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ASSESSMENT OF ANTIOXIDANT DEFENCE SYSTEM AS A SELECTION CRITERION AGAINST TO OXIDATIVE STRESS DURING THE EARLY GROWTH PERIOD OF COMMON WHEAT

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ABSTRACT

Plants are negatively impacted by high levels of reactive oxygen species (ROS). The objective of the study was to find out the effect of exogenous hydrogen peroxide (H_2O_2) induced oxidative stress on the antioxidant defence system of common wheat seedlings and to screen the genotypes for tolerance or susceptibility to stress. In this study, three varieties (Flamura-85, Selimiye, and Esperia) and three advanced lines (TDE-45-1, TDE-84-5, and TDE-111-9) were used as experimental materials. The experiment was conducted in accordance with a randomized split-plot design with three replicates. In the experiment, wheat genotypes were allocated to the main plots, and different H_2O_2 applications (0-control, 50, 100 mM) were applied to the subplots. Wheat plants grown in pots were irrigated with different H_2O_2 solutions to induce oxidative stress at the two- to three-leaf stage. One week after application, the antioxidant enzyme levels, thiobarbituric acid reactive substance (TBARS) content, H_2O_2 content, plant growth and leaf water status of the seedlings were examined. The differences between the means of the wheat genotypes were statistically significant for all studied traits. The results indicated that the response of common wheat genotypes to oxidative stress differed. Flamura-85 and TDE-45-1 were identified as the genotypes with the best antioxidant defence system against H_2O_2. The resistance of wheat to oxidative stress may be improved by using these genotypes as novel genetic resources. In addition, biochemical analysis results indicated that superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), TBARS and H_2O_2 could be used as selection parameters in future wheat breeding studies to screen the resistance of wheat genotypes to oxidative stress during the early growth period.

Keywords: Oxygen radicals; *Triticum aestivum*; hydrogen peroxide; oxidative stress; shoot dry weight

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INTRODUCTION

As one of the earliest domesticated crops, wheat plays an important role in the diet of people worldwide (Pour-Aboughadareh *et al.*, 2022). Additionally, wheat has a substantial amount of several nutrients that are good for human nutrition and health (Ali *et al.*, 2023). Currently ranked first among the world's cultivated cereal species, with a harvested area of 219 million hectares, wheat is a strategically significant crop that produced 808 million tons in 2022, ranking second only to corn (1.1 billion tons) (FAO, 2024). By the end of 2050, there will be a 50% increase in the demand for wheat (Khan *et al.*, 2022). Wheat is also an essential food for Türkiye and about 20 million tons of wheat are produced on an area of 6.6 million hectares in Türkiye (FAO, 2024).

Wheat is globally subjected to stress factors that affect its yield (Ali *et al.*, 2023). In addition, climate change exacerbates the stress factors that diminish wheat grain yield and quality. Reactive oxygen species (ROS) are primarily involved for oxidative stress in plants (Chowdhury *et al*., 2021). During agricultural production activities carried out under stress conditions, severe losses occur, especially in terms of the yield of susceptible plants. ROS (such as hydrogen peroxide (H_2O_2)) are atmospheric oxygen molecules that are partially reduced or excited. In addition to functioning as signalling molecules in cells, they are also regarded as harmful results of aerobic metabolism (Mittler, 2017). At the cellular level, oxidative cell damage is caused by an increase in ROS and/or reactive nitrogen species (Baidya *et al*., 2023). H_2O_2 works as a signalling molecule and promotes plant growth and regulation processes and many physiological responses under stress conditions (Hossain *et al.*, 2015). But, excessive accumulation of H_2O_2 in cells causes oxidative stress, and as a result, the cell dies (Koç *et al.*, 2024). Under stressful circumstances, plants use their antioxidant defense

system to combat oxidative cell damage. This defense system contains enzymatic antioxidants and nonenzymatic antioxidants (Hasanuzzaman *et al*., 2020). The plant antioxidant defense system keeps cell membranes and organelles safe from harm because of increased ROS due to increased environmental stress (Noctor *et al*., 2014). The suppressive effect of the stress factors that come with global warming is severe today, and abiotic stress factors reduce crop production worldwide (Hamani *et al*., 2023). Producing the worldwide demand for wheat will only be possible by improving novel wheat genotypes that are resistant to oxidative stress caused by stress factors. This can be achieved by determining the physiological and biochemical selection parameters that can be used in selection in early generations in wheat breeding programs. Several physiological and morphological traits, including erectness, greening time, leaf chlorophyll content, and grain filling time, have been found to be significantly correlated with yield components in some studies. These traits can be used as selection parameters for resistance to stressful conditions (Okan *et al.*, 2023). Al-Ashkar *et al.* (2021) emphasized that CAT can be used as a selection parameter for tolerance of salt stress in wheat. Wheat's capacity to survive oxidative stress has been the subject of numerous physiological and biochemical investigations; nevertheless, little is known about the utilization of biochemical features related to the antioxidant defense system as selection criteria. The present study aimed to test three bread wheat varieties commonly grown in Türkiye (Flamura-85, Selimiye, and Esperia) as well as three promising advanced bread wheat lines (TDE-45-1, TDE-84-5, and TDE-111-9) to investigate the effects on physiological and biochemical parameters of oxidative stress resulting from the external application of H_2O_2 during the seedling growth period. Additionally, this study aimed to identify the selection parameters that may be applied in the early generation process in oxidative stress resistance research considering the knowledge gained from this work.

