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BREEDING-BASED SCREENING OF WINTER WHEAT GENOTYPES FOR DROUGHT TOLERANCE AND ANTIOXIDANT ACTIVITY

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SUMMARY

The evaluation of six winter wheat (*Triticum aestivum* L.) cultivars and 15 F7 lines for drought tolerance cultivated under rainfed conditions comprised studies of their morphophysiological and biochemical traits. Determining the relative water content (RWC) of flag leaves, antioxidant activity, and the soluble proteins proceeded at the flowering, milk, and wax ripeness stages of ontogenesis. The F7 lines Yubiley 90, Erytrospermum 100, 21 FAWWON9/Gaudio, 29th SAWYT N395, 21 FAWWON 9/Gallio, and Gobustan/Gyrmyzy gul 1 showed the highest performance for studied indicators, resulting in a recommendation as promising genotypes for use in breeding programs. Thus, a comprehensive assessment of drought tolerance traits allows for increasing the effectiveness of breeding programs, developing cultivars tolerant to climate change, and managing biotechnological and genomic strategies to improve the genotypes' drought tolerance.

Keywords: Wheat (*T. aestivum* L.), drought tolerance, rainfed conditions, productivity, antioxidant defense system

Key findings: The integrative evaluation of morphophysiological and biochemical traits enhanced the efficiency of breeding strategies aimed at developing climate-resilient wheat (*T. aestivum* L.) cultivars.

INTRODUCTION

Drought is one of the most critical abiotic stresses limiting wheat productivity worldwide,

particularly in arid and semi-arid regions. In Azerbaijan, where a significant proportion of winter wheat (*Triticum aestivum* L.) reaches cultivation under rainfed conditions, irregular

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precipitation and increasing temperature variability frequently expose crops to water deficit during key developmental stages. This results in substantial yield instability and poses a serious challenge to sustainable grain production. Improving drought tolerance in wheat is therefore a major priority for national breeding and agricultural research programs. Drought tolerance is a complex trait involving the interaction of morphological, physiological, and biochemical mechanisms, including maintenance of plant water status, efficient use of available moisture, and activation of antioxidant defense systems. Identifying genotypes with enhanced adaptive capacity under water-limited conditions is essential for ensuring yield stability and food security in the context of ongoing climate change.

With the combined pressure of ecological and climate change, soil degradation of arable lands, and rapid population growth, higher productivity of food crops worldwide is necessary. Moreover, fostering a healthy lifestyle in the community requires focusing not only on crop yield but also on the quality traits of important crop species (Topa *et al.*, 2025). For ensuring the sustainable development of agriculture, one of the main priorities is to enhance the tolerance level of winter wheat (*Triticum aestivum* L.) genotypes to abiotic stress factors, particularly drought. Handling the said problem requires guiding breeding programs and studying genetic diversity for identifying and obtaining valuable initial breeding materials (Leng and Hall, 2019).

Morphological traits, such as high tillering capacity, short vegetation period and early maturity, robust root system, medium plant height, elongated upper internode of the stem, and leaf surface area, were the key indicators considered for drought tolerance (Lal *et al.*, 2024). In water deficit conditions, the considerable variations in these parameters were seemingly adaptive mechanisms aimed at reducing water loss and preserving plant viability. Especially, the preservation of chlorophyll content in the flag leaf has become one of the main indicators of productivity (Sallam *et al.*, 2019).

Numerous studies revealed that wheat genotypes with higher antioxidant activity and those that synthesize more antioxidant compounds in stress conditions exhibited greater tolerance to oxidative stress settings (Aliyeva *et al.*, 2023). The wheat-tolerant genotypes can manage the level of reactive oxygen species (ROS) due to the highest activity of antioxidant enzymes. The increased activity of enzymes, such as ascorbate peroxidase, catalase, and superoxide dismutase, has been evident in wheat genotypes in response to water deficit conditions (Huseynova *et al.*, 2014; Aliyeva *et al.*, 2020, 2023). These enzymes regulate the levels of ROS that accumulate in cells in stress conditions, thereby reducing oxidative damage. In general, the antioxidant defense system's response to different stress conditions depends on the initial activity level of antioxidant enzymes, which, in turn, becomes determined by the physiological state of crop plants.

At the global level, extensive research has been progressive to elucidate the morphological, physiological, and molecular mechanisms that determine the drought tolerance potential in wheat genotypes (Gurbanova 2023). Global initiatives, such as the International Wheat Improvement Program (IWIP) and organizations like the International Maize and Wheat Improvement Center (CIMMYT), have emphasized the need to develop drought-tolerant genotypes to ensure sustainable wheat production under changing climatic conditions (Bapela *et al.*, 2022).

Based on the above circumstances, the relevant study also contributes to these intensive efforts by examining morphophysiological and biochemical traits of locally adapted winter wheat cultivars and advanced lines. Therefore, the concerned study aimed to evaluate the relationship between certain morphophysiological and biochemical traits in winter wheat cultivars and advanced F7 lines under drought stress conditions and identify the drought stress-tolerant genotypes.

