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RESEARCH ARTICLE

Changes in Some Carbon and Nitrogen Metabolism Enzymes in Field-Grown Wheat

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Abstract

Objective: The activities of alanine and aspartate aminotransferases, NAD-malate dehydrogenase in the ontogenesis have been determined in leaves of durum wheat genotypes (Barakatli 95, Garagylchyg 2) with contrasting drought tolerance, cultivated under natural soil drought conditions. **Methods/analysis:** Enzymatic activities of aspartate and alanine transferases, NAD-malate dehydrogenase were determined spectrophotometrically (Ultrospec 3300 pro, Amersham, USA). Measurements were taken at 340 nm for 1 min and the obtained results were expressed as μmol mg^{-1}*protein min^{-1}. The unequal variance two-tailed Student's t-test was applied for the analysis of the significance of differences between plants cultivated under irrigated and natural drought conditions. $P \leq 0.05$ was considered statistically significant. We used three samples for each treatment and performed the analysis twice. **Findings :** In flag leaves of the drought-tolerant Barakatli 95 genotype, the alanine aminotransferase activity increased ~2.7 and ~2.2 fold compared to the drought-sensitive Garagylchyg 2 genotype, under irrigated and natural drought conditions. According to the results, catabolism of amino acids is faster in the drought-sensitive Garagylchyg 2 genotype compared to the drought-tolerant Barakatli 95 genotype under stress. Although the activity of all three enzymes studied varies in parallel in the ontogenesis of flag leaves, it is mostly dependent on leaf water content during the day. **Novelty/improvement:** The obtained data suggest that high enzyme activities in the Barakati 95 genotype play a role in achieving drought tolerance.

Keywords: Wheat; flag leaf; natural drought conditions; aspartate aminotransferase; alanine aminotransferase; daytime

1 Introduction

Irrigation is widely used in most of the wheat fields in arid and semi-arid areas. The combination of heat stress and water deficiency in such areas during the generative development of wheat leads to a decrease in grain yield and quality indices. Most of

the substances transported to the grain are synthesized in the flag leaves of wheat.

Abiotic stresses such as drought, high temperatures inactivate photosynthetic electron transfer, and photophosphorylation, adversely affect metabolic processes of photosynthesis [1]. Due to photosynthetic limitations imposed by stomatal and non-stomatal processes [2] drought is considered the major abiotic stress impairing crop production [3,4]. Wheat, which is strategically important in terms of meeting the needs of the world population for food, is exposed to a number of stressors, including drought, during development. Drought stress is expected to reduce wheat yields by up to 20% in the future [5]. In order to prevent possible food shortages, global wheat production should reach a new record of 780 million tons in 2021 (FAO).

We aimed to study the ontogenetic and diurnal changes in NAD-MDH, AlaAT, and AspAT activities in the flag leaves of drought-tolerant and drought-sensitive durum wheat genotypes cultivated under irrigated and natural drought conditions.

Aminotransferases catalyze the interconversion of amino acids, which link carbon and amino acid metabolism, and the reversible transamination of keto-acids into amino acids. As a result, the synthesis of amino acids is enhanced [6–10]. The reversible transfer of an amino group between glutamate and oxaloacetate/pyruvate is catalyzed by aspartate aminotransferase (AspAT) and alanine aminotransferase (AlaAT) leading to the formation of 2-oxoglutarate and aspartate/alanine, respectively [11]. AspAT is involved also in the primary nitrogen assimilation, the transport of reducing equivalents, and the exchange of nitrogen (N) among subcellular compartments [12].

NAD-malate dehydrogenase (NAD-MDH, EC 1.1.1.37) is involved in metabolic processes such as TCA and glyoxylate cycle, synthesis of amino acids, glyconeogenesis, and exchange of metabolites between cytosol and subcellular organoids [13]. Maintaining a correct N: C ratio is important for plants. Therefore, various biochemical processes that enable plants to adjust their metabolism and accommodate environmental stress conditions have been developed [14].

Recent studies have shown that the increased activity of these enzymes due to abiotic stress factors has an anaplerotic function by providing the Krebs cycle with intermediate compounds. Therefore, the study of the role of the activity of these enzymes in the development of drought tolerance of durum and bread wheat genotypes grown under field conditions is of scientific and practical importance.

2 Materials and methods

Durum wheat genotypes (Barakatli 95 and Garagylchyg 2) cultivated in the experimental field of the Research Institute of Crop Husbandry located in the Absheron peninsula were used as the study materials. The plot dimensions were 1.05 m×10 m, with 15.0 cm row spacing. Irrigated samples were watered every 5 days. Natural drought condition samples were not irrigated during the entire vegetation. Samples for the study were taken on the 5^{th},9^{th}, 13^{th}, 17^{th}, and 21^{st} days of the flag leaf ontogenesis. To evaluate the daily dynamics of the enzyme activities, samples were taken also at the solar time in three-hour intervals (07:00, 10:00, 13:00, and 16:00 hours) on the 13^{th} day of the flag leaf ontogenesis. Field conditions (light intensity and temperature) are described in Table 1.

Table 1. Light intensity and air temperature during the flag leaf ontogenesis under field conditions.