MATERIALS AND METHODS

Plant material: Wheat (*Triticum aestivum* L.) varieties (Flamura-85, Selimiye, and Esperia) widely grown in Türkiye and promising advanced common wheat lines (TDE-45-1 [Victoria × Bezostaja-I], TDE-84-5 [Selianka × Syrena] and TDE-111-9 [Sagittario × Sadova-I]) were used as plant materials.

Plant growth method, H_2O_2 application and plant harvesting: The plant growth study was made in the plant growth room of the Field Crops Department at Tekirdağ Namik Kemal University (TNKU). Surface-sterilized seeds were sown in plastic pots (13 cm × 13 cm) containing perlite, with 20 seeds per pot. A pot experiment was performed in pots with perlite to ascertain how the genotypes responded to oxidative stress during the seedling development stage. The experiment was adjusted in a split-plot design with three replicates. Wheat genotypes constituted the main plots, and different (0-control (distilled water), 50, 100 mM) concentrations of H_2O_2 solutions constituted the subplots.

The pots were subsequently transferred to the plant growth room under 250 µmol m^{-2} s^{-1} light, with a 16/8-hour photoperiod (light/dark), 25±2°C/15±2°C (day/night) temperature, and 60±5% humidity.

The pots were irrigated with 50% Hoagland solution until the seeds germinated and the plants reached the 2-leaf stage. H_2O_2 solutions of different concentrations (0-control (50% Hoagland solution), 50, 100 mM) were added to the Hoagland solution to induce oxidative stress in the seedlings that reached the 2-leaf stage. The plants were grown until they reached the Zadoks growth scale (ZGS): 13-15 (Zadoks *et al*., 1974). Leaf samples taken for biochemical tests were kept at -18°C, and analyses were carried out by taking samples. The morphological, physiological, and biochemical parameters were determined in 10 plant samples randomly taken from the H_2O_2-treated pots after seven days.

Changes in growth and biochemical parameters were determined in the laboratories of the Agricultural Biotechnology Department at TNKU.

Biomass: Analysis of plant biomass was performed on ten randomly chosen plants from each group. To monitor the change in plant biomass, root and shoot dry weight values (mg) were founded after the plant parts were kept at 70°C until constant weight with balance (Koch *et al.*, 2021).

Relative water content (RWC): The leaves of ten plants were removed and weighed, and their fresh weights were determined in milligram units. Then, these leaves were turned into turgor by keeping them in water containers between distilled water and completely wetted filter paper for three hours. Leaf turgor weight was determined. After that, they were dried at 70°C for 48 hours, after which their dry weights were determined. The method proposed by Smart and Bingham (1974) was used to compute the RWC of the leaves.

TBARS content: Leaves (0.2 g) were homogenized using a 0.1% solution of trichloroacetic acid (TCA). Then, the homogenates were centrifuged at 4°C. The reaction mixture, which included TCA and thiobarbituric acid (TBA), was added to the supernatants of the centrifuged samples, which were then maintained at 95°C for 30 min in a hot water bath. Samples from the water bath were immediately cooled by submerging them in an ice bath. Next, the samples were centrifuged once more, and absorbance measurements at 532 and 600 nm were

made spectrophotometrically. The extinction coefficient (Є=155 mM^{-1} cm^{-1}) was used to calculate the concentration of malondialdehyde (MDA). The findings are given in terms of nmol g fresh $weight^{-1}$ (Madhava Rao and Sresty, 2000).

H_2O_2 content: Two millilitres of 100 mM potassium phosphate buffer (pH 6.8) were used to homogenize leaves (0.2 g) that had been treated with liquid nitrogen. The extract underwent a 30 min 4°C centrifugation at 13.000 rpm. After mixing the supernatant with the peroxide reagent, it was incubated for 10 min at 30°C. The process was finally stopped after adding 1 M perchloric acid. The absorbance at 436 nm was measured with a spectrophotometer, and the H_2O_2 standard curve was utilized to determine the H_2O_2 content of the leaves (Bernt and Bergmeyer, 1974).

Total protein content: Leaves (0.2 g) treated with liquid nitrogen were homogenized with 2 ml of 50 mM sodium phosphate (Na-P) buffer (pH 7.8) containing 1 mM EDTA.Na_2. The extract was centrifuged at 13000 rpm for 30 min at 4°C. One hundred microliters of supernatant and 5 ml of reagent were mixed in a test tube. After vortexing the mixture, the resulting color was read at 595 nm in a spectrophotometer against the blank. Bovine serum albumin (BSA) was used during the preparation of the protein standard plot. The total protein amount of the samples was calculated on the BSA standard graph (Bradford 1976). The specific enzyme activity was calculated using the protein values.

