STAGE-DEPENDENT MORPHOPHYSIOLOGICAL AND BIOCHEMICAL RESPONSES REVEAL DROUGHT-ADAPTED WINTER WHEAT GENOTYPES

Ulduza Gurbanova[1*]

[1]Institute of Molecular Biology, Ministry of Science and Education of the Republic of Azerbaijan, AZ1073, Baku, Azerbaijan

Abstract: The drought responses of six modern winter wheat varieties and 15 stabilized F7 lines grown under rainfed conditions were evaluated using morphophysiological and biochemical traits. Relative water content, soluble protein content, enzyme activities, plant height, heading time, and yield were assessed at different developmental stages. Gobustan, Yubiley 90, and several F7 lines were identified as early heading genotypes, while a group of varieties and lines showed medium plant height associated with favorable productivity. Drought escape and tolerance mechanisms contributed positively to yield performance. Yubiley 90, Erytrospermum 100, 21 FAWWON9/Gaudio, 29th SAWYT N395, 21 FAWWON9/Gallio, and Gobustan/Gyrmyzy gul 1 were identified as promising genotypes for breeding. Gobustan/Gaudio, characterized by strong antioxidant defense, may serve as a donor for improving physiological drought tolerance.
Keywords: wheat, drought, productivity, antioxidant defense system, metabolic enzymes.

***Corresponding author:** ulduzagurbanova@gmail.com

Received: 6 January 2026; Accepted: 4 February 2026; Published: 30 March 2026

DOI: 10.54414/GVGR1353

Introduction

Drought tolerance is a complex trait governed by plant–environment interactions and therefore requires integrated evaluation. With the increasing frequency of drought under climate change, improving drought tolerance in winter wheat has become a major breeding priority. Selection based solely on grain yield may be insufficient; combining morphological, physiological, and biochemical traits can provide a more reliable assessment. Drought adversely affects wheat throughout vegetative and reproductive development, including germination, heading, flowering, and grain filling. Traits such as high tillering capacity, early maturity, a strong root system, moderate plant height, elongated upper internodes, and optimized leaf area contribute to drought adaptation by limiting water loss and maintaining plant viability. Physiological responses include stomatal closure and reductions in photosynthesis, transpiration, stomatal conductance, and relative water content, together with cell-wall modification, enhanced oxidative stress, and activation of alternative mitochondrial electron transport

pathways (Chaouachi et al. 2023). At the biochemical level, the efficiency of the Rubisco enzyme and photochemical efficiency decline; antioxidant defense systems are activated; abscisic acid, proline, polyamines, carbohydrates, and stress proteins accumulate (Nezhadahmadi et al. 2013).

Drought stress greatly influences photosynthesis in plants, resulting in decreased photosynthetic activity and negatively impacting plant development and productivity (Karami et al. 2025). As a C3 plant, wheat exhibits lower photosynthetic efficiency under water-deficient conditions compared to C4 plants. Studying the role of C4 photosynthetic enzymes, which are crucial for initial CO_2 fixation and the metabolism of carbon acids, under stress conditions in C3 plants is highly relevant. C4 photosynthetic enzymes play an important role in the non-photosynthetic organs of C3 plants by participating in the Krebs and Calvin cycles. They contribute to the biosynthesis of amino acids and help meet the NADPH demand of the antioxidant system.

Under drought stress conditions, the metabolic balance in plants is disrupted, leading to an increased demand for energy and, consequently, an enhancement of defense mechanisms. In such situations, AAT contributes to amino acid synthesis and the redistribution of nitrogen, thereby promoting the production of osmoprotectants, maintaining energy balance, and preventing the accumulation of harmful compounds during stress.

Metabolites produced via phosphoenolpyruvate carboxylase (PEPC) contribute to the reduction of oxidative stress and the detoxification of ROS. PEPC supports the metabolic adaptation of plants under drought conditions, maintains carbon and energy balance, and enhances drought stress tolerance (Li et al. 2024).

Under water-deficit conditions, a significant increase in the activity of C4 photosynthetic enzymes in wheat has been demonstrated in studies conducted. In genotypes with high enzyme activities, photosynthetic efficiency, stress tolerance, and metabolic plasticity are enhanced. Under drought conditions, due to the reduction in photosynthesis, NAD-MDH contributes to mitochondrial respiration by synthesizing ATP and NADH, thereby enhancing the activity of antioxidant enzymes and mitigating oxidative stress (Kambona et al. 2023).

The study aimed to evaluate the relationship between certain morphophysiological and biochemical traits under drought conditions in winter bread wheat and to screen for tolerant genotypes.

Materials and Methods

Experimental site and plant material

The study was conducted under rainfed conditions at the Gobustan Experimental Station of the Research Institute of Crop Husbandry, Ministry of Agriculture of the Republic of Azerbaijan. The site is located at an altitude of 800 m above sea level. Twenty-one bread wheat (*Triticum aestivum* L.) genotypes were used as study materials. The 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 seeds of bread wheat genotypes were planted at the end of October, at an average density 400 seeds per m^2, at 1m x 10 m plots, consisting of 7 rows placed 15 cm apart. During the vegetation period, phenological observations were conducted, plant height was measured at full maturity, and grain productivity was determined for each genotype.

Productivity

The harvested spikes from a 1 m² area were threshed using a threshing machine, the grains were separated and weighed. The mean value of three replications was calculated, and the resulting value was considered as the yield per 1 m² (g/m²).

Plant height

Before harvesting, the height of 10 plants in each plot was measured, and the average value was used to determine the plant height for each genotype (cm).

Determination of Relative Water Content

Relative water content (RWC) in leaves was measured based on the method proposed by Turner (1981) and calculated using the following formula:

$$RWC = [(FW - DW) / (TW - DW)] \times 100$$

where: FW – fresh weight of the leaf, TW – turgid weight, DW – dry weight.

Preparation of enzyme extract

A 0.5 g leaf sample was ground in liquid nitrogen and homogenized in 100 mM sodium phosphate buffer (pH 7.8) containing 1 mM EDTA, 2 mM PMSF, 1% PVP, and 0.1% Triton X-100. The homogenate was then centrifuged, and the resulting supernatant was used for the analysis of antioxidant enzyme activities. Enzyme activity was calculated based on the following formula:

$$A = \Delta OD \cdot V / \varepsilon \cdot b \cdot \tau$$

A – activity in the International System of Units; ΔOD – change in optical density per minute; V – volume of the reaction mixture (ml); τ – time of the reaction; ε – molar extinction coefficient, b – volume of the enzyme extract added to the reaction mixture (µl). The specific activities of the enzymes were calculated relative to protein content and per 1 g of fresh weight.

