doi:10.14720/aas.2024.120.3.18268 Original research article / izvirni znanstveni članek

Biochemical and molecular characterization of bread wheat genotypes under drought stress: Implications for antioxidant defense mechanisms and genomic analysis

Numan ECZACIOGLU [1], Begum Terzi AKSOY [1], Ozlem Ates SONMEZOGLU [1], Yakup ULUSU [1, 2]

Received February 22, 2024; accepted June 12. 2024.
Delo je prispelo 22. februar 2024, sprejeto 12. junij 2024

Biochemical and molecular characterization of bread wheat genotypes under drought stress: Implications for antioxidant defense mechanisms and genomic analysis

Abstract: Plants face abiotic stresses like drought, salinity, and high temperature, which adversely affect growth and induce physiological and metabolic changes. Drought is a complex stress controlled by many genes, requiring investigation through molecular markers and biochemical characterization in wheat genotypes. This study involved eight bread wheat cultivars and two controls: drought-tolerant 'Gerek-79' and drought-sensitive 'Sultan-95'. These were grown for 40 days and then subjected to 10 days of drought stress. Antioxidants and antioxidant enzyme activities, which neutralize ROS, are key resistance mechanisms against oxidative stress. Levels of polyphenol oxidase (PPO), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT) were measured. Plant responses to stress included changes in photosynthetic pigments, total proteins, hydrogen peroxide, lipid peroxidation (MDA), and proline levels. POD showed the highest change in enzyme activity, while PPO was least affected. Chlorophyll b levels increased under stress across all varieties. Notably, proline levels, an abiotic stress marker, significantly rose by the 10th day of drought. Additionally, wheat genotypes were analyzed using drought-related SSR markers (Xwmc 89, Xwmc 118, Xwmc 304, Xgwm 337). This allowed evaluation of the impact of molecular characterization on biochemical changes under drought stress.

Key words: bread wheat, drought stress, antioxidant enzymes, proline, lipid peroxidation, hydrogen peroxide, photosynthetic pigments

Prepoznavanje biokemičnih in molekularnih označevalcev krušne pšenice v razmerah sušnega stresa, ki so vključeni v mehanizme antioksidacijske obrambe in genomske analize

Izvleček: Rastline se izpostavljene abiotskim stresom kot so suša, slanost in visoke temperature, ki negativno vplivajo na rast in vzpodbudijo fiziološke in med njimi presnovne spremembe. Suša je kompleksni stres, ki ga uravnavajo številni geni, kar pri genotipih krušne pšenice zahteva preučevanje molekularnih označevalcev in biokemičnih procesov. V to raziskavo je bilo vključenih osem genotiopov krušne pšenice in dve kontroli: na sušo tolerantna sorta Gerek-79 in občutljiva sorta sorta Sultan-95. Vse rastline so bile gojene 40 dni v standardnih razmerah in nato izpostavljene sušnemu stresu za 10 dni. Antioksidanti in antioksidacijski emcimi, ki nevtralizirajo aktivne zvrsti kisika (ROS) so ključni mehanizmi za odpravo oksidacijskega stresa. Izmerjene so bile vsebnosti polifenol oksidase (PPO), peroksidaze (POD), askorbat peroksidaze (APX) in katalaze (CAT). Analiza odziva rastlin na stres je obsegala še meritve sprememb v vsebnosti fotosinteznih pigmentov, celokupnih beljakovin, vodikovega peroksida, spremembe v peroksidaciji maščob (MDA) in v vsebnosti prolina. POD je pokazala največjo spremembo v aktivnosti med tem, ko je bila PPO najmanj prizadeta. Vsebnost klorofila b se je v stresnih razmerah povečala pri vseh sortah. Značilno se je po desetih dneh suše povečala vsebnost prolina kot označevalca abiotičnega stresa. Dodatno so bili v genotipih krušne pšenice anlizirani na s sušo povezani SSR označevalci (Xwmc 89, Xwmc 118, Xwmc 304, Xgwm 337). To je omogočilo ovrednotenje biokemičnih sprememb v razmerah sušnega stresa na molekularni osnovi.

Ključne besede: krušna pšenica, sušni stres, antioksidacijski encimi, prolin, peroksidacija lipidov, vodikov peroksid, fitosintezna barvila

1 Faculty of Engineering, Department of Bioengineering, Karamanoglu Mehmetbey University, Karaman, Turkey
2 Corresponding author, e-mail: yakupulusu@kmu.edu.tr

1 INTRODUCTION

Global crop production is threatened by the increased frequency of long-term water and heat stress caused by climate change (Arora, 2019). Drought caused by climate change is one of the important natural disasters that significantly impact the agricultural sector. Due to its geographical size and range, it has a complex structure and affects a large population (Javadinejad et al., 2021).

Plants can face very different stress conditions throughout their lives. These stress factors can be biotic (pathogen, competition with other organisms, etc.) or abiotic (drought, salinity, radiation, chemical substances, high temperature or frost etc.). The ability of a plant to grow in a limited area of water is referred to as drought resistance (Kadam et al., 2012). Under drought stress, plants exhibit various metabolic changes such as protein synthesis, ROS accumulation, enzyme activity change, and pigment content (Chen et al., 2016).

The response of plants to drought stress depends on plant growth (development), stress period and plant genetics (Beltrano & Ronco, 2008; Khan et al., 2012). Drought stress induces many common cellular reactions in plants. The stress leads to cellular dehydration, which causes osmotic stresses resulting in reduced cytosolic and vacuolar bulk (Wang et al., 2003). Except for ionic compounds, the early response of this plant to the plant is largely the same. The response of the plant to the stress condition is quite complex in terms of cellularity, and plants respond to stress by changing many biochemical and molecular mechanisms (genes, etc.) at tissue and plant levels (Bohnert & Jensen, 1996; Wang et al., 2003). As a result, there are numerous changes to the plant adaptation, such as reduction in growth, slowing of photosynthesis, closing of stomata, transient increases in ABA levels, induction of gene expressions, accumulation of coherent soluble and protective proteins, increased levels of antioxidants and suppression of energy consumption pathways (Bartels & Sunkar, 2005).