Ontogenesis of the flag leaf	Light intensity (μmol (photons) m^{-2} s^{-1})	Temperature (°C)
5^{th}	150±4.0	23±2
9^{th}	600±11	31±1
13^{th}	1000±18	30±1
17^{th}	1800±15	36±2
21^{st}	2000±21	38±2

The plant material frozen in liquid nitrogen was thawed, and 200 mg of it was subjected to quick extraction using a chilled mortar and pestle with 1 ml of medium containing 100 mM Tris-HCl (pH 7.8), 8 mM MgCl2, 2 mM EDTA, 10 mM 2-Mercaptoethanol, 2 mM phenylmethylsulphonyl fluoride (PMSF) and 2% (w/v) insoluble polyvinylpyrrolidone (PVP). The supernatant obtained by centrifugation (at 13000 g for 15min) of the suspension containing cell fragments was used for the enzyme activity assays.

The AlaAT enzyme activity was determined at 25°C in a continuous assay by coupling the reaction of lactate dehydrogenase (LDH) to nicotinamide adenine dinucleotide (NADH) oxidation. The reaction mixture contained 50 mM alanine, 8 mM 2-OG, 5 μM pyridoxal 5- phosphate, 0.2 mM NADH, 2mM EDTA, 50 mM Tris-HCl (pH 8.2) and 5 units of LDH. [15]

The activity of AspAT was measured in 1 ml volume at 25 mM Tris-HCl (pH 8.5), containing 2mM EDTA, 2.5 mM 2-oxoglutarate, 5 μg/ml pyridoxal 5-phosphate, 10 mM DTT, 12 U/ml malate dehydrogenase, 0.1 mM NADH [16].

The activity of NAD-MDH was determined by adding the substrate (1 mM oxaloacetate) to the reaction medium containing 0.1 M Tris-HCl (pH 8.8), 8mM MgCl2, and 0.2 mM NAD [8]. The activity was monitored as a change in absorbance at 340 nm due to NAD+ reduction or NADH oxidation. A molar extinction coefficient for NADH of 6.22 mM-1cm-1 was used for the calculation of amounts of catalyzed NAD(H). Enzymatic activities of transferases were determined spectrophotometrically.

The method of Tambussi et al. (2005) [17] was used in the determination of relative water content at the solar time in three-hour intervals on the 13th day of ontogenesis. Protein was measured by the method of Rekowski (2021), using bovine serum albumin as the standard [18].

The significance of differences between mean values was compared by Student's t-test. Differences at $P \leq 0.05$ were considered significant.

3 Results and Discussion

The study of biochemical changes in contrasting durum wheat genotypes, caused by adverse environmental factors, which lead to a decline in plant productivity and grain quality is of great importance. For this purpose, dynamics of the activities of NAD-MDH, AlaAT, and AspAT playing an important role in carbon and nitrogen metabolism has been studied at various stages of ontogenesis, during the light period of the day, in the flag leaves of the Barakatli 95 and Garagylchyg 2 genotypes grown under irrigated and natural drought conditions [Table 2]. The same AlaAT activity was observed in the flag leaves of the drought-tolerant Barakatli 95 genotype at the beginning of ontogenesis. It was slightly higher in the flag leaves of the Garagylchyg 2 genotype cultivated under natural drought conditions compared to the irrigated variant. Under irrigated and natural drought conditions, the AlaAT activity in the flag leaves, increased ~2.7 and ~2.2 fold, respectively, compared to the drought-sensitive Garagylchyg 2 genotype.

The enzyme activity in the drought-sensitive Garagylchyg 2 genotype increased ~1.3 fold, whereas in other variants, in the middle of the ontogenesis (day 15) of flag leaves, the activity decreased sharply. At the end of the ontogenesis of flag leaves, the enzyme activity increased in both variants. In the Garagylchyg 2 genotype, AlaAT activity increased ~1.5 and ~1.8 fold, respectively, in the plants cultivated under irrigated and natural drought conditions, compared to the beginning of ontogenesis. Thus, the maximum activity was observed at the end of the ontogenesis of flag leaves (day 21), at the grain ripening stage.

Table 2. Changes in the AlaAT and AspAT activities at various stages of the ontogenesis of flag leaves in the durum wheat genotypes cultivated under irrigated and natural drought conditions

Genotype	Treatment	Ontogenesis of flag leaves				
		Day5	**Day9**	**Day15**	**Day17**	**Day21**
		AlaAT activity, μmol mg^{-1}.protein min^{-1}				
Barakatli 95	irrigated	0.97±0.12	0.76±0.09	0.54±0.06	0.93±0.11	1.13±0.14
	natural drought conditions	0.82±0.09^{ns}	1.04±0.12*	0.87±0.09*	1.65±0.19**	1.94±0.23**
Garagylchyg2	irrigated	0.35±0.04	0.32±0.04	0.28±0.03	0.45±0.05	0.56±0.07
	natural drought conditions	0.54±0.06*	0.58±0.07**	0.55±0.07**	0.79±0.09**	0.75±0.09*
		AspAT activity, μmol mg^{-1}.protein min^{-1}				
Barakatli 95	irrigated	0.11±0.01	0.21±0.03	0.34±0.04	0.12±0.01	0.18±0.02
	natural drought conditions	0.14±0.02^{ns}	0.31±0.04*	0.53±0.06*	0.34±0.04**	0.32±0.04**
Garagylchyg2	irrigated	0.27±0.03	0.17±0.02	0.13±0.02	0.13±0.02	0.17±0.02
	natural drought conditions	0.31±0.04^{ns}	0.32±0.04**	0.29±0.04**	0.33±0.04**	0.24±0.03*