Extraction of metabolic enzymes from leaf samples

Leaf samples were homogenized in 100 mM Tris-HCl (pH 7.8) containing 5 mM DTT, 5 mM $MgCl_2 \cdot 6H_2O$, 1 mM EDTA, 0.5% Triton X-100, and 1% PVP. The homogenate was centrifuged at 1000g for 10 minutes and then at 5000g for 30 minutes at 4°C. The activity of enzymes was determined by measuring the decrease in absorbance at 340 nm resulting from NADH oxidation within 1 min (Ultrospec 3300 spectrophotometer). Spectrophotometric measurements were carried out in 1.0 ml cuvettes.

Aspartate aminotransferase (AAT, EC 2.6.1.1) activity assay

AAT activity was determined according to the method of Yang et al. (2019). For the assay, the reaction mixture contained 100 mM Tris-HCl buffer (pH 8.5), 2 mM EDTA, 2.5 mM 2-oxoglutarate, 10 µg/ml pyridoxal phosphate, 10 mM DTT, 12 U/ml MDH and 0.2 mM NADH. To this mixture, 20 µl of leaf extract and 2.5 mM L-aspartate were added. The activity was calculated using a molar extinction coefficient of ε = 6.22 $mM^{-1} \cdot cm^{-1}$ and expressed as µmol aspartate mg^{-1} protein min^{-1}.

NADP-malic enzyme (NADP-ME, EC 1.1.1.40) activity assay

NADP-ME activity was determined by the method of Arias et al. (2018). The reaction mixture consisted of 2.5 mM Tris-HCl buffer (pH 8.3), 0.5 mM EDTA, 0.75 µM CoA, 0.25 mM NADP, 2.5 mM malate, and 5.0 mM $MnCl_2$. The activity was calculated using a molar extinction coefficient of ε = 6.22 $mM^{-1} \cdot cm^{-1}$ and expressed as µmol malate mg^{-1} protein min^{-1}.

Malate dehydrogenase (NAD-MDH, EC 1.1.1.38) activity assay

The activity of NAD-MDH was determined according to the method of

Aliyeva et al. (2023). The reaction mixture consisted of 100 µM Tris-HCl buffer (pH 8.0), 1 mM oxaloacetic acid (OAA), 10 mg/ml bovine serum albumin (BSA), 10 mM $MgCl_2$, 0.15 µM NADH, and 10 µl of enzyme extract. The activity was calculated using a molar extinction coefficient of ε = 6.22 $mM^{-1} \cdot cm^{-1}$ and expressed as µmol OAA mg^{-1} protein min^{-1}.

Phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) activity assay

PEPC enzyme activity was determined according to the method of Aliyeva et al. (2023). The reaction mixture consisted of 60 mM HEPES-NaOH buffer (pH 8.0), 10 mM $MgCl_2$, 2 mM DTT, 10 mM $NaHCO_3$, 0.2 mM NADH, 10 units of MDH, 10 mM phosphoenolpyruvate, and 40 µl of enzyme extract. The activity was calculated using a molar extinction coefficient of ε = 6.22 $mM^{-1} \cdot cm^{-1}$ and expressed as µmol PEP mg^{-1} protein min^{-1}.

Determination of the amount of soluble protein

Protein content was determined spectrophotometrically at 595 nm according to the method proposed by Bradford (1976). During the construction of the calibration curve, different concentrations of bovine serum albumin (BSA) diluted in water were used as the standard protein.

Statistical analysis

Data from three independent biological replicates were analyzed using one-way analysis of variance (ANOVA). When significant differences were observed, Tukey's Honest Significant Difference (HSD) post hoc test was applied for pairwise comparisons.

Results

Drought is one of the environmental factors that negatively affects plant development at the early stages of vegetative growth, ultimately leading to a reduction in productivity. In this study, we determined a range of morphophysiological and biochemical parameters in 21 bread wheat genotypes cultivated under rainfed conditions at the Gobustan Experimental Station of the Research Institute of Crop Husbandry of the Azerbaijan Republic.

In the 2023–2024 vegetation period, plant heights of the genotypes were measured before harvest, and the results are presented in Fig. 1A. Statistical analysis revealed significant differences in plant height among the genotypes at the 0.01 significance level (CV 2.7%, LSD 4.3**). As shown in the figure, the plant height of the studied genotypes varied widely, ranging from 83 to 115 cm, with an average value of 97.3 cm. Among the studied genotypes, the tallest plant heights were observed in Gobustan/Gyrmyzy gul 1 (115 cm) and Gobustan/Gallio (110 cm). The shortest plant heights were recorded in the varieties Yubiley 90, Gyrmyzy gul 1, and Gaudio (96, 95, and 95 cm, respectively), and in the lines Quality/Balaton, Lutescens 76, 21FAWWON61/Balaton, Erytrospermum 100, and Gobustan/AZE026-10-4 (95, 92, 85, 84, and 83 cm, respectively). The remaining genotypes exhibited intermediate plant heights.

At the same time, the yield performance of the studied genotypes was evaluated, and the results are presented in Fig. 1B. The analysis of variance revealed a statistically significant difference among the genotypes for this parameter at the 0.01 significance level (CV 4.5%, LSD 40.6**).

As shown in the Fig. 1B, the yield of the varieties Lider and Yubiley 90 was 726 and 709 g/m², respectively, which was higher than that of the other genotypes. Among the studied lines, Erytospermum 100 (625 g/m²), Gobustan/Balaton (592 g/m²), and 29th SAWYT N~~o.~~ 395 (612 g/m²) demonstrated high performance. In terms of yield, the lowest values among the varieties were observed in Gobustan and Gallio, while among the lines, Gobustan/AZE 026-

10-4, Gyrmyzy gul 1/Gobustan, Lutescens 76, Quality/Balaton, Gobustan/Gallio, Gobustan/Gaudio and Quality/Gaudio 1 exhibited the lowest results. The remaining genotypes showed intermediate yield performance. Thus, along with other parameters, the yield data of genotypes should also be considered in the continuation of the breeding program.

