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THE STUDY OF NADP-MALATE DEHYDROGENASE IN AMