

Research Article

Physiological and Biochemical Insights into NADP-Dependent Malate Metabolism under Drought Stress in Bread Wheat

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Abstract

The diverse and essential functions of NADP-MDH and NADP-ME highlight their important roles in carbon allocation and energy metabolism in higher plants. These enzymes provide valuable insights into the biochemical and molecular mechanisms linking photosynthesis, respiration, and photorespiration. Therefore, the activities of two key enzymes involved in malate metabolism, NADP-malate dehydrogenase (NADP-MDH) and NADP-malic enzyme (NADP-ME), were evaluated in the leaves of bread wheat (*Triticum aestivum* L.) genotypes differing in drought tolerance. Among the genotypes studied, the highest NADP-MDH activity was recorded in Murov 2 and Zirva 85. These results indicate that Murov 2 may possess enhanced metabolic adaptation to drought conditions and could serve as a promising genetic resource for breeding programs aimed at developing drought-tolerant wheat varieties, particularly for rainfed and semi-arid environments.

Keywords: drought stress, malate metabolism, NADP-MDH, NADP-ME.

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1. Introduction

In addition to being a period of economic growth, the 21st century also plays an important role in increasing the productivity of cultivated species as a period of intensive study of metabolic processes in plants and the development of methodology in plant research [1]. In the globalized world, the rapid increase in population, the decrease in biodiversity, and the limitation of suitable and productive land areas for agriculture pose a serious threat to meeting people's food needs [2]. In this regard, the direct or indirect effects of abiotic stress factors, including drought, on photosynthesis remain a subject of active discussion among scientists in this field in recent decades [3].

Wheat is an important food product in the world and constitutes 18-20% of human dietary protein [4]. In this context, studying malate dehydrogenase system enzymes with oxidoreductase and decarboxylase activity, which play an important role in increasing wheat productivity, is important [5]. The enzyme NADP-ME reduces NAD(P)⁺ to NAD(P)H in the oxidative decarboxylation of L-malate (MAL) to pyruvate (PYR) [6]. NADP-malic enzyme participates in numerous metabolic processes and plays an important role in plant tolerance to various stress factors. It is known that stress factors such as drought, salinity, and cold affect the entire metabolism by impairing the development of plants. Being a product of the reaction catalyzed by NADP-ME, NADPH, as one of the main factors determining cell development, is involved in the biosynthesis of osmotically active substances such as proline, mannose-6-phosphate, and mannitol. Stress affects the water balance, leading to loss of turgor and membrane breakdown. This condition, caused by stress in the plant cell, requires the regeneration of membrane

lipids. NADP-ME participates in the regeneration process by directing pyruvate and NADPH to the biosynthesis of fatty acids [7].

The NADP-MDH enzyme converts oxaloacetate into malate using excess NADPH to regenerate the NADP electron acceptor. The activity level of the enzyme is higher in the young leaves of the plant and decreases as the leaves age. This fact indicates that the enzymatic activity of NADP-MDH is closely related to active metabolism. The activation of NADP-MDH is inhibited by its product, NADP. Therefore, when NADPH is used for assimilation processes in the chloroplast, NADP-MDH "turns off" its activity. Due to this, the export of reducing equivalents such as malate does not occur. Because ATP formation continues as electrons are transferred to malate, the malate valve plays a crucial role in maintaining the ATP/NADPH balance in chloroplasts as an indirect export system of reducing equivalents.

The study of the regulation mechanisms of metabolic processes creates conditions for understanding the biochemical mechanisms of plant adaptation to stress. From this point of view, the study of malate dehydrogenases and malic enzymes in the active stages of plant development is of particular importance. The study of the effect of soil drought on plant organisms is still of great theoretical and practical importance as an important scientific direction and opens wide prospects for the creation of plant varieties tolerant to unfavorable external environmental factors.

2. Materials and Methods

The genotypes stored in the wheat gene pool of the Research Institute of Crop Husbandry of the Ministry of Agriculture were compared, and 4 genotypes were selected: Murov 2, Aran, Gyzyl bughda, and Zirva 85. Bread wheat genotypes were cultivated in an artificial climate laboratory with a light period of 16/8 hours, 50% relative humidity, and a temperature regime of 24°C/18°C day/night, respectively. Drought stress was applied to 14-day-old seedlings. Measurements were made in 10 biological and 3 technical replicates in 2 variants.

