Baku State University Journal of Life Sciences & Biology ***2024****, v.1 (3), p. 7-13*

journal homepage: http://bsuj.bsu.edu.az/en

https://doi.org/10.30546/300045.2024.1.3.22

ENZYMATIC RESPONSE OF MATURING GRAINS TO DROUGHT STRESS IN DIFFERENT WHEAT GENOTYPES

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Received 23 october 2024; accepted 30 october 2024

Abstract

One of the major challenges in agriculture is the loss of productivity in bread and durum wheat genotypes cultivated in Azerbaijan due to water scarcity. As a result, the development of wheat genotypes that are resistant to both abiotic and biotic stresses remains a key focus of research. This study examined the effect of drought stress on the activity of key metabolic enzymes-phosphoenolpyruvate carboxylase (PEPC), aspartate aminotransferase (AspAT), and NAD-malate dehydrogenase (NAD-MDH) in durum and bread wheat genotypes during the milky stage (MS), dough stage (WS), and physiological stage (PS) of grain development. Results indicated that PEPC activity significantly increased during the WR stage in the drought-tolerant durum wheat variety Barakatli 95, showing a threefold increase under drought conditions. In contrast, the drought-sensitive Garagylchyg 2 exhibited a smaller increase of 1.5 times. In bread wheat varieties, PEPC activity remained unchanged in Tale 38, while it decreased by twofold in the ripening seeds of the drought-sensitive variety Gobustan in the dough stage. AspAT activity was stable in durum wheat but significantly decreased in bread wheat, particularly in the Gobustan variety. NAD-MDH activity increased in durum wheat, while it decreased in bread wheat under drought conditions. Further biochemical studies on sensitive and tolerant wheat varieties could provide valuable insights into drought tolerance and help identify promising genotypes for future breeding programs.

Keywords: wheat, phosphoenolpyruvate carboxylase, aspartate aminotransferase, NAD-malate dehydrogenase.

1. Introduction

The 20th century was not only a period of economic growth but also a crucial time for intensive studies of plant metabolic processes and the development of research methodologies in plant science [1, 2, 3, 4, 5]. In the

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face of globalization, a rapidly growing population, declining biodiversity, and limited fertile land for agriculture, meeting the food demands of humanity has become increasingly challenging [6]. Within this context, the impact of abiotic stress factors, particularly drought stress, on photosynthesis continues to be a key focus of current research [7].

According to the Food and Agriculture Organization of the United Nations (FAO), wheat occupies the largest area of cultivation globally, with 220-225 million hectares planted annually, producing an average of 685 million tons of wheat grain [https://www.fao.org/worldfoodsituation/csdb/en/]. In Azerbaijan, local wheat production currently meets 65.1% of the population's needs. The grain-filling phase is critical for determining grain weight and plays a major role in wheat yield [8, 9, 10].

Studying the enzymes involved in carbon and energy metabolism during various stages of grain maturation is of significant importance. To this end, the activities of phosphoenolpyruvate carboxylase (PEPC), aspartate aminotransferase (AsAT), and NAD-malate dehydrogenase (NAD-MDH) were analyzed in wheat grains at different stages of maturation.

Phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31) is a versatile enzyme that plays a central role in carbon fixation, energy metabolism, and adaptation to environmental stress. In plant tissues that are not involved in photosynthesis, PEPC is essential for anaplerotic reactions. In C3 plants, PEPC is non-photosynthetic and participates in various functions such as replenishing the tricarboxylic acid cycle, managing carbon-nitrogen interactions, carbon storage, and maintaining pH balance [11, 12].

Aspartate aminotransferase (AspAT; EC 2.6.1.1) catalyzes the reverse transamination reaction, forming aspartate and 2-oxoglutarate from glutamate and oxaloacetate in various cellular compartments [13]. Multiple isoforms of AsAT are localized in different subcellular organelles, including the cytosol, chloroplasts, mitochondria, and peroxisomes [14]. AsAT plays a key role in primary nitrogen assimilation, transferring reducing equivalents, and distributing carbon and nitrogen among cellular components [15, 16].

