{79}	Chlorophyll a	23.91±2.49c	15.74±0.78b	7.62±0.91a	6.19±0.67a
	Chlorophyll b	6.72±0.61b	4.44±0.50a	3.18±0.47a	3.20±0.34a
	Total Chlorophyll	23.24±2.30b	16.49±3.11ab	10.80±1.38a	9.39±1.01a
T_{98-9}	Chlorophyll a	17.79±1.14b	21.11±1.34c	12.02±0.45a	15.48±1.11b
	Chlorophyll b	5.25±0.38b	5.33±0.17b	3.63±0.19a	5.18±0.41b
	Total Chlorophyll	23.04±1.53b	23.49±0.83b	15.65±0.56a	20.66±1.52b
PM ME_1	Chlorophyll a	10.20±2.49a	16.21±2.03ab	9.42±1.22a	23.68±3.30b
	Chlorophyll b	3.93±1.08a	4.76±1.02a	3.26±1.08a	12.88±1.05b
	Total Chlorophyll	14.13±3.62a	18.20±4.38a	12.68±1.76a	36.55±6.17b
GRK	Chlorophyll a	24.59±4.59a	24.83±0.91a	27.64±1.15a	*
	Chlorophyll b	16.42±0.41b	8.76±0.72a	17.1±1.18b	*
	Total Chlorophyll	41.01±2.54a	33.59±1.60a	44.87±1.21a	*
S_{95}	Chlorophyll a	25.77±2.33c	29.16±0.55c	16.55±2.75b	7.17±0.48a
	Chlorophyll b	27.1±0.62c	13.63±1.61b	10.12±2.38b	2.68±0.73a
	Total Chlorophyll	42.31±8.28c	42.78±0.15c	26.67±3.53b	9.85±1.15a
Pastor	Chlorophyll a	16.6±2.52c	9.12±0.90b	3.96±0.23a	7.34±0.53ab
	Chlorophyll b	5.61±0.81b	3.03±0.44ab	1.49±0.29a	3.68±0.13ab
	Total Chlorophyll	22.26±2.66c	12.16±1.10b	5.45±0.31a	10.03±0.63b
G_{79}	Chlorophyll a	13.62±1.02c	8.133±0.60b	2.44±0.11a	1.93±0.03a
	Chlorophyll b	4.45±0.04c	2.67±0.43b	0.81±0.09a	0.78±0.02a
	Total Chlorophyll	18.07±0.30c	10.80±0.60b	3.25±0.08a	2.71±0.01a

4. Conclusions

This study provides a significant illustration of how wheat genotypes display diversity in their reaction to drought, covering aspects such as the commencement and severity of drought pressure, water-based mechanisms, capacity for recuperation, and plant biochemical responses. Our findings highlight that G79 and H79 emerge as the most physiologically sensitive to drought among the seven distinct wheat genotypes studied. In contrast, Pastor and S_{95} genotypes display a higher degree of drought tolerance than the other genotypes. The data on antioxidative enzyme activities and proline production in wheat can be instrumental as selection criteria in drought tolerance breeding programs. Ultimately, the selection of drought-tolerant wheat genotypes aims to increase the productivity of the targeted crops.

Conflict of Interest

Authors declare conflict of interest.

Authors' Contributions

The authors contributed equally.

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