Proteins are essential components of living organism. The most important indicator of wheat quality is the amount of protein and the quality of the existing protein (Finney et al., 1987).

As a result of water loss, the interactions of hydrophobic and hydrophilic amino acids in the structure of proteins with water are disrupted. So, proteins denaturation and enzymes inhibition occur. Proline is a molecular chaperone that protects proteins from degradation and regulates different enzyme activities. It has been suggested that proline accumulation in high plants is an antioxidant because it contributes to osmotic compliance and is effective in preserving the integrity of the plasma membrane by against to drought and salinity, metal toxicity, oxidative stress and biotic stress factors (Mansour, 1998; ÖZDEMİR et al., 2012). Causing cross-linking and polymerization of membrane components, aldehydes inactivate receptors and membrane bound enzymes at

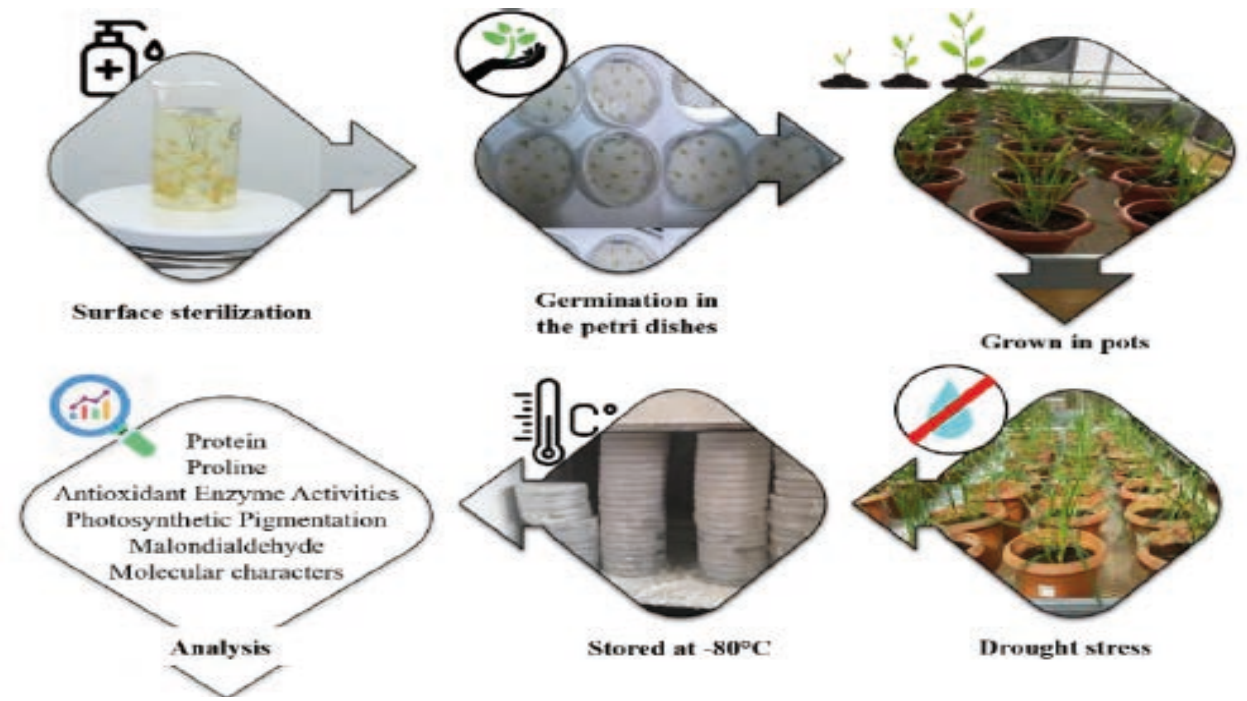

Figure 1: Schematic diagram of the study

membrane. So, serious damage can also occur in membrane proteins. Malondialdehyde (MDA), a reactive aldehyde, is formed as a result of the oxidation of lipids in the biological system. MDA inhibits the production of peroxidized unsaturated fatty acids in plant membranes, triggering transcriptional stress responses (Tenikecier, 2013). In biotic or abiotic stressed plants, the inactivation of reactive oxygen species, which damage plant cells to vital components such as membrane lipids, proteins, enzymes, pigments and nucleic acids, is provided by the increase of antioxidant enzymes (Maheshwari & Dubey, 2009). ROS, such as hydrogen peroxide, superoxide anion, hydroxyl radical and singlet oxygen, are formed by partial reduction of molecular oxygen (Kireçci, 2012). ROSs cause degradation of membrane components, oxidation of protein sulfhydryl groups, formation of cytoplasmic gel phase, and loss of membrane functions. Antioxidants protect cells from oxidative damage by capturing and detoxifying ROS. This system consists of antioxidants such as ascorbate, glutathione, α tocopherol and carotenoids with small molecular weight and enzymatic antioxidants such as SOD, CAT, ASPX and GR (Ahmad et al., 2008; Keleş & Öncel, 2002; Zhang Feng et al., 2004).

Wheat is an important grain product that meets a large portion (22 %) of global food needs (Naderi et al., 2020). Due to population growth worldwide (Faostat, 2017), wheat production needs to be supported to meet food needs around the world. Determining the crop tolerance mechanism in drought is important for wheat breeding programs (Gálvez et al., 2019). Landraces are more tolerant to stressful environments and temporary climatic conditions thanks to their agro-physiological characteristics and genetic structure (Mohammadi et al., 2015). For this reason, determining suitable wheat varieties against drought stress can contribute to creating new varieties and benefit from creating new product plans with improved stress tolerance (Naderi et al., 2020; Zhou et al., 2015). In this study, ten different bread wheat genotypes were subjected drought stress by deficient of water for 10 days. Changes in some antioxidant enzymes and molecular indicator of drought stress such as MDA, prolin, pigments etc. were measured in different wheat genotypes to develop relationship between antioxidant enzymes, molecular indicators and mechanisms of drought tolerance.

