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The effect of soil drought on malate-aspartate shuttle enzyme levels in wheat genotypes

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One of the major agricultural issues is the decrease in productivity of bread wheat varieties grown in Azerbaijan due to water scarcity caused by climate change. In this regard, drought, one of the abiotic stress factors that can directly or indirectly affect plant metabolism, has remained an active discussion topic of plant metabolism research in recent decades. The highest ratio between the variants was observed in the Murov 2 variety. As a result of studying the enzymes (AspAT and NAD-MDH) of the malate-aspartate shuttle, which plays an important role in increasing the productivity of wheat plants, it was found that the activity of both enzymes in drought-tolerant genotypes is higher compared to sensitive genotypes. AspAT, NAD-MDH, and NAD+/NADH play important roles in plant development and stress response.

Keywords: *Wheat, malate-aspartate suttle, stress, drought*

INTRODUCTION

Rapid population growth, declining biodiversity, and limited suitable and productive agricultural land pose a serious threat to meeting the food needs of the population in a globalized world (Ghatak et al., 2022, Padhan et al., 2020). In this regard, drought, one of the abiotic stress factors that can directly or indirectly affect plant metabolism, has remained an active topic of discussion in recent decades of research in this area (Chen et al., 2019; Kapoor et al., 2020). The enzymes aspartate aminotransferase (AspAT/ASAT/AAT, EC 2.6.1.1) and NAD-malate dehydrogenase (L-malate-NAD-oxidoreductase, NAD-MDH, EC 1.1.1.37) the primary enzyme in the malate–aspartate shuttle. provide a connection between carbohydrate metabolism and amino acid metabolism, playing an important role in both processes of catabolism and anabolism. The enzyme malate dehydrogenase, which catalyzes the mutual conversion of malate and oxaloacetate, is found in all living organisms and subcellular organelles (mitochondria, glyoxysomes, peroxisomes, chloroplasts) (Liszka et al., 2020). AspAT mediates the transport of α-amine groups between aspartate and glutamate. It catalyzes the reversible reaction converting aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. Aspartate is an intermediate metabolite of amino acid metabolism and the Krebs cycle. It is involved in the growth and development of plants and in the formation of the mechanism of stress tolerance (Han et al., 2021; Jia et al., 2016; Ullah et al., 2017; Gargallo-Garriga et al., 2018; Khan et al., 2019; Barickman et al., 2020; Zhang et al., 2017). The main role of aspartate in eukaryotic cells is to transport reducing equivalents synthesized as a result of glycolysis across the mitochondrial membrane and to participate in the generation of ATP (Borst, 2020). Aspartate synthesized in mitochondria is transported to the cytosol using special transporters. Aspartate in the cytosol is converted back to malate by AspAT and MDH (Singh et al., 2022), resulting in an increase in the NAD+/NADH ratio (Easlon et al., 2008, Borst, 2020). The NAD+/NADH ratio integrates many

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aspects of metabolism and plays an important role in plant development and stress response. A detailed study and investigation of aspartate anabolism and catabolism and associated pathways (i.e. Asp family amino acids, nucleotides, NAD, Krebs cycle and glycolysis) is extremely important to expand our knowledge of cell division, growth, and self-renewal.