NAD-malate dehydrogenase (NAD-MDH; EC 1.1.1.37) encompasses multiple isoforms of malate dehydrogenase that catalyze the interconversion of malate and oxaloacetate in plant tissues. These isoforms, encoded by different genes, exhibit distinct kinetic properties, subcellular localizations, and physiological roles [17]. NAD-MDH reactions are reversible and aim to decrease oxaloacetate levels. The direction of the reaction *in vivo* depends on the substrate-to-product ratio and the redox state, which can vary depending on physiological conditions, even within the same tissue.

2. Materıals and Methods

Mature grains of durum wheat genotypes (Barakatli 95 and Garagylchyg 2) and bread wheat genotypes (Gobustan and Tale 38) were collected from an experimental field located on the Absheron Peninsula at the Research Institute of Crop Husbandry for research purposes.

To prepare enzyme extracts, the plant material was homogenized in a cold homogenizer with quartz sand in a buffer containing 100 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid (EDTA), 5 mM dithiothreitol (DTT), 2 mM phenylmethylsulfonyl fluoride (PMSF), and 2% (w/w) insoluble polyvinylpyrrolidone (PVP). The resulting homogenate was filtered and centrifuged at 12,000 g for 5 minutes, and the supernatant was used to assess enzyme activity.

To measure the activity of phosphoenolpyruvate carboxylase (PEPC), the reaction medium consisted of 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 2 mM DTT, 10 mM $NaHCO_3$, 0.2 mM NADH, 10 U/ml malate dehydrogenase (MDH), 10 mM phosphoenolpyruvate (PEP), and 40 µl of enzyme extract. The reaction was initiated by adding 10 mM PEP [13].

For aspartate aminotransferase (AspAT) activity, the reaction medium included 100 mM HEPES-KOH (pH 7.4) and 100 mM Tris-HCl (pH 8.5), 2 mM EDTA, 2.5 mM 2-oxoglutarate, 10 µg/ml pyridoxal phosphate, 10

mM DTT, 12 U/ml MDH, 0.2 mM NADH, 20 µl of enzyme extract, and 2.5 mM L-aspartate. The reaction commenced upon the addition of L-aspartate [18].

To determine the activity of NAD-malate dehydrogenase (NAD-MDH) for oxaloacetate reduction, the reaction medium contained 1 mM oxaloacetate (OA), 10 mg/ml bovine serum albumin (BSA), 10 mM $MgCl_2$, 0.15 mM NADH, and 5-10 µl of enzyme preparation in 100 mM Tris-HCl buffer (pH 8.0). The reaction was initiated by adding 1 mM oxaloacetate. For the direct reaction, the medium composition included 100 mM Tris-HCl (pH 9.0), 30 mM malate, and 0.2 mM NAD. Spectrophotometric measurements were conducted using 1.0 ml cuvettes, and enzyme activities were quantified spectrophotometrically (Ultrospec 3300 Pro, Amersham, USA) at a wavelength of 340 nm.

The total soluble protein content was assessed using a 0.12% Coomassie Brilliant Blue G-250 solution in a spectrophotometer [20].

3. Results and Discussion

The activity of the enzymes studied in the ripening seeds of durum wheat varieties (Barakatli 95 and Garagylchyg 2) increased under drought stress during the dough stage. In contrast, the activity of all three enzymes decreased in the seeds of bread wheat Gobustan during the same phase.

Specifically, the activity of phosphoenolpyruvate carboxylase (PEPC) in the drought-tolerant variety Barakatli 95 was three times higher under drought stress compared to normal irrigation conditions. In the drought-sensitive variety Garagylchyg 2, the enzyme's activity increased by 1.5 times during the dough stage. For the bread wheat varieties, PEPC activity remained unchanged in Tale-38, while it decreased by twofold in the ripening seeds of the drought-sensitive variety Gobustan (Fig. 1).

