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Determination of the Activities of Some Photosynthetic Enzymes in *Sorghum Bicolor* Depending on Environmental Factors

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During the natural day-night cycle, light is the most important environmental factor controlling the diurnal changes of photosynthesis in C_4 plants. Activities of NADP-MDH and NADP-ME have been determined in *Sorghum bicolor* leaves at various stages of the plant development in relation to environmental parameters. A positive correlation has been found between the plant age and NADP-MDH activity.

Keywords: *C_4 photosynthesis, photosynthetic enzymes, temperature, NADP-MDH, NADP-ME*

INTRODUCTION

C_4 plants are more productive under hot climatic conditions compared with C_3 plants. Photosynthetic apparatus in leaves of these plants is distributed between bundle sheath and mesophyll cells with contrasting anatomic and biochemical properties. These cells differ in morphological and structural properties, as well as in the differentiation of thylakoid membranes) (Igamberdieva and Bykovab, 2018; Friso et al., 2010). *Sorghum bicolor* is a NADP-malic enzyme type C_4 plant, having high photosynthetic activity. This plant is of great agricultural importance. Sorgo is the fifth most important cereal plant in the world after rice, wheat, maize and barley, it is rich in nutrients, fibers, and biocomponents.

The main aim of the research was the diel dynamics of the activities of photosynthetic enzymes. To this end, the activities of NADP-malate dehydrogenase and NADP-malic enzyme have been determined in leaves of the mature *Sorghum bicolor* plant depending on the plant age and environmental parameters.

MATERIALS AND METHODS

Plant material and growth conditions: *Sorghum bicolor* was cultivated in the experimental field of the Institute of Molecular Biology and Biotechnologies. Activities of the photosynthetic enzymes were determined at the various stages of the plant growth. Diel dynamics of the NADP-MDH and NADP-ME activities was studied during the reproductive stage of the plant development.

Extraction of plant materials: To determine the activities of the enzymes leaves were ground using pestle and mortar. Homogenization was performed by adding 2 ml of 50 mM Tris-HCl (pH 8.0) buffer, containing 0.01% BSA, 0.5% Triton, 14 mM β-ME, 1 мM ethylenediaminetetraacetic acid (EDTA), and 0.5% polyvinyl pyrrolidone to 0.5g leaves in the presence of quartz sand. Homogenization continued for 5 min, at 10,000g. Supernatant was used for the enzyme activity assays.

Enzyme activity assays: Tris-HCl buffer (100 mM, pH 8.0) containing 10 mg/ml BSA, 0.5 M EDTA, 20 mM $MgCl_2$, 0.2 mM NADP·H and 50 µl activated enzyme preparation was used to determine NADP-malate dehydrogenase activity. The reaction was initiated by adding 1 mM oxaloacetate. To activate NADP-MDH, the enzyme preparation was kept in the reaction medium containing 1 M Tris-HCl (pH 8.0), 1M DTT and 50 µl enzyme preparation for 15 min (Scheibe and Stitt, 1988).

NADP-ME activity was determined spectrophotometrically by following NADPH production at 340 nm in the spectrophotometer Ultrospec 3300 pro. The standard assay medium contained 50 mM Tris-HC1, (pH 8.0), 10 mM $MgCl_2$, 0.5 mM NADP, and 4 mM L-malate in a final volume of 1 ml (Maurino et al., 1997).

RESULTS AND DISCUSSION

Activities of NADP-MDH and NADP-ME have been determined in *Sorghum bicolor* leaves at various stages of the plant development in relation to environmental parameters. Photosynthetic enzymes in C_4 plants are regulated by a number of factors, including light. The photosynthetic enzyme activity *in vivo* in plant leaves at any given point during photosynthesis reflects the combined effects of light, metabolites and other factors at that time (Cousins et al., 2003).

NADP-malate dehydrogenase (NADP-MDH; EC

1.1.1.82) is a crucial enzyme of the C_4 pathway. Playing an important role in the photosynthetic carbon assimilation, this enzyme catalyzes the conversion of oxaloacetate into malate. In Sorghum leaves, NADP-MDH is activated in the light and inactivated in the dark and this apparently depends on interconversion between dithiol and disulfide groups on the enzyme. *In vivo*, the light activation (reduction) process probably occurs via thioredoxin reduced in turn by the photosynthetic electron transport system through ferredoxin (Rebeille and Hatch, 1987). In C_4 plants such as sorghum and maize, it is located in the chloroplasts of mesophyll cells where the produced malate is exported to the bundle-sheath cell chloroplasts, thus delivering reducing equivalents that are needed for the photosynthetic fixation of carbon dioxide into organic molecules. Among all the malate dehydrogenases, the chloroplastic NADP-dependent form exhibits the unique property of being strictly regulated by light, while the NAD-dependent MDHs are permanently active. It is totally inactive in the dark and activated by the ferredoxin-thioredoxin system only when the chloroplasts are illuminated (Johansson et al., 1999).

