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The Dynamics of Some Carbon and Nitrogen Metabolism Enzymes in Daytime in Various Wheat Genotypes during Drought

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Abstract

PEPC plays a pivotal role in various metabolic processes in C3 plants including by providing intermediates for the Krebs cycle, maintaining intracellular pH and osmotic pressure, regulating the movement of stomatal guard cells, refixing CO2 formed by respiration, forming a carbon skeleton for lipid synthesis during grain development and nitrogen assimilation. Aspartate aminotransferase is essential to primary nitrogen assimilation, the transportation of reducing equivalents and the exchange of carbon and nitrogen resources among cellular subcompartments. Durum (Barakatli 95 and Garagylchyg 2) and bread wheat genotypes (Gobustan and Tale 38) cultivated in the experimental field of the Research Institute of Crop Husbandry were used as study materials. The high level of activity of PEPC and NAD-MDH during morning hours and a positive correlation between them in daytime suggest that, functioning mutually, these enzymes participate in the biosynthesis of malic acid.

Keywords: PEP-carboxylase, aspartate aminotransferase, NAD-malate dehydrogenase

Introduction

The annual production of wheat, which meets one fourth of global population demand for protein and calories, is ~700 million tons. This crop is cultivated on an area of 215 million ha, which is more than 16% of the world's sowing fields. The main goals of contemporary and future biotechnology are to produce high-quality bread and biofuel, and to create varieties of wheat convenient for human consumption (Huppe and Turpin, 1994).

Playing a crucial role in carbon and nitrogen metabolism in plants, PEPC catalyses the irreversible carboxylation of phosphoenolepyruvate (PEP) and converts it into oxaloacetate (OAA) (Huppe and Turpin, 1994). The studies conducted are *still not sufficient* to elucidate the physiological role and regulatory mechanisms of C3 PEPCs (O'Leary et al., 2009). Currently, the role of PEPC in carbon and nitrogen metabolism in plants has not been studied sufficiently under in vivo conditions.

Aspartate aminotransferase (AsAT, EC 2.6.1.1) plays an important role in primary nitrogen assimilation, the transportation of reducing equivalents and the exchange of carbon and nitrogen resources between cellular subcompartments (Gaufichon et al., 2016). Several isoforms of AsAT are localized in subcellular organoids – cytosol, chloroplasts, mitochondria and peroxisomes - of plants (Duff et al., 2011).

NAD-malate dehydrogenase (NAD-MDH, EC 1.1.1.37) participates in some metabolic processes, including the tricarboxylic acid and glyoxylate cycles, the synthesis of amino acids, glyconeogenesis and the exchange of metabolites between cytosol and subcellular organoids (Nunes-Nesi et al., 2005; Scheibe, 2004; Schertl and Braun, 2014).

Materials and methods

The Durum (Barakatli 95 and Garagylchyg 2) and bread wheat genotypes (Gobustan and Tale 38) used as study materials were cultivated in the experimental field of the Research Institute of Crop Husbandry located on the Absheron peninsula. Samples were taken at three-hourly intervals. The plant material was ground in the presence of quartz sand in a cooled mortar and pestle. The 100 mM Tris-HCl buffer used in homogenization contained 10 мM MgCl2, 1мM ethylenediaminetetraacetic acid *(EDTA),* 5 mM dithiothreitol (DTT), 2mM phenylmethanesulfonyl fluoride (PMSF) and 2% (w/v) insoluble polyvinylpyrrolidone (PVP).

PEPC activity was determined in a 1 ml reaction medium containing 50 mM Tris-HCl (pH 8.0), 10 mM MgCl2, 2mM DTT, 10 mM NaHCO3, 0.2 mM NADH, 10 U/ml MDH, 10 mM PEP and 40 µl enzymatic extract. The reaction was initiated by adding a substrate (10 mM PEP) to the reaction medium (Pyankov et al., 2000).

AsAT activity was measured in a reaction medium containing 100 mM HEPES-KOH (pH 7.4) and 100 mM Tris-HCl (pH 8.5), 2 mM EDTA, 2.5 mM 2-oxoglutorate, 10µg/ml pyrodoxalphosphate, 10 mM DTT, 12 U/ml MDH, 0.2 mM NADH, 20µl leaf extract and 2.5 mM L-aspartate. The reaction was initiated by adding L-aspartate (Alfonso Brüggemann, 2012).

NAD-malate dehydrogenase activity was determined by adding a substrate (1 mM oxaloacetate) to a reaction medium containing 100 mM Tris-HCl (pH 9.0), 30 mM malate and 0.2 mM NAD.

