KSÜ Tarım ve Doğa Derg 28 (4), 893-902, 2025
KSU J. Agric Nat 28 (4), 893-902, 2025
https://doi.org/10.18016/ksutarimdoga.vi.1651818

Superoxide Dismutase and Isozyme Analysis in Two Bread Wheat Genotypes Under Osmotic Stress

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ABSTRACT
Climate change, driven by human activity, has accelerated global warming, leading to heightened water scarcity and drought globally. In regions like Turkey and the Mediterranean basin, changing climate patterns threaten key sectors such as agriculture, where wheat is a staple crop. This study investigated the osmotic stress tolerance of two bread wheat genotypes, Bezostaya-1 and Karahan-99, by analyzing their superoxide dismutase (SOD) enzymes and isoenzymes under hydroponic conditions. Plants were exposed to osmotic stress at the vegetative and generative growth stages, alongside a control group. SOD enzyme activities and isozyme profiles were analyzed. The results showed that both genotypes produced different activities of Mn-SOD, Cu/Zn-SOD, and Fe-SOD isoenzymes under osmotic stress. Cu/Zn-SOD and Fe-SOD demonstrated peak activity, highlighting their vital role in mitigating oxidative damage. Total SOD activity increased significantly, especially during the generative stage, highlighting the importance of antioxidant defense mechanisms during critical growth phases under osmotic stress. Overall, the study highlights the importance of understanding how wheat genotypes respond biochemically to osmotic stress, particularly in the context of changing climate conditions. The results provide valuable information for the development of resistant wheat genotypes.

Biochemistry

Research Article

Article History
Received : 05.03.2025
Accepted : 29.05.2025

Keywords
Bread wheat
Osmotic stress
Antioxidant defence mechanisms
Superoxide dismutase enzymes and isoenzymes

Osmotik Stres Altındaki İki Ekmeklik Buğday Genotiplerinde Süperoksit Dismutaz ve İzozim Analizi

ÖZET
İnsan faaliyetlerinin yol açtığı iklim değişikliği, küresel ısınmayı hızlandırarak küresel ölçekte su kıtlığı ve kuraklığın artmasına neden olmuştur. Türkiye ve Akdeniz havzası gibi bölgelerde değişen iklim modelleri, buğdayın temel bir ürün olduğu tarım gibi kilit sektörleri tehdit etmektedir. Bu çalışmada, Bezostaya-1 ve Karahan-99 adlı iki ekmeklik buğday genotipinin ozmotik stres toleransı, hidroponik koşullar altında süperoksit dismutaz (SOD) enzimleri ve izoenzimleri analiz edilerek araştırılmıştır. Bitkiler, bir kontrol grubu ile birlikte vejetatif ve generatif büyüme aşamalarında ozmotik strese maruz bırakılmıştır. SOD enzim aktiviteleri ve izozim profilleri analiz edilmiştir. Sonuçlar, her iki genotipin ozmotik stres altında farklı Mn-SOD, Cu/Zn-SOD ve Fe-SOD izoenzim aktiviteleri ürettiğini göstermiştir. Cu/Zn-SOD ve Fe-SOD en yüksek aktiviteyi göstererek oksidatif hasarın azaltılmasındaki hayati rollerini vurgulamışlardır. Toplam SOD aktivitesi, özellikle generatif aşamada önemli ölçüde artarak, ozmotik stres altındaki kritik büyüme aşamalarında antioksidan savunma mekanizmalarının önemini vurgulamıştır. Genel olarak çalışma, özellikle değişen iklim koşulları bağlamında buğday genotiplerinin ozmotik strese biyokimyasal olarak nasıl tepki verdiğini anlamanın önemini vurgulamaktadır. Sonuçlar, dayanıklı buğday genotiplerinin geliştirilmesi için değerli bilgiler sağlamaktadır.