Results of Student's t – test **, *– significance at the 0.01, 0.05 probability levels, respectively

The AsAT activity was higher in the flag leaves of the drought-tolerant Barakatli 95 genotype compared to the other variants at the beginning of the ontogenesis. Thus, at the beginning of the flag leaf ontogenesis of both genotypes, the AsAT activity was higher in the variants grown under natural drought conditions compared to the irrigated plants. In Barakatli 95, the enzyme activity was 2.7 fold higher under natural drought conditions compared to the irrigated variant. Similar to the AlaAT enzyme, the activity of AsAT sharply decreased in the middle of the ontogenesis of the flag leaves (except for the variants of the drought-sensitive Garagylchyg 2 genotype grown under natural drought conditions). At the end of the ontogenesis of the flag

leaf, the enzyme activity increased again. According to the results, catabolism of amino acids is faster in the drought-sensitive Garagylchyg 2 genotype compared to the drought-tolerant Barakatli 95 genotype under water stress.

Similar change patterns of NAD-malate dehydrogenase (NAD-MDH) localized in mitochondria and activities of AspAT and AlaAT playing an important role in the biosynthesis of amino acids were observed in the flag leaves of the studied genotypes during ontogenesis.

The main role of aspartate in the cell is the transport of reducing equivalents formed by glycolysis to the membranes of mitochondria via malate-aspartate shuttle. ASAT and NAD-MDH are the main enzymes of the malate–aspartate shuttle, which converts malate to Asp in the mitochondria, and the reverse reaction occurs in the cytosol[19]. Aspartate is transported from the cytosol to mitochondria by specific aspartate carriers and changes in the NAD^+ / NADH ratio catalyze the conversion of malate to ASAT and NAD-MDH[20]. In the cytosol, oxaloacetate is synthesized in a reaction catalyzed by AspAT and reduced by NADH to malate. Then, malate is transported across the mitochondrial membrane by the malate-α-ketoglutarate carrier to be oxidized by NAD^+ and converted again to oxaloacetate[21].

Table 3. Changes in the activity of NAD-MDH in flag leaves of durum wheat genotypes grown under irrigated and natural drought conditions, during various stages of ontogenesis.

Genotype	Treatment	Ontogenesis of flag leaves				
		Day 5	**Day 9**	**Day 13**	**Day 17**	**Day 21**
		NAD-MDH activity, μmol mg^{-1} . protein min^{-1}				
Barakatli 95	irrigated	79.2±3.2	50.1±2.0	39.3±1.6	43.0±1.7	48.0±1.9
	natural drought conditions	74.0±3.0	61.0±2.4**	43.7±1.8*	53.7±2.2**	60.9±2.4**
Garagylchyg 2	irrigated	40.2±1.6	24.2±1.4	28.5±1.1	33.6±1.3	47.7±1.9
	natural drought conditions	46.7±1.8*	42.2±1.7**	22.3±0.9**	45.8±1.8**	58.6±2.3**

Results of Student's t – test **, *– significance at the 0.01, 0.05 probability levels, respectively.

Activities of NAD-MDH, AlaAT, and AspAT increased in parallel from the beginning to the end of ontogenesis in both variants [Table2 and Table 3]. According to the results of our experiments, NAD-MDH, AlaAT, and AspAT activities were higher in drought-exposed plants compared to watered genotypes.

The activities of the measured enzymes changed differently over the light period of the day. The identical tendency in AlaAT activity change was observed in the flag leaves of both durum wheat genotypes under irrigated and drought conditions. Whereas, in the drought-sensitive genotype (Garagylchyg 2), its activity increased in the variant grown under natural drought conditions. AspAT activity in the studied genotypes was always higher in the afternoon hours compared to morning and evening hours [Figure 1].

The activities of the studied enzymes changed differently during the light period of the day [Figure 1]. Wheat genotypes manifested the same tendency of the change in the AlaAT activity in the flag leaves under both irrigated and drought conditions.

However, in the drought-sensitive Garagylchyg 2 genotype, AlaAT activity at 10:00 increased by 2.5 fold in the irrigated plants and ~3.7 fold in the variants grown under natural drought conditions compared to the activity detected at 7:00. At 13:00, the enzyme activity was found to decrease.

The distribution of the AlaAT enzyme in various plant tissues, in roots, stems, flowers, and fruits indicates its important role in plant growth and development. The enzyme AlaAT was previously studied in Hordeum vulgare, Medicago truncatula, and Arabidopsis plants under hypoxia[22,23]. AlaAT was studied in relation to the glycolysis and tricarboxylic acid cycle in Lotus japonicus under hypoxia caused by flooding[10]. AlaAT is a limiting factor in alanine synthesis under low oxygen conditions. Thus, the main role of AlaAT in A. thaliana was found to be the destruction of excess alanine[24].

The AlaAT enzyme activity was found to be regulated not only by hypoxia but also by light and nitrogen[25]. Many metabolic processes take place in the leaves, such as the synthesis of organic compounds, photosynthesis, and photorespiration. According to some authors, the concentration of N in leaves generally decreases with age. Genes associated with N metabolism may play an important role in the regulation of short-term N metabolism and may influence morphological changes in poplar roots by regulating fermentation, glycolysis, and tricarboxylic acid cycle (TCA), secondary metabolism, hormone metabolism, and transport processing[25].