Table 1. Soil chemical properties of the experimental field (0–30 cm depth).

Parameter	Unit	Value
Soil type	–	Light chestnut (calcareous)
pH	–	8.6
Humus content	%	1.25–2.17
Nitrogen	mg kg ⁻¹	42.7
Phosphorus	mg kg ⁻¹	23.4
Potassium (as K ₂ O)	mg kg ⁻¹	170.5

Soil samples collected before sowing entailed analysis using standard procedures.

MATERIALS AND METHODS

Experimental site and breeding materials

The study took place under rainfed conditions at the Gobustan Experimental Station of the Research Institute of Crop Husbandry, Ministry of Agriculture, Azerbaijan. The site location has an altitude of 800 m above sea level. Twenty-one winter wheat (*T. aestivum* L.) genotypes comprising six cultivars and 15 F7 lines served as the study material. The wheat genotypes Gaudio and Gallio originated from Austria, Lutescens 76 and Erytrospermum 100 from Russia, while the remaining genotypes were of Azerbaijani origin.

Planting and experimental design

The experimental soil was slightly alkaline, with medium humus content, low available phosphorus, and normal potassium levels (Table 1). Before sowing, 50 kg ha⁻¹ of phosphorus (P) and 40 kg ha⁻¹ of potassium (K) fertilizers were the active ingredients applied, while the treatment of 70 kg ha⁻¹ nitrogen (N) fertilizer served as the top dressing in spring. The seeds of different wheat genotypes, planted at the end of October, had an average density of 400 seeds per m² at 1 m × 10 m subplots, comprising seven rows placed at 15 cm apart. During the vegetation period, phenological observations commenced, with plant height measured at full maturity and the grain productivity determined for each genotype.

Climatic conditions

According to environmental data obtained from the Gobustan Hydrometeorological Station, the average annual temperature during the growing season of 2023–2024 was 13.1 °C. The maximum temperature reached 33.8 °C in May, while the minimum dropped to –11.3 °C in February. Precipitation during autumn of 2023 totaled 192.0 mm, creating favorable conditions for plant emergence and early growth. Precipitation during winter and spring months was 245.4 mm, exceeding the long-term average. In total, a recorded 437.4 mm of precipitation was successful during the growing season of 2023–2024.

Plant height and productivity

Before harvesting, the height of 10 plants in each subplot succeeded in their measurements, using the average value to determine the plant height for each genotype. The harvested spikes from a 1-m² area entailed the use of a threshing machine, with the grains separated and weighed. The average value of three replications, as calculated, had the resulting value considered as the yield per 1 m² (g/m²).

Relative water content (RWC)

In wheat genotype leaves, the relative water content (RWC) measurement relied on the method proposed by Turner (1981).

Preparation of enzyme extract

A 0.5 g leaf sample sustained grinding in liquid nitrogen and homogenization in 100 mM sodium phosphate buffer (pH 7.8) containing 1 mM EDTA, 2 mM PMSF, 1% PVP, and 0.1% Triton X-100. Then the homogenate underwent centrifugation, with the resulting supernatant used for the analysis of antioxidant enzyme activities. The specific activities of the enzymes obtained calculations relative to protein content and per 1 g of fresh weight.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity assay

The activities of antioxidant enzymes underwent spectrophotometric determination by measuring variations in optical density using an Ultrospec 3300 pro spectrophotometer (Amersham, USA). The APX activity measurement was at the wavelength of 290 nm based on the decomposition of H_2O_2 by APX in the reaction mixture (Nakano and Asada, 1981).

Guaiacol peroxidase (GPX, EC 1.11.1.7) activity assay

GPX activity detection was dependent on the change in optical density of the reaction solution at 470 nm over a 3-minute period (Peñuelas and Munne-Bosch, 2005).

Catalase (CAT, EC 1.11.1.6) activity assay

Catalase activity determination spectrophotometrically was according to the rate of hydrogen peroxide decomposition at 240 nm over a 1-minute interval (Torres *et al.*, 2019).

Statistical analysis

The recorded data on various wheat parameters underwent a one-way analysis of variance (ANOVA). By getting significant mean differences, the application of Tukey's Honest Significant Difference (HSD) post hoc test aided pairwise comparisons. The means

significance consisted of $p < 0.05$ (* significant), $p < 0.01$ (** very significant), and $p < 0.001$ (***) highly significant), with the results at $p \geq 0.05$ considered nonsignificant (ns). All the analyses performed used GraphPad Prism (v. 10.6.1 for Mac; GraphPad Software). Determining the differences among the winter wheat genotypes, the study performed an analysis of variance, with the least significant differences ($LSD_{0.05}$) test helping to identify significant mean differences.

RESULTS

Drought is one of the crucial environmental factors that negatively affects plant growth and development at the early stages of vegetative growth, ultimately leading to considerable reduction in productivity. In the presented study, various morphophysiological and biochemical parameters entailed assessment in 21 bread wheat (*T. aestivum* L.) genotypes cultivated under rainfed conditions.