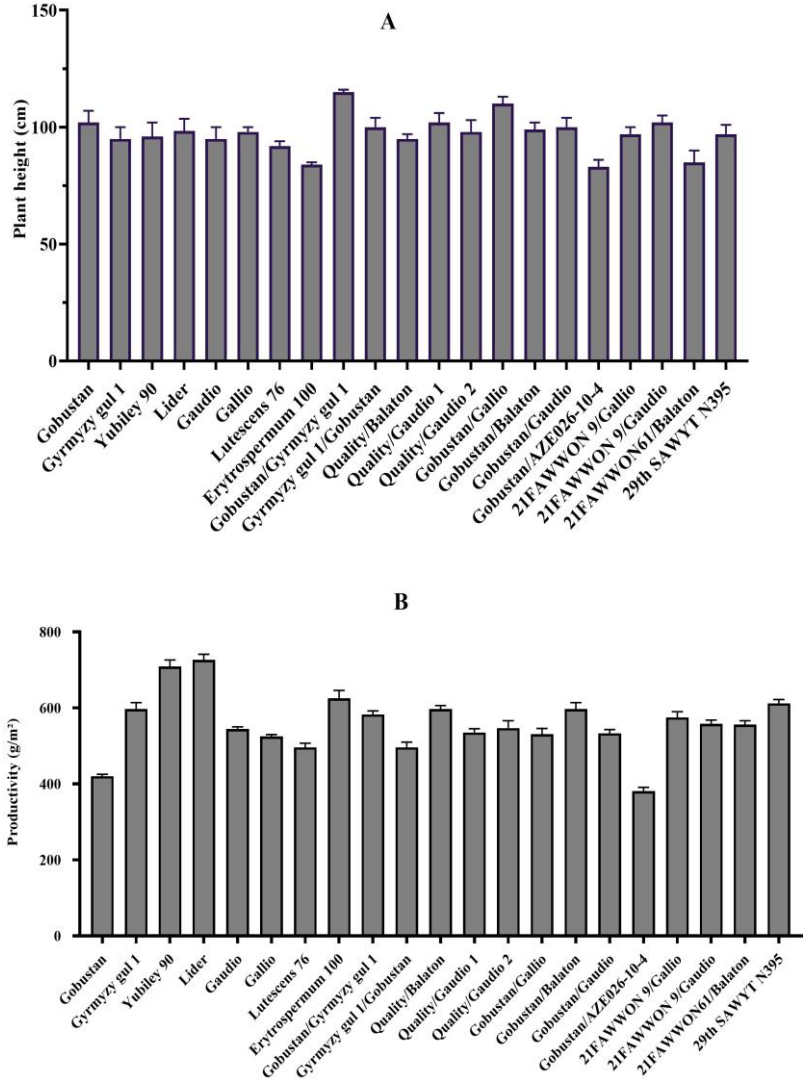

Figure 1. Plant height of the studied genotypes: CV 2.7%, LSD 4.3** (A); Productivity of the studied genotypes: CV 4.5%, LSD 40.6** (B). The data are presented as the means ± SD and are obtained from 3 biological replicates.

Heading dates for the studied genotypes in the 2023–2024 vegetation period are presented in Table. As shown in the table, the heading dates of the genotypes ranged from April 29 to May 7. Considering that the heading date is an important phenological trait of wheat genotypes and plays a significant role in productivity under

rainfed conditions, it was recorded as the number of days from May 1, and statistical analysis was carried out accordingly. The analysis of variance revealed statistically significant differences among genotypes for this parameter. Among the studied varieties, Gobustan and Yubiley 90 exhibited early heading on April 30 and May 1, respectively, while Gaudio, Gyrmyzy gul 1, Gallio and Lider were classified as late-heading varieties (Table). Among the lines, Gyrmyzy gul 1/Gobustan, 21FAWWON61/Balaton, Gobustan/ Gyrmyzy gul 1 and Quality/Gaudio 1 exhibited early heading. In contrast, 29th SAWYT N395 and 21FAWWON9/Gaudio showed late heading. It should be noted that the late-heading varieties mentioned above were developed for irrigated and moisture-sufficient rainfed regions.