The activity of AspAT in the studied durum wheat genotypes was higher in the afternoon hours compared to the morning and evening hours. The AspAT activity was higher in the irrigated variants of both genotypes compared to the variants grown under natural drought conditions.

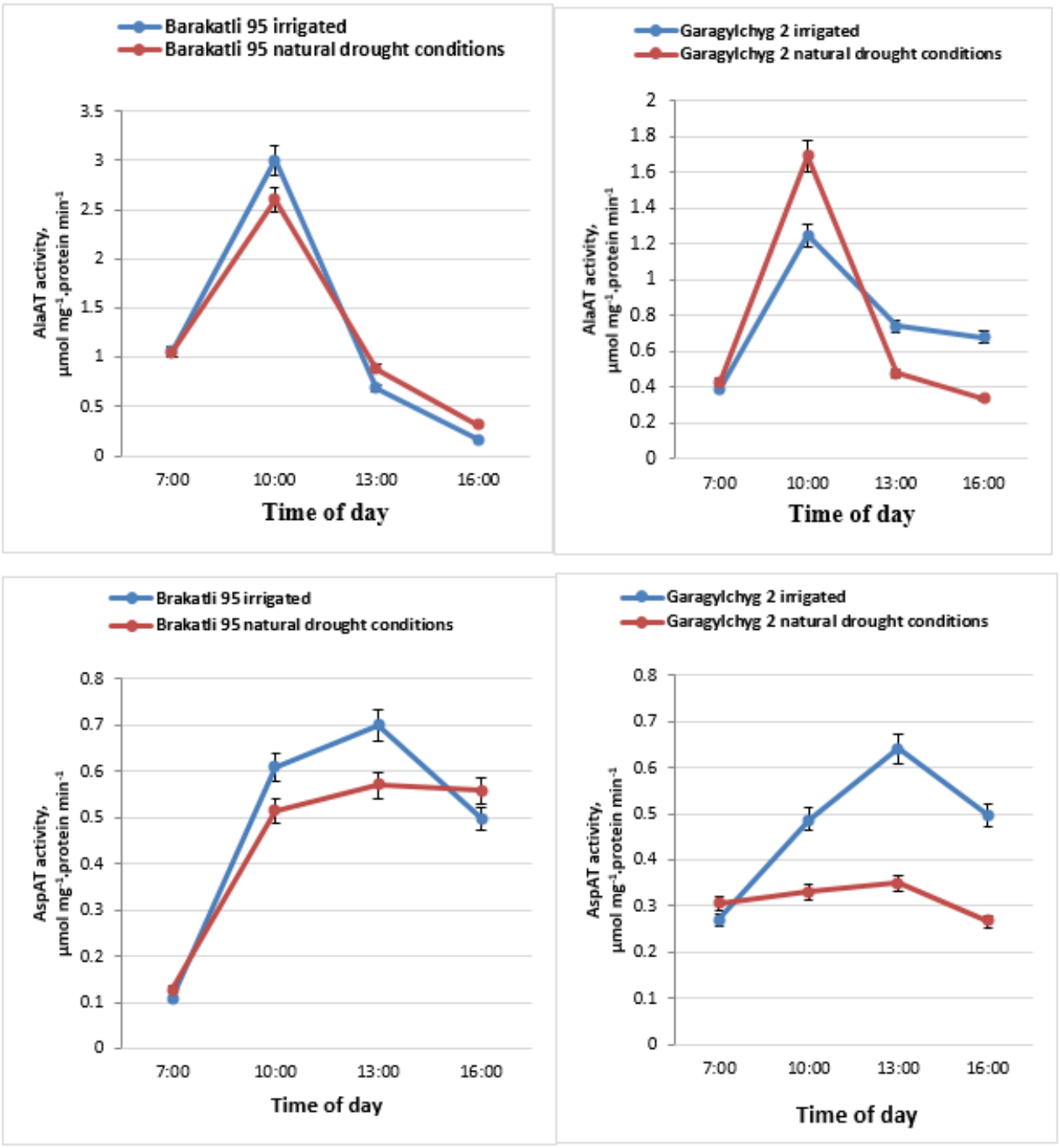

Fig 1. Changes in the AlaAT and AspAT activities during the light phase of the day, at the beginning of the ontogenesis of flag leaves, in durum wheat genotypes cultivated under irrigated and natural drought conditions.

As seen in Figure 2, the maximum NAD-MDH activity was observed in the flag leaves of both genotypes in the morning hours. The NAD-MDH activity in the flag leaves of the studied wheat genotypes was similar in the irrigated plants and variants grown under natural drought conditions during the morning hours. The enzyme activity decreased sharply in the afternoon hours (at 10:00 and 13:00) with the increasing light intensity and temperature. In the afternoon hours, the enzyme activity decreased ~2.2 fold in the flag leaves of the irrigated plants and variants of the drought-tolerant Barakatli 95 genotype grown under natural drought conditions. It decreased ~2.2 fold in the flag leaves of the drought-sensitive Garagylchyg genotype grown under irrigated conditions similar to the Barakatli 95 genotype. In the variant of the Garagylchyg 2 genotype grown under natural drought conditions, the NAD-MDH activity decreased only ~1.6 fold in all variants. Contrary to Barakatli 95, in the Garagylchyg 2 genotype, NAD-MDH activity increased again at 16.00 and approached values obtained in the morning hours. Under natural drought conditions, the enzyme activity was 1.6 fold higher in flag leaves of the Garagylchyg 2 genotype at 16.00 compared to that at 13.00, while no change was observed in the irrigated variant.