In the wheat growing season of 2023–2024, plant height measurement of genotypes occurred before harvest, with the results presented in Figure 1A. The wheat genotypes revealed significant ($p < 0.01$) differences for plant height. Genotypes expressed varied values for plant height, ranging from 83 to 115 cm, with an average value of 97.3 cm. Among the studied genotypes, the tallest plants resulted in wheat genotypes Gobustan/Gyrmyzy gul 1 (115 cm) and Gobustan/Gallio (110 cm). The shortest plants recorded were the cultivars Yubiley 90, Gyrmyzy gul 1, and Gaudio (96, 95, and 95 cm, respectively), and the advanced F7 lines Quality/Balaton, Lutescens 76, 21FAWWON61/Balaton, Erytrospermum 100, and Gobustan/AZE026-10-4 (95, 92, 85, 84, and 83 cm, respectively). However, the remaining wheat genotypes exhibited a moderate plant height.

About the yield performance of the studied wheat genotypes, the analysis of variance revealed a significant ($p < 0.01$) difference among the genotypes for this important parameter (Figure 1B). Cultivars

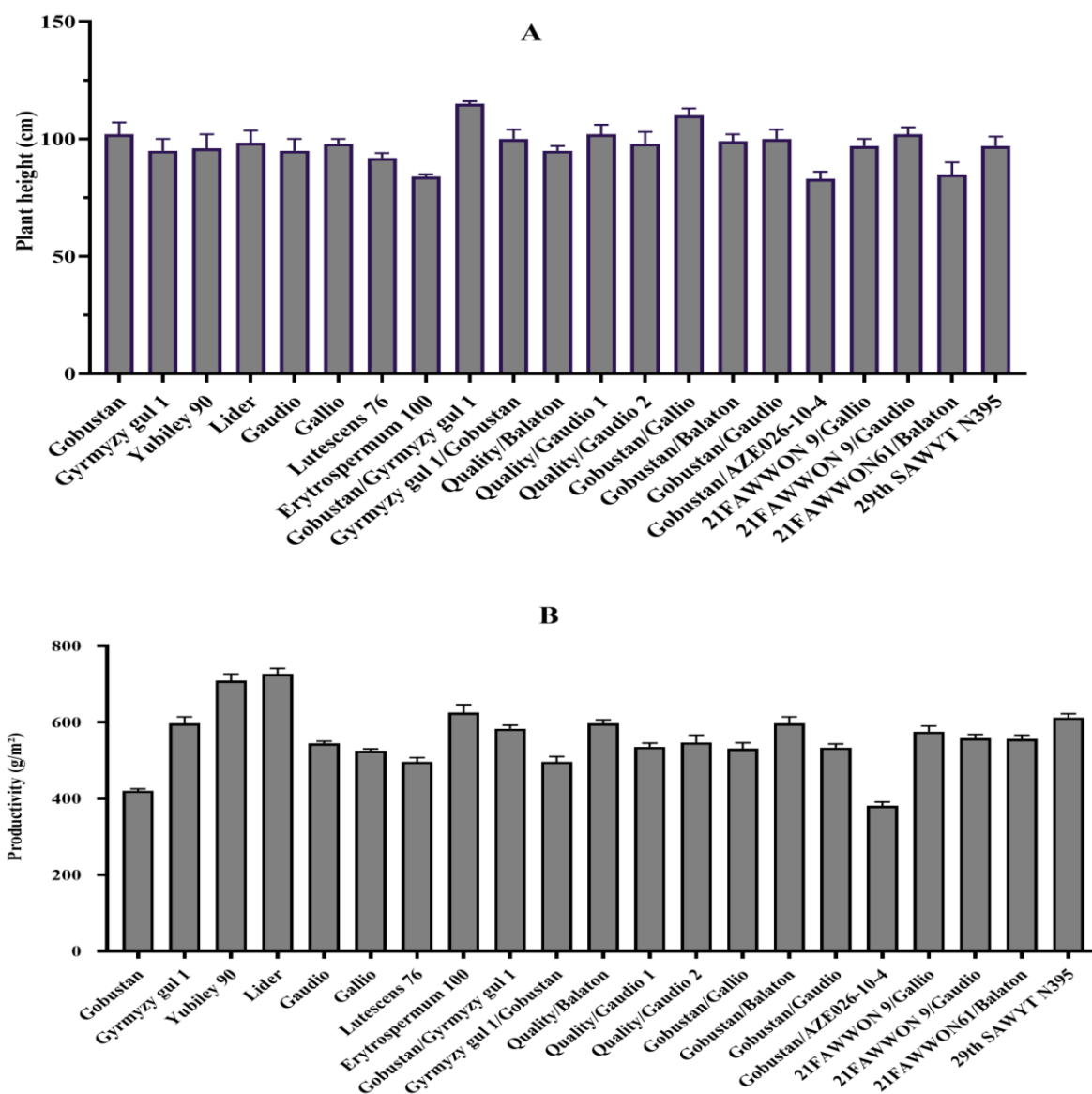


Figure 1. Plant height of the studied genotypes: CV (2.7%), $LSD_{0.05}$ (4.3**); A) Productivity of the studied genotypes: CV (4.5%), $LSD_{0.05}$ (40.6**), and B) The data presented are as means $\pm$ SD, as obtained from three biological replications.