Malic enzymes catalyze the oxidative decarboxylation of L-malate to yield pyruvate, CO_2, and NAD(P)H in the presence of a bivalent metal ion. In plants, different isoforms of the NADP-malic enzyme (NADP-ME) are involved in a wide range of metabolic pathways. The C_4-specific NADP-ME has evolved from C_3-type malic enzymes to represent a unique and specialized form of NADP-ME as indicated by its particular kinetic and regulatory properties. The photosynthetic C_4 NADP-ME, which is involved in the CO_2 concentrating mechanism that increases the photosynthetic yield of NADP-ME type C_4 plants, is compartmentalized in bundle sheath chloroplasts. In NADP-ME type C_4 plants, these organelles show a gradation of structure from chloroplasts with rudimentary grana (in maize and crabgrass) to completely agranal (in sugarcane and sorghum) (Detarsio et al., 2000).

Sorghum development has been separated into three major divisions: vegetative (GS-1), reproductive (GS-2), and grain fill (GS-3), with about a third of the life cycle spent in each (Wood et al., 2006). Stages shown and discussed range from emergence until physiological maturity. Time required to reach each stage depends both on the hybrid and the environment in which it is growing.

Dynamics of the activities of NADP-MDH and NADP-ME has been studied in the leaves of *Sorghum bicolor* at various stages of the plant development (Figure 1).

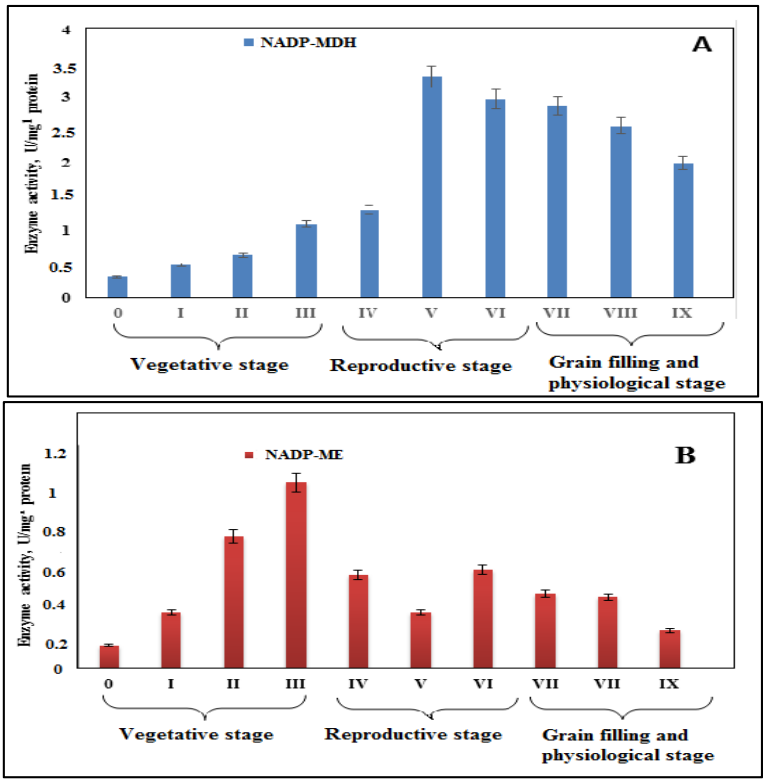

Fig. 1. Dynamics of the activities of NADP-MDH (A) and NADP-ME (B) in the *Sorghum bicolor* plants of various ages. ((0-Emergence; I- 3 three–leaf stage; II- five–leaf stage; III- Growing Point Differentiation)- vegetative stage; (IV- Final Leaf Visible in the Whorl; V- Boot Stage; VI- Half Bloom)- reproductive stage; (VII- Soft Dough; VIII- Hard Dough; IX- Physiological Maturity))- grain filling and physiological maturity stage.