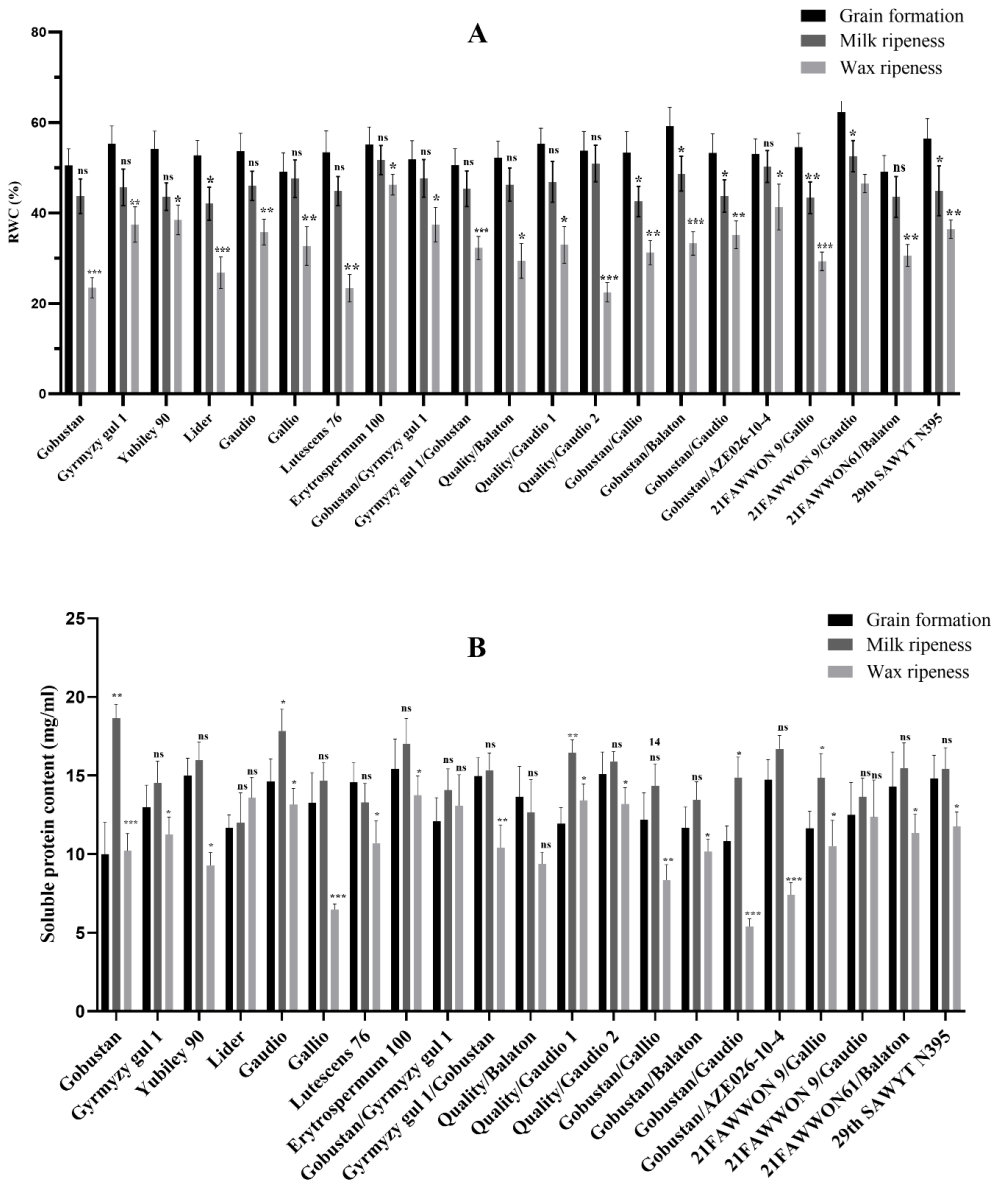

Figure 2. Relative water content (A), and soluble protein content (B) in bread wheat genotypes. Statistical significance was set at $p < 0.05$ (*, significant) and $p < 0.01$ (**, very significant), $p < 0.001$ (***, highly significant); results with $p \geq 0.05$ were considered not significant (ns).

From the perspective of heading time, the early spike emergence of Gobustan and Yubiley 90 varieties makes them more suitable for cultivation in arid rainfed regions, where drought stress typically occurs in the later stages of the vegetation period. On the other hand, the early-heading characteristics of lines such as Gyrmyzy gul 1/Gobustan, 21FAWWON61/Balaton, Gobustan/ Gyrmyzy gul 1, and Quality/Gaudio 1 suggest their potential use in breeding programs targeting moisture-deficient rainfed environments.

The results of 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 are presented in Fig. 2A.

The analysis of variance revealed statistically significant differences among genotypes for this parameter. As shown in the table, the RWC of the flag leaf varied between 49.1% and 62.3% (CV 13.2%) during the grain formation stage, between 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.

As seen from the presented results, during the grain formation and milk ripeness stages, the variation in RWC was within a narrower range due to the presence of a certain level of soil moisture and the relatively young physiological state of the genotypes. In other words, the genotypes showed less differentiation in this parameter during these stages. However, in the wax ripeness stage, the reduction in soil moisture led to a more pronounced expression of differences among genotypes. Thus, at this stage, the RWC values in the lines 21FAWWON 9/Gaudio and Erytrospermum 100 were considerably higher than those of other genotypes (46.5% and 46.3%, respectively), indicating their better regulation of water balance under conditions of declining soil moisture. In contrast, the lowest RWC values at this stage were recorded in the line Quality/Gaudio 2 (22.5%), the line Lutescens 76 (23.4%), and the variety Gobustan (23.5%), suggesting water regime regulation in these genotypes.

Thus, the study of RWC in several wheat varieties and lines revealed significant differences among genotypes for this parameter and it is advisable to use genotypes with higher flag leaf RWC in future breeding programs.

The results of the analyses showed that reduced water content in plants significantly decreases the amount of proteins in the cells. In the samples taken during the initial stage, the amount of soluble proteins ranged between 10.00 mg/ml and 15.41 mg/ml (Fig. 2B).

The AAT enzyme is one of the key regulatory enzymes that enhance plant tolerance under drought conditions (Fig. 3A). High activity levels of this enzyme were observed in the Erytrospermum 100 and Gobustan/Gaudio genotypes during the studied growth phases. According to the study results, the lowest activity levels were recorded in the Gobustan/Gallio genotype and in the Lutescens 76 genotype. In the parental varieties, Gobustan, Gyrmyzy gul 1, Yubiley 90, Gaudio, and Gallio, enzymatic activity remained at a moderate level. However, some hybrid forms, such as Gyrmyzy gul 1/Gobustan and Quality/Gaudio 2, exhibited higher enzyme activity compared to their parental lines.