Lider and Yubiley 90 (726 and 709 g/m², respectively) were notable with a higher yield than the other genotypes. Among the studied F7 lines, Erytrospermum 100 (625 g/m²), Gobustan/Balaton (592 g/m²), and 29th SAWYT N 395 (612 g/m²) displayed better performance for grain yield. However, the lowest grain yield appeared in the cultivars Gobustan and Gallio, while the advanced lines Gobustan/AZE 026-10-4, Gyrmyzy gul

1/Gobustan, Lutescens 76, Quality/Balaton, Gobustan/Gallio, Gobustan/Gaudio, and Quality/Gaudio 1 also exhibited the lowest yield. The remaining genotypes showed an intermediate yield performance. Thus, along with other parameters, the yield performance of the winter wheat genotypes should also be an indicator in the continuation of the breeding program.

Table 2. Heading time in bread wheat genotypes.

Genotypes	Heading date
Gobustan	April 30, 2024
Gyrmyzy gul 1	May 07, 2024
Yubiley 90	May 01, 2024
Lider	May 06, 2024
Gaudio	May 07, 2024
Gallio	May 06, 2024
Lutescens 76	April 30, 2024
Erytrospermum 100	May 03, 2024
Gobustan/ Gyrmyzy gul 1	April 30, 2024
Gyrmyzy gul 1 /Gobustan	April 29, 2024
Quality/Balaton	May 02, 2024
Quality/Gaudio 1	April 30, 2024
Quality/Gaudio 2	May 02, 2024
Gobustan/Gallio	May 02, 2024
Gobustan/Balaton	May 01, 2024
Gobustan/Gaudio	May 04, 2024
Gobustan/AZE026-10-4	May 01, 2024
21FAWWON 9/Gaudio	May 06, 2024
21FAWWON 9/Gallio	May 03, 2024
21FAWWON61/Balaton	April 30, 2024
29 th SAWYT N395	May 07, 2024
CV (%)	8.3
LSD _{0.05}	0.4**

Note: The presented data are as means $\pm$ SD, as obtained from three biological replications.

For the studied wheat genotypes in the growing season of 2023–2024, Table 2 shows the heading dates. Overall, for heading days, the genotypes ranged from April 29 to May 07, 2024. Considering that the heading date is an influential phenological trait of wheat genotypes and plays a considerable role in productivity under rainfed conditions, its recording commenced as the number of days from May 01, simultaneously carrying out the analysis. The results revealed significant differences among the wheat genotypes for heading days. Among the studied cultivars, Gobustan and Yubiley 90 exhibited early heading on April 30 and May 01, respectively, while the genotypes Gaudio, Gyrmyzy gul 1, Gallio, and Lider received a classification as late-heading cultivars. Among the advanced F7 lines, Gyrmyzy gul 1/Gobustan, 21FAWWON61/Balaton, Gobustan/Gyrmyzy gul 1, and Quality/Gaudio 1 demonstrated an early heading. In contrast, the lines 29th SAWYT N395 and 21FAWWON9/Gaudio showed the late heading. One should also note that the late-heading cultivars as mentioned above

attained development for the irrigated and moisture-sufficient rainfed regions.

From the perspective of heading time, the early spike emergence of the cultivars Gobustan and Yubiley 90 proved them to be more suitable for cultivation in arid rainfed regions. Such a region is where drought stress typically occurs in the later stages of the vegetation period. However, the early-heading traits of the wheat lines, such as Gyrmyzy gul 1/Gobustan, 21FAWWON61/Balaton, Gobustan/Gyrmyzy gul 1, and Quality/Gaudio 1, suggested their potential use in developing wheat genotypes for moisture-deficient rainfed environments.

The relative water content (RWC) measurements of the flag leaf during the grain formation (May 21, 2024), milk ripeness (May 30, 2024), and wax ripeness (June 11, 2024) stages for the studied genotypes in the vegetation period appear in Figure 2A. The results disclosed significant differences among the wheat genotypes for RWC. In genotypes, the RWC of the flag leaf varied between 49.1% and 62.3% during the grain formation stage,