Biyokimya

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 05.03.2025
Kabul Tarihi : 29.05.2025

Anahtar Kelimeler
Ekmeklik Buğday
Ozmotik stres
Antiosidan savunma mekanizması
Süperoksit dismütaz enzim ve izozimleri

Atıf Şekli: Keleş, R., & Küçüködük, M., (2025). Osmotik Stres Altındaki iki Ekmeklik Buğday Genotiplerinde Süperoksit Dismutaz ve İzozim Analizi. *KSÜ Tarım ve Doğa Derg 28* (4), 893-902. https://doi.org/10.18016/ksutarimdoga.vi.1651818

To Cite : Keleş, R., & Küçüködük, M., (2025). Superoxide Dismutase and Isozyme Analysis in Two Bread Wheat Genotype Under Osmotic Stress. *KSU J. Agric Nat 28* (4), 893-902. https://doi.org/10.18016/ksutarimdoga.vi.1651818

INTRODUCTION

Although the climate is a naturally variable system, today's climate change is unprecedented in its speed and the extent of human influence. Although the phenomenon, in which human activity plays a significant role, is commonly known as "global warming", its most significant impact is water scarcity and resulting drought. Drought, defined as a prolonged period of below-average precipitation (Schneider, 2011), limits crop production globally (Kutlu, 2010), accounting for 26% of stress factors in cultivated areas (Blum & Jordan, 1985). Due to global warming and climate change, Turkey and the Mediterranean basin are expected to experience significant drought. A major cause of this trend is the poleward shift of the 30° latitude high-pressure belt (Şahin & Kurnaz 2014). In Turkey, wheat (*Triticum* sp.) is a critical cereal, cultivated on 7.3 million hectares and producing ~20 million tons annually. A decline in wheat productivity due to drought could exacerbate socio-economic challenges linked to food security.

One of the climatic phenomena that occurs under growing conditions with global climate change is the change in precipitation regime rather than the amount of precipitation. The severity of osmotic stress effects on plants varies depending on the developmental stage, in addition to the effect of irregular rainfall regime (Gupta et al., 2001). Osmotic stress tolerance varies considerably among genotypes of the same species, and some osmotic stress-tolerant varieties are less affected by osmotic stress in terms of yield components (Majer, 2008). Recent studies emphasize that drought-tolerant wheat genotypes exhibit heightened antioxidant enzyme activity (e.g., SOD, CAT, APX) under water deficits, underscoring their role in stress mitigation (Aliyeva et al., 2023). Under osmotic stress, plants undergo a significant biochemical change, resulting in the formation of reactive oxygen species (ROS), including superoxide and hydroxyl radicals, as well as non-radical ROS such as singlet oxygen and hydrogen peroxide (Bhargava& Sawant, 2013). The majority of the damage that occurs in plants under abiotic stress conditions, such as osmotic stress, is closely related to oxidative damage at the cellular level, and enhanced antioxidant protection is very important in terms of increasing osmotic stress tolerance. Specifically, the upregulation of mitochondrial Mn-SOD has been associated with improved drought tolerance in certain wheat genotypes, suggesting its pivotal role in ROS detoxification (Sheoran et al., 2015). Under optimal growth conditions, the plant's antioxidant defense system maintains a tight balance between the formation and consumption of reactive oxygen species (ROS). Primary antioxidant enzyme systems, such as SOD, react with existing radicals to convert them into less harmful forms or to limit the formation of new free radicals.

This study aims to evaluate the osmotic stress tolerance levels of two bread wheat genotypes, Bezostaya-1 and Karahan-99, under hydroponic conditions during different growth stages, specifically the vegetative and generative phases. The primary focus is on the activity and isoenzyme profiles of the superoxide dismutase (SOD) enzyme system, which plays a critical role in mitigating oxidative damage caused by osmotic stress in wheat leaves. By identifying and analyzing key SOD isoenzymes—Mn-SOD, Cu/Zn-SOD, and Fe-SOD—the research seeks to elucidate their distinct contributions and adaptive responses to osmotic stress conditions, offering insights into genotypic variations in biochemical responses. Previous research has identified multiple SOD isoenzymes with varying subcellular localizations in wheat, and their differential expression under stress conditions underscores the complexity of the antioxidant response (Miri-Hesar et al., 2019). Additionally, the study examines changes in SOD activity and isoenzyme dynamics during critical growth periods to better understand the antioxidant defense mechanisms employed by these genotypes. The findings are expected to advance the molecular and biochemical understanding of osmotic stress tolerance in wheat, particularly by highlighting the roles of specific SOD isoenzymes in stress adaptation. This study aims to address the growing challenges posed by water scarcity and climate change. It seeks to establish a foundation for targeted genetic improvement strategies and sustainable agricultural practices in water-limited environments. The findings will guide breeding strategies to enhance drought resistance. This is particularly relevant for Turkey and similar regions.