2 MATERIAL & METHOD

2.1 PLANT MATERIALS

Ten bread wheat cultivars (Sarıcanak-98, Karahan, Sonmez2001, Kate-1, Altay2000, Bayraktar, Harmankaya99, Izgi2001, Gerek-79, Sultan-95) that differ in drought tolerance were used as plant material. Gerek 79 cultivars for drought tolerance and Sultan 95 cultivars for drought sensitivity were used as control cultivars. The wheat cultivars and lines were kindly provided by the GAP International Agricultural Research Institute, Eskişehir Transition Zone Agricultural Research Institute, Bahri Dağdaş International Agricultural Research Institute, Ankara Field Crops Central Research Institute and Thrace Agricultural Research Institute.

Surface sterilization was applied before the wheat seeds were planted in petri dishes. Briefly, seeds were treated for 1 min with 70 % ethanol and 5 % sodium hypochlorite for 10 min. Finally, the seeds were rinsed three times with distilled water.

The seeds were placed in the petri dishes with 10-12 seeds each. The petri dishes were closed with para-film and were kept at +4 °C for 4-5 days. Then, the germination was observed for 3-4 days at room temperature. Seven- and ten-day seedlings pots were grown in pots for thirty days. Irrigation was left in the application groups after the thirtieth day. On the third, seventh, and tenth days, tissue samples were collected and stored at -80 °C for analysis.

2.2 DETERMINATION OF ANTIOXIDANT ENZYME ACTIVITIES

0.25 g of plant tissue was homogenized with potassium phosphate (pH = 7) and centrifuged at 10000 x G for 15 minutes. The supernatant was used to determine enzyme activities.

Depending on the consumption of the prepared substrates or the product formation, the enzyme activities were examined at the optimum pH and temperature for the enzymes by spectrophotometric method at specific wavelengths. All characterization operations were performed in triplicate.

2.2.1 CATALASE ASSIGNMENT

In the activity measurement, 3 ml of reaction buffer was prepared which including 50 mM phosphate buffer (pH = 7), 30 mM H_2O_2 and 30 µl homogenate. The absorbance was measured during 2 minutes. For the calculations, the amount of enzyme that cleaved 1 µmol of H_2O_2 was determined in one minute at 240 nm from using the A = Є.b.c formula by taking the light path as 10 mm and the extinction coefficient (Є H_2O_2: 0.0394 $mmol^{-1}x\ mm^{-1}$).

2.2.2 PEROXIDASE ASSIGNMENT

To determine the peroxidase activity 3 ml of the reaction mixture was prepared consisting of 50 mM phosphate buffer (pH = 7), 22.5 mM H_2O_2, 30 mM guaiacol and 20 µl enzyme extract. The activity to the reaction was initiated by the addition of the enzyme solution to the assay medium and optical density was recorded at 470 nm for 2 minutes. An enzyme unit was calculated as the amount of enzyme catalyzing 1 µmol guaiacol per minute.

2.2.3 ASCORBATE PEROXIDASE ASSIGNMENT

To determine the ascorbate peroxidase activity, 3 ml of the reaction mixture was prepared in the activity measurement to consist of 100 mM phosphate buffer (pH = 7), 10 mM H_2O_2, 2.5 mM ascorbic acid and 100 µl enzyme extract. The reaction was initiated by adding hydrogen peroxide to the activity measurement medium last and the absorbance was recorded at 290 nm for 2 minutes. Enzyme activity was calculated using ascorbate extinction coefficient (2.8 $mM^{-1}xcm^{-1}$).

2.2.4 POLYPHENOL OXIDASE ASSIGNMENT

0.3 g of the tissue was taken and 2 ml of buffer (0.2 M Na_2HPO_4 and 5 mM ascorbic acid pH: 6.5) was added to prepare the homogenate. Homogenate was centrifuged at 12000 x G for 10 minutes. Enzyme activity measurement is based on the observation of the increase in absorbance at 420 nm caused by the conversion of catechol to brownish-yellow colored quinone in the presence of oxygen.

2.2.5 THE HYDROGEN PEROXIDE (H_2O_2) ASSIGNMENT

Leaf tissues were homogenized in 5 ml of ice-bath with 0.1 % (w / v) TCA. The homogenate was centrifuged for 15 min at 12000 x G. 0.5 ml of supernatant were added to 0.5 ml of 10 mM potassium phosphate (pH: 7.0) buffer and 1 ml of 1 M KI. The absorbance of the mixture was then determined at 390 nm and the amount of H_2O_2 was determined by using the standard curve.

2.3 PHOTOSYNTHETIC PIGMENTATION ASSIGNMENT

Chlorophyll a, b and carotenoid amounts were determined according to Arnon after extraction with 80 % acetone of plant leaves (Arnon, 1949). The amount of carotenoid was determined according to the Jaspars formula (Ulusu et al., 2017). 0.2 g of leaf tissue was homogenized in 5 ml porcelain mortar with 80 % acetone. The homogenate was transferred to a 15 ml centrifuge tube and centrifuged at 3000 x G for 5 minutes. The absorbance of the supernatant at 645, 663 and 450 nm was measured by spectrophotometer and the amount of pigment/ml extract was calculated from the following formulas with the help of these absorbance values.

Chlorophyll a = 12.7 x A663 – 2.69 x A645

Chlorophyll b = 22.9 x A645 – 4.68 x A663

Total Chlorophyll = 20.2 x A645 + 8.02 x A663

Carotenoid = 4.07 x A450 – (0.0435 x amount of Chlorophyll a + 0.3367 x amount of Chlorophyll b)

2.4 PROTEIN ASSIGNMENT

The amount of protein in the leaves was determined by Bradford method (Bradford, 1976). In order to determine the amount of protein, 0.25 g leaf tissue was homogenized in 2.5 ml of 50 mM KH_2PO_4 (pH = 7) buffer in porcelain mortar. homogenate was transferred to eppendorf tubes and centrifuged at 15000 x G for 20 minutes at +4 °C. 20 µl of supernatant was added in 2.5 ml of Coomassie brilliant blue G-250 and vortexed. After 10 minutes of incubation, the absorbance at 595 nm was measured and the amount of protein in the leaves was determined by means of a standard graphic.