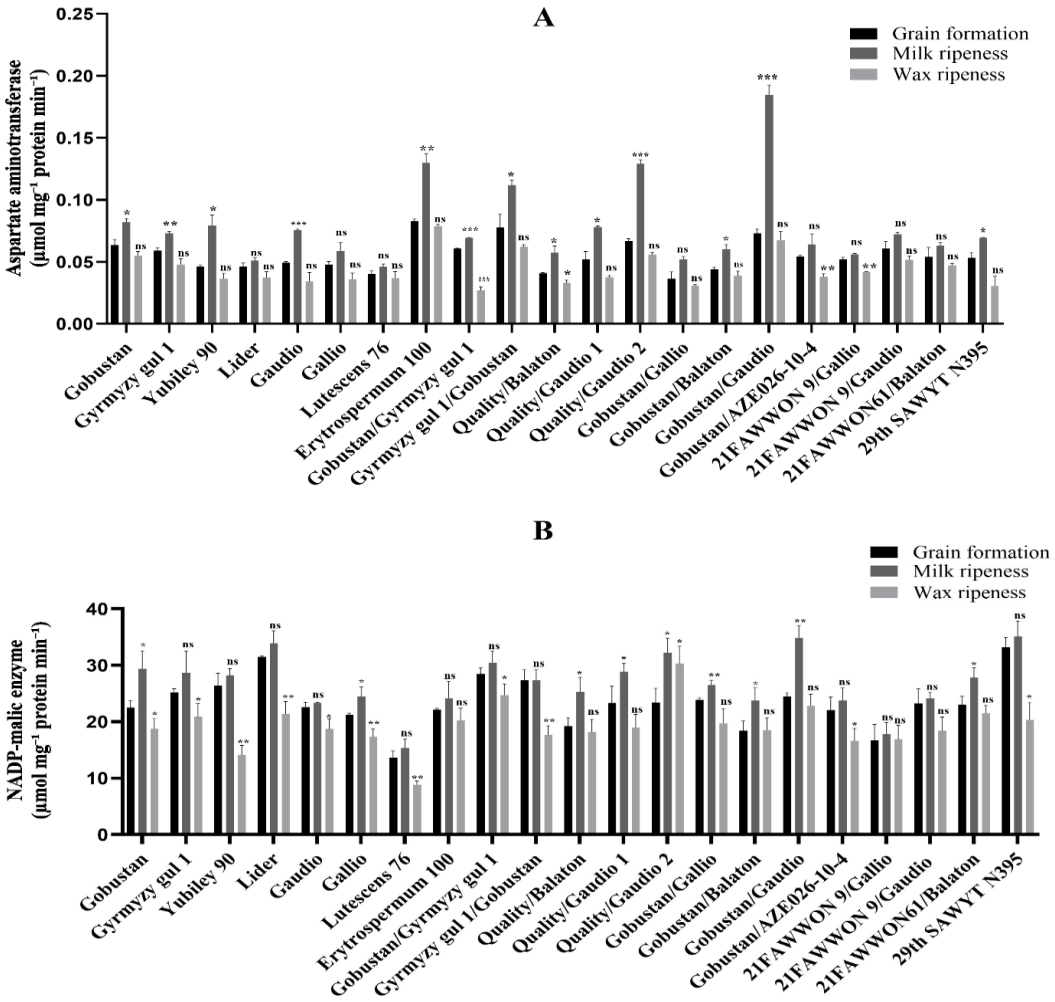

Figure 3. Activity of AAT (A) and NADP-ME (B) in bread wheat genotypes. Statistical significance was set at $p < 0.05$ (*, significant) and $p < 0.01$ (**, very significant), $p < 0.001$ (***, highly significant); results with $p \geq 0.05$ were considered not significant (ns).

In general, the enzyme activity was highest during the milk ripeness stage. At this stage, the highest AAT activity was recorded in the Gobustan/Gaudio genotype. This value differed significantly from those of the other genotypes in statistical terms ($p \leq 0.05$).At the wax ripeness stage, AAT activity decreased in almost all genotypes, indicating a decline in enzyme activity toward the later stages of development. The results of the study revealed that the activity of AAT in bread wheat genotypes varied significantly depending on the phenological stage and genotype. The Gobustan/Gaudio and Erytrospermum 100 genotypes exhibited high enzymatic activity across all stages of the study. In contrast, the Gobustan/Gallio and Lutescens 76 genotypes showed relatively low activity. Notably, in hybrid forms, a marked increase in activity was observed, particularly during the milk ripeness stage.

The 29th SAWYT N395 hybrid exhibited the highest NADP-ME activity across all developmental stages (Fig. 3B). High NADP-ME activity was also observed in the Lider, Gobustan/Gaudio, and Quality/Gaudio 2 specimens, whereas the lowest enzymatic activity was recorded in Lutescens 76 and 21FAWWON 9/Gallio.

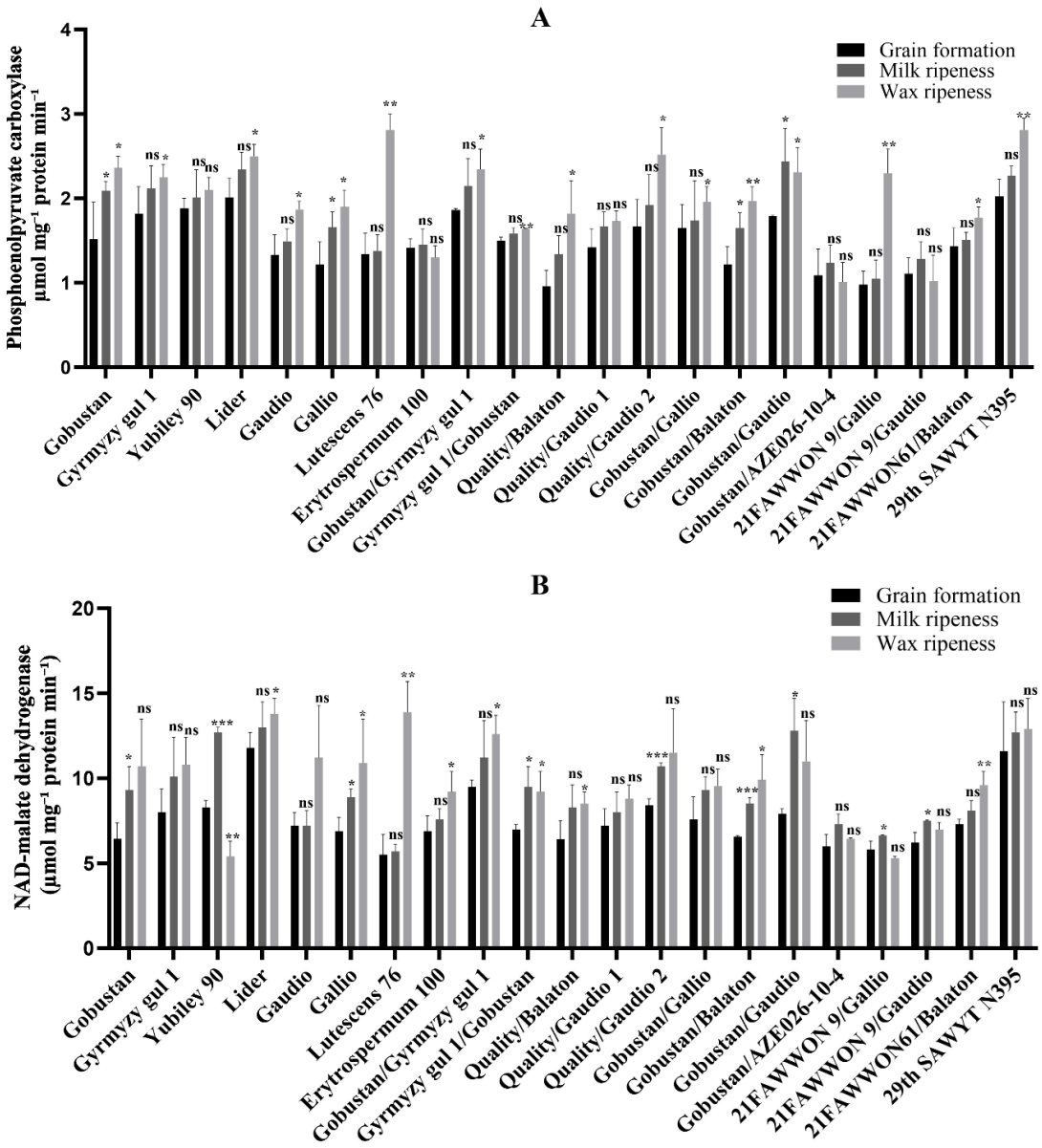

Figure 4. Activity of PEPC (A) and NAD-MDH (B) in bread wheat genotypes. Statistical significance was set at $p < 0.05$ (*, significant) and $p < 0.01$ (**, very significant), $p < 0.001$ (***, highly significant); results with $p \geq 0.05$ were considered not significant (ns).