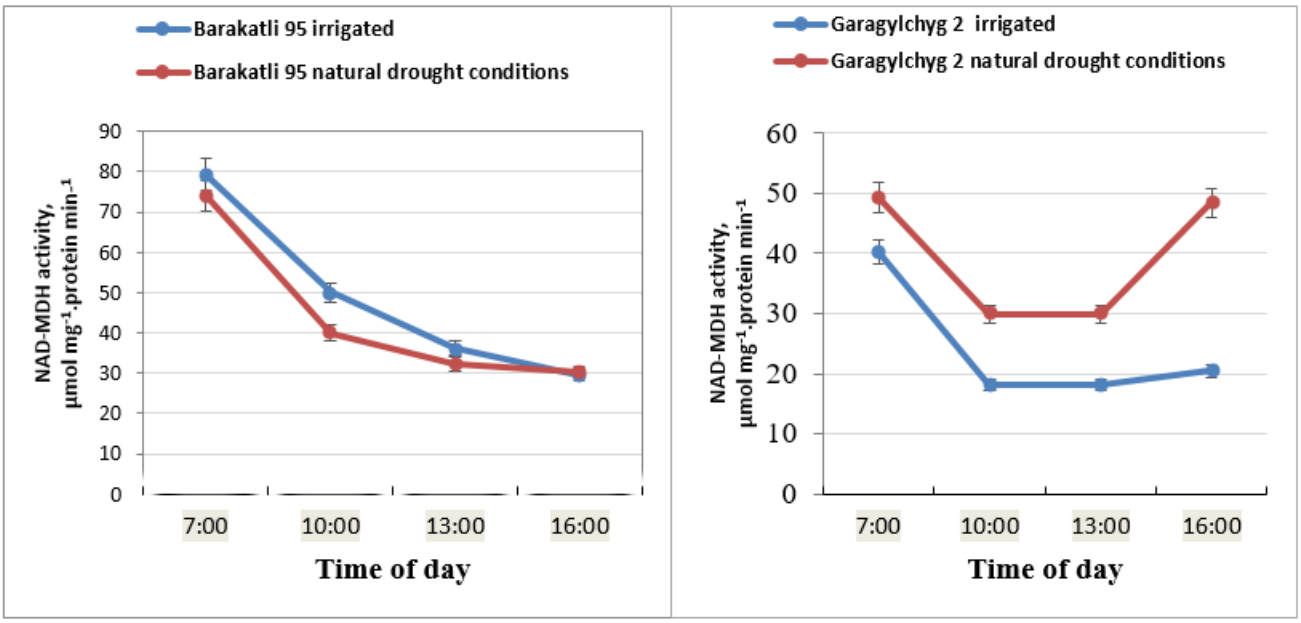

Fig 2. Changes in the NAD-MDH activities during the light phase of the day, at the beginning of the ontogenesis of flag leaves, in durum wheat genotypes cultivated under irrigated andnatural drought conditions.

The NAD-MDH activity in the flag leaves of the drought-sensitive Garagylchyg 2 genotype, contrary to other objects of the study, increased in the evening hours approaching the values detected in the morning hours.

Relative water content, relative humidity of the air, and soil humidity are known to regulate water balance among plant leaves, soil, and the atmosphere. The decrease of these indices plays a role in the signal for launching a plant protection system [26].

Similar changes in RWC during the day were observed in both genotypes and RWC was found to be higher in the morning and evening hours compared to the values obtained in the afternoon [Figure 3]. However, when the plants are under high solar intensity and temperature, relatively low RWC was observed in the drought-sensitive Garagylchyg 2. Besides, the total activity of the enzymes is relatively higher in Barakatli 95 compared to Garagylchyg 2. It is known that there is a connection between changes in the RWC of wheat leaves and plant tolerance to drought.

Higher plants cannot escape adverse environmental conditions that are a constant threat throughout their life cycle. Unfavorable growth conditions such as extreme temperatures (cold, freezing, heat), drought (deficient precipitation, drying winds), and contamination of soils with high salt concentrations are considered the major abiotic environmental stressors that limit plant growth and development [10]. Thus, we can conclude that mitochondrial NAD-MDH catalyzes the oxidation of NADH, reduced in the reaction of glycine decarboxylation during photorespiration thereby intensifying the biosynthesis of 2-oxoglutarate, involved in the synthesis of amino acids playing the role of the carbon skeleton. Amino acid catabolism is important during normal senescence as well as in the stress tolerance of wheat. Besides, until the photosynthetic apparatus is fully functional, amino acid oxidation and degradation of other storage compounds, such as fatty acids and starch, have to meet

the energy demand of the young plants[11]. To maintain the carbon-nitrogen balance throughout plants, AlaAT activity may be important for the translocation of either Ala or pyruvate. Amino acid biosynthesis is up-regulated to provide substrates for the highly active protein synthesis in growing photosynthetically active cells. During this stage, protein turnover and amino acid degradation are less important. In addition to their role as a protein constituent, which requires tightly controlled steady-state levels, amino acids have several functions.