2.5 PROLINE ASSIGNMENT

To determine the amount of proline, 0.4 grams of leaf was homogenized in 4 % sulfosalicylic acid and then filtered through homogenate filter paper. 0.5 ml was taken from the filtrate and diluted 10 times with distilled water. 1 ml of sample, 1 ml of 96 % glacial acetic acid and 1 ml of acid ninhydrin solution were added to the tubes, and all the tubes were allowed to incubate for 60 minutes in a water bath at 100 °C. After incubation, the tubes were held for 10 minutes in ice cubes, and then 2 ml of toluene was added to each tube and vortexed. After this process, five minutes were waited and the absorbance of the pink phase formed at the upper part of each tube was measured at 520 nm and recorded. The results were calculated as the amount of prolin per gram of fresh tissue using standard graphics prepared from pure proline.

2.6 MALONDIALDEHYDE ASSIGNMENT

1 g of plant tissue was homogenized with 0.1 % (w / v) TCA and centrifuged at 10000 x G for 5 minutes.

TCA containing 0.5 % TBA was added to the supernatant and incubated at 95 °C for 30 minutes and then quickly cooled in ice bath. The mixture was centrifuged again at 10000 x G for 15 minutes. The amount of malondialdehyde was determined by measuring the absorbance of the supernatant at 532 nm in the spectrophotometer.

2.7 DNA EXTRACTION AND PCR AMPLIFICATION

Young leaves reaching the two-leaf stage were used for DNA extraction, and it was performed according to the method of Doyle and Doyle (1990) with some modifications. The SSR primers used for this study were selected from the SSR primers that showed polymorphic properties according to the previous drought characterization study (Ateş Sönmezoğlu & Terzi, 2018). PCR conditions used for Xwmc 89, Xwmc 118, Xwmc 304 and Xgwm 337 primers are as specified in the source article.

Agarose gel concentrations were 2 % for PCR products and 1 % for genomic DNAs. Ethidium bromide (10 mg ml^{-1}) was used as an imaging dye in electrophoresis. Electrophoresis was performed in 1 % TBE buffer at 100 V constant for 60 min. PCR reactions were carried out with BIO-RAD C1000 Touch Thermal Cycler. Biorad ChemiDoc MP was used as a gel imaging system, and polymorphic bands were determined.

2.8 STATISTICAL ANALYSIS

Polymorphic bands obtained from PCR were scored 1 (presence) and 0 (no band) for molecular characterization. DendroUPGMA (D-UPGMA) software (//genomes.urv.es/UPGMA) was used for the molecular comparisons of genotypes, and Interactive Tree of Life (iTOL) (https://itol.embl.de) was used to construct the interactive tree.

3 RESULTS AND DISCUSSIONS

3.1 ANTIOXIDANT ENZYME ACTIVITIES

In Bayraktar, Sönmez, Gerek and İzgi cultivars, the amount of H_2O_2 and CAT varied inversely according to the stress days, while in Sultan, Harmankaya, Karahan, Kate and Sarıçanak cultivars, both were parallel. In this case, H_2O_2 produced as a result of stress factors was tried to be kept under control by CAT, but these plants had a

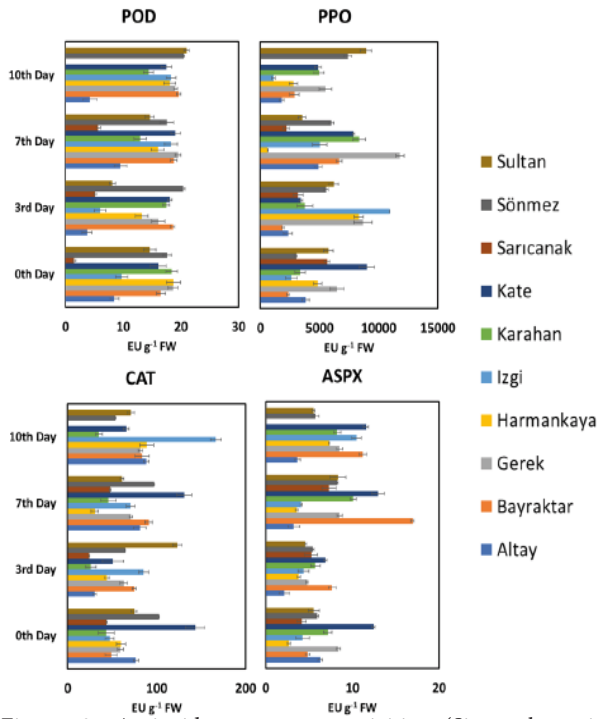

Figure 2: Antioxidant enzyme activities (Since there is not enough vegetative tissue left in the Sarıcanak variety, the analyzes on the 10th day could not be studied)

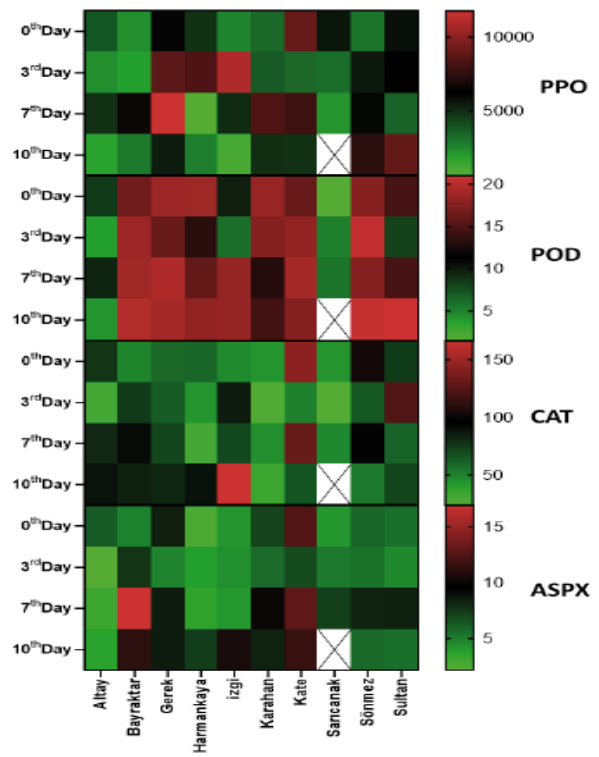

Figure 3: Heatmap analysis of the antioxidant enzyme activities (Since there is not enough vegetative tissue left in the Sarıcanak variety, the analyzes on the 10th day could not be studied).