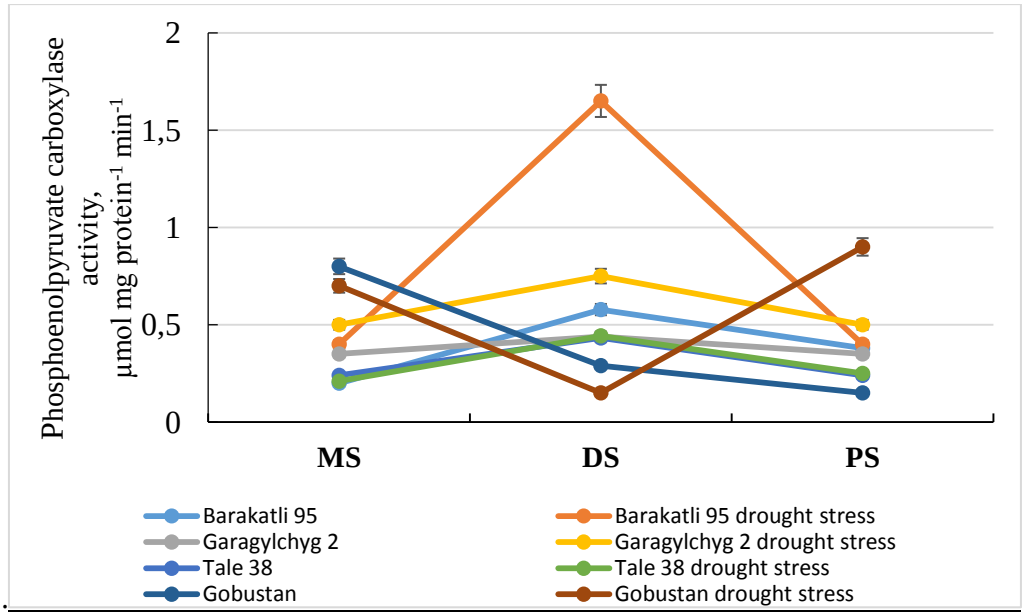

Fig 1. PEPC activity in developing grains of durum (*T. Durum*) and bread (*T. aestivum*) wheat genotypes. Milky stage (MS), dough stage (DS) and physiological stage (PS) phase of grain development.

The significantly higher activity of phosphoenolpyruvate carboxylase (PEPC) in the drought-tolerant Barakatli 95 variety (more than threefold increase under drought stress compared to normal irrigation) suggests that this enzyme plays a central role in the plant's drought tolerance mechanisms. This finding aligns with previous research emphasizing PEPC's role in stress adaptation through carbon fixation and metabolic regulation under adverse conditions [21].

In wheat, PEPC activity is known to peak around 15 days of post-anthesis, primarily contributing to grain development [22]. In our study, the ripening grains of Barakatli 95 showed a notable increase in PEPC activity under drought stress, indicating an adaptive metabolic shift aimed at sustaining grain development despite water limitations. Conversely, the lower activity observed in Garagylchyg 2 suggests that the regulation of PEPC during development is less efficient in this variety.

The increase in PEPC activity in Barakatli 95 likely reflects a metabolic adjustment to ensure continued carbon fixation and the production of metabolites like malate, essential for maintaining cellular functions during drought. This suggests that Barakatli 95 has a stronger capacity to redirect carbon metabolism under stress. In contrast, the more modest increase in Garagylchyg 2 indicates that its metabolic pathways may lack the same flexibility under stress, contributing to its drought sensitivity.

Transgenic studies have shown that increasing PEPC activity in crops enhances photosynthetic performance and water use efficiency [23]. This suggests that manipulating PEPC expression could be a viable strategy for further improving drought tolerance in wheat varieties like Barakatli 95. Our findings support the notion that selecting or engineering genotypes with elevated PEPC activity may lead to the development of more resilient crops.

Among bread wheat cultivars, PEPC activity in the drought-tolerant variety Gobustan was reduced twofold under drought stress, while it remained unchanged in Tale 38 in the dough stage. This indicates that different species may regulate PEPC differently, depending on their genetic background and adaptive strategies. While there is an increase in PEPC activity under stress in drought-tolerant cultivars like Barakatli 95, the decrease in Gobustan suggests that these varieties may employ different control mechanisms, possibly involving other metabolic or maintenance pathways during drought conditions [21].

The activity of aspartate aminotransferase (AspAT) in the durum wheat genotypes remained virtually unchanged. In contrast, similar to PEPC, a decrease in AsAT activity was observed in the bread wheat genotypes. While no significant difference in AsAT activity was noted in the Tale-38 genotype between irrigated and drought-stressed conditions, the enzyme activity in the maturing grains of the Gobustan genotype decreased by 2.4 times in the physiological stage (Fig 2).