SOD (EC 1.15.1.1) activity: After determining the total protein content of the samples, different concentrations of supernatant and the reaction mixture containing 50 mM Na-P buffer (pH 7.8), 0.1 M L-methionine, 1 mM nitro blue tetrazolium (NBT), 1 mM riboflavin and 0.1 mM EDTA.Na_2 were used, and the mixture was incubated at 300 µmol m^{-2} s^{-1} at 25°C for 10 min. The mixture's absorbance was measured at 560 nm (Beauchamp and Fridovich, 1971; Giannipolities and Ries, 1977).

APX (EC 1.11.1.11) activity: Ascorbate oxidation was started when the supernatant was combined with a reaction mixture that contained 50 mM Na-P buffer (pH 7.0), 0.5 mM ascorbate, and 0.1 mM EDTA.Na_2, and 1.2 mM H_2O_2. After three minutes at 290 nm, the reaction was observed in a spectrophotometer (Nakano and Asada, 1981). The activity was determined using the extinction coefficient (2.8 mM^{-1} cm^{-1}). Accordingly, 1 enzyme unit is the amount of 1 µmol ml^{-1} ascorbate oxidized per min.

GR (EC 1.6.4.2) activity: After the supernatant was collected, a reaction mixture including glutathione disulfide (GSSG) buffer, 25 mM Na-P buffer (pH 7.8), and β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate buffer was mixed. The reaction was monitored in a spectrophotometer for 3 min at 340 nm (Foyer and Halliwell, 1976). For GR, the extinction coefficient was 6.2 mM^{-1} cm^{-1}. The GSSG level was founded by using this coefficient during the calculation. One enzyme unit is the amount of GSSG (µmol ml^{-1}) per min.

CAT (EC 1.11.1.6) activity: The supernatant was mixed with a reaction mixture containing Na-P buffer (pH 7), 1 mM EDTA, 3%H_2O_2 and dI H_2O. The reaction was monitored in a spectrophotometer for 3 min at 240 nm for the consumption of H_2O_2 (µmol) per min (Bergmeyer, 1970).

POX (EC 1.11.1.7) activity: Liquid nitrogen-treated leaves (0.2 g) were homogenized using 50 mM sodium acetate (NaOAc) buffer (pH 6.5). The extract underwent a 15 min centrifugation at 13.000 rpm and 4°C. A reaction mixture including 90 mM H_2O_2, 0.1 M pyrogallol, and 50 mM NaOAc buffer (pH 6.5) was mixed with the supernatant. For two minutes, the reaction was observed at 300 nm in a spectrophotometer (Kanner and Kinsella, 1983).

A Mecasys Optizen Pop device was used for all spectrophotometric readings. The specific enzyme activities are reported as enzyme units (U mg $protein^{-1}$).

Statistical analysis: Using a randomized split-plot design with genotypes as the main plot and H_2O_2 application as the subplot, analysis of variance was made to examine the effects of genotype and H_2O_2 application on the studied traits. With the use of MSTAT-C software, the mean values of genotypes and H_2O_2 applications for the traits examined in the study were compared using the least significant difference (LSD) test at a probability level of 5% (Steel and Torrie, 1984). In addition, Pearson's analysis was applied to investigate the correlation between physiological and biochemical traits investigated in the study (Hawkins, 2009).

RESULTS

H_2O_2 level: To investigate the H_2O_2 levels in the leaves of wheat seedlings, seven days after H_2O_2 application, the H_2O_2 levels of the genotypes were analysed. The differences between the means of the genotypes for the H_2O_2 level were significant at $P \leq 0.01$. The highest level was detected in the Flamura-85 genotype, followed by Selimiye and TDE-84-5. The Esperia, TDE-45-1 and TDE-111-9 genotypes had the lowest H_2O_2 levels (Table 1). The genotype x H_2O_2 application interaction was found to be statistically significant at the 1% level. The highest content was detected in the 50 mM H_2O_2 treatment of the Flamura-85 genotype, and the lowest content was detected in the 100 mM H_2O_2 treatment of the Selimiye genotype. The H_2O_2 content was significantly greater in the Flamura-85 genotype (9.1%) treated with 50 mM H_2O_2 than in the control plants for

each genotype. This value was followed by that of the Selimiye (6.7%) and TDE-111-9 (3.7%) genotypes treated with 50 mM H_2O_2 (Fig. 1a).

RWC: There were statistically significant variations in RWC between the genotype means at 5% (Table 1). The highest value was detected for the TDE-45-1 genotype, followed by the TDE-111-9 and TDE-84-5 genotypes. The lowest RWC was detected in the Flamura-85 genotype. When comparing RWC to that of the control plants, there was no noticeable variation under H_2O_2 stress (Fig. 1b).

Shoot and root dry weight: There was a significant difference ($P \leq 0.05$) for the SDW among the genotypes (Table 1). The highest SDW was detected for the TDE-45-1 genotype, followed by Flamura-85 and TDE-111-9. The TDE-84-5 genotype had the lowest SDW. H_2O_2 application suppressed the SDW; however, there was no noticeable variation in the values between genotypes and groups (Fig. 1c). This result showed that genotypes were similarly affected by H_2O_2 application. Likewise, a significant variation ($P \leq 0.01$) in RDW was detected among the genotypes. The highest value was determined for the TDE-45-1 genotype, followed by Selimiye, in the same statistical group. Among the genotypes, the TDE-84-5 genotype had the lowest RDW (Table 1).