little more difficulty in maintaining the balance than the others. ASPX activity tended to decrease in Altay variety due to stress, while it increased in other wheat varieties compared to the control group. On the other hand, polyphenol oxidase activity increased until the middle of the stress application in the other varieties except Sarıçanak variety, while it showed a decreasing trend as of the 10th day due to the deterioration of the internal balance (Fig. 2).While an increase in POD activity was observed in Bayraktar, Sultan, Izgi and Sarıçanak varieties, no significant change in POD activity was observed in other treatment groups due to stress (Fig. 3).

3.2 PHOTOSYNTHETIC PIGMENTS

Photosynthesis is one of physiological processes, which is the most sensitively affected by abiotic stress conditions in plants. Drought stress also causes structural and functional effects on chloroplasts. In this study, changes in chlorophyll a, b, total chlorophyll and carotenoid contents were investigated in different wheat varieties under water stress. While no significant pigment change was observed in the samples during the early drought period, a relative decrease occurred in chlorophyll a, b and total chlorophyll amounts in parallel with the increasing stress conditions. (Fig.4, Fig. 5).

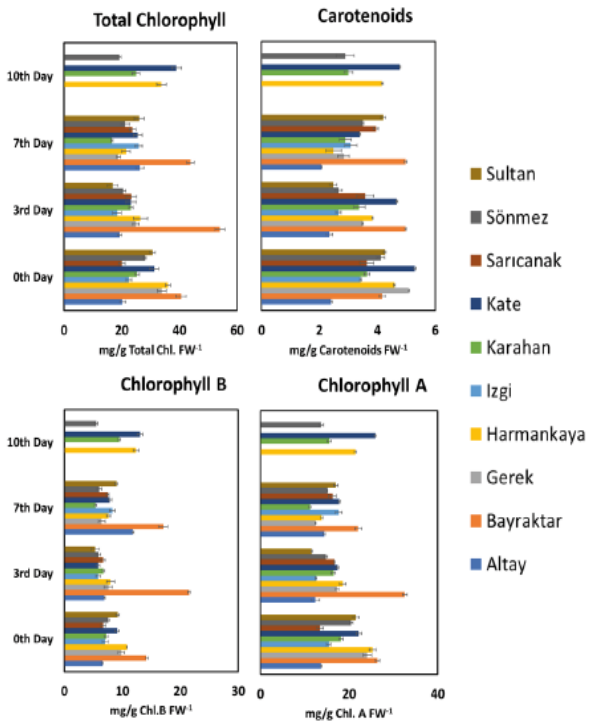

Figure 4: Photosynthetic pigment analysis (Since there is not enough vegetative tissue left in the Altay, Bayraktar, Gerek, Izgı, Sarıcanak, Sultan varieties the analyzes on the 10th day could not be studied)

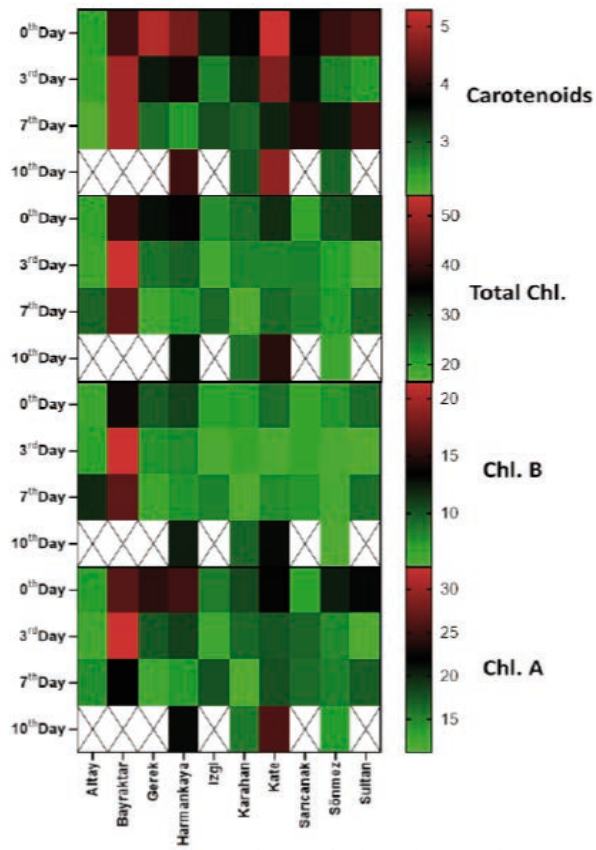

Figure 5: Heatmap analysis of the photosynthetic pigments (Since there is not enough vegetative tissue left in the Altay, Bayraktar, Gerek, Izgı, Sarıcanak, Sultan varieties the analyzes on the 10th day could not be studied)

3.3 PROTEIN

Drought tolerance of plants is usually achieved by changes in antioxidant enzymes and maintenance of H_2O_2 levels within certain limits. Water stress is associated with increased oxidative stress due to increased accumulation of ROS, especially O^{2-} and H_2O_2 in chloroplasts, mitochondria and peroxisomes. Induction of antioxidant enzyme activities is a general adaptation strategy used by plants to overcome oxidative stresses (Fig. 6) (Foyer & Noctor, 2003).