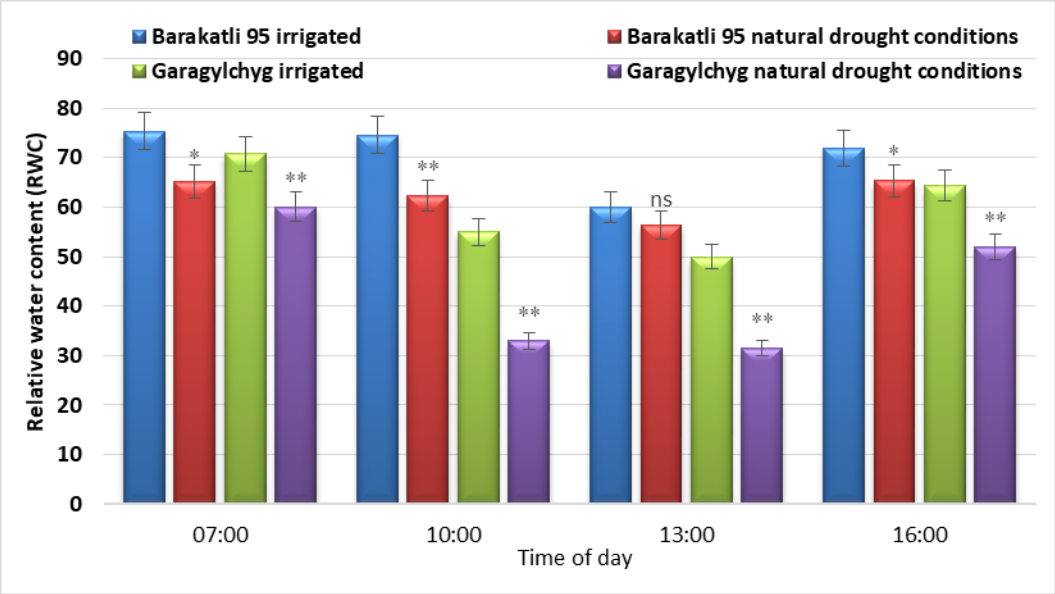

Fig 3. Effect of drought stress on flag leaf relative watercontent. Each value represents the mean of 3 replicates. Results of Student's t – test **, *–significance at the 0.01, 0.05 probability levels, respectively.

Cytosolic NAD-MDH, which catalyzes the conversion of malate to oxalate acetate, regulates relations between glycolysis and the TCA cycle[27,28]. Previous studies showed that metabolic processes in mitochondria, including the TCA cycle, are regulated depending on the hours of the day[29]. By studying NAD-MDH, which plays an important role in the distribution of carbon and energy in plants, it is possible to come up with new ideas in terms of ensuring the connection between rapid plant growth, respiration, and photosynthesis.

Our previous research on wheat genotypes, grown under field conditions, revealed an increase in the activity and intensity of the mitochondrial izoform of NAD-MDH under drought stress. Besides, the activity of the pyruvate dehydrogenase complex isolated from mitochondria of the pea (*Pisum sativum* L.) and barley (*Hordeum vulgare* L.) leaves was stimulated upon adding malate to the medium[30]. According to the authors, the effect of malate is likely not due to a direct activation but to the involvement of malate dehydrogenase in the recycling of NADH formed in the reaction of the pyruvate dehydrogenase complex. Whereas, other authors believe that the alterations of the MDH level in mitochondria significantly decrease the capacity for photorespiration and also affect respiratory rates in leaves[31].

A significant increase in the activities of AlaAT and AspAT, observed in the drought-tolerant Barakatli 95 genotype, is attributed to the high glutamate demand and maintenance of the Krebs cycle to achieve the correct C: N status. Glutamate is a major amino group donor that enables the AlaAT and AspAT functions. During the recovery period, the production of amino acid-derived secondary metabolites was up-regulated specifically. This indicates that synthesis rates of the precursor amino acids increased. Thus, it is suggested the existence of a strong relationship between amino acid metabolism and stress responses. AlaAT and AspAT activities have previously been reported to increase under salt stress in wheat seedlings[32]. Different strategies to minimize the adverse effects of abiotic stress factors have been evolved by plants and some of them are connected to amino acid metabolism[1]. Reactions catalyzed by aminotransferases are reversible. Alanine and aspartate may also be involved in the replenishment of the glutamate pool[18]. The important role of the studied aminotransferases in nitrogen mobilization is indisputable. Because the main part of the nitrogen is transported from flag leaves to seeds in the form of glutamate. Thus, according to the results of our research, mitochondrial NAD-MDH catalyzes the oxidation reaction of NADH, reduced by glycine decarboxylation during photorespiration, and this enzyme intensifies the biosynthesis of 2-oxoglutarate which is involved in the synthesis of amino acids playing a role of the carbon skeleton.