During the daytime, malate is accumulated in leaf cells, particularly, in the vacuole, whereas the cytosolic malate concentration is maintained at a relatively constant, low level (Gerhardt et al., 1987). Some cytosolic malate is imported into the peroxisomes, where it is used by peroxisomal NAD-MDH to recycle OAA and regenerate the NADH required for hydropyruvate reduction (Selinski and Scheibe, 2019). In mitochondria, both the malate-OAA shuttle (Kramer and Evans, 2011; Vishwakarma et al., 2015; Yoshida et al., 2007; Noguchi and Yoshida, 2008; Zakhartsev et al. 2016; Zhao et al. 2018; Pastore 2007) and photorespiration (Gardeström and Wigge, 1988; Igamberdiev et al., 2001) have been proposed to provide reducing equivalents to the mitochondrial electron transport chain (mETC) for ATP production. In a flux balance model of the mature leaves of C3 plants, both photorespiration and the malate OAA shuttle are predicted to contribute to feeding NADH into the mETC (Cheung et al., 2014). Experimental data obtained from barley leaf protoplasts (Gardeström and Wigge 1988; Igamberdiev et al., 2001; Gardeström and Igamberdiev 2016) and isolated mitochondria (Lee et al., 2010) suggest that photorespiration is the major source of reducing equivalents to the mETC. However, this has not been examined at a whole plant level, and the direction of the flow of reducing equivalents between different subcellular compartments during photosynthesis has not yet been fully resolved (Shameer et al., 2019). Here, by employing NADPH and NADH/NAD+ sensors, we examined plant dynamic changes in the NADPH pools and NADH/NAD+ ratio in the stroma and cytosol upon illumination.

The study aimed to look into the role of malate-aspartate shuttle enzymes (AspAT and NAD-MDH) in increasing the productivity of wheat.

MATERIALS AND METHODS

Four wheat genotypes from the gene bank of the Research Institute of Crop Husbandry of the Ministry of Agriculture were chosen for the study: Murov 2, Aran, Gyzyl Bugda, and Zirva 85. The bread wheat genotypes were grown in a laboratory with an artificial climate under a photoperiod of 16h/8 h and a temperature of 240C/180C, day/night mode, respectively, and a relative humidity of 50%. 14-day-old seedlings were subjected to drought stress. The measurements were carried out in two variants, 10 biological and 3 technical replicates.

To determine the enzymatic activity, the leaves were washed with distilled water, dried on filter paper, and crushed for 3 minutes in a mortar using 100 mM Tris-HCl (pH 7.8) buffer containing 5 mM DTT, 5 mM $MgCl_2 \cdot 6H_2O$, 1 mM EDTA ·4Na, 0.5% Triton X-100 and 1% PVP. After filtering the resulting homogenate, the filtrate was first centrifuged at 1000 g for 10 minutes and then at 5000 g for 30 minutes. This process was carried out at a temperature of +4°C. The supernatant was used to determine the activity of the enzymes.

NAD-MDH activity was determined by the spectrophotometric method (Ultrospec 3300 pro, Amersham, USA) (Scheibe and Stitt, 1988). The reaction medium consisted of 100 mM Tris-HCl (pH 8.0) buffer containing 1 mM oxaloacetate, 10 mg/ml bovine serum albumin (BSA), 10 mM MgCl2, 0.15 μM NADH and 5-10 μl of the enzyme preparation. The reaction was initiated by adding 1 mM oxaloacetate to the medium. The medium for the direct reaction consisted of 100 mM Tris-HCl (pH 9.0), 30 mM malate, 0.2 mM NAD. Measurements were carried out in spectrophotometric cuvettes with a volume of 1.0 ml. The amount of NADH was determined by the decrease in the optical density of the molar concentration of this compound at a wavelength of 340 nm for 1 minute.

The reaction medium for determining aspartate aminotransferase activity consisted of 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, and 0.2 mM NADH. The reaction was initiated by adding 20 μl of leaf

extract and 2.5 mM L-aspartate to the medium (Alfonso and Brüggemann 2012).

Total soluble protein was determined using 0.12% Coomassie Brilliant Blue G-250 solution. Optical density measurements were carried out using a spectrophotometer (Ultrospec 3300 pro, Amersham) at a wavelength of 610 nm. Bovine serum albumin was used to construct the calibration curve (Sedmak and Grossberg, 1977).

All experiments were performed in triplicate and errors were determined using the Student's t-test statistical analysis program. Differences between mean values were considered significant at P values <0.01, 0.005.