In plants subjected to drought stress, changes in the amount of hydrophilic total protein within the cells were observed as a response to drought. A statistically significant increase in the amount of protein was observed in 7 out of 10 different varieties (Altay, Bayraktar, Gerek, Sultan, İzgi, Karahan and Kate) analyzed. This increase is thought to have been provided by the activities in the

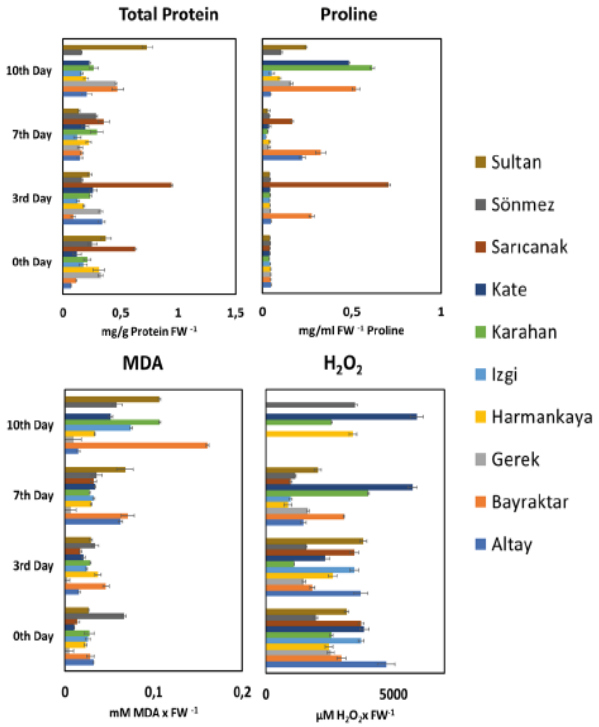

Figure 6:Total protein, proline, MDA and H_2O_2 levels of treatment groups (Since there is not enough vegetative tissue left in the Altay, Bayraktar, Gerek, Izgı, Sarıcanak, Sultan varieties the H_2O_2 analyzes on the 10th day could not be studied)

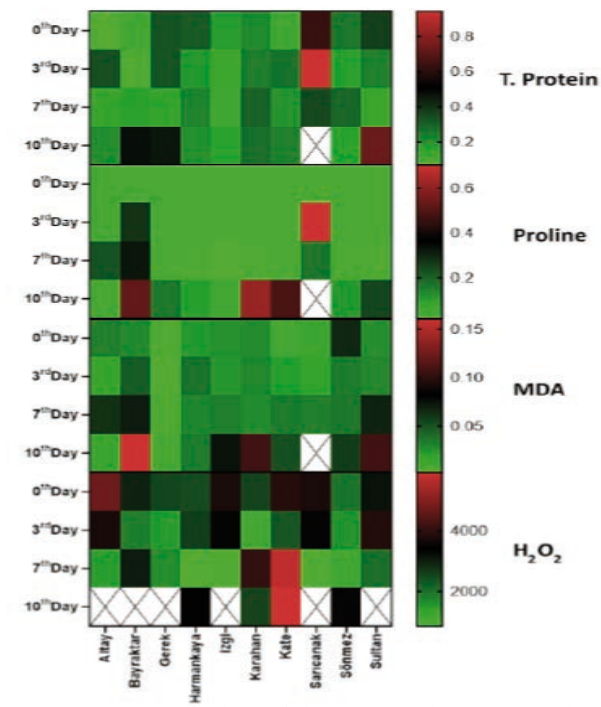

Figure 7: Heatmap analysis of al protein, Proline, MDA and H^2O^2 levels (Since there is not enough vegetative tissue left in the Altay, Bayraktar, Gerek, Izgı, Sarıcanak, Sultan varieties the H^2O^2 analyzes on the 10th day could not be studied)

synthesis of antioxidant enzymes, which are one of the intracellular defense systems. On the other hand, while no significant change was observed in Harmankaya variety, in Sönmez and Sarıçanak varieties, the plant was out of the hemostasis state and in parallel with this, a decrease in total protein amount was observed.

3.4 PROLINE

In the present study, proline levels increased significantly in all treatment groups except Altay and Sarıçanak, especially on the 10th drought day. This indicates that the plants were stressed after drought and this provided a sufficient signal for the activation of many biochemical and physiological antioxidant defense mechanisms within the organism. In Altay, the amount of free proline increased about five times after the 7th treatment day compared to the control group; however, it decreased back to its previous level by the 10th day. A similar situation was observed in Sarıçanak varietal as of the 3rd day (Fig. 7).

3.5 MALONDIALDEHYDE

In Altay varietal, the amount of MDA, which reached its highest level on the 7th day, decreased on the 10th day. In this case, it is thought to be an indication that especially membrane lipids started to abound due to stress and the hemostasis state of the plant gradually started to disappear.

3.6 ANALYSES OF MOLECULAR CHARACTERS

In this study, the dendrogram of the genotypes was conducted by using 4 SSR primers (Xwmc 89, Xwmc 118, Xwmc 304, and Xgwm 337), which show polymorphic features in drought-related gene regions (Ateş Sönmezoğlu & Terzi, 2018; Kirigwi et al., 2007; Kumar et al., 2012; Mdluli et al., 2020). According to *Figure 8*, bread-wheat genotypes were divided into two main groups. While the first of these main groups were the Sultan-95 genotype, which is sensitive to drought, the second main group included the Gerek-79 genotype, which is resistant to drought. Group 1 contained the Sultan-95 and Karahan genotypes, while the other group contained all remaining genotypes. The second group was also divided into subgroups within itself. According to the dendrogram values, the closest genotypes to each other were Kate-1 and Sönmez 2001. The results of this study showed that the genotypes used have a high genetic diversity.