MATERIAL and METHODS

Materials

The bread wheat genotypes Bezostaja-1 and Karahan-99 were used as plant material in the study. Both bread wheat varieties were preferred because of their different responses to environmental stresses. While Bezostaja-1

is recommended for irrigated and rainy regions, Karahan-99 is highly drought-resistant and suitable for barren areas. In this context, Karahan is often used as a reference variety in international drought breeding programmes. These different physiological and morphological characteristics make it possible to compare the performance of varieties under different environmental conditions.

Bezostaya-1: The registered variety, recommended for irrigated areas with high rainfall and possessing a winter character, was developed by the Transitional Zone Agricultural Research Institute in 1968. It has an awnless and white spike, with red, hard, and coarse grains. The variety is sensitive to drought but resistant to cold. Depending on growing conditions, the grain yield ranges from 320-580 kg/da (Sakin et al., 2015)

Karahan-99: The registered variety, recommended for barren, semi-bottom, and bottom areas, has a winter character. It was registered by the Bahri Dağdaş International Agricultural Research Institute in 1999.

The recommended variety for cultivation in arid regions with rainfall-based agriculture has excellent drought tolerance capacity. Depending on growing conditions, the grain yield ranges from 200-500 kg/da (Mazid et al., 2009).

Methods

The research was conducted under hydroponic conditions. The materials were standardized by passing them through a 2.25 mm sieve, followed by surface sterilization in 5% sodium hypochlorite for 15 minutes, and then washed with sterile deionized water.

Seeds were sown in petri dishes and allowed to germinate under controlled conditions in a climate chamber set at 24±1°C with a 16-hour light/8-hour dark cycle. Germinated seeds were transferred to 5-litre pots containing Hoagland's nutrient solution. The plants were subjected to a 16-hour light cycle with a light intensity of 350 µmol $m^{-2}s^{-1}$, at a temperature of 25°C and 65% humidity, followed by an 8-hour dark cycle at a temperature of 24°C and 75% humidity. Each pot contained 20 plants, and the nutrient solution was changed every 5 days. During the following periods, an equal number of dilutions were made from each pot to ensure sufficient air circulation between the plants.

The study consisted of two treatments, vegetative period osmotic stress (D1) and generative period osmotic stress (D2), and a control group (C) for each treatment.

Vegetative Period Osmotic Stress (D1): Artificial drought conditions were created with PEG-6000 application between the beginning of emergence (Zadoks 30) and the beginning of spike (Zadoks 50) periods.

Generative Period Osmotic Stress (D2): Artificial drought conditions were created with PEG-6000 application between spike initiation (Zadoks 50) and milk maturity (Zadoks 70) periods.

Control: In order to determine the optimum performance for each of the genotypes in the experiment, osmotic stress was not applied. For the D1 and D2 treatments, an osmotic stress was induced using 15% PEG 6000 (-2.95 MPa) for 5 days at the specified times according to Zadoks (Ullah et al., 2010). To conduct SOD enzyme and isozyme analyses, leaf samples were collected from each group (D1, D2) on the same date as their respective control group.

Laboratory analyses

Enzyme extractions were conducted at ±4 °C. For both the treatment and control groups, 0.5 g of leaf samples were homogenized using liquid nitrogen in a chilled mortar with 50 mM Tris-HCl (pH 7.8) buffer containing 0.1 mM EDTA, 0.1% (v/v) Triton-X 100, 1 mM PMSF, and 2% (w/v) PVPP. The protein content and enzyme activities were determined using the supernatant obtained by centrifuging the homogenate at 4°C and 10.000 rpm for 20 minutes. Spectrophotometric measurements were carried out using the Shimadzu UV-1800 and Thermo Multiskan GO.

Electrophoretic separation of superoxide dismutase (SOD) isoenzymes was performed by staining with riboflavin and NBT (Beauchamp & Fridovich, 1971). Samples containing 40 µg of protein underwent non-denaturing polyacrylamide gel electrophoresis (PAGE) (Laemmli, 1970). SOD samples were run at 4°C under a constant current of 120 mA on a 5% stacking gel and 12% separating gel. Following electrophoresis, the SOD isozymes were identified by adding inhibitors to the dye solution. To inhibit Cu/Zn-SOD, potassium cyanide (KCN) was added to the dye solution, while H_2O_2 was used to inhibit Fe-SOD and Cu/Zn-SOD. Mn-SOD activity, on the other hand, remained resistant to both inhibitors. To determine SOD isozymes, we used 0.5 units of SOD standard with samples containing 60 µg protein for each genotype. After separating the proteins in non-denaturing PAGE (PAGE-polyacrylamide gel electrophoresis), we determined total SOD activities using densitometric method with Biorad Image Lab. Version 4.01 software programme.