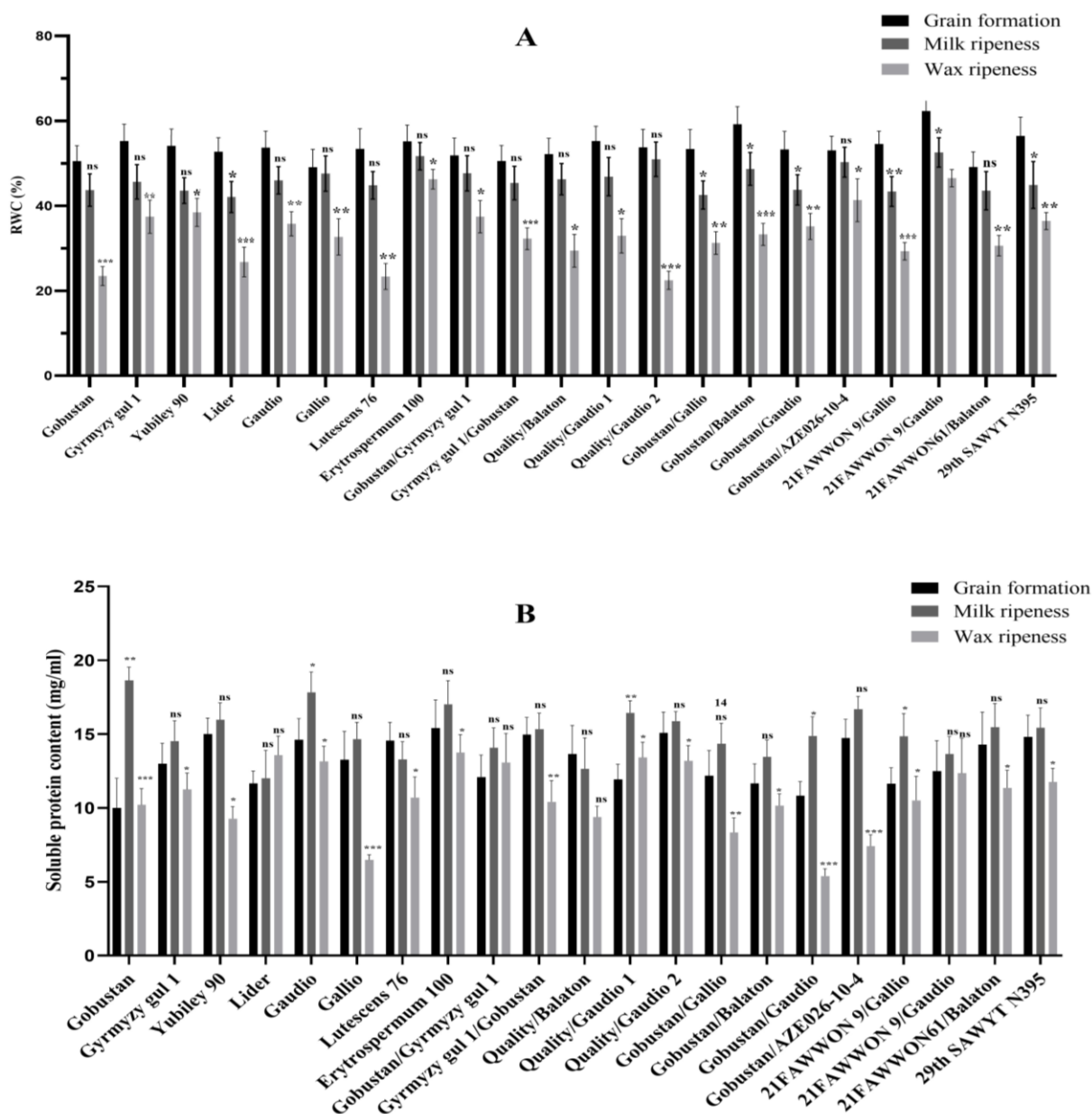


Figure 2. Relative water content (A) and soluble protein content (B) in bread wheat genotypes. Statistical significance was set at $p < 0.05$ (*: Significant), $p < 0.01$ (**: very significant), and $p < 0.001$ (***: highly significant); results with $p \geq 0.05$ were considered nonsignificant (ns).

42.1% and 52.6% (CV 10.5%) during the milk ripeness stage, and between 22.5% and 46.3% (CV 23.8%) during the wax ripeness stage.

During the grain formation and milk ripeness stages, the variation in RWC was within a narrower range due to the presence of soil moisture to some extent and the relatively

young physiological state of wheat genotypes. However, the genotypes showed fewer differences for this parameter during these stages. In the wax ripeness stage, the reduction in soil moisture led to a more pronounced expression of differences among the genotypes. Thus, at this stage, in the wheat lines 21FAWWON 9/Gaudio and

Erytrospermum 100, the RWC values were considerably higher than those of other genotypes (46.5% and 46.3%, respectively).

In the flowering stage, the highest RWC values resulted in the genotypes 21FAWWON 9/Gaudio (62.3%) and Gobustan/Balaton (59.2%), while in the milk ripeness stage, the highest values surfaced in the wheat genotypes 21FAWWON9/Gaudio (52.6%) and Erytrospermum 100 (51.7%). Toward the end of ontogenesis, in the wax ripeness stage, the highest RWC values were notable in the wheat genotypes Erytrospermum 100 (46.27%), 21FAWWON 9/Gaudio (46.52%), Gobustan/AZE026-10-4 (41.32%), Gobustan/Gyrmyzy gul 1 (37.44%), and Gobustan/Gaudio (35.17%). Thus, several wheat cultivars and lines revealed significant differences for RWC, making them advisable for use as genotypes with higher RWC in future breeding programs.

The results showed the reduced water content in wheat plants significantly affect protein content in the cells. In the samples taken during the initial stage, the soluble protein ranged between 10.00 and 15.41 mg/ml (Figure 2B). At the milk ripeness stage, an increase in protein content was evident in all samples as compared with the previous stage. As the drought stress conditions intensified, a considerable decrease in soluble protein content occurred in all samples during the wax ripeness stage. At this stage, the wheat genotypes Lider, Gaudio, Erytrospermum 100, Gobustan/Gyrmyzy gul1, and 21FAWWON 9/Gaudio demonstrated relatively higher protein values than the other genotypes.

Given that the drought-induced water deficiency disrupts the osmotic balance, this increases the accumulation of ROS in cells and triggers the activation of the antioxidant defense system in plants. During the grain-filling stage, the activity of APX varied between 0.496 and 1.472 μmol ascorbate/mg protein (Figure 3A). At this stage, APX activity was relatively low in the wheat genotypes Gyrmyzy gul 1 and Quality/Balaton. Toward the end of ontogenesis, a considerable decrease was apparent in the enzyme activity compared to the grain-filling stage in the wheat advanced

lines Gyrmyzy gul 1/Gobustan and Gobustan/Gyrmyzy gul 1. However, nonsignificant ($p \geq 0.05$) differences were noticeable in the genotypes Gyrmyzy gul 1 and 21FAWWON61/Balaton. In the other analyzed samples, a significant increase in enzyme activity emerged. The highest APX activity during the wax ripeness stage resulted in wheat genotypes Gobustan/Gaudio, Yubiley 90, Gallio, Lutescens 76, and 21FAWWON 9/Gallio, indicating that these genotypes possess a robust antioxidant defense system. However, at this stage, the recorded lowest enzyme activity was in the cultivar Gyrmyzy gul 1.

Among the wheat genotype samples, the significant ($p \leq 0.05$) differences recorded for GPX activity were particularly during the grain formation and wax ripeness stages (Figure 3B). During the advanced phase of drought stress, in the wax ripeness stage, the GPX activity enhanced and reached its peak. At this stage and as compared with the milk ripeness stage, the GPX activity was considerably higher (1.95–2.29 times) in the wheat genotypes Gobustan/Gyrmyzy gul 1, Gobustan/Gaudio, Yubiley 90, Gaudio, Gallio, and Gobustan/AZE026-10-4. However, in the genotypes Lider, Quality/Gaudio 1, Quality/Gaudio 2, Gobustan/Gallio, and Gobustan/Balaton specimens, the GPX activity at the wax ripeness stage was lower than at the onset of the drought stress conditions.

One of the enzymes involved in the utilization of hydrogen peroxide accumulated in cells in stress conditions is catalase. At the onset of drought stress, during the grain formation stage, catalase activity was higher in cultivars Gobustan, Gyrmyzy gul1, Lider, and Lutescens 76 and in lines Gobustan/Gyrmyzy gul 1, Quality/Gaudio1, and Gobustan/Gallio (Figure 3C). During the milk ripeness stage, a significant decrease in enzyme activity appeared in all wheat samples. According to the results, the activity of the catalase enzyme decreased during the milk ripeness stage compared with the grain formation stage in all the analyzed wheat specimens.

At the flowering stage, the CAT activity showed a significant decrease in the specimens of genotypes Gallio, Erytrospermum 100, Gyrmyzy gul 1/Gobustan, Quality/Gaudio2,

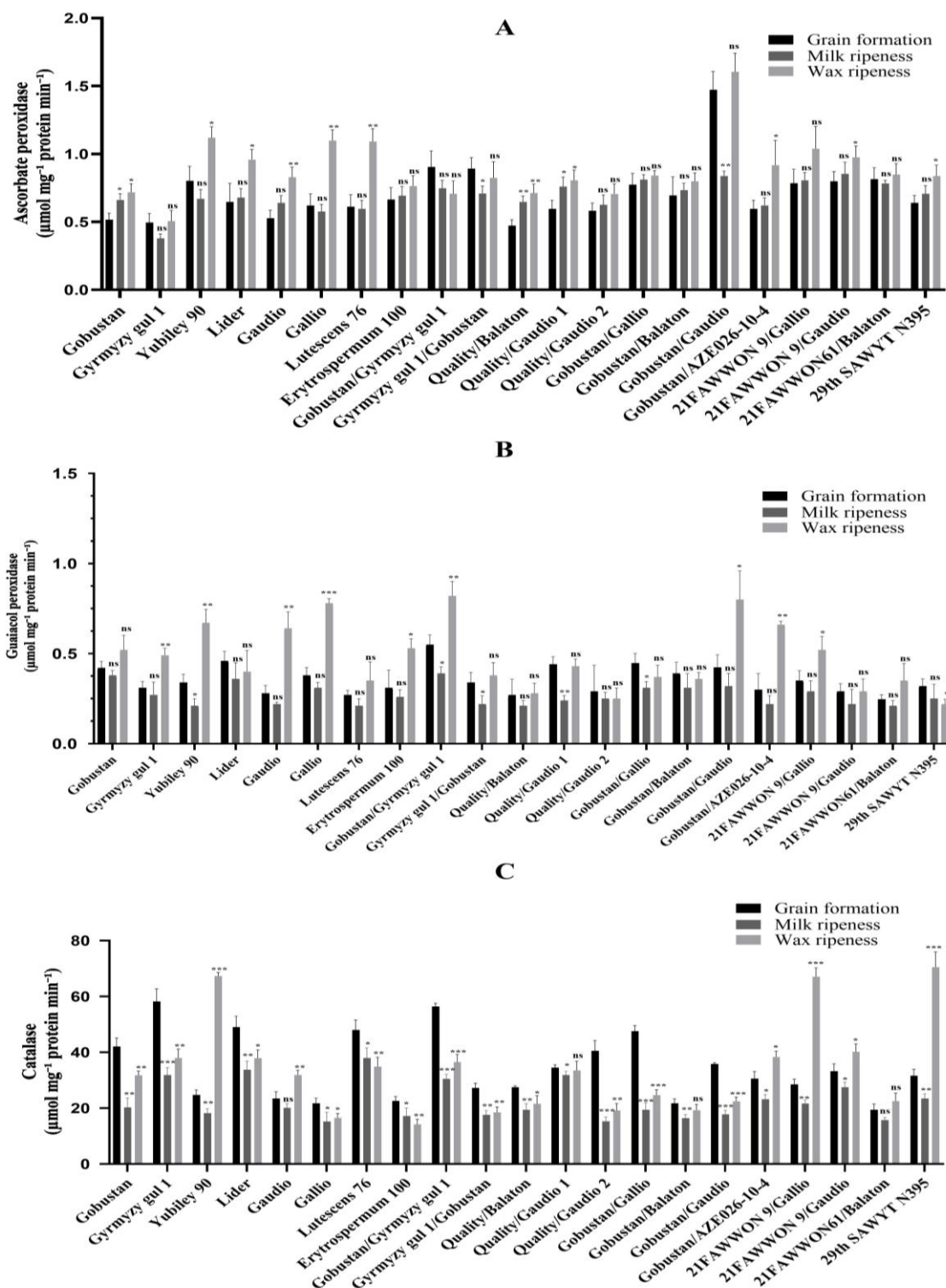


Figure 3. Activity of ascorbate peroxidase (A), guaiacol peroxidase (B), and catalase (C) in bread wheat genotypes. Statistical significance was set at $p < 0.05$ (*: Significant), $p < 0.01$ (**: very significant), and $p < 0.001$ (***: highly significant); results with $p \geq 0.05$ were considered nonsignificant (ns).

Gobustan/Balaton, Gobustan/Gaudio, and 21FAWWON61/Balaton. However, the most pronounced decrease was remarkable in the genotype Quality/Gaudio 2, where a 2.6-fold reduction occurred compared with the first-day observation. It was noteworthy that under drought stress conditions, the wheat cultivars exhibited distinct antioxidant enzyme activity profiles. These differences suggested wheat cultivars use different strategies to cope with the effects of water stress conditions.

DISCUSSION

Drought stress conditions negatively affect wheat productivity by causing variations at the morphological, physiological, and biochemical levels (Pour-Aboughadareh *et al.*, 2017). Under rainfed conditions with limited moisture availability, plant height plays a viable role in drought tolerance and productivity in wheat. However, the increased plant height can negatively alter grain yield by reducing the harvest index; it may also contribute to some extent to grain formation during extremely dry growing seasons. Drought stress occurrence during the wheat stem elongation stage causes the most significant decrease in plant height, depending on its severity (Sarto *et al.*, 2017). Considering all these factors, tall plant height has historically been serving as a selection criterion in developing wheat cultivars for arid regions. Similar to our findings, several studies have reported that plant height is a complex trait influencing yield stability under drought, where moderate plant height often indicates an association with improved performance due to better assimilate partitioning (Yu *et al.*, 2025).

In rainfed arid regions, drought stress does not occur consistently every year, and in some seasons, favorable conditions also appear. Therefore, to improve wheat productivity in rainfed areas, it is necessary to develop cultivars with drought stress tolerance and higher yield potential in favorable years. However, since taller cultivars reduce lodging resistance under favorable conditions and lower the harvest index, thereby limiting grain yield, it is deemed a negative selection criterion. Moreover, studies conducted in arid

and semi-arid regions have indicated that taller genotypes may maintain biomass accumulation under severe drought, partially supporting flowering (Zhang *et al.*, 2022). Past studies reported the wheat genotypes with tall stature and large leaf surface area tend to have a lower drought tolerance (Su *et al.*, 2019). By examining the productivity of the studied wheat genotypes in relation to plant height, this conclusion receives further support. In this study, the genotypes Yubiley 90, Lider, Erytrospermum 100, 29th SAWYT N395, and Quality/Balaton, which belong to the short and medium-height group, produced a higher grain yield than the other wheat genotypes. This observation is consistent with previous reports indicating that semi-dwarf genotypes generally outperform tall genotypes under optimal conditions due to improved harvest index and lodging resistance (Fellahi *et al.*, 2023).

In partial drought escape mechanism, heading time plays a crucial role in yield formation under rainfed conditions. The heading time is not only a specific genotypic trait but also depends on the hydrometeorological factors of the vegetation period. According to past studies, in rainfed regions where drought tends to occur during the later stages of vegetation, early flowering and heading can serve as a mechanism to avoid drought stress conditions (Shavrukov *et al.*, 2017). Drought stress during the flowering stage reduced the pollen viability, which can lead to spike sterility. In wheat, drought stress occurring from the heading to grain-formation period negatively affects the number and weight of grains per spike, as well as the 1000-grain weight, resulting in a sharp decline in grain yield (Ihsan *et al.*, 2016). In agreement with our observations, Shavrukov *et al.* (2017) reported that early heading and flowering can help plants escape terminal drought in dry environments (Shavrukov *et al.*, 2017). Similarly, Reynolds *et al.* (2009) emphasized that phenological adaptation is one of the key mechanisms for improving wheat yield under drought conditions (Reynolds *et al.*, 2009).

However, it is noteworthy that early-heading genotypes do not necessarily guarantee higher yields. Genotypes with advanced developmental traits and prolonged

senescence gene (SG) period of leaves tend to be more advantageous in achieving higher grain yields. Studies by Kamal *et al.* (2019) have shown that genotypes with extended grain-filling duration and prolonged stay-green (SG) traits tend to achieve higher yields, even under water-limited conditions. The relative water content (RWC) reflects the proportion of water in a leaf relative to its full water-holding capacity. It serves as an indicator of the balance between water absorption from the soil and water loss through transpiration under identical conditions. Several studies have also reported genotypic differences for RWC in wheat genotypes (Tanaka and Ito, 2025). Our findings are consistent with those of Ashrafi *et al.* (2018), who highlighted RWC as a reliable physiological indicator of drought tolerance, with higher RWC values associating with better cellular hydration and sustained metabolic activity in stress.

Differences in the activity of enzymatic components of the antioxidant defense system within stress conditions could become key indicators for assessing drought tolerance levels in wheat cultivars and for the identification and selection of promising genotypes in breeding programs. The process of plant adaptation to unfavorable environmental conditions is highly complex and requires flexible, rather than static, responses. This also attains support from Mittler (2002), who described oxidative stress regulation as a dynamic and tightly controlled process.

One of the main enzymes involved in the detoxification of hydrogen peroxide accumulated in plant cells during stress is catalase. Under prolonged soil drought conditions, a significant increase in catalase activity has been evident in wheat and rice plants (Huseynova *et al.*, 2016). Similar increases in CAT activity under drought conditions entailed reporting from Aliyeva *et al.* (2023), suggesting its crucial role in ROS scavenging. However, based on the severity and duration of drought stress, a report of decrease in enzyme activity has also emerged. In experiments conducted with *Mentha pulegium* L. and *Cicer arietinum* L., a reduction in CAT activity was notable under drought stress conditions (Uluslu *et al.*, 2022). This

decrease is consistent with reports indicating that severe stress can inhibit enzyme synthesis or enhance proteolytic degradation (Apel and Hirt, 2004). This decrease may have an association with the inhibition of enzyme synthesis under drought stress conditions and the degradation of the enzymes by peroxisomal proteases.

Peroxidases play a prominent role in plant growth and development, influencing processes such as lignification, suberization, and the formation of structural components of the cell wall (Li *et al.*, 2024). Additionally, the POD enzyme breaks down H_2O_2 into H_2O , preventing its accumulation in cells, thereby protecting plants from oxidative damage and reducing the toxic effects of water stress conditions. Studies conducted on *Carthamus tinctorius* L., *Brassica napus* L., tomatoes, and wheat plants have shown that peroxidases activity considerably increased under drought stress conditions (Huseynova *et al.*, 2016; Niyazova and Huseynova, 2024). Similarly, Ashraf (2009) and Gill and Tuteja (2010) reported enhanced peroxidase activity as part of the adaptive response to oxidative stress induced by drought.

Thus, the presented research revealed that the winter wheat genotypes Yubiley 90, Erytrospermum 100, 21FAWWON 9/Gaudio, Gobustan/Gaudio, Gobustan/Gyrmyzy gul 1, 29th SAWYT N395, and 21FAWWON 9/Gallio demonstrated notable drought tolerance, considering them suitable for cultivation in moisture-deficient arid regions. Among these genotypes, the genotype Gobustan/Gaudio showed the robust antioxidant defense system and could serve as a valuable source material for antioxidant analysis and biochemical screening. These findings are consistent with global breeding efforts emphasizing the importance of combining physiological resilience and yield stability under drought (Ashrafi *et al.*, 2018; Reynolds *et al.*, 2009).

CONCLUSIONS

The results enhanced the understanding of drought tolerance mechanisms under rainfed conditions and clarified the role of antioxidant

enzymes in stress protection. Winter wheat (*T. aestivum* L.) cultivars and advanced lines were visible with significant differences in drought tolerance. High relative water content and stable antioxidant enzyme activities could serve as key traits associated with improved adaptation and productivity under drought stress conditions. The results support the identification of promising genotypes for breeding and provide a sound scientific basis for developing climate-resilient wheat cultivars.

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