4 Conclusion

The results of the study of the physiological functions and biochemical properties of NAD-MDH, AspAT, ALaAT enzymes in cell metabolism, which play an important role in the formation of adaptation mechanisms against the effects of drought in wheat genotypes can be used in the selection of drought-tolerant, productive new plant varieties and in solving many environmental problems. The results obtained are of fundamental importance in terms of a deeper understanding of the plant adaptation mechanisms to abiotic stressors. They can be very useful in understanding the mechanisms of changes in plant carbon and nitrogen metabolism during drought. According to the results, the Barakatli-95 genotype, which demonstrates high physiological performance during drought, can be used as a starting material for developing stress-tolerance wheat varieties in practical breeding programs.

Abbreviations

DTT - Dithiothreitol, EDTA - Ethylenediaminetetraacetic acid, PVPP –polyvinylpolypyrrolidone, LDH - lactate dehydrogenase, NADH - nicotinamide adenine dinucleotide, AspAT - aspartate aminotransferase, AlaAT - alanine aminotransferase, NAD -MDH - NAD-malate dehydrogenase, OAA- Oxaloacetate, 2-OG- 2-Oxoglutarate, TCA -Tricarboxylic acid

References

1) Baslam M, Mitsui T, Sueyoshi K, Ohyama T. Recent Advances in Carbon and Nitrogen Metabolism in C3 Plants. *International Journal of Molecular Sciences*. 2020;22(1):318–318. Available from: https://dx.doi.org/10.3390/ijms22010318.
2) Dahal K, Wang J, Martyn GD, Rahimy F, Vanlerberghe GC. Mitochondrial Alternative Oxidase Maintains Respiration and Preserves Photosynthetic Capacity during Moderate Drought in Nicotiana tabacum. *Plant Physiology*. 2014;166(3):1560–1574. Available from: https://dx.doi.org/10.1104/pp.114.247866.
3) Michae M, Ankerst DP, Annette M. Interactions between temperature and drought in global and regional crop yield variability during 1961-2014. *PLoS One*. 1961;12:178339–178339. Available from: 10.1371/journal.pone.0178339.
4) Ali R, Ali R, Saher MS, Xiling Z, Xuekun Z, Yan L. Xu Jinsong Impact of Climate Change on Crops Adaptation and Strategies to Tackle Its Outcome: A Review. *Plants (Basel)*. 2019;8(2):34–34. doi:doi: 10.3390/plants8020034.
5) Leng G, Hall J. Crop yield sensitivity of global major agricultural countries to droughts and the projected changes in the future. *Science of The Total Environment*. 2019;654:811–821. Available from: https://dx.doi.org/10.1016/j.scitotenv.2018.10.434.
6) Batista-Silva W, Heinemann B, Rugen N, Nunes-Nesi A, Araújo WL, Braun HP, et al. The role of amino acid metabolism during abiotic stress release. *Plant, Cell & Environment*. 2019;42(5):1630–1644. Available from: https://dx.doi.org/10.1111/pce.13518.
7) Hildebrandt TM, Nesi AN, Araújo WL, Braun HP. Amino Acid Catabolism in Plants. *Molecular Plant*. 2015;8(11):1563–1579. Available from: https://dx.doi.org/10.1016/j.molp.2015.09.005.
8) Liszka A, Schimpf R, Zaruma KIC, Buhr A, Seidel T, Walter S, et al. Three cytosolic NAD-malate dehydrogenase isoforms of Arabidopsis thaliana: on the crossroad between energy fluxes and redox signaling. *Biochemical Journal*. 2020;477(19):3673–3693. Available from: https://dx.doi.org/10.1042/bcj20200240.
9) McAllister CH, Good AG. Alanine Aminotransferase Variants Conferring Diverse NUE Phenotypes in Arabidopsis thaliana. *PLOS ONE*. 2015;10(4):e0121830–e0121830. Available from: https://dx.doi.org/10.1371/journal.pone.0121830.
10) Marcio R, Francesco L, Wagner LA, Adriano NN, Ladaslav S, FernieAlisdair, et al. Fernie Alisdair, and van Dongen Joost Glycolysis and the Tricarboxylic Acid Cycle Are Linked by Alanine Aminotransferase during Hypoxia Induced by Waterlogging of Lotus japonicas. *Plant Physiology*. 2010;152(3):1501–1513. Available from: https://doi.org/10.1104/pp.109.150045.
11) Gaufichon L, Rothstein SJ, Suzuki A. Asparagine Metabolic Pathways in Arabidopsis. *Plant and Cell Physiology*. 2016;57(4):675–689. Available from: https://dx.doi.org/10.1093/pcp/pcv184.
12) Guo R, Shi L, Ding X, Hu Y, Tian S, Yan D, et al. Effects of Saline and Alkaline Stress on Germination, Seedling Growth, and Ion Balance in Wheat. *Agronomy Journal*. 2010;102(4):1252–1260. Available from: https://dx.doi.org/10.2134/agronj2010.0022.
13) Schertl P, Braun HP. Respiratory electron transfer pathways in plant mitochondria. *Frontiers in Plant Science*. 2014;5:163–163. Available from: https://dx.doi.org/10.3389/fpls.2014.00163.
14) Galili G, Avin-Wittenberg T, Angelovici R, Fernie AR. The role of photosynthesis and amino acid metabolism in the energy status during seed development. *Frontiers in Plant Science*. 2014;5:447–447. Available from: https://dx.doi.org/10.3389/fpls.2014.00447.
15) Naliwajski MR, Skłodowska M. The relationship between carbon and nitrogen metabolism in cucumber leaves acclimated to salt stress. *Plant Biology*. 2018;6:e6043–e6043. Available from: https://dx.doi.org/10.7717/peerj.6043.
16) Alfonso SU, Brüggemann W. Photosynthetic responses of a C3 and three C4 species of the genus Panicum (s.l.) with different metabolic subtypes to drought stress. *Photosynthesis Research*. 2012;112(3):175–191. Available from: https://dx.doi.org/10.1007/s11120-012-9763-4.
17) Tambussi EA, Nogués S, Araus JL. Ear of durum wheat under water stress: water relations and photosynthetic metabolism. *Planta*. 2005;221(3):446–458. Available from: https://dx.doi.org/10.1007/s00425-004-1455-7.
18) Rekowski A, Langenkämper G, Dier M, Wimmer MA, Scherf KA, Zörb C. Determination of soluble wheat protein fractions using the Bradford assay. *Cereal Chemistry*. 2021;98(5):1059–1065. Available from: https://dx.doi.org/10.1002/cche.10447.
19) Borst P. The malate–aspartate shuttle (Borst cycle): How it started and developed into a major metabolic pathway. *IUBMB Life*. 2020;72(11):2241–2259. Available from: https://dx.doi.org/10.1002/iub.2367.
20) Easlon E, Tsang F, Skinner C, Wang C, Lin SJ. The malate–aspartate NADH shuttle components are novel metabolic longevity regulators required for calorie restriction-mediated life span extension in yeast. *Genes & Development*. 2008;22(7):931–944. Available from: https://dx.doi.org/10.1101/gad.1648308.
21) Monné M, Miniero DV, Bisaccia F, Fiermonte G. The mitochondrial oxoglutarate carrier: from identification to mechanism. *Journal of Bioenergetics and Biomembranes*. 2013;45(1-2):1–13. Available from: https://dx.doi.org/10.1007/s10863-012-9475-7.