Statistical Analyses

The study was conducted using a 'split plots in randomized blocks' experimental design with four replications. The varieties were the main plots, and the osmotic stress application period was the sub-plots. The study data underwent variance analysis, and the mean values of the data with significant differences, as determined by the 'F' test, were classified using the 'LSD' multiple comparison test.

The t-test was used to determine the differences and significance levels between the values of the treatment and control groups in our study (Mendeş, 2012). The statistical analyses were performed using the JMP 11.2.1 statistical package programme to evaluate all the obtained data.

RESULTS and DISCUSSION

The results of the analysis of variance of total superoxide dismutase (SOD) enzyme activity (U mg protein-1) with its isozymes obtained in the study carried out to determine the effects of vegetative and generative osmotic stress treatments on Bezostaya-1 and Karahan-99 bread wheat varieties are given in Table 1.

Table 1. Analysis of variance of total superoxide dismutase (SOD) enzyme activity (U mg protein^{-1}) values of Bezostaya-1 and Karahan-99 bread wheat total superoxide dismutase (SOD) enzyme activity values of vegetative and generative drought treatments

Çizelge 1. Bezostaya-1 ve Karahan-99 ekmeklik buğdaylarının vejetatif ve generatif kuraklık uygulamalarına ait toplam süperoksit dismutaz (SOD) enzim aktivitesi (U mg protein^{-1}) değerlerine ait varyans analizi

Source	DF	Sum of Squares	F
Replications	3	0.00022	0.3329
Variety	1	0.12426	2326.17**
Error$_1$	3	0.00017	0.43
Drought App. Period	1	0.00801	149.96**
Variety* Drought App. Period	1	0.00731	136.85**
Error$_2$	6	0.00032	
C.Total	15	0.14029	
CV (%)			3.88

** *Significant difference at (P<0.01); DF: Degrees of Freedom; CV: Coefficiend of Variations*

The mean values of superoxide dismutase enzyme activity (U mg protein-1) and multiple comparison test "LSD" groups of Bezostaya-1 and Karahan-99 bread wheat varieties at different osmotic stress application periods are given in Table 2.

For the Bezostaya-1 cultivar, the mean total SOD activity value (U mg protein-1) was 0.100±0.00303. The values for D1 and D2 treatments were 0.099±0.006944 and 0.101±0.000943, respectively (Table 2). For the Karahan-99 variety, the mean value of total SOD activity (U mg protein-1) was 0.276±0.017836, 0.233±0.002887 for the D1 treatment and 0.320±0.004714 for the D2 treatment (Table 2).

Table 2. Mean superoxide dismutase (SOD) enzyme total activity (U mg protein-1) values and LSD groups of Bezostaya-1 and Karahan-99 bread wheat varieties at different drought treatment periods

Çizelge 2. Bezostaya-1 ve Karahan-99 ekmeklik buğday çeşitlerinin farklı kuraklık uygulama dönemlerindeki ortalama süperoksit dismutaz (SOD) enzimi toplam aktivite (U mg protein^{-1}) değerleri ve LSD grupları

	Karahan-99	Bezostaya-1	Mean
D1	0.233±0.002887$_b$**	0.099±0.006944^c	0.166±0.027613^B**
D2	0.320±0.004714^a	0.101±0.000943^c	0.211±0.044293^A
Mean	0.276±0.017836^A**	0.100±0.00303^B	0.188±0.024971

** *Significant difference at (P<0.01);* $LSD_{Var.}$:0.009; $LSD_{Drought\ App.\ Per.}$: 0.009; $LSD_{Var\ X\ Drought\ App.\ Per.}$:0.013

Both varieties were found to contain Mn-SOD, Cu/Zn-SOD, and Fe-SOD isozymes, which are part of the SOD enzyme system and can be regulated during development and are highly reactive to stress conditions (Figure 1, Figure 2).

Regarding the average activity values of isozymes, it was found that Cu/Zn-SOD isozymes had the highest activity values during the D1 osmotic stress treatment period for the Bezostaya-1 variety, whereas Fe-SOD isozymes had the highest activity values during the D2 period (Table 3). For tThe Karahan-99 cultivar, Fe-SOD isozymes had the highest activity values during both osmotic stress treatment periods (D1, D2) (Table 3).