RESULTS AND DISCUSSION

The activity of NAD-malate dehydrogenase was determined in the leaves of bread wheat varieties grown under artificial climate conditions. NAD-MDH activity in the leaves of the Zirva 85 variety increased 1.5 times under stress (17.38±2.0 µmol OA/protein min^{-1}) compared to samples grown under normal watering (11.4±1.2 µmol OA /protein min^{-1}). The activity of the NAD-MDH enzyme in the varieties Aran and Murov 2 was close to that of the variety Zirva 85 (Figure 1).

Unlike other varieties, in the leaves of the Gyzyl Bugda variety, the activity of the NAD-MDH enzyme decreased by 1.2 times (17.6 ± 1.8 µmol OA/protein min^{-1}) under stress conditions compared to samples (15.0 ±1.45 µmol OA/protein min^{-1}) grown under normal watering. The highest NAD-MDH enzyme activity was observed in leaves of the Aran variety (18.08 ± 2.0 µmol OA/protein min^{-1}) under stress (Figure 2). Malate is involved in many physiological processes, such as providing NADH for the nitrate reduction reaction, fatty acid biosynthesis for the carbon chain and photorespiration, stomatal movement through regulation of osmotic pressure, control of cellular pH, redox hemostasis, as well as transport and exchange of reduced equivalents between cellular compartments. The synthesis of malate is the result of the sequential action of PEPC and MDH. In this work, we studied the activity of the enzyme aspartate aminotransferase in flag leaves of wheat varieties (Gurbanova et al., 2021).

The activity of the AspAT enzyme was higher in stress variants of all studied wheat varieties compared to plants grown under normal watering. The activity of the AspAT enzyme increased 2 times in leaf samples of the Zirva 85 variety under stress conditions (0.116±0.02 µmol mg^{-1} protein min^{-1}) compared to the watering variant (0.059±0.007 µmol mg^{-1} protein min^{-1}). Among the studied varieties of bread wheat, the highest indicator was found in the Zirva 85 variety grown under drought conditions. The enzyme activity in the Aran variety under drought (0.056±0.007 µmol mg^{-1} protein min^{-1}) was slightly higher compared to the control plants (0.054±0.008 µmol mg^{-1} protein min^{-1}).

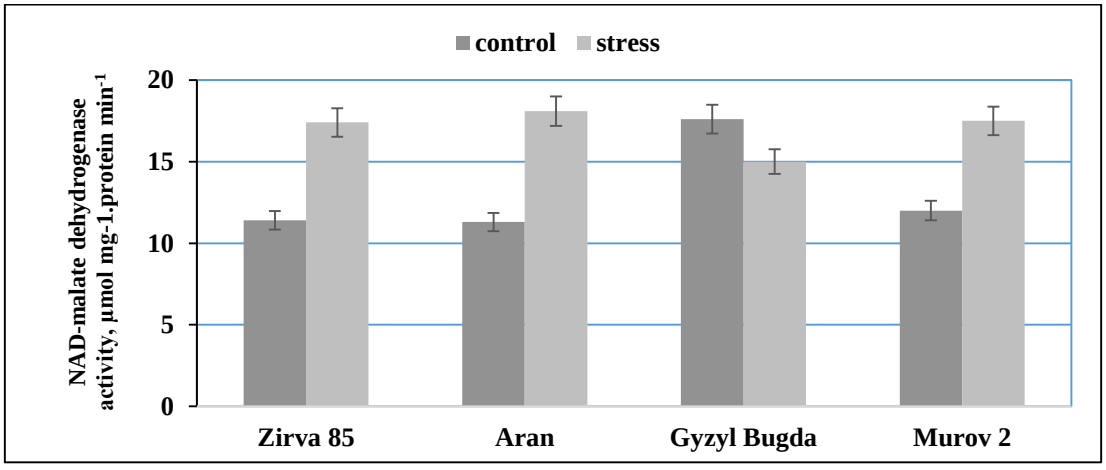

Fig. 1. Changes in NAD-malate dehydrogenase activity in leaves of bread wheat varieties grown under artificial climate conditions