Table 1. Average values of genotypes for H_2O_2, RWC, SDW, RDW, TBARS, SOD, POX, APX, CAT, and GR in wheat seedlings.

Genotypes	H_2O_2 (μM)	RWC (%)	SDW (mg)	RDW (mg)	TBARS (nmol/g FW)	SOD (U mg^{-1})	POX (U mg^{-1})	APX (U mg^{-1})	CAT (U mg^{-1})	GR (U mg^{-1})
Flamura-85	0.104 ^{a}	94.74 ^{c}	440.11 ^{ab}	40.89 ^{bc}	3.84 ^{a}	411.81 ^{b}	3.51 ^{ab}	5.40 ^{b}	18.31 ^{a}	189.30 ^{c}
Selimiye	0.102 ^{a}	95.44 ^{bc}	402.89 ^{bc}	49.89 ^{a}	2.96 ^{c}	234.63 ^{c}	3.30 ^{ab}	5.56 ^{b}	15.62 ^{b}	148.23 ^{d}
Esperia	0.086 ^{b}	95.54 ^{bc}	425.56 ^{bc}	42.44 ^{b}	3.41 ^{b}	606.74 ^{a}	4.40 ^{a}	7.53 ^{a}	15.83 ^{b}	251.62 ^{b}
TDE-45-1	0.086 ^{b}	96.58 ^{a}	479.00 ^{a}	52.00 ^{a}	4.01 ^{a}	331.36 ^{bc}	3.40 ^{ab}	5.64 ^{b}	11.02 ^{d}	276.35 ^{b}
TDE-84-5	0.100 ^{a}	96.39 ^{ab}	381.67 ^{c}	35.00 ^{c}	3.07 ^{bc}	339.77 ^{bc}	2.50 ^{b}	2.11 ^{c}	13.22 ^{c}	160.96 ^{cd}
TDE-111-9	0.086 ^{b}	96.48 ^{ab}	437.56 ^{ab}	40.11 ^{bc}	2.96 ^{c}	343.70 ^{bc}	4.38 ^{a}	3.06 ^{c}	17.59 ^{ab}	330.12 ^{a}
LSD	0.010**	1.50*	48.01*	5.90**	0.35**	117.36**	1.23**	0.96**	2.11**	32.21**

H_2O_2 Hydrogen peroxide, RWC Relative water content, RDW Root dry weight, SDW Shoot dry weight, TBARS Thiobarbituric acid reactive substance, SOD Superoxide dismutase, POX Peroxidase, APX Ascorbate peroxidase, CAT Catalase, GR Glutathione reductase

Note: Lowercase letters (a–d) indicate the level of significance between the averages. Averages with the same letters within a column are not significantly different ($P \leq 0.05$). *Significant correlations at $P \leq 0.05$ and **Significant at $P \leq 0.01$

The effect of the genotype×H_2O_2 interaction on RDW was statistically significant at the 1% level. The highest RDW was detected in the control Esperia genotype, and the lowest RDW was detected in the 100 mM H_2O_2 treatment of the TDE-45-1 genotype. The results showed that H_2O_2 application to all the genotypes significantly inhibited root growth. In particular, 100 mM H_2O_2 had a greater suppressive effect on root growth. Compared with the control, 100 mM H_2O_2 had a greater effect on the Selimiye genotype. Compared with that of the control plants, the RDW of the Flamura-85 genotype decreased by 24.1% at this concentration (Fig. 1d).

TBARS content: The TBARS content variations between the genotypes were statistically significant ($P \leq 0.01$). The highest content was detected in the TDE-45-1 genotype, followed by Flamura-85, in the same statistical group. However, the Selimiye and TDE-111-9 genotypes, which had the same mean values, had the lowest TBARS contents (Table 1). The effect of the genotype×H_2O_2 interaction on TBARS was statistically significant at the 1% level. Compared with those of the control plants for each genotype, the TBARS contents of the Flamura-85 (~20%) and TDE-45-1 (~36%) genotypes decreased more in response to H_2O_2 application. These decreases can indicate that these genotypes are more tolerant to oxidative stress than others. TDE-111-9 had a significant increase (17.8%) in TBARS concentration as compared to the control plants (Fig. 2a).

SOD activity: The average genotypes differed significantly in their SOD activity. The highest SOD activity was detected in the Esperia genotype, followed by the Flamura-85 genotype. The Selimiye genotype had the lowest SOD activity. The findings indicated that the advanced lines did not differ significantly from one another (Table 1). The genotypexH_2O_2 application interaction for SOD activity was statistically significant at the 1% level. Compared with those of the control plants, the SOD activities of the Flamura-85 (249.9%), TDE-45-1 (119.1%) and TDE-84-5 (100.0%) genotypes increased in response to 100 mM H_2O_2. The Esperia genotype was more affected by H_2O_2 application than the other genotypes. The activity decreased by 62.8% in the

50 mM H_2O_2 treatment group and 70.1% in the 100 mM H_2O_2 treatment group compared to that in the control group (Fig. 2b).

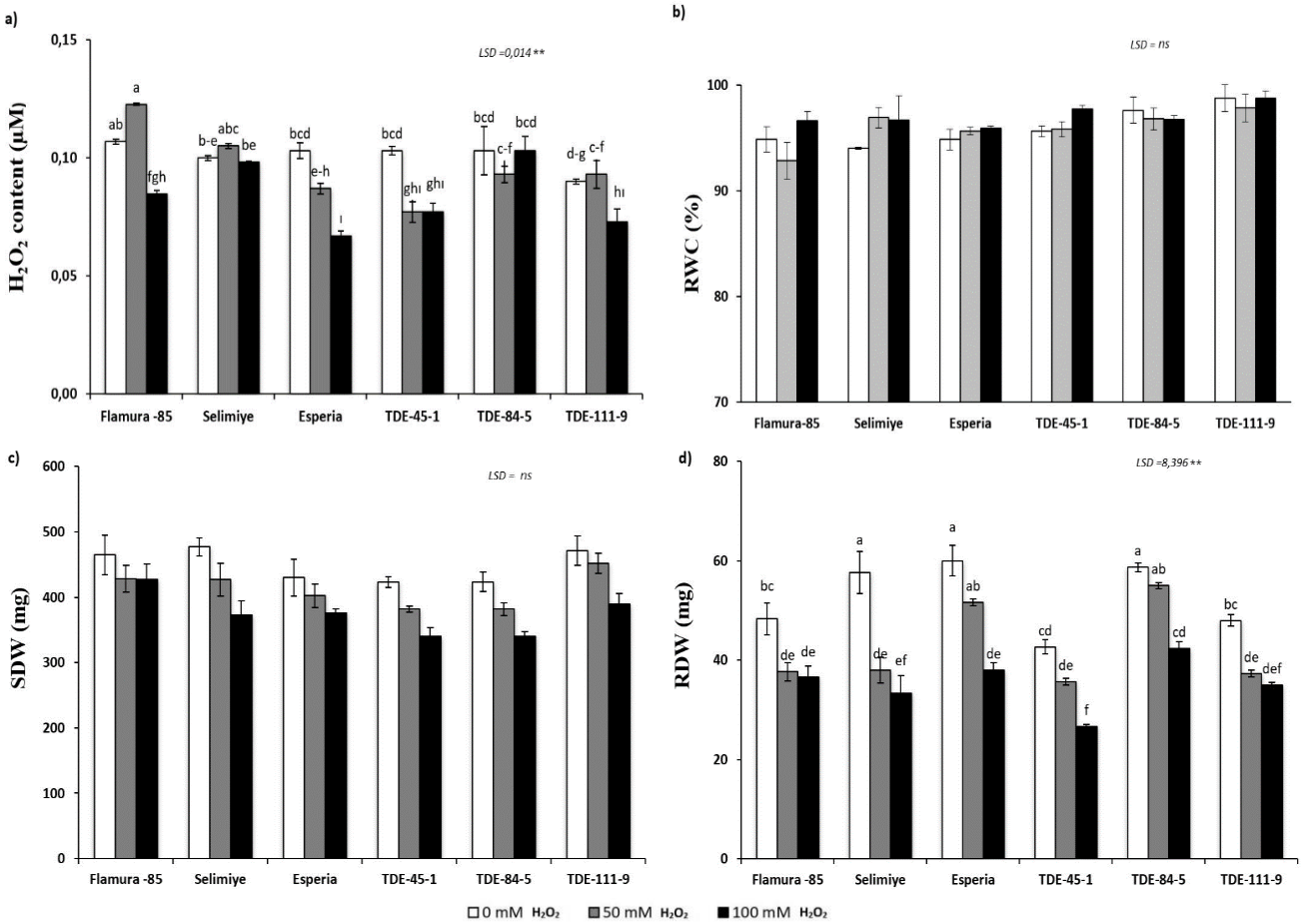

Fig. 1. Changes in H_2O_2 content (a), RWC (b), SDW (c) and RDW (d). Lowercase letters (a–ı) indicate the level of significance between the averages. Averages with the same letters within a bar are not significantly different ($P \leq 0.05$). **Significant correlations at $P \leq 0.01$; ns not significant

POX activity: The findings indicated that genotype had a significant ($P \leq 0.01$) impact on POX activity. In the same statistical group, the TDE-111-9 genotype had the second-highest POX activity, after the Esperia genotype. The lowest POX activity was obtained for the TDE-84-5 genotype (Table 1). The effect of the genotype×H_2O_2 interaction on POX activity was statistically significant at the 1% level. The two-way interactions showed that POX activity in the TDE-45-1 genotype (164.3%) increased in response to 100 mM H_2O_2 application compared to that in the control plants. In the other genotypes, the activities decreased in response to H_2O_2 application (Fig. 2c).

APX activity: Variations in APX activity between the genotype means were statistically significant ($P \leq 0.01$). The Esperia genotype had the highest APX activity, followed by the TDE-45-1, Selimiye, and Flamura-85 genotypes. The TDE-84-5 genotype had the lowest APX activity, followed by the TDE-111-9 genotype, in the same statistical group (Table 1). The APX activity under the genotype×H_2O_2 application interaction was statistically significant. Considering the reactions of genotypes to H_2O_2 application, the APX activity in all the genotypes decreased in response to H_2O_2 application compared to that in the control plants. TDE-45-1 was more strongly affected by 50 mM H_2O_2 application. The decrease was 91.4%. However, the activity of TDE-84-5 increased by 27.6% in the 100 mM H_2O_2 treatment compared to that in the control (Fig. 2d).

CAT activity: The effect of genotype on CAT activity was statistically significant ($P \leq 0.01$). The Flamura-85 genotype showed greater CAT activity than did the TDE-111-9 genotype according to mean comparisons. The lowest CAT activity was recorded for the TDE-45-1 genotype, followed by the TDE-84-5 genotype (Table 1). The genotype x H_2O_2 application interaction was found to be statistically significant at the 1% level. The control plants of the TDE-111-9 genotype showed the highest activity, while the Esperia genotype control plants

showed the lowest activity after applying a 100 mM H_2O_2 treatment. The Flamura-85 and TDE-45-1 genotypes' CAT activities increased in response to H_2O_2 application compared to those of the control plants. It was significantly greater in the Flamura-85 genotype treated with 50 mM H_2O_2 (68.6%) and 100 mM H_2O_2 (101.8%). The increases for TDE-45-1 were 16.9% and 34.6% in the 50 mM and 100 mM H_2O_2 treatments, respectively. The most inhibited activity was in the Esperia genotype (65.3%) treated with 100 mM H_2O_2 (Fig. 2e).

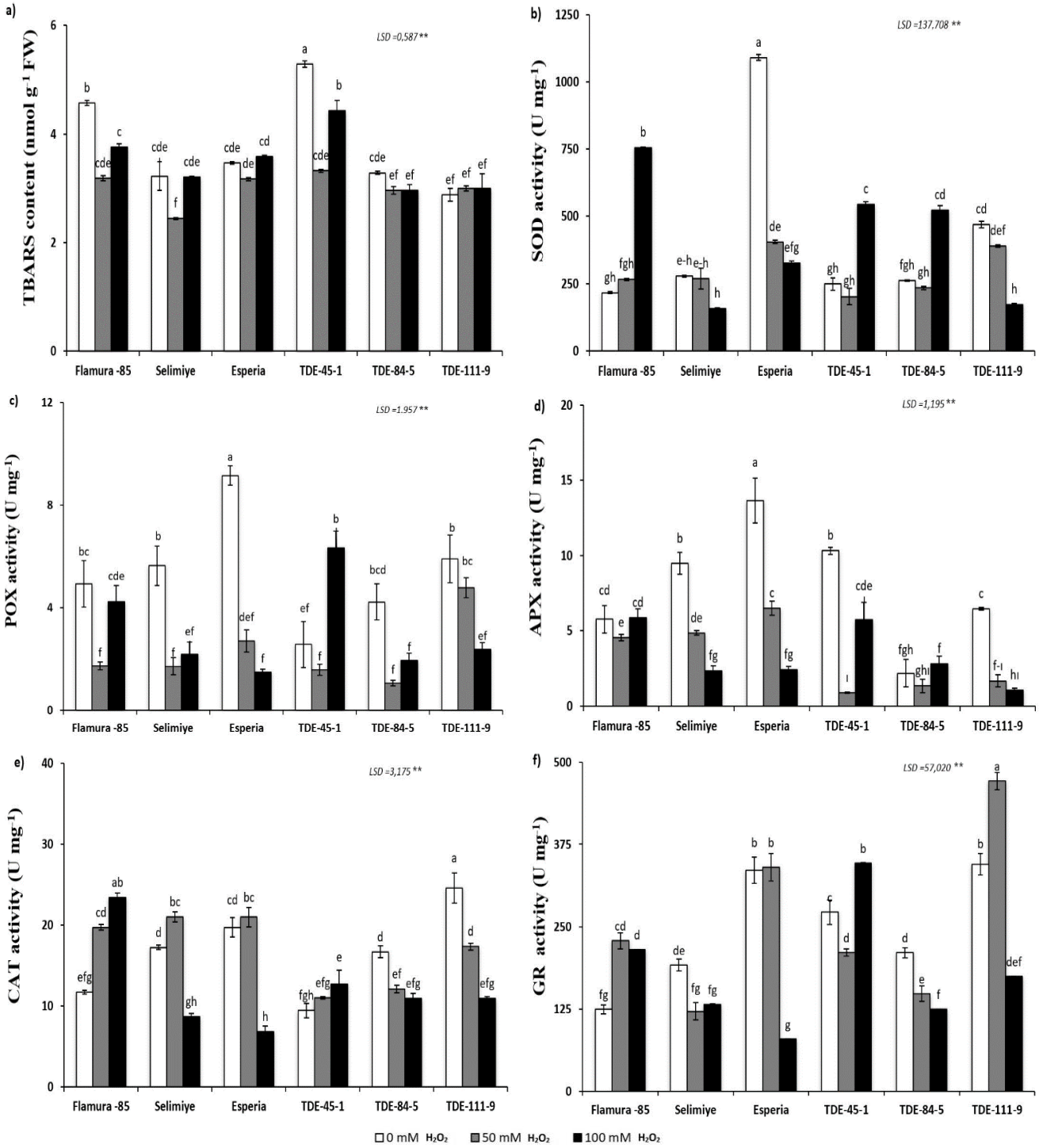

Fig. 2. Changes in the TBARS (a), SOD (b), POX (c), APX (d), CAT I and GR (f) activities. Lowercase letters (a–ı) indicate the level of significance between the averages. Averages with the same letters within a bar are not significantly different ($P \leq 0.05$). **Significant correlations at $P \leq 0.01$

GR activity: GR activity was significantly affected by genotype. The TDE-45-1 genotype was shown to have lower GR activity than the TDE-111-9 genotype. The lowest GR activity occurred in the Selimiye genotype, followed by the TDE-84-5 genotype (Table 1). The differences between the means of the genotype×H_2O_2 application interactions were statistically significant. The highest activity was detected in the 50 mM H_2O_2 treatment of the TDE-111-9 genotype, and the lowest activity was detected in the 100 mM H_2O_2 treatment of the Esperia genotype. Compared with those of the control plants, the GR activity of the Flamura-85 genotype increased (83.7% and 72.9%, respectively) in response to the 50 and 100 mM H_2O_2 treatments. In the Selimiye (36.75% and 30.9%) and TDE-84-5 (29.5% and 40.9%) genotypes, the activity gradually decreased with increasing H_2O_2 concentration (Fig. 2f).

Interrelationships between traits: The correlation analyses between the variables used in our study are given in Table 2. SOD activity contributed to the formation of H_2O_2 because of its reaction of superoxide anions with POX ($r=0.617^{**}$), GR ($r=0.406^{**}$) and CAT ($r=0.431^{**}$). A positive correlation was found between POX activity, which contributes to a decrease in H_2O_2 levels, and GR ($r=0.525^{**}$), CAT ($r=0.389^{**}$), and APX ($r=0.637^{**}$) activities and RDW ($r=0.400^{**}$) and SDW ($r=0.386^{**}$values, where biomass was monitored. In addition, a positive correlation was found between GR activity and CAT ($r=0.451^{**}$), APX ($r=0.314^{*}$) and SDW ($r=0.431^{**}$). A positive correlation was detected between APX activity and H_2O_2 ($r=0.300^{**}$), RDW ($r=0.611^{**}$), and SDW ($r=0.448^{**}$) values. The relationship between RWC and APX activity was negative ($r=-0.333^{*}$). TBARS was negatively related to CAT activity (r=-0337**) and positively related to APX activity ($r=0.381^{**}$), RDW ($r=0.314^{*}$) and SDW ($r= 0.397^{**}$).

Table 2. Correlation matrix for the examined traits in wheat seedlings.

Variables	SOD	POX	GR	CAT	APX	TBARS	H_2O_2	RDW	SDW	RWC
SOD	1.000	0.617^{**}	0.406^{**}	0.431^{**}	0.575^{**}	-0.010^{ns}	-0.032^{ns}	0.071^{ns}	0.178^{ns}	-0.142^{ns}
POX		1.000	0.525^{**}	0.389^{**}	0.637^{**}	0.069^{ns}	0.080^{ns}	0.400^{**}	0.386^{**}	-0.172^{ns}
GR			1.000	0.451^{**}	0.314^{*}	-0.018^{ns}	-0.033^{ns}	0.197^{ns}	0.474^{**}	0.104^{ns}
CAT				1.000	0.357^{**}	-0.337^{**}	0.239^{ns}	0.164^{ns}	0.213^{ns}	-0.136^{ns}
APX					1.000	0.381^{**}	0.300^{*}	0.611^{**}	0.448^{**}	-0.333^{*}
TBARS						1.000	0.100^{ns}	0.314^{*}	0.397^{**}	-0.162^{ns}
H_2O_2							1.000	0.262^{*}	0.070^{ns}	-0.457^{**}
RDW								1.000	0.589^{**}	-0.277^{*}
SDW									1.000	-0.217^{ns}
RWC										1.000

H_2O_2 Hydrogen peroxide, RWC Relative water content, RDW Root dry weight, SDW Shoot dry weight, TBARS Thiobarbituric acid reactive substance, SOD Superoxide dismutase, POX Peroxidase, APX Ascorbate peroxidase, CAT Catalase, GR Glutathione reductase

*Significant correlations at $P \leq 0.05$; **Significant at $P \leq 0.01$; ns not significant

DISCUSSION

Environmental stress conditions cause yield and quality losses in wheat production. Breeding studies on wheat contribute to the introduction of new varieties to the market every day. The greatest obstacle to classical breeding methods is that the process takes many years (Poggi *et al*., 2023). Plant tolerance is mostly determined by the metabolic reactions of plants to environmental stressors. Plants can withstand a variety of stressful situations because of their antioxidant defense system (Baidya *et al*., 2023). ROS cause lipid peroxidation, which results in membrane damage, protein degradation, enzyme deactivation, etc. (Fridovich, 1986; Liebler *et al*., 1986; Mittler, 2017). In our study, we applied H_2O_2, which is a low-cost product of natural metabolic activities that cause oxidative stress, to wheat plants. The defense responses of three common wheat varieties widely grown in Türkiye and three promising high-quality advanced bread wheat lines to oxidative stress applied in the early development stages were investigated. According to the ANOVA results, there were significant differences in H_2O_2 levels between genotypes for each trait that was investigated. This indicates that there is a genetic difference among wheat genotypes, and they are affected differently by H_2O_2 levels.

In our study, the Flamura-85 variety and TDE-45-1 advanced line stand out in terms of tolerance to oxidative stress. The Romanian variety Flamura-85 is known to have high yield stability under changing climatic conditions. In addition, the high tolerance to fungal diseases, cold and drought of Ukrain origin Victoria (♀) and high cold tolerance of Russia origin Bezostaja-I (♂), which are the rootstocks of the TDE-45-1 advanced line, may have increased the tolerance of this line to oxidative stress. In support of our results, Al-Ashkar *et al.* (2021) decelerated that the difference in the

responses of wheat genotypes to oxidative stress was due to their different genetic structures.

H_2O_2 can be utilized as an indicator when plants are stressed (Asaeda *et al*., 2018). An increase in the H_2O_2 content in wheat leaves was negatively correlated with the RWC, and a decrease in H_2O_2 content can indicate that wheat plants have water problems. The TBARS can also be used as a stress indicator parameter (Fridovich, 1986; Liebler *et al*., 1986). We found a nonsignificant interaction between H_2O_2 and the TBARS content in this study (Table 2). Some differences between the genotypes were found in our results. The responses of the Esperia genotype to H_2O_2 application in terms of the mean SOD, POX, APX and H_2O_2 values showed that this variety seems to be more tolerant than other genotypes. The TDE-111-9 promising genotype, which had the highest mean POX, GR, CAT, SDW and RWC values and the lowest mean TBARS and H_2O_2 content, had the closest response to the Esperia genotype, and it came to the forefront in terms of tolerance among the other genotypes used in the experiment (Table 1). This is because the decrease after H_2O_2 application was greater than that of the control plants. As the responses of the genotypes to H_2O_2 application were examined, changes in the tolerance levels of the genotypes were detected. In this evaluation, the Flamura-85 and TDE-45-1 genotypes had higher enzyme activities (SOD, CAT, and GR) after H_2O_2 application than did the control plants, and this increase in activity was also supported by decreased TBARS and H_2O_2 values (Fig. 1). Similarly, Sadeghi *et al*. (2022) showed that SOD activity increased and MDA decreased in tolerant wheat seedlings during drought stress. The authors determined that there were different responses between the two wheat varieties under drought stress. Koç *et al.* (2024) also reported that tolerant wheat genotypes had high antioxidant enzyme activities and low MDA contents during drought stress. Al-Ashkar *et al.* (2021) also highlighted that CAT can be utilized as a selection parameter for salinity tolerance of wheat in breeding programs. Khan *et al.* (2022) founded that SOD and POX activities increased under water stress, and a related study with *Elymus* spp. revealed close taxonomic relationships with wheat, rye, and barley. According to Demirbas and Balkan (2020), triticale seedlings' ability to withstand saline stress was facilitated by increases in GR, APX, and POX activities.

The ascorbate–glutathione pathway may prevent H_2O_2 from accumulating in cells (Noctor *et al*., 2014). As previously documented, in both resistant and sensitive wheat plants, APX and GR activities increase with water stress; however, these increases in sensitive plants do not prevent the increase in H_2O_2 production or TBARS content caused by dehydration (Gietler *et al*., 2016). There are similar results in wheat under water (Loggini *et al*., 1999), salt (Li *et al*., 2018), UV-B (Alexieva *et al*., 2001) and heat (Gupta *et al*., 2013) stresses in the literature. We found that the application of H_2O_2 decreased the RDW and SDW in all the genotypes in our study. These findings are comparable to those of studies conducted on wheat (Lu *et al.*, 2013) and rice (Lin and Kao, 2001).

Conclusion: In wheat breeding programmes, the responses of genotypes to stress conditions can be determined by applying high-dose H_2O_2. Thus, plant stress responses obtained in a short time can reduce the loss of time in breeding studies. In our study, the Flamura-85 and TDE-45-1 genotypes were more tolerant than the other genotypes. These genotypes may be used as novel genetic resources in wheat breeding programs in the future to boost oxidative stress tolerance. In addition, increased SOD, CAT, and GR activity and decreased TBARS and H_2O_2 values can be used as selection parameters for physiological breeding studies in wheat.

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