The Karahan-99 cultivar, Fe-SOD isozymes had the highest activity values during both osmotic stress treatment periods (D1, D2) (Table 3).

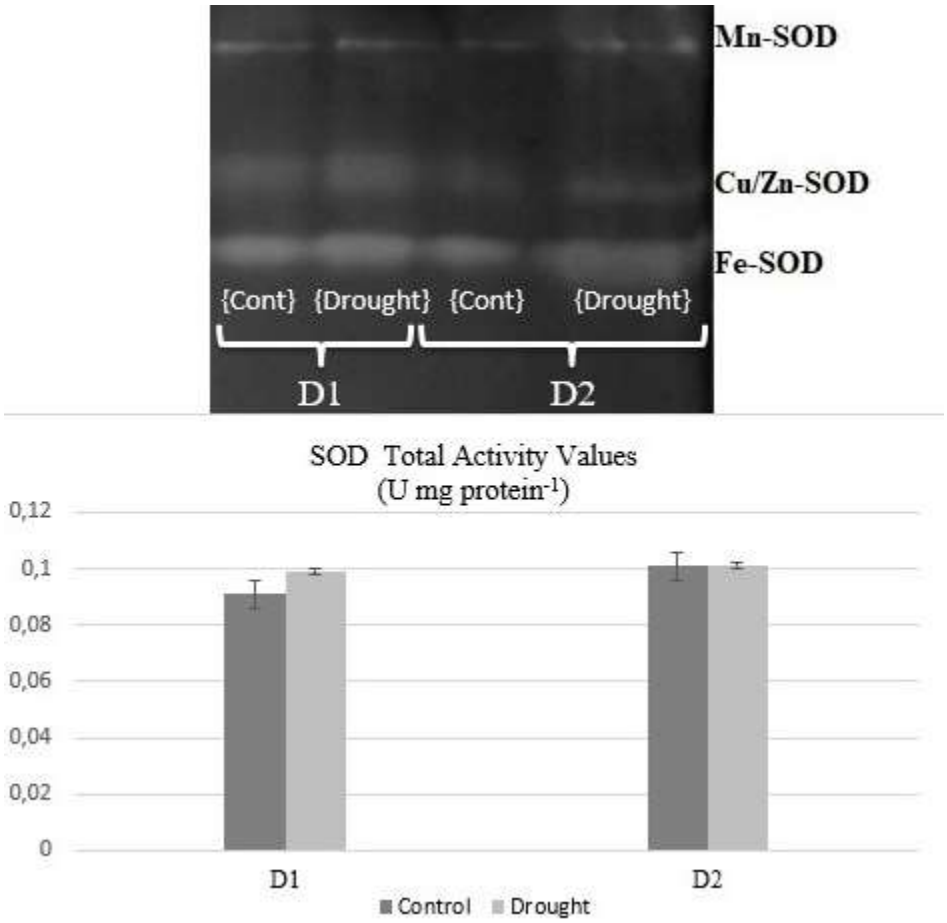

Figure 1. SOD isozymes and total activity (U mg protein^{-1}) of Bezostaya-1 bread wheat variety during vegetative and generative drought treatment periods with control groups

Şekil 1. Bezostaya-1 ekmeklik buğday çeşidinin kontrol grupları ile vejetatif ve generatif dönem kuraklık uygulaması SOD izozimleri ve toplam aktivitesi (U mg protein^{-1})

Table 3. Mean activity (U mg protein^{-1}) values of different SOD isozymes of Bezostaya-1 and Karahan-99 cultivars at different drought application periods

Çizelge 3. Bezostaya-1 ve Karahan-99 çeşitlerinin farklı kuraklık uygulama dönemlerinde farklı SOD izozimlerinin ortalama aktivite (U mg protein-1) değerleri

	Bezostaya-1		Karahan-99	
SOD izozim	**D1**	**D2**	**D1**	**D2**
Mn-SOD	0.013	0.057	0.043	0.080
Cu/Zn-SOD	0.045	0.274	0.081	0.055
Fe-SOD	0.041	0.388	0.110	0.186

The Bezostaya-1 cultivar exhibited an 8.08% increase in total SOD activity during the D1 treatment period compared to the control groups. However, there was no change observed during the D2 treatment period (Figure 1). Similarly, when comparing the total SOD activity values of the Karahan-99 cultivar with the control groups, significant increases of 13.6% and 78.3% were observed in both osmotic stress treatment periods (D1, D2) compared to the control groups. However, the increase in the D2 treatment period was particularly high (Figure 2). The t-test was conducted to determine the significance level of the difference between the SOD total activity values of both osmotic stress application periods (D1, D2) and the control group values. The results showed that the increase in the D1 osmotic stress application period and the stability in the D2 period were not statistically significant for the Bezostaya-1 variety (Table 4). For the Karahan-99 variety, there was a statistically significant difference ($P<0.01$) between the SOD total activity values of the osmotic stress application periods (D1, D2) and the control group values (Table 4).