The results of the study demonstrated that the activity of PEPC in bread wheat genotypes varied significantly depending on the phenological development stages and genotypes (Fig. 4A). In general, the highest enzyme activity was observed during the wax ripeness stage. At this stage, the 29th SAWYT N395 and Lutescens 76 genotypes exhibited enzyme activity levels of 2.9 and 2.8 µmol mg^{-1} protein min^{-1}, respectively, which were statistically significantly higher than those of all other genotypes. In addition, Quality/Gaudio, Gobustan/Gaudio, and Yubiley 90 genotypes also showed high PEPC activity. The lowest activity was recorded in the 21FAWWON 9/Gaudio, 21FAWWON 9/Gallio, and 21FAWWON61/Balaton genotypes,

particularly during the f grain formation and milk ripeness stages. High NAD-MDH activity was observed in the 29th SAWYT N395 and Gobustan/Gyrmyzy gul 1 genotypes (Fig. 4B). These genotypes not only exhibited strong metabolic activity but also showed increasing enzyme levels throughout the vegetation period. These characteristics indicate their potential for high productivity and tolerance to abiotic stress. The lowest NAD-MDH activity was recorded in the 21FAWWON 9/Gallio hybrid.In the Yubiley 90 variety, a sharp decrease in enzyme activity was observed during the wax ripeness stage. Although NAD-MDH activity in the Lutescens 76 genotype was low during the grain formation and milk ripeness stages (5.5 and 5.7 µmol mg^{-1} protein min^{-1}, respectively), it increased during the wax ripeness stage, reaching its maximum level (13.9 µmol mg^{-1} protein min^{-1}). In the Gobustan/Gaudio and Quality/Gaudio2 hybrids, an increase in enzyme activity was also observed during the wax ripeness stage. These results indicate that these genotypes possess a more stable and progressively adaptive metabolic mechanism.

The heatmap analysis revealed that the increase in PEPC and NADP-ME activities observed in tolerant genotypes indicates enhanced metabolic flexibility, supporting carbon fixation and energy supply under drought stress (Fig. 5).

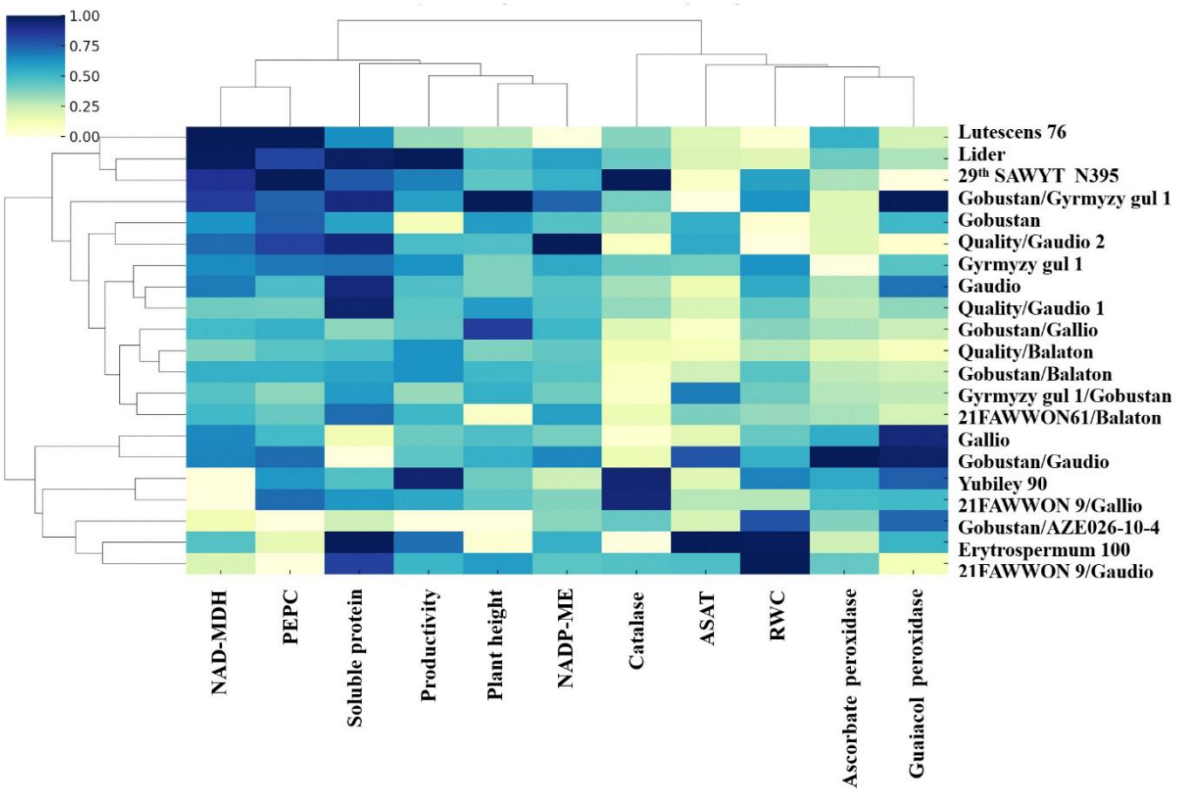

Figure 5. Clustered heatmap with dendrograms - genotypes are now grouped according to their overall biochemical and physiological similarity.

Based on the overall analysis, the investigated genotypes were grouped into three main clusters (Fig. 5). Cluster 1 included Yubiley 90 and Lider, which were characterized by high productivity and strong antioxidant enzyme activities.

Cluster2 comprised Gobustan and Gobustan/AZE026-10-4 genotypes, which exhibited relatively lower productivity but showed high RWC and strong stress adaptation capacity, indicating their potential drought tolerance. Cluster 3 consisted of genotypes with intermediate and diverse characteristics, including Gyrmyzy gul 1, Gaudio, Gallio, Lutescens 76, Erytrospermum 100, Gobustan/Gyrmyzy gul 1, Gyrmyzy gul 1/Gobustan, Quality/Balaton, Quality/Gaudio 1, Quality/Gaudio 2, Gobustan/Gallio, Gobustan/Balaton, Gobustan/Gaudio, 21FAWWON 9/Gallio, 21FAWWON 9/Gaudio, and 21FAWWON61/Balaton. The genotypes in this group exhibited a wide range of productivity and enzyme activity levels, representing valuable genetic material for future breeding programs aimed at improving both yield potential and stress tolerance.