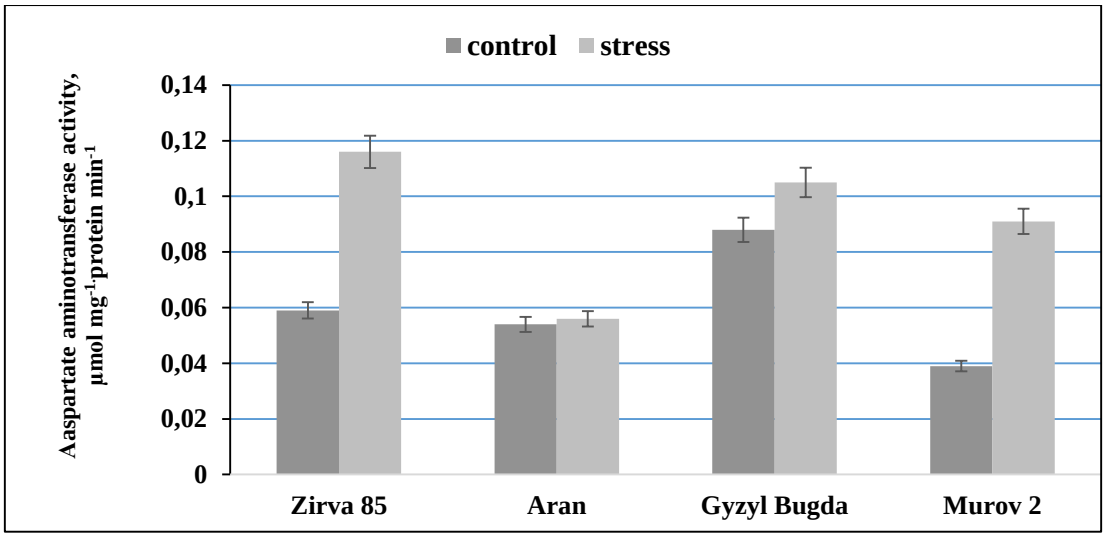

Fig. 2. Changes in aspartate aminotransferase activity in leaves of bread wheat varieties grown under artificial climate conditions.

The activity of the AspAT enzyme in the control and experimental variants was, respectively, 0.088±0.01 µmol mg^{-1}.protein min^{-1} and 0.105±0.001 µmol mg-1.protein min-1. In this variety, the activity of the AspAT enzyme in the experimental variant was two times higher than the value of the control variant. The activity of the AspAT enzyme was 2.3 times higher in plants of the Murov 2 variety (0.091±0.001 µmol mg^{-1}protein min^{-1}) under drought compared to the control plants (0.039 µmol mg^{-1}.protein min^{-1}). The highest ratio between the variants was observed in the Murov 2 variety. As a result of studying the enzymes (AspAT and NAD-MDH) of the malate-aspartate shuttle, which plays an important role in increasing the productivity of wheat plants, it was found that the activity of both enzymes in drought-tolerant genotypes is higher compared to sensitive genotypes. AspAT, NAD-MDH, and NAD^+/NADH play important roles in plant development and stress response. Lim and colleagues suggest that, at the light intensities we used, photorespiration supplies a large amount of reducing equivalents to mitochondria during photosynthesis, which exceeds the NADH dissipating capacity of the mETC. Consequently, the surplus NADH must be exported from the mitochondria to the cytosol through the mitochondrial malate-OAA shuttle (Lim et al., 2020).

The malate shuttle, which contributes to photorespiration at multiple levels, is involved in the transamination of glyoxylate into glycine coupled with the conversion of glutamate to 2-oxoglutarate in the peroxisome. This reaction is catalyzed by glutamate:glyoxylate aminotransferase (GGT). The plastidial malate/2-oxoglutarate shuttle transports glutamate from the chloroplast, which acts as an NH3 donor for glyoxylate transamination. According to the results obtained, the studied enzymes of the malate-aspartate shuttle play an important role in the adaptation processes of higher plants - the distribution of carbon and energy.

CONFLICT OF INTEREST

There is no conflict of interest in the present study.

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