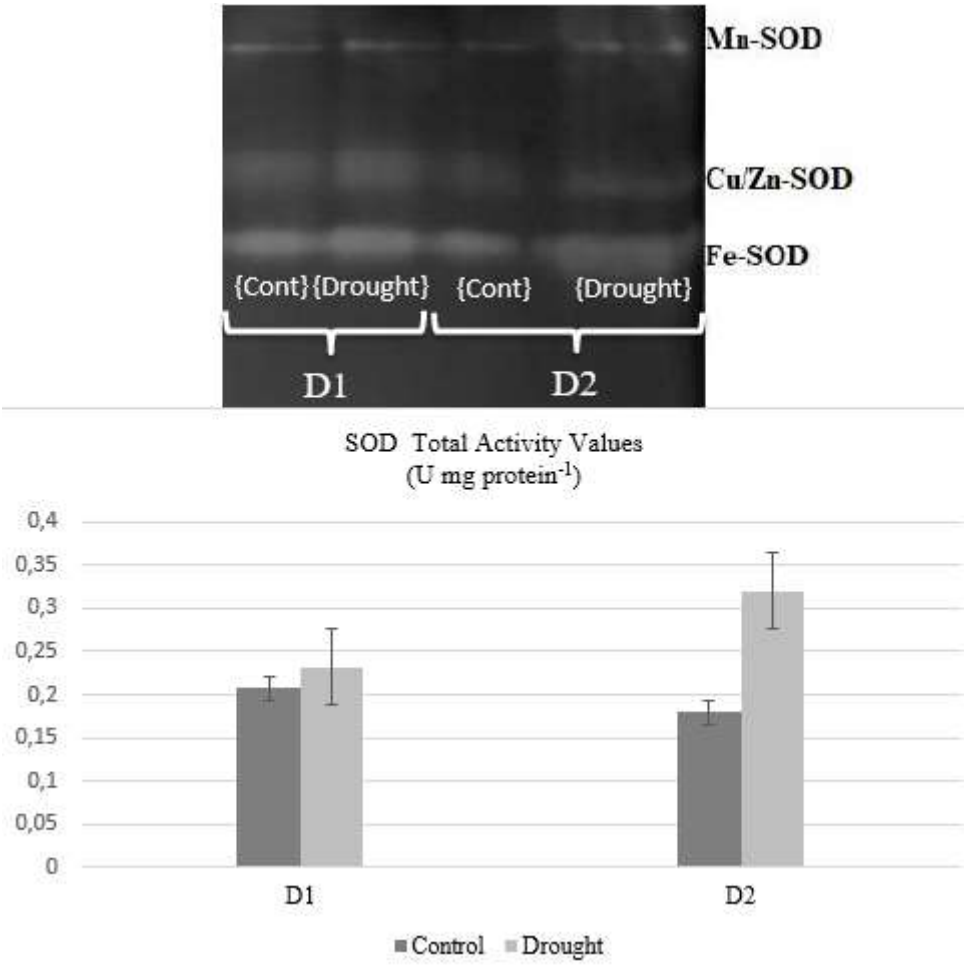

Figure 2. SOD isozymes and total activity (U mg protein-1) of Karahan-99 bread wheat variety during vegetative and generative drought treatment periods with control groups

Şekil 2. Karahan-99 ekmeklik buğday çeşidinin kontrol grupları ile vejetatif ve generatif dönem kuraklık uygulaması SOD izozimleri ve toplam aktivitesi (U mg protein^{-1})

Table 4. "t" test scores and significance levels of total SOD activity values (U mg protein^{-1}) between drought treatments and control groups

Çizelge 4. SOD toplam aktivitesi değerlerinin (U mg protein^{-1}) kuraklık uygulamaları ve kontrol grupları arasındaki "t" testi değerleri ve anlamlılık düzeyleri