Discussion

Drought stress negatively affects wheat productivity by causing changes at morphological, physiological, and biochemical levels (Ayed et al. 2021). Under rainfed conditions with limited moisture availability, plant height plays a significant role in both drought tolerance and productivity in wheat.

As a partial drought escape mechanism, heading time plays a crucial role in yield formation under rainfed conditions. The timing of heading is not only a genotypic trait specific to the plant, but also depends on the hydrometeorological factors of the vegetation period. According to the literature, in regions where drought tends to occur during the later stages of vegetation, early heading and flowering can serve as mechanisms to avoid drought. Several studies have also emphasized that the heading and fertilization stages are among the most drought-sensitive phases in wheat (Dong et al. 2017).

RWC is an important indicator of drought tolerance that ensures metabolic activity in plant tissues under water-deficient conditions. This parameter tends to be high during the early stages of plant development but decreases as the plant's dry biomass begins to increase. Although a plant's water potential depends on the water status of the soil, plant, and atmosphere, leaf RWC specifically reflects the plant's internal water status, making it a more accurate indicator of water balance in the plant. A decline in RWC in response to drought stress has been observed in various plant species. Several studies have also reported genotypic differences in leaf RWC in wheat plants (Tanaka and Ito 2025). Leaf RWC is associated with drought tolerance and may be considered a better indicator of drought stress than other morphophysiological and biochemical parameters of plants. RWC reflects the proportion of water in a leaf relative to its full water-holding capacity at the time of measurement, and it serves as an indicator of the balance between water absorption from the soil and water loss through transpiration under identical conditions. Since transpiration typically occurs during midday hours, the RWC measured at this time depends on the plant's ability to absorb and retain water from the soil. Therefore, midday RWC values represent the plant's overall capacity for water uptake and evaporation, while also characterizing the water deficit in the leaf.

AAT is one of the key enzymes involved in the transamination of amino acids, and its activity is closely associated with the efficiency of nitrogen metabolism. The high activity of the enzyme during the milk ripeness stage indicates intensive protein synthesis and redistribution of nitrogen at this stage. This was particularly pronounced in the Gobustan and Ertyospermum 100 genotypes, suggesting

that these genotypes exhibit more efficient nitrogen metabolism.

In many cases, hybrid forms showed higher NADP-ME activity compared to their parental lines. These results reflect potential differences in the Krebs cycle and mitochondrial respiration. The NADP-ME enzyme has been studied in some C3 plants such as *Arabidopsis thaliana* and *Oryza sativa* (Liu 2007).

The NADP-ME enzyme also plays a key role in plant defense mechanisms against abiotic stresses such as water deficiency and high temperatures (Li et al. 2025). From this perspective, genotypes exhibiting high enzyme activity should be considered for selection programs as indicators of stress tolerance. Besides, the reaction catalyzed by NADP-ME results in the release of CO_2 (Sun et al. 2019), which can enter the Calvin cycle and contribute to an increase in photosynthetic products. In general, the presented results suggest that certain hybrid genotypes demonstrated higher enzymatic activity compared to their parental lines. This strengthens the likelihood that these hybrids are better adapted to abiotic stresses and enhances their potential for use in breeding programs.

PEPC catalyzes the conversion of PEP to oxaloacetic acid OAA, providing a link between carbohydrate and nitrogen metabolism (Wei et al. 2025). In our study, PEPC activity increased significantly during the wax ripeness stage. The 29th SAWYT N395 and Lutensens 76 genotypes, which showed high enzyme activity at this stage, probably indicated a greater need for energy supply and regulatory metabolism at the final stage of development. Since the wax ripeness stage represents the final phase of seed development, the increase in enzymatic activity may be associated with the redirection of carbon skeletons into biosynthetic processes. PEPC plays a crucial role in plant adaptation to abiotic stresses, including drought and salt stress. Increasing enzyme activity may support enhanced photosynthetic efficiency and anabolic processes (Chen et al. 2019). The lowest activity observed in the FAWWON series genotypes suggests a weaker metabolic response, indicating that these genotypes should be carefully evaluated in breeding programs. In contrast, the high enzyme activity observed in genotypes such as Quality/Gaudio and Gobustan/Gaudio indicates that they are metabolically more active and potentially more tolerant to stress conditions.

NAD-MDH catalyzes the conversion of malate to OAA and NADH, thereby acting as a bridge between carbon metabolism and energy production (de Lorenzo et al. 2024). In our study, the variation in NAD-MDH enzyme activity across developmental stages indicates that the enzyme is dynamically regulated in accordance with the plant's metabolic demands. In most genotypes, the peak activity observed during the wax ripeness stage is associated with the accumulation of energy and carbon reserves in the seed at this phase. This trend is particularly evident in the genotypes Lider, Lutescens 76, and 29th SAWYT N395, highlighting their high potential in energy metabolism. These genotypes are recommended for careful evaluation in breeding programs. Differences in NAD-MDH activity may also be linked to adaptation mechanisms under stress conditions. Studies have shown that this enzyme supports mitochondrial energy flux and enhances ROS balance during abiotic stresses, especially salt and drought stress (Monteiro-Batista et al. 2025).

Thus, the comprehensive research conducted indicates that the 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 and are considered suitable for cultivation in moisture-deficient arid regions. Among them, the Gobustan/Gaudio genotype, which possesses a strong antioxidant defense system, may serve as a valuable source material for antioxidant analysis and biochemical screening.

Conclusion

The results obtained provide a better understanding of the mechanisms underlying drought tolerance under rainfed conditions and help clarify the role of antioxidant enzymes in stimulating a broad spectrum of protective responses. The research revealed significant differences in drought tolerance levels among modern winter wheat varieties and lines. Based on the analysis of biochemical indicators, it was found that high RWC and sustained activity of antioxidant and metabolic enzymes are key traits contributing to better adaptation under drought conditions. Varieties possessing these traits are less affected by environmental stress factors and are able to maintain their productivity. The findings of this study enable the identification of promising genotypes for use in breeding programs. A comprehensive analysis of drought tolerance creates a scientific basis for more efficient breeding programs and the development of wheat varieties tolerant to climate change.

Conflict of Interest

Authors declare that there is no conflict of interest.

Acknowledgement

This work was supported by the Ministry of Science and Education of the Republic of Azerbaijan under the project titled"Creation of infrastructure for the development of new directions of genetic engineering in Azerbaijan, application of genome editing methods in biomedical research and genome selection" (2023–2024).