2.1. NADP-MDH activity assay

The determination of the NADP-MDH activity started with the activation of the enzyme. Activation was performed for 15 minutes using 1 M Tris-HCl buffer (pH 8.0) containing 10 mg/ml BSA, 100 mM DTT, and the enzyme preparation. The reaction medium for NADP-MDH was as follows: 100 mM Tris-HCl buffer (pH 8.0), 10 mg/ml BSA, 0.5 M EDTA·4Na, 20 mM MgCl₂, 10 mM NADP-H, and 30 µl activated enzyme preparation. The reaction started by adding 10 µl of 1 mM OA to the reaction medium. The determination of the enzyme activity is based on the decline in optical density for 1 min of the reaction course due to the expenditure of NADH. The millimolar equivalent of ε-extinction for NADPH at maximum absorption amounted to 6.22 mM·cm⁻¹ at 340 nm [8].

2.2. NADP-ME activity assay

The enzyme activity was determined based on an increase in the optical density due to the formation of NADPH. The reaction medium for the determination was as follows: 50 mM Tris-HCl (pH 8.2), 1 mM EDTA·4Na, 20 mM MgCl₂, 0.5 mM NADP⁺, 5 mM L-malate·Na and the enzyme extract. The reaction started by adding malate to the reaction medium.

2.3. Relative water content (RWC) Determination

Relative water content (RWC) in leaves was measured based on the method proposed by Turner [9] 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.

Protein was measured by the method of Rekowski, using bovine serum albumin as the standard [10].

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

NADP-MDH is redox-regulated, activated by light, and is inactive in the dark. NADH-MDH, localized in chloroplasts, plays an important role in maintaining a balance of reduced equivalents between cytosol and stroma. Considering this, we determined the NADP-MDH activity in irrigated and drought-exposed variants of chosen wheat genotypes.

The highest NADP-MDH activity was observed in the genotypes Murov 2 and Zirva 85. The enzyme activity changed similarly in the leaves of irrigation and drought variants. The enzyme activity decreased gradually in the leaves of the drought-exposed Gyzył bughda genotype. A decrease in the amount of photosynthetic pigments under long-term soil drought resulted in a gradual decrease in the NADP-MDH activity.

The highest NADP-MDH activity was detected in the genotypes Murov 2 and Zirva 85. After four and six days of irrigation, the enzyme activity increased by 40.0% and 57.2%, respectively. In the leaves of the Zirva 85 genotype, the NADP-MDH activity changed similarly in the irrigated and trial variants. In the leaves of the Gyzył bughda genotype, the enzyme activity decreased gradually under drought (Table 1). The gradual decrease in the amount of photosynthetic enzymes under long-term soil drought caused a gradual decrease in the activity of the light-regulated enzyme NADP-MDH.

Table 1. Dynamics of NADP-MDH activity changes in wheat leaves.

Genotypes	Variants	NADP-MDH activity, EU/mg protein				
		Drought days				
		2	4	6	8	10
Murov 2	irrigation	15.69±1.4	16.71±1.7	17.9±2.0	18.71±1.7	18.9±2.0
	drought	13.04±1.3 ^{ns}	18.3±2.0*	20.5±2.1**	21.7±2.3**	18.9 ±1.8*
Zirva 85	irrigation	18.71±1.4	18.9±2.2	20.71±1.7	24.9±2.7	22.0±2.3
	drought	17.33±1.5*	19.1±2.0**	25.71±1.7*	24.9±2.5**	15.9±1.3*
Aran	irrigation	8.51±0.8	8.9±0.6	10.8±1.2	14.9±1.3	15.9±1.5
	drought	7.1±0.7 ^{ns}	7.9±0.9*	8.7±1.0*	7.9±0.6*	6.7±0.4*
Gyzył bughda	irrigation	13.1±1.8	18.9±1.6	19.8±1.9	24.9±2.3	16.9±1.5
	drought	13.1±1.9**	13.9±1.1*	13.7±1.3*	11.9±1.1*	4.7±0.1 ^{ns}

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

NADP-ME is involved in many processes related to plant responses to stress at the metabolic level. Thus, the enzyme plays an important role in maintaining the pH of the cytoplasm, regulating the function of the stomatal apparatus, and creating tolerance to stress in plants. Pyruvate, a product of the reaction, is used as a precursor in the biosynthesis of flavonoids and lignin.

The heatmap analysis revealed distinct genotype-dependent patterns in NADP-MDH activity under drought stress. Among the evaluated genotypes, Murov 2 and Zirva 85 exhibited the strongest activation of NADP-MDH during drought exposure. In Murov 2, enzyme activity progressively increased from 13.0 EU mg⁻¹ protein at day 2 to 21.7 EU mg⁻¹ protein at day 8, indicating enhanced metabolic adjustment during prolonged water deficit. Similarly, Zirva 85 showed a marked increase in NADP-MDH activity, reaching the highest value (25.7 EU mg⁻¹ protein) on the sixth day of drought treatment (Figure 1).