	Bezostaya-1	Karahan-99
D1	1.25^{ns}	7.31**
D2	0.00^{ns}	21.80**

Superoxide dismutase (SOD; EC 1.15.1.1) catalyzes the dismutation reaction of superoxide ($O_2^{\cdot-}$) radical anions to O_2 and H_2O_2 and is the first step in the key components of the cell's defense system against oxidative stress (Ren et al., 2016). Superoxide dismutase comprises three main isozymes: Mn-SOD, Cu/Zn-SOD, and Fe-SOD (Elshafei, 2020). These isozymes can actively dismutase superoxide radicals in various plant organelles. The same species, different genotypes within the same species, plant growth period, stress severity and duration can affect the expression rates of total SOD activity and isoenzymes separately for each at high levels and cause significant differences.

Furthermore, the total superoxide dismutase (SOD) activity values during the D2 osmotic stress treatment period were higher than those during the D1 osmotic stress treatment period for both cultivars. This is in agreement with Simova-Stoilova et al. (2008), who reported that good antioxidant protection during generative development is likely to play an important role in osmotic stress resistance.

The assessment of individual isoenzymes, which contribute differently and in varying proportions to the total activity of the SOD enzyme in wheat leaves during osmotic stress, is a crucial aspect (Huseynova et al., 2014). Furthermore, SOD isoenzymes also play an important role in maintaining redox homeostasis under abiotic stress

(Dumanović et al., 2021). SOD is a metalloprotein that acts as a catalyst in chloroplasts, mitochondria, stisols, peroxisomes and apoplasts with Cu, Zn, Mn and Fe cofactors (Miller, 2012). It has three isoenzymes that can be developmentally regulated according to their functional and structural roles (Bowler et al., 1994). Among these isozymes, Fe-SODs are found in plastids (Quartacci & Navari-Izzo, 1992), Mn-SODs in mitochondria (Kuźniak & Skłodowska, 2004) and peroxisomes (Palma et al., 1998), Cu/Zn-SODs in chloroplasts, stromal surface of thylakoid membranes (Ogawa et al., 1995), cytoplasm and apoplast (Ogawa et al., 1996; Del Río et al., 2002; Mittler, 2002). In addition, all of these isoenzymes can be targeted to organelles via NH_2-terminal targeting sequences (Bowler et al., 1994). Although Fe-SOD and Mn-SOD are structurally similar due to their electrical properties, Cu/Zn-SOD differs significantly from the other two isozymes because of its distinct electrical properties (Bannister et al., 1991).

The isoenzymes with high activity values in Bezostaya-1 and Karahan-99 cultivars were Cu/Zn-SOD and Fe-SOD isoenzymes, while Mn-SOD isoenzyme activity remained at low levels for both cultivars in both osmotic stress periods. The response of isozyme activity levels to oxidative stress may be related to genotypic variations and different subcellular locations of the isozymes. It is therefore important to consider the site of action of the different oxidative stresses encountered. The induction of widespread and high Cu/Zn-SOD and then Fe-SOD activities in subcellular structures for Bezostaya-1, Karahan-99 genotypes in the zone of influence of oxidative stress sources occurring depending on wheat growth periods, especially in the chloroplast, stisol, and apoplasm, tends to increase to prevent damage due to ROS under osmotic stress.