Enzyme activity was determined by spectrophotometry (Ultrospec 3300 Pro, Amersham, USA). The measurements were taken in a 1ml cuvette, at 340 nm wavelength (Scheibe and Stitt, 1988).

Total protein assay: Total soluble protein was determined by spectrophotometry, using 0.12% Coomassie Brilliant Blue G-250 (Sedmak and Grossberg, 1977).

Results and discussion

Phosphoenolpyruvate carboxylase, aspartate aminotransferase and NAD-malate dehydrogenase activity was studied comparatively in durum and bread wheat genotypes with contrasting drought tolerance. In both variants of the durum wheat genotypes (Barakatli 95 and Garagylchyg 2) the highest level of PEPC activity was observed at 8^{00}. Whereas, in the bread wheat genotypes (Gobustan and Tale 38) the highest level of PEPC activity was recorded at 17^{00} in flag leaves. There were no significant changes in enzyme activity during morning and afternoon hours.

The highest level of AsAT activity was registered in the watered variant of the Barakatli 95 variety, the lowest level of activity in flag leaves of the stressed Garagylchyg 2 variety, which is drought sensitive (Fig. 1).

The highest level of AsAT activity in flag leaves of both variants of the drought-tolerant Gobustan variety was observed at 8^{00}. At 11^{00}, enzyme activity declined by factors of 1.3 and 1.5 in watered and drought-exposed plant leaves, respectively. AsAT activity decreased gradually during the day, but began to increase from 17^{00}, reaching the values observed in morning hours. Performing transamination in various cell compartments, AsAT catalyses the formation of aspartate and 2-*oxoglutorate* from glutamate and oxaloacetate (Fig. 2).

AsAT plays an important role in primary nitrogen assimilation, the transportation of reducing equivalents and the exchange of carbon and nitrogen resources between cellular subcompartments.

The highest level of NAD-MDH activity was found in all samples taken from Barakatli 95 at 17^{00} with the exception of the drought-exposed variant. In the bread wheat varieties, the highest level of NAD-MDH activity was found in the morning hours (8^{00} and 11^{00}) of the day (Fig. 3). A gradual decrease in NAD-MDH activity

was observed in flag leaves of the drought-exposed Gobustan variety and watered Tale 38 variety during the day. Malate displayed a diurnal rhythm in the leaves of C_3 plants.