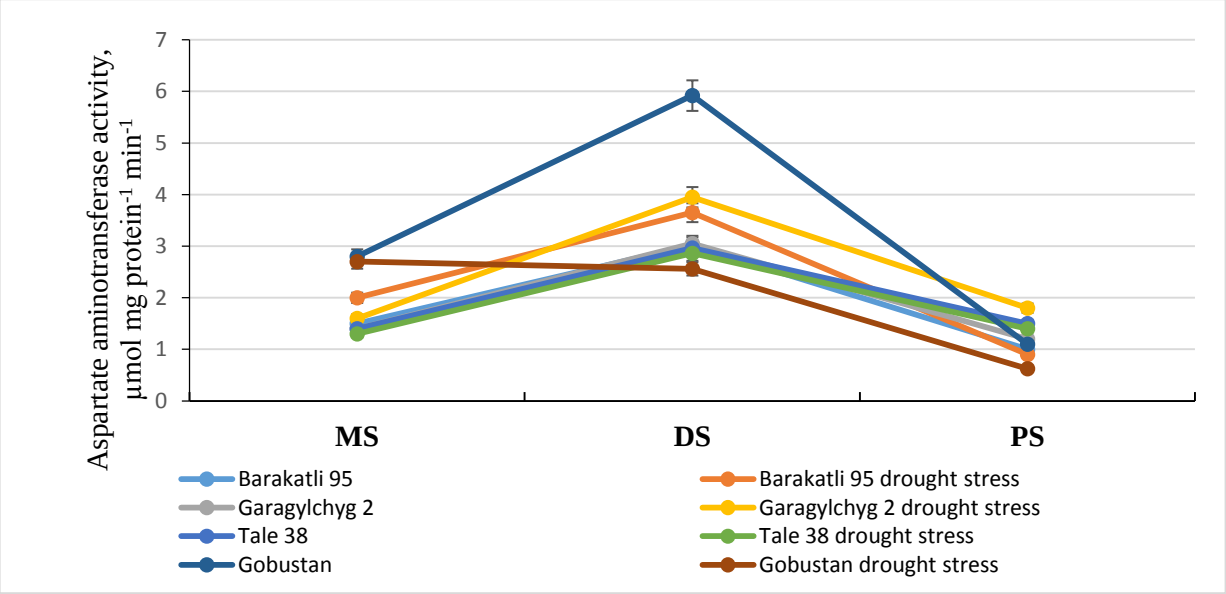

Fig 2. AspAT activity in developing grains of durum (*T. Durum*) and bread (*T. aestivum*) wheat genotypes. Milky stage (MS), dough stage (DS) and physiological stage (PS) phase of grain development.

The finding that aspartate aminotransferase (AspAT) activity remained constant in durum wheat aligns with observations of varying enzymatic responses among cultivars under drought stress. This may indicate that some durum wheat varieties possess a nitrogen barrier during grain filling, even under water-limited conditions, which could contribute to enhanced drought tolerance. In contrast, the reduction of AspAT activity in bread wheat - particularly the significant 2.4-fold decrease observed in the Gobustan variety-confirms studies indicating that drought stress typically reduces the activity of enzymes involved in nitrogen metabolism [24; 25]. This reduction may reflect disturbances in amino acid synthesis and nitrogen uptake, ultimately leading to decreased grain filling under drought conditions.

While the reduction of AspAT activity in bread wheat is consistent with general observations of wheat under drought stress, the lack of a significant difference in the Tale 38 genotype suggests that genetic factors may play a role in drought tolerance. Although drought generally decreases AspAT activity, as seen in common wheat varieties, some cultivars, such as certain durum wheat varieties, maintain elevated levels of this enzyme. This finding could inform breeding programs aimed at enhancing drought tolerance.

In durum wheat varieties subjected to drought, the activity of NAD-malate dehydrogenase (NAD-MDH) increased by 1.3 to 1.4 times. Conversely, in bread wheat varieties, the enzyme activity in ripening grains under drought conditions slightly decreased (Fig. 3).