In contrast, Aran and Qizil buğda displayed lower NADP-MDH activity throughout the stress period, with a continuous decline observed at later stages of drought. Particularly, Qizil buğda showed a sharp reduction in enzyme activity after 8–10 days of stress, suggesting limited metabolic capacity for maintaining malate turnover under prolonged drought conditions. Overall, the heatmap demonstrates that drought-tolerant genotypes, particularly Murov 2 and Zirva 85, maintain or enhance NADP-MDH activity during water deficit, which may contribute to improved carbon redistribution, redox balance, and metabolic adaptation under drought stress.

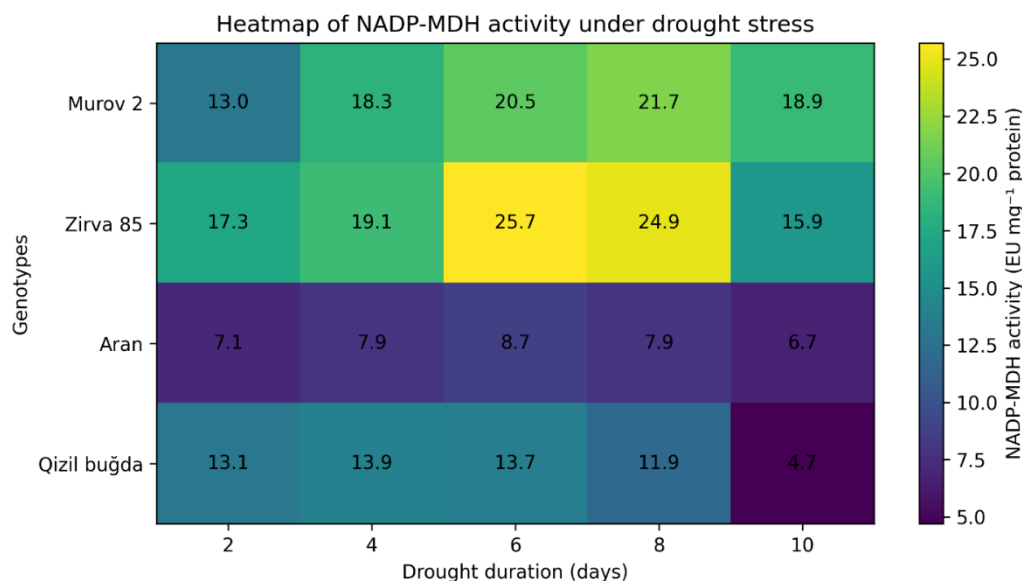


Figure 1. Heatmap visualization of NADP-MDH activity dynamics in bread wheat genotypes under drought stress.

The highest NADP-ME activity was observed in leaves of the Zirva 85 genotype 8 days after irrigation (3.5 ± 0.35 EU/mg protein). In the samples taken from the irrigated variant of the Murov 2 genotype, the activity of the enzyme gradually increased. Significantly lower activity was observed in the leaves of Aran and Gyzył bughda genotypes compared to Murov 2 and Zirva 85 genotypes. The results of our experiments confirm the tolerance of the genotypes Murov 2 and Zirva 85 (Table 2). The responses of plants to water stress differ depending on the plant species, the type of organ and tissue, the compartmentalization of the cell, the intensity and duration of water deficit, and the stage of ontogenesis.

Table 2. Dynamics of changes in NADP-ME activity in wheat leaves.

Genotypes	Variants	NADP-ME activity EU/mg protein				
		Drought days				
		2	4	6	8	10
Murov 2	irrigation	1.38 ± 0.12	1.76 ± 0.18	1.88 ± 0.12	2.27 ± 0.12	2.68 ± 0.12
	drought	$1.24 \pm 0.14^*$	$1.54 \pm 0.16^*$	1.68 ± 0.19^{ns}	2.54 ± 0.25^{ns}	$3.14 \pm 0.36^*$
Zirva 85	irrigation	1.18 ± 0.1	1.3 ± 0.02	1.45 ± 0.15	1.5 ± 0.16	1.8 ± 0.2
	drought	$1.54 \pm 0.18^*$	$1.7 \pm 0.2^*$	$2.45 \pm 0.15^*$	$3.5 \pm 0.35^*$	2.3 ± 0.1^{ns}
Aran	irrigation	0.28 ± 0.02	0.8 ± 0.01	0.84 ± 0.07	1.24 ± 0.05	1.36 ± 0.05
	drought	0.24 ± 0.02^{ns}	$0.44 \pm 0.02^*$	$0.52 \pm 0.02^*$	$0.4 \pm 0.05^*$	$0.76 \pm 0.05^*$
Gyzyl bughda	irrigation	0.31 ± 0.04	0.34 ± 0.04	0.44 ± 0.04	0.74 ± 0.05	1.48 ± 0.05
	drought	$0.24 \pm 0.05^*$	0.36 ± 0.05^{ns}	0.59 ± 0.05^{ns}	$0.24 \pm 0.05^*$	$0.16 \pm 0.05^*$