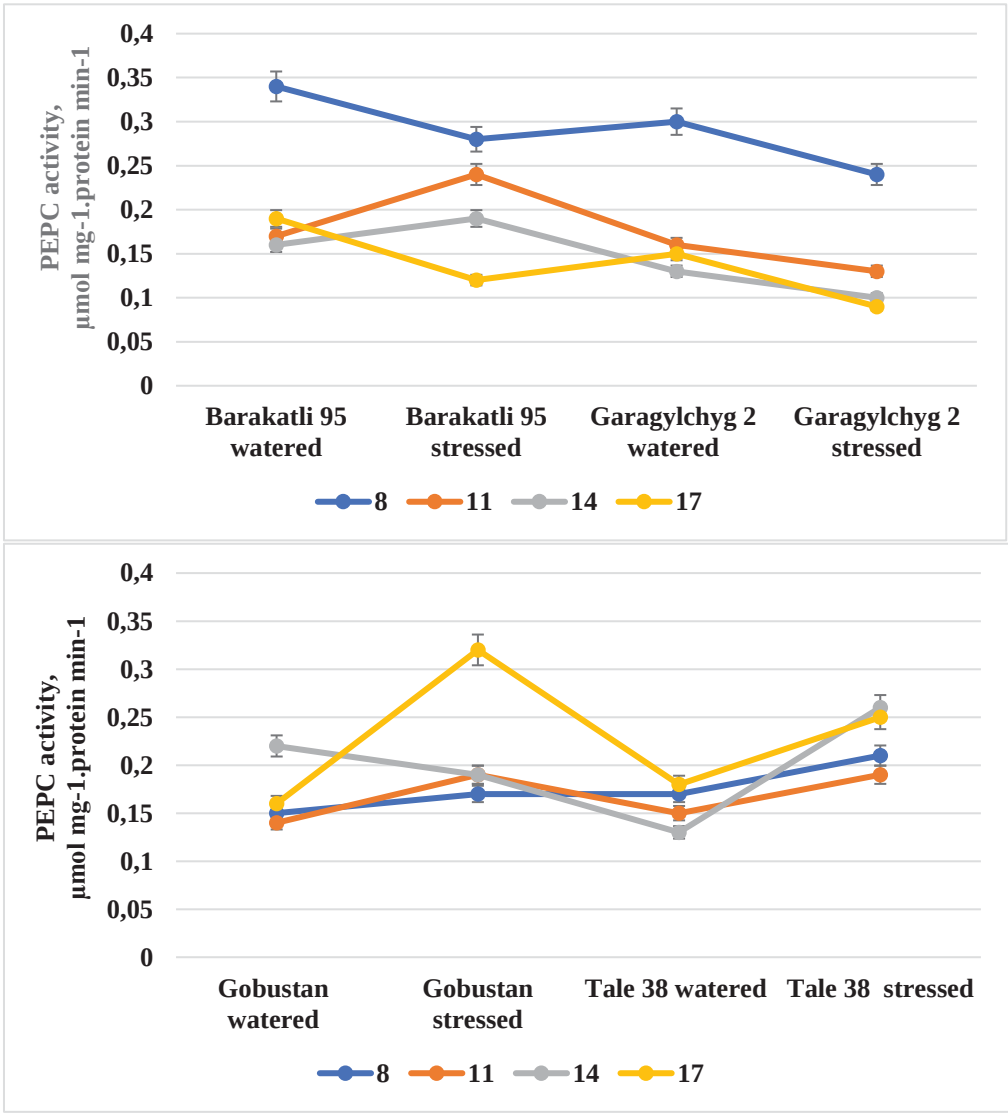

Fig. 1. Dynamics of PEPC activity in flag leaves of durum and bread wheat genotypes during light phases of the day