22) de Sousa CAF, Sodek L. Alanine metabolism and alanine aminotransferase activity in soybean (Glycine max) during hypoxia of the root system and subsequent return to normoxia. *Environmental and Experimental Botany*. 2003;50(1):1–8. Available from: https://dx.doi.org/10.1016/s0098-8472(02)00108-9.
23) Claudie R, Orcaray EL, Jean-Bernard C. Limami Anis M. Characterization of alanine aminotransferase (AlaAT) multigene family and hypoxic response in young seedlings of the model legume Medicago truncatula. *Journal of Experimental Botany*. 2006;57(12):3079–3089. doi:10.1093/jxb/erl069.
24) Miyashita Y, Dolferus R, Ismond KP, Good AG. Alanine aminotransferase catalyses the breakdown of alanine after hypoxia in Arabidopsis thaliana. *The Plant Journal*. 2007;49(6):1108–1121. Available from: https://dx.doi.org/10.1111/j.1365-313x.2006.03023.x.
25) Xu Z, Ma J, Qu C, Hu Y, Hao B, Sun Y, et al. Identification and expression analyses of the alanine aminotransferase (AlaAT) gene family in poplar seedlings. *Scientific Reports*. 2017;7(1):45933–45933. Available from: https://dx.doi.org/10.1038/srep45933.
26) Driesen E, Van Den Ende W, Proft MD, Saeys W. Influence of Environmental Factors Light, CO2, Temperature, and Relative Humidity on Stomatal Opening and Development: A Review. *Agronomy*. 2020;10(12):1975–1975. doi:10.3390/agronomy10121975.
27) Yongqiang C, Zhiyuan F, Hui Z, Runmiao T, Huili Y, Canran S, et al. Tang Jihua Cytosolic malate dehydrogenase 4 modulates cellular energetics and storage reserve accumulation in maize endosperm. *Plant Biotechnology Journal*. 2020;18(12). Available from: https://doi.org/10.1111/pbi.13416.
28) Selinski J, Scheibe R. Malate valves: old shuttles with new perspectives. *Plant Biology*. 2019;21(S1):21–30. Available from: https://dx.doi.org/10.1111/plb.12869.
29) Omini J, Wojciechowska I, Skirycz A, Moriyama H, Obata T. Association of the malate dehydrogenase-citrate synthase metabolon is modulated by intermediates of the Krebs tricarboxylic acid cycle. *Scientific Reports*. 2021;11(1):18770–18770. Available from: https://dx.doi.org/10.1038/s41598-021-98314-z.
30) Abir I, George R, Gupta K, Kapuganti J. Plant mitochondria: source and target for nitric oxide. *Mitochondrion*. 2014;19:329–333. doi:10.1016/j.mito.2014.02.003.
31) Tomaz T, Bagard M, Pracharoenwattana I, Lindén P, Lee CP, Carroll AJ, et al. Mitochondrial Malate Dehydrogenase Lowers Leaf Respiration and Alters Photorespiration and Plant Growth in Arabidopsis. *Plant Physiology*. 2010;154(3):1143–1157. Available from: https://dx.doi.org/10.1104/pp.110.161612. doi:10.1104/pp.110.161612.
32) Häusler RE, Ludewig F, Krueger S. Amino acids – A life between metabolism and signaling. *Plant Science*. 2014;229:225–237. Available from: https://dx.doi.org/10.1016/j.plantsci.2014.09.011. doi:10.1016/j.plantsci.2014.09.011.