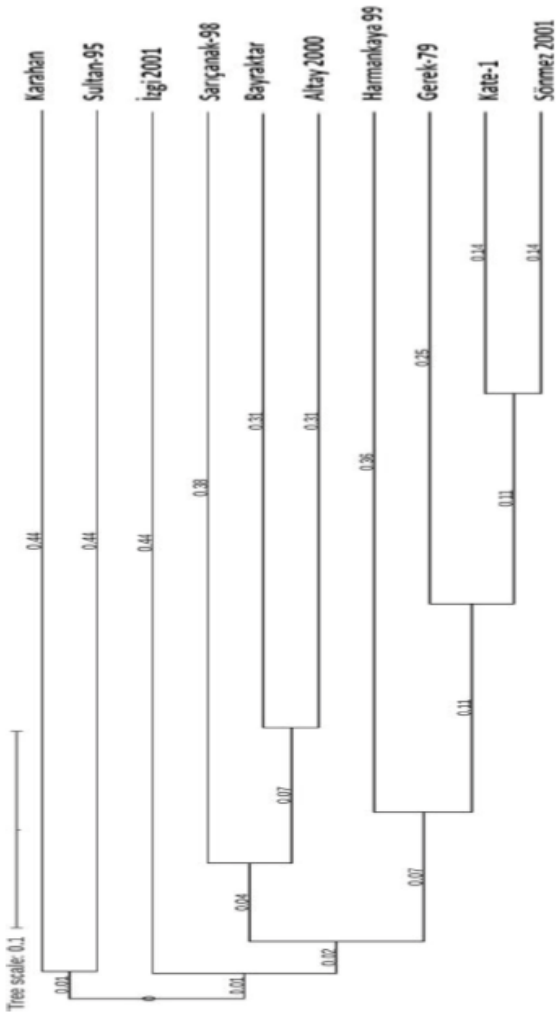

Figure 8: Dendrogram based on molecular data.

The antioxidant response is often enhanced in relation to plant tolerance when abiotic stress conditions occur because enhanced antioxidant enzyme activities contribute to drought tolerance to reduce oxidative damage (Gill & Tuteja, 2010). It has been stated in the literature that high antioxidant enzyme activities contribute to stress tolerance (Mehrabad Pour-Benab et al., 2019), but different studies may show different results (Cruz de Carvalho, 2008).

In this study, catalase enzyme activity increased gradually from day 0 in the tolerant genotype (Gerek-79), while it decreased significantly after the 3rd day for the sensitive genotype (Sultan-95). Catalase activities increased on the 10th day for Altay 2000, Harmankaya 99, and Izgi 2001 varieties. While the peroxidase enzyme activity did not show a big difference for Gerek-70, it decreased and then increased for the Sultan on the 3rd day. Izgi 2001 and Altay 2000 cultivars were also reduced on the 3rd day the other days, like the Sultan-95. Ascorbate peroxidase activity decreased on the 3rd day for Gerek-79 but increased for the following days, it decreased on the 10th day in the Sultan-95 cultivar. Bayraktar, Altay 2000, Sönmez 2001 and Karahan also decreased on the 10th day.

When evaluated biochemically, some of the wheat genotypes selected in different genotypes under drought stress are more resistant, and some are more sensitive. However, this varies for different biochemical analyzes. Therefore, no significant correlation was observed between genotypes when the biochemical results were examined in our study.

Vuković et al. (2022) did not find a linear correlation between the results on the biochemical and molecular responses of winter wheat varieties under drought stress. They stated that the mechanism of drought tolerance is quite complex. Accordingly, factors such as the period of the plant and the intensity or duration of the stress to which the plant is exposed should be considered (Vuković et al., 2022).

Since genotypes under drought stress exhibit various biochemical properties, it is not enough to generalize the molecular data according to biochemical results. The reason for this can be explained as follows: 1) wheat has a large genome (16,000 Mb for bread wheat), and drought is a quantitative trait controlled by many genes. In this regard, the molecular markers may not be specific to the gene regions controlling the relevant biochemical processes; 2) molecular markers can also cover non-coding regions of the genome.

4 CONCLUSIONS

In this study, the characterization of different wheat genotypes for drought tolerance as well as detailed biochemical analyzes, was evaluated. The tolerance mechanism of drought, which has a complex mechanism, was examined in terms of different genotypes. This study is expected to provide molecular and biochemical preliminary information for local wheat varieties in our country. In addition, we propose a detailed molecular, biochemical, and morphological examination of the drought mechanism for essential crop plants with subsequent studies.

5 ACKNOWLEDGEMENT

This work was supported by the Karamanoglu Mehmetbey University Unit for Scientific Research Projects, project no: 31-M-16.

6 REFERENCES

Ahmad, P., Sarwat, M., & Sharma, S. (2008). Reactive oxygen species, antioxidants and signaling in plants. *Journal of Plant Biology*, *51*, 167-173.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiology*, *24*(1), 1.

Arora, N. K. (2019). Impact of climate change on agriculture production and its sustainable solutions. *Environmental Sustainability*, *2*(2), 95-96.

Ateş Sönmezoğlu, Ö., & Terzi, B. (2018). Characterization of some bread wheat genotypes using molecular markers for drought tolerance. *Physiology and Molecular Biology of Plants*, *24*(1), 159-166.

Bartels, D., & Sunkar, R. (2005). Drought and salt tolerance in plants. *Critical Reviews in Plant Sciences*, *24*(1), 23-58.

Beltrano, J., & Ronco, M. G. (2008). Improved tolerance of wheat plants (*Triticum aestivum* L.) to drought stress and rewatering by the arbuscular mycorrhizal fungus Glomus claroideum: Effect on growth and cell membrane stability. *Brazilian Journal of Plant Physiology*, *20*, 29-37.

Bohnert, H. J., & Jensen, R. G. (1996). Strategies for engineering water-stress tolerance in plants. *Trends in Biotechnology*, *14*(3), 89-97.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248-254.

Chen, Y. E., Liu, W. J., Su, Y. Q., Cui, J. M., Zhang, Z. W., Yuan, M., Zhang, H. Y., & Yuan, S. (2016). Different response of photosystem II to short and long- term drought stress in *Arabidopsis thaliana*. *Physiologia Plantarum*, *158*(2), 225-235.