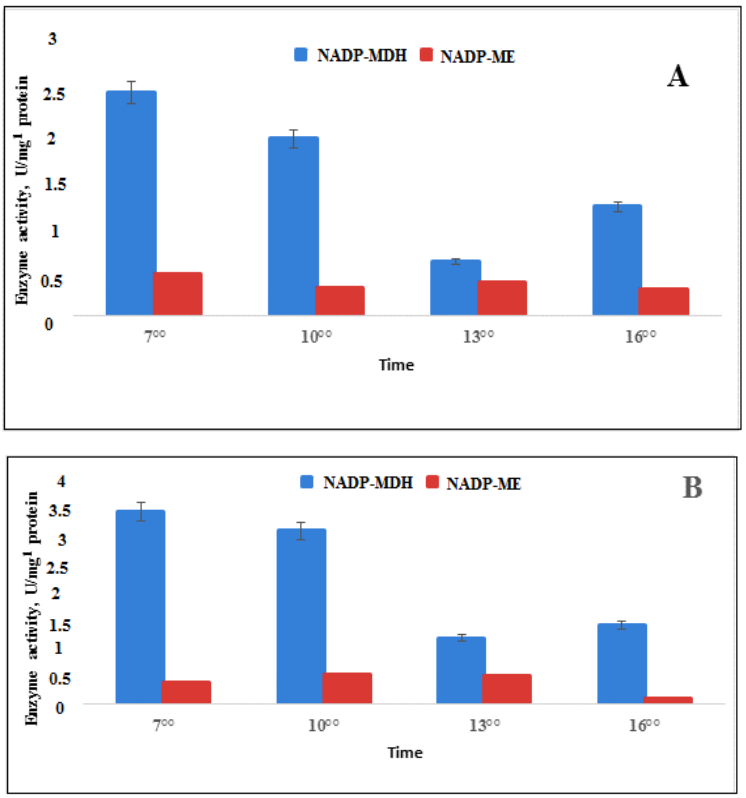

Fig. 2. Time-dependent dynamics of the NADP-MDH and NADP-ME activities in *Sorghum bicolor* leaves. A- Final Leaf Visible in the Whorl (reproductive stage); B- grain filling and physiological maturity stage.

According to the results of the research, a positive correlation exists between the NADP-MDH activity and the plant age. As seen in Figure 1 (A), the highest activity of the enzyme was observed in the tube formation, i.e. reproductive stage. Thus, the enzyme activity during flag-leaf formation stage was 3 times higher compared with the five–leaf stage, and 1.5 times higher compared with the grain formation and physiological maturity stages. The positive correlation observed between the enzyme activity and the plant age can be related to the plant development and the grain formation process.

As seen in Figure 1(B), the NADP-MDH activity during the vegetative stage (Growing Point Differentiation Stage) was higher than in the reproductive stage. Thus, the enzyme activity was 0.5 times higher in the last phase of the vegetative stage compared with the flag leaf formation phase.

Time-dependent dynamics of the activities of photosynthetic enzymes in the leaves of the mature *Sorghoum biclor* plant is shown in Figure 2.The activity of NADP-MDH was found to be higher during both flag leaf formation and physiological maturity stages, in the morning hours (7.00). The enzyme activity was 2 times higher at 7.00 compared with 16.00. Moreover, during both stages the enzyme activity gradually decreased until 16.00 with a subsequent increase. However, there was no pronounced difference in the NADP-ME activity during both stages.

The higher activity of NADP-MDH observed in the morning hours is suggested to relate to the high PEPCase activity. Thus, it is known that oxaloacetate, which is the product of PEPC, is converted into malate by NADP-MDH. According to previous reports, under hot climatic conditions the activity of PEPC in C_4 plants was higher during the morning hours and it decreased in the afternoon hours (Du et al., 2000). The decrease in the PEPC activity under hot is assumed to cause a decrease in the oxaloacetate amount, which is the product of PEPC. This is accompanied by the decrease in the NADP-MDH activity that catalyzes the conversion of oxaloacetate into malate.

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Ətraf Mühit Amillərindən Asılı Olaraq *Sorghum Bicolor* Bitkisində Bəzi Fotosintetik Fermentlərin Aktivliklərinin Təyini

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AMEA Molekulyar Biologiya və Biotexnologiyalar İnstitutunun Karbonun fotosintetik assimilyasiyasının enzimologiyası laboratoriyası

Təbii gecə və gündüz tsikli ərzində işıq C_4 bitkilərdə fotosintez prosesini gündəlik tənzimləyən ətraf mühitin mühüm faktorlarından biridir. İnkişafın müxtəlif mərhələlərində Sorghum bicolor bitkisinin yarpaqlarında ətraf mühit parametrlərindən asılı olaraq, NADP-MDH və NADP-ME fermentlərinin aktivliyi təyin olunmuşdur. Aparılan tədqiqatlar nəticəsində müəyyən olunmuşdur ki, NADP-MDH fermentinin aktivliyi ilə bitkinin yaşı arasında müsbət korrelyasiya mövcuddur.

Açar sözlər: *C_4 fotosintez, fotosintetik fermentlər, temperatur, NADP-MDH, NADP-ME*

Определение Активности Некоторых Фотосинтетических Ферментов в *Sorghum Bicolor* в Зависимости от Факторов Окружающей Среды

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Свет является одним из самых важных факторов окружающей среды, регулирующих процесс фотосинтеза в С4 растениях в период естественного дневного/ночного цикла . Активность ферментов NADP-MDH и NADP-ME была определена в растении *Sorghum Bicolor*. Установлена положительная корреляция между возрастом растений и активностью NADP-MDH.

Ключевые слова: *C_4 фотосинтез, ферменты фотосинтеза, температура, NADP-MDH, NADP-ME*