In plants under various stress conditions, such as osmotic stress, free radicals tend to accumulate in association with the stress conditions (Smirnoff, 1993). Plant cells have the ability to detoxify ROS through various mechanisms that can be classified into three general categories (Jahnke et al., 1991): lipid-soluble and membrane-associated antioxidants (such as alpha-tocopherol), water-soluble reductants (such as glutathione and ascorbate), and enzymatic antioxidants like SOD. The ability of genotypes to utilise both enzymatic and non-enzymatic antioxidant systems is crucial in determining their tolerance levels (Molassiotis et al., 2006; Hasanuzzaman et al., 2021). SOD catalyzes the dismutation of the superoxide anion radical with high efficiency, resulting in the production of hydrogen peroxide and oxygen (Smirnoff, 1993). The activity of SOD enzyme varies depending on the severity and duration of osmotic stress, the genotype (Alscher et al., 2002; Seleiman et al., 2021), the rate of change of SOD activity (Menezes-Benavente et al., 2004), and the growth stage of the plant (Simova-Stoilova et al., 2009; Huseynova et al., 2016; In our study, the response of the SOD enzyme activity values to the control groups was quite different according to the varieties. This change was in the form of an increase of 13.6-78.3% in the D1 and D2 drought application periods for the Karahan-99 variety, and similarly, Kavuncu (2019) reported this rate as 70.0%. The Bezostaya-1 cultivar showed an increase in the D1 treatment, while no change was observed in the D2 treatment, which remained constant. This difference in response to stress conditions may be attributed to variations in the biochemical responses (Quitadamo et al. 2021) of the genotypes. In fact, it has been found that there are different trends of change in antioxidant enzyme activities depending on the osmotic stress loading pattern, duration, and severity of osmotic stress (Sairam & Srivastava, 2001). Many studies have reported that these trends are in the form of increased (Khaleghi et al., 2019), decreased (Huseynova et al., 2014) or unchanged (Bano et al., 2012) SOD activity under osmotic stress. Increases in SOD synthesis, as the primary $O_2^{\cdot-}$ radical dismutation response step in the photosynthetic architecture, are associated with enhanced protective adaptation against oxidative damage, including lipid peroxidation associated with oxidative stress (Khaleghi et al., 2019). A decline in SOD activity may compromise the O_2 scavenging system of cells and promote its accumulation. Given that the measured enzyme activity is a result of both synthesis and degradation, a decrease in net SOD activity may be attributed to reduced enzyme synthesis or increased enzyme degradation intensity (Khayatnezhad & Gholamin, 2021). Similarly, excessive stimulation of H_2O_2 accumulation during osmotic stress can cause significant decreases in SOD activity by inhibiting the enzyme structure.

Another reason for the unchanged or decreased SOD activity may be due to the maintenance of stomatal aperture, which keeps ROS formation and associated SOD activity at the same or low level. This helps to maintain the excitation energy of the genotype at an optimum level during osmotic stress.

CONCLUSION

Osmotic stress response: Both genotypes exhibited Mn-SOD, Cu/Zn-SOD, and Fe-SOD isoenzymes, with Cu/Zn-SOD and Fe-SOD showing the highest activity levels under osmotic stress. This highlights the key role of these isoenzymes in scavenging reactive oxygen species (ROS) and mitigating oxidative damage.

Differential response to osmotic stress Although total SOD activity increased in response to osmotic stress in both genotypes, the extent of this response varied between developmental stages. Karahan-99 showed a

significant elevation in total SOD activity during both the vegetative and generative periods, indicating a stronger antioxidative defense. In contrast, Bezostaya-1 exhibited a moderate increase during the vegetative stage, but remained relatively unchanged during the generative stage, suggesting a less consistent response. Future studies: Transcriptomic and proteomic approaches may reveal the upstream signaling pathways involved in SOD regulation. Furthermore, integrating CRISPR-Cas9 mediated editing of candidate SOD genes with field trials under simulated Mediterranean drought conditions can validate their functional relevance and accelerate the development of climate resilient wheat varieties. Understanding the molecular mechanisms underlying SOD gene function, combined with well-designed field studies, will critically support breeding programs aiming at osmotic stress tolerance. These findings highlight the potential of genotype-specific antioxidant strategies and gene-editing technologies in improving drought and osmotic stress tolerance in wheat.

Future studies: Transcriptomic and proteomic approaches may reveal the upstream signaling pathways involved in SOD regulation. Furthermore, integrating CRISPR-Cas9 mediated editing of candidate SOD genes with field trials under simulated Mediterranean drought conditions can validate their functional relevance and accelerate the development of climate resilient wheat varieties. Understanding the molecular mechanisms underlying SOD gene function, combined with well-designed field studies, will critically support breeding programs aiming at osmotic stress tolerance. These findings highlight the potential of genotype-specific antioxidant strategies and gene-editing technologies in improving drought and osmotic stress tolerance in wheat.

Summary of researcher contribution rate declaration

This study was carried out within the scope of the PhD project titled 'Determınatıon of Physıologıcal and Bıochemıcal Change Levels of Some Bread Wheat Genotypes Under Different Growth Period Drought Stress in Field and Laboratory Conditions' at Selçuk University, Institute of Science and Technology, Department of Biotechnology. This study was financially supported by Selcuk University Scientific Research Projects Coordination Office (Project No: 20211010).

Summary of researcher contribution rate declaration

As authors, we declare that we have each made an equal contribution to the article.

Conflict of interest

The authors of this manuscript declare that there are no conflicts of interest associated with this manuscript.

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