References

Aliyeva D.R., Gurbanova U.A., Rzayev F.H., Gasimov E.K., Huseynova I.M. (2023) Biochemical and ultrastructural changes in wheat plants during drought stress. *Biochemistry*, 88(11):1944–1955. Doi: 10.1134/S0006297923110226

Arias C.L., Pavlovic T., Torcolese G., Badia M.B., Gismondi M., Maurino V.G., Andreo C.S., Drincovich M.F., Gerrard Wheeler M.C., Saigo M. (2018) NADP-Dependent malic enzyme 1 participates in the abscisic acid response in *Arabidopsis thaliana*. *Front Plant Sci*, 9:1637. Doi: 10.3389/fpls.2018.01637

Ayed S., Bouhaouel I., Othmani A., Bassi F.M. (2021) Use of wild relatives in durum wheat (*Triticum turgidum* L. var. *durum* Desf.) breeding program: Adaptation and stability in context of contrasting environments in Tunisia. *Agron*, 11:1782. Doi: 10.3390/agronomy11091782.

Bradford M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2):248–254. Doi: 10.1006/abio.1976.9999

Chaouachi L., Marín-Sanz M., Kthiri Z., Boukef S., Harbaoui K., Barro F., Karmous Ch. (2023) The opportunity of using durum wheat landraces to tolerate drought stress: screening morpho-physiological components, AoB. *Plants*, 15(3):plad022. Doi: 10.1093/aobpla/plad022

Chen Q., Wang B., Ding H., Zhang J., Li S.C. (2019) The role of NADP-malic enzyme in plants under stress. *Plant Sci*, 281:206–212. Doi: 10.1016/j.plantsci.2019.01.010.

de Lorenzo L., Stack T.M.M., Fox K.M., Walstrom K.M. (2024) Catalytic mechanism and kinetics of malate dehydrogenase. *Essays Biochem*, 68(2):73–82. Doi: 10.1042/EBC20230086

Dong B., Zheng X., Liu H., Able J.A., Yang H., Zhao H., Zhang M., Qiao Y., Wang Y., Liu M. (2017) Effects of drought stress on pollen sterility, grain yield, abscisic acid and protective enzymes in two winter wheat cultivars. *Front. Plant Sci*, 8:1008. Doi: 10.3389/fpls.2017.01008

Huseynova I.M., Rustamova S.M., Suleymanov S.Y., Aliyeva D.R., Mammadov A.C., Aliyev J.A. (2016) Drought-induced changes in photosynthetic apparatus and antioxidant components of wheat (*Triticum durum* Desf.) varietes. *Photosynth. Res*, 130(1-3):215–223. Doi: 10.1007/s11120-016-0244-z

Kambona C.M., Koua P.A., Léon J., Ballvora A. (2023) Stress memory and its regulation in plants experiencing recurrent drought conditions. *Theor Appl Genet*, 136(2):26. Doi: 10.1007/s00122-023-04313-1.

Karami S., Shiran B., Ravash R. (2025) Molecular investigation of how drought stress affects chlorophyll metabolism and photosynthesis in leaves of C3 and C4 plant species: A transcriptome meta-analysis. *Heliyon*, 11(3):e42368. Doi: 10.1016/j.heliyon.2025.e42368.

Li S., Zheng H., Sui N., Zhang F. (2024) Class III peroxidase: an essential enzyme for enhancing plant physiological and developmental process by maintaining the ROS level: A review. *International Journal of Biological Macromolecules*, 283: 137331. Doi: 10.1016/j.ijbiomac.2024.137331

Li H., Xiao J., Chen J., Shen X., Luo J., Guo F., Wang S., Xu L., Guo X., Wang S., Wang H., Wang W. (2025) Identification of the cassava NADP-ME gene family and its response and regulation in photosynthesis. *Front Plant Sci*, 16:1525193. Doi: 10.3389/fpls.2025.1525193

Liu S., Cheng Y., Zhang X., Guan Q., Nishiuchi Sh., Hase K., Takano T. (2007) Expression of an NADP-malic enzyme gene in rice (*Oryza sativa*. L) is induced by environmental stresses; over-expression of the gene in *Arabidopsis* confers salt and osmotic stress tolerance. *Plant Mol Biol*, 64: 49–58. Doi: 10.1007/s11103-007-9133-3

Monteiro-Batista R.C., Siqueira J.A., da Fonseca-Pereira P., Barreto P., Feitosa-Araujo E., Araújo W.L., Nunes-Nesi A. (2025) Potential roles of mitochondrial carrier proteins in plant responses to abiotic stress. *Journal of Experimental Botany*, eraf032. Doi: 10.1093/jxb/eraf032

Nezhadahmadi A., Prodhan Z.H., Faruq G. (2013) Drought tolerance in wheat. *Sci. World J.*, 12 p. Doi: 10.1155/2013/610721

Niyazova N.N., Huseynova I.M. (2024) The antioxidant defense system of tomato (*Solanum lycopersicum* L.) varieties under drought stress and upon post-drought rewatering. *Biochemistry*, 89(6):1146–1157. Doi: 10.1134/S0006297924060130.

Sun X., Han G., Meng Z., Lin L., Sui N. (2019) Roles of malic enzymes in plant development and stress responses. *Plant Signal Behav.*,

14(10):e1644596. Doi: 10.1080/15592324.2019.1644596

Tanaka A., Ito H. (2025) Chlorophyll degradation and its physiological function. *Plant Cell Physiol*, 66(2): 139–152. Doi: 10.1093/pcp/pcae093

Turner N.C. (1981) Techniques and experimental approaches for the measurement of plant water status. *Plant Soil*, 58: 339–366. Doi: 10.1007/BF02180062

Wei H., Lu Z., Jiang H., Xue C., Xu X., Liu G., Lian B., Zhong F., Movahedi A., Zhang J. (2025) Comprehensive analysis of PEPC gene family in *Populus trichocarpa*: characterization, evolutionary insights, and the role of PtPEPC4-PtLTPG14 interaction in carbon metabolism. *Plant Physiol Biochem*, 220:109573. Doi: 10.1016/j.plaphy.2025.109573

Yang M., Geng M., Shen P., Chen X., Li Y., Wen X. (2019): Effect of post-silking drought stress on the expression profiles of genes involved in carbon and nitrogen metabolism during leaf senescence in maize (*Zea mays* L.), *Plant Physiol. Biochem*, 135:304-309. Doi: 10.1016/j.jhazmat.2021.127159