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

As a result of the increase in temperature, the upper layer of the soil dries up, and osmotic stress occurs around the roots; the concentration of salts and ions that have a toxic effect increases. First of all, this leads to a decrease in the volume and number of leaves [11]. As a result of stress, relative water content in cells and tissues decreases. During drought, due to osmotic adaptation, cells can reduce the osmotic potential and increase the water gradient, maintaining the turgor of the cell by elasticity. This contributes to maintaining the physiological balance of the plant during long-term drought. The weakening of the water storage capacity of plants is more pronounced in drought-sensitive genotypes [12]. Relative water content (RWC) of the leaf, stomatal resistance, transpiration rate, and leaf temperature are important indicators that affect water exchange in the plant. This indicator is high in the

early stages of leaf development and decreases when the dry biomass of the plant begins to increase. The value of RWC in a plant depends on the adsorption of water through the roots and the loss of water through transpiration [13].

Relative water content of the flag leaves of bread wheat genotypes was measured depending on the days of drought in 3 cultivation variants. This parameter was highest in Murov 2 and Aran genotypes, and lower in the Gyzyly bughda genotype compared to others. In the Murov 2 genotype, RWC remained relatively stable. On the 2nd day of the drought, RWC in the control and stress variants was 90% and 89%, and on the 10th day, 85% and 79%, respectively. In the Aran genotype, RWC at the beginning of the drought was 92% in the control variant and 89% in the stress variant, while on the 10th day of the drought, it amounted to 84% in the control variant and 80% in the stress variant. On the 4th day of drought, RWC was 89% in the control variant and 88% in the stress variant of the Murov 2 genotype, while in the Gyzyly bughda genotype, this parameter amounted to 70% and 64%, respectively. Thus, the RWC in the Gyzyly bughda genotype was sharply reduced due to the effect of stress. This can be attributed to shorter roots of the Gyzyly bughda genotype and a relatively stronger osmotic effect (Figure 2).

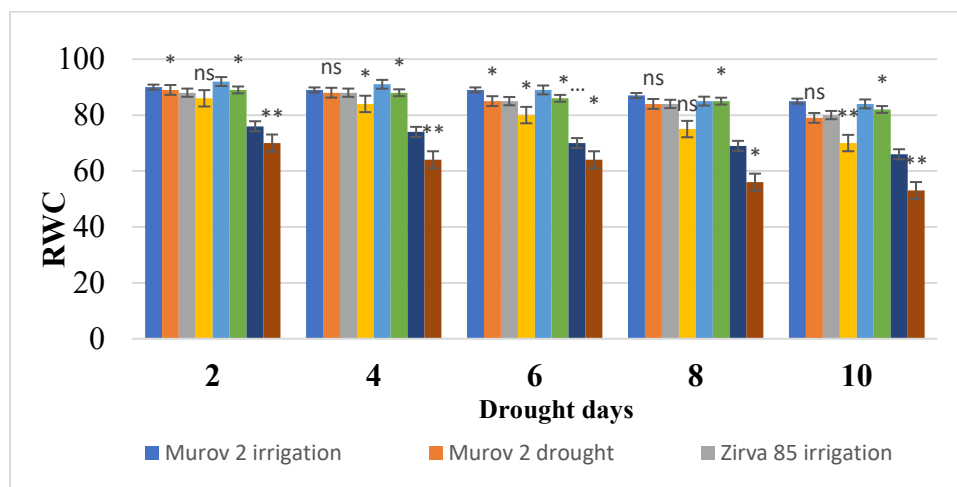


Figure 2. Effect of drought stress on flag leaf relative water content. Each value represents the mean of three replicates.

The relative stability of RWC during drought can be explained by a deeper extension of the root system contributing to less osmotic influence, which has been confirmed by our experiments. NADP-ME participates in the regeneration process by directing pyruvate and NADPH to the biosynthesis of fatty acids. Stress factors that affect water balance also cause oxidative stress by inducing the regeneration of free oxygen radicals. Drought stress, being one of the most common environmental factors that seriously affect plant development and productivity, causes many physiological, biochemical, and molecular changes in plants. Furthermore, the results indicated that organic acids such as malate, oxaloacetic acid, citric acid, and fumaric acid play an important role in the primary carbon metabolism in plants. Thus, the research revealed that the Murov 2 genotype can be cultivated in rainfed and semi-arid regions and can be used as a starting material for creating stress-tolerant wheat varieties in practical breeding programs.

Author Contributions

U. Gurbanova - Conceptualization, Writing; A. Afandiyeva - Methodology, Writing, Submission.

Conflict of Interest

The author(s) declare no conflicts of interest.

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