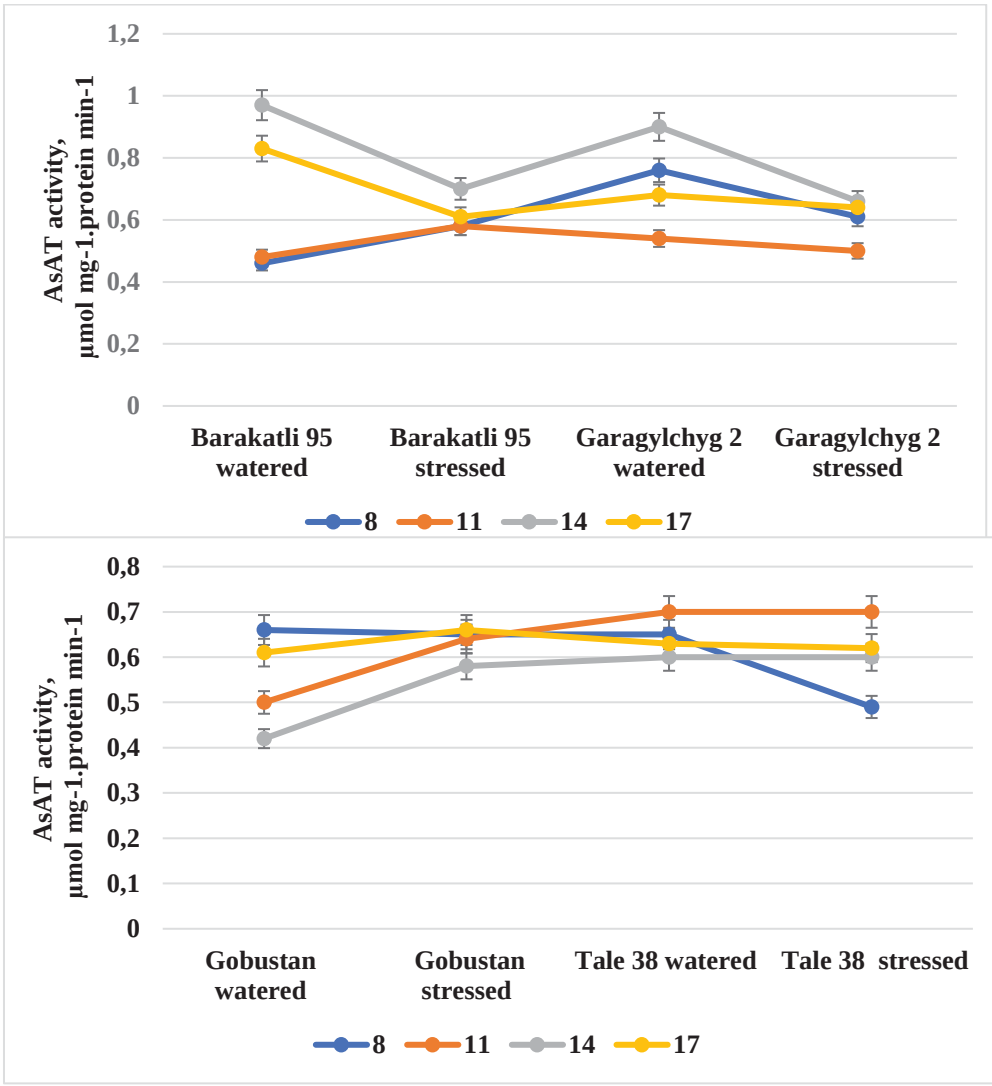

Fig. 2. Dynamics of AsAT activity in flag leaves of durum and bread wheat genotypes during light phases of the day

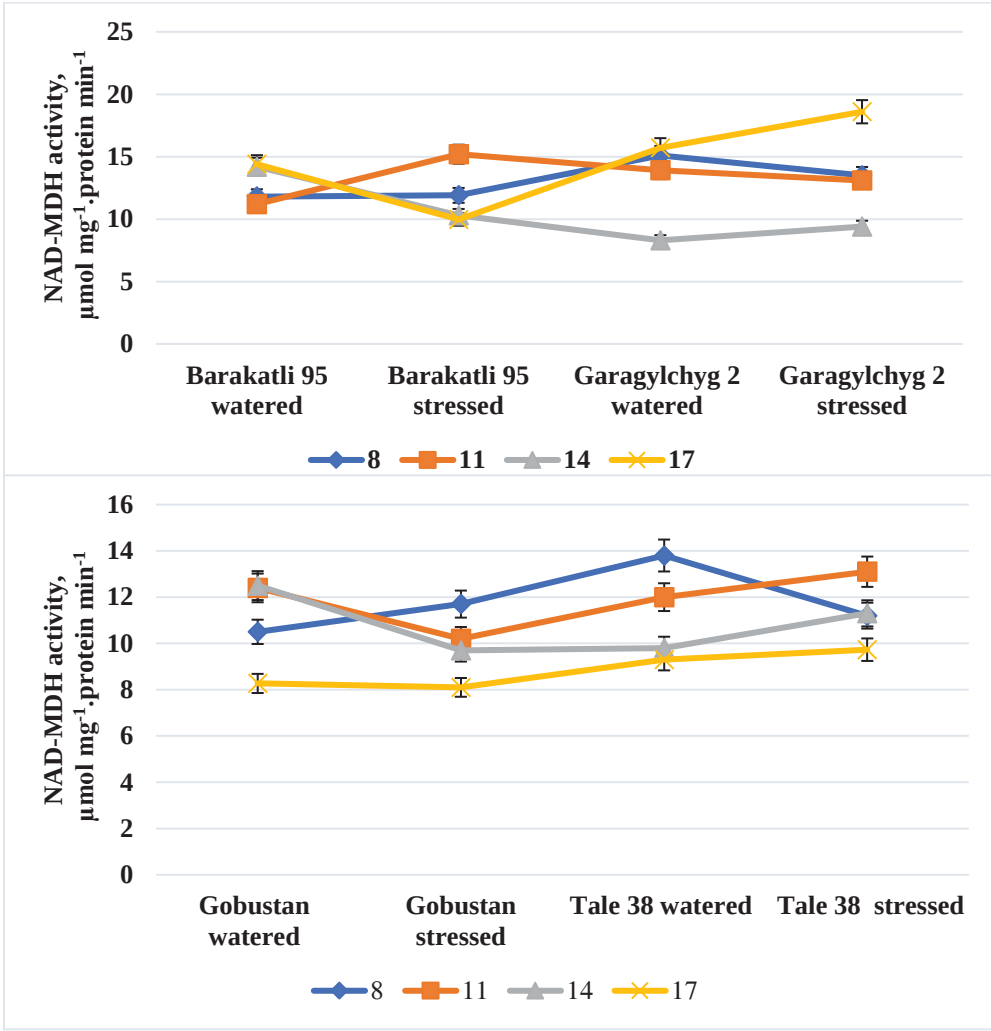

Fig. 3. Dynamics of NAD-MDH activity in flag leaves of durum and bread wheat genotypes during light phases of the day

Conclusion

Increasing during periods of light, enzyme levels were highest at the end of the day and decreased during the night. Malate synthesis during periods of light is the result of the sequential functioning of PEPC and MDH. Thus, acting as a primary carboxylating enzyme, PEPC combines CO2 with PEP and forms 4-carbon, dibasic oxaloacetic acid.

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