Cruz de Carvalho, M. H. (2008). Drought stress and reactive oxygen species: production, scavenging and signaling. *Plant Signaling & Behavior*, *3*(3), 156-165.

Faostat, F. (2017). Available online: http://www. fao. org/faostat/en/# data. *QC (accessed on January 2018)*.

Finney, P., Gaines, C., & Andrews, L. (1987). Wheat quality: a quality assessor's view.

Foyer, C. H., & Noctor, G. (2003). Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiologia Plantarum*, *119*(3), 355-364.

Gálvez, S., Mérida-García, R., Camino, C., Borrill, P., Abrouk, M., Ramírez-González, R. H., Biyiklioglu, S., Amil-Ruiz, F., IWGSC, & Dorado, G. (2019). Hotspots in the genomic architecture of field drought responses in wheat as breeding targets. *Functional & Integrative Genomics*, *19*, 295-309.

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, *48*(12), 909-930.

Javadinejad, S., Dara, R., & Jafary, F. (2021). Analysis and prioritization the effective factors on increasing farmers resilience under climate change and drought. *Agricultural Research*, *10*, 497-513.

Kadam, S., Singh, K., Shukla, S., Goel, S., Vikram, P., Pawar, V., Gaikwad, K., Khanna-Chopra, R., & Singh, N. (2012). Genomic associations for drought tolerance on the short arm of wheat chromosome 4B. *Functional & Integrative Genomics*, *12*, 447-464.

Keleş, Y., & Öncel, I. (2002). Response of antioxidative defence system to temperature and water stress combinations in wheat seedlings. *Plant Science*, *163*(4), 783-790.

Khan, A. L., Hamayun, M., Khan, S. A., Kang, S.-M., Shinwari, Z. K., Kamran, M., ur Rehman, S., Kim, J.-G., & Lee, I.-J. (2012). Pure culture of Metarhizium anisopliae LHL07 reprograms soybean to higher growth and mitigates salt stress. *World Journal of Microbiology and Biotechnology*, *28*, 1483-1494.

Kireçci, O. A. (2012). Kuraklık stresine maruz bırakılan *Triticum aestivum* L.(buğday) çeşitlerinde sinyal iletiminde rol oynayan bazı biyomoleküllerin araştırılması.

Kirigwi, F., Van Ginkel, M., Brown-Guedira, G., Gill, B., Paulsen, G. M., & Fritz, A. (2007). Markers associated with a QTL for grain yield in wheat under drought. *Molecular Breeding*, *20*, 401-413.

Kumar, S., Sehgal, S. K., Kumar, U., Prasad, P. V., Joshi, A. K., & Gill, B. S. (2012). Genomic characterization of drought tolerance-related traits in spring wheat. *Euphytica*, *186*, 265-276.

Maheshwari, R., & Dubey, R. (2009). Nickel-induced oxidative stress and the role of antioxidant defence in rice seedlings. *Plant Growth Regulation*, *59*, 37-49.

Mansour, M. M. F. (1998). Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress. *Plant Physiology and Biochemistry*, *36*(10), 767-772.

Mdluli, S. Y., Shimelis, H., & Amelework, A. B. (2020). Genetic diversity and population structure of elite drought tolerant bread wheat (*Triticum aestivum* L.) genotypes. *Australian Journal of Crop Science*, *14*(9), 1362-1371.

Mehrabad Pour-Benab, S., Fabriki-Ourang, S., & Mehrabi, A.-A. (2019). Expression of dehydrin and antioxidant genes and enzymatic antioxidant defense under drought stress in wild relatives of wheat. *Biotechnology & Biotechnological Equipment*, *33*(1), 1063-1073.

Mohammadi, R., Sadeghzadeh, B., Ahmadi, H., Bahrami, N., & Amri, A. (2015). Field evaluation of durum wheat landraces for prevailing abiotic and biotic stresses in highland rainfed regions of Iran. *The Crop Journal*, *3*(5), 423-433.

Naderi, S., Fakheri, B.-A., Maali-Amiri, R., & Mahdinezhad, N. (2020). Tolerance responses in wheat landrace Bolani are related to enhanced metabolic adjustments under drought stress. *Plant Physiology and Biochemistry*, *150*, 244-253.

ÖZDEMİR, E., Bayram, S., SOYLU, S., & ATALAY, E. (2012). Ekmeklik buğday (*Triticum aestivum* L.)'da priming uygulamalarının kurak ve normal ortam koşullarında büyüme parametreleri ile bağıl su içeriği değerleri üzerine etkileri. *Selcuk Journal of Agriculture and Food Sciences*, *26*(2), 25-30.

Tenikecier, H. S. (2013). *Buğdayda (Triticum aestivum L. em Thell.) endosprem ve tane iriliğinin çimlenme ve fide özellikleri ile verim ve kalite unsurlarına etkisi* Namık Kemal Üniversitesi].

Ulusu, Y., Öztürk, L., & Elmastaş, M. (2017). Antioxidant capacity and cadmium accumulation in parsley seedlings ex-

posed to cadmium stress. Russian Journal of Plant Physiology, 64, 883-888.

Vuković, R., Čamagajevac, I. Š., Vuković, A., Šunić, K., Begović, L., Mlinarić, S., Sekulić, R., Sabo, N., & Španić, V. (2022). Physiological, biochemical and molecular response of different winter wheat varieties under drought stress at germination and seedling growth stage. Antioxidants, 11(4), 693.

Wang, W., Vinocur, B., & Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta, 218, 1-14.

Zhang Feng, Z. F., Guo JinKui, G. J., Yang YingLi, Y. Y., He Wen-Liang, H. W., & Zhang LiXin, Z. L. (2004). Changes in the pattern of antioxidant enzymes in wheat exposed to water deficit and rewatering.

Zhou, Q., Ravnskov, S., Jiang, D., & Wollenweber, B. (2015). Changes in carbon and nitrogen allocation, growth and grain yield induced by arbuscular mycorrhizal fungi in wheat (*Triticum aestivum* L.) subjected to a period of water deficit. Plant Growth Regulation, 75, 751-760.