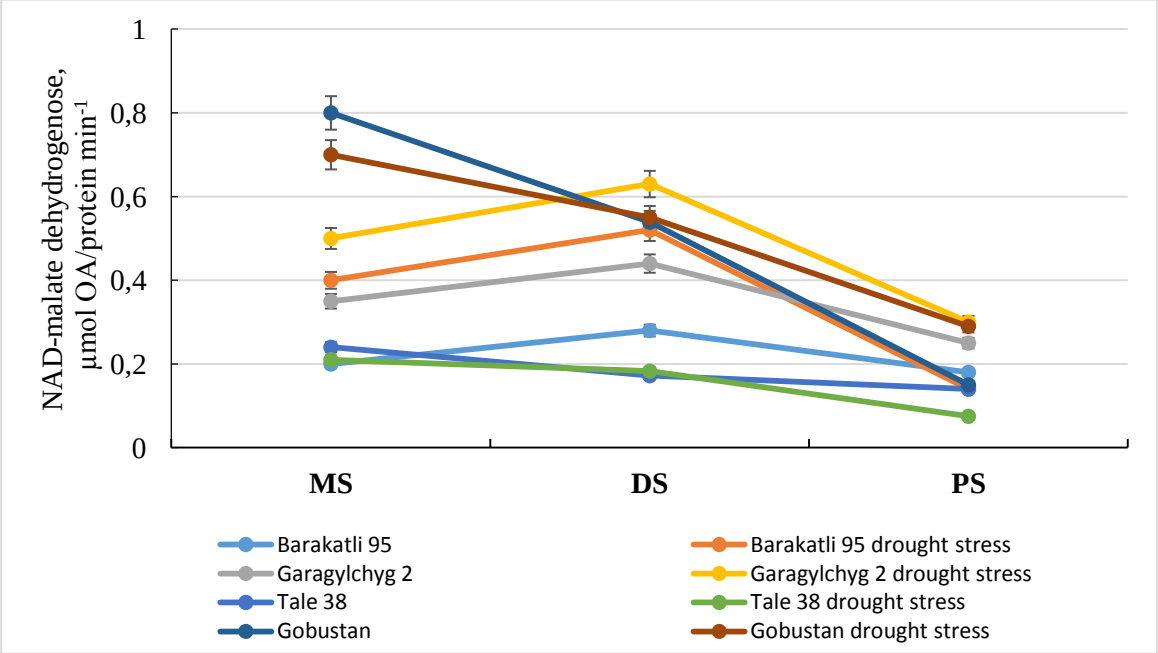

Fig 3. NAD-MDH activity in developing grains of durum (*T. Durum*) and bread (*T. aestivum*) wheat genotypes. Milky stage (MS), dough stage (DS) and physiological stage (PS) phase of grain development.

The observed increase in NAD-malate dehydrogenase (MDH) activity in drought-stressed durum wheat (1.3 to 1.4 times) supports the idea that MDH activity can be regulated by multiple nuclear loci, such as Mdh1, Mdh2, and Mdh3 in maize, which are critical for mitochondrial function [26]. While these loci are specific to maize, this suggests that a similar genetic regulation mechanism may operate in wheat, contributing to the increase in MDH activity observed in durum wheat. This implies that durum wheat genotypes may possess a stronger genetic capacity to upregulate MDH activity under stress, thereby maintaining energy production and metabolic balance.

In contrast, the slight decrease in MDH activity in drought-stressed bread wheat during grain ripening may be attributed to reduced mitochondrial efficiency under stress. Since MDH plays a key role in malate oxidation and

energy production, this reduction could indicate a decline in the efficiency of metabolic processes. Given that MDH activity is influenced by the redox state of NAD+, the decrease in bread wheat may reflect a shift in the cellular redox environment during drought, potentially leading to reduced metabolic efficiency and grain filling.

Research on the role of malate dehydrogenase (MDH) in mitochondrial function and energy balance in maize [26] and wheat [27] provides a solid foundation for understanding metabolic changes under drought conditions. Our findings extend these theories, indicating that different wheat types exhibit varying responses to drought stress. This variation may be due to the more adaptable mitochondrial system in durum wheat, which allows it to maintain higher MDH activity under drought conditions, whereas common wheat varieties show reduced enzyme activity.

4. Conclusions

The response of enzyme activity in wheat seeds to drought stress varies by genotype. In durum wheat, aspartate aminotransferase (AspAT) activity was maintained, while NAD-malate dehydrogenase (MDH) activity increased, indicating enhanced metabolic flexibility under drought conditions. On the contrary, bread wheat showed reduced activities of both AspAT and MDH, particularly in the Gobustan variety, demonstrating greater sensitivity to drought stress. These findings highlight the importance of transcriptional regulation in drought responses and suggest that durum wheat has more adaptive mechanisms to sustain growth under low-water conditions.

Funding: This work was supported by the Ministry of Science and Education of the Republic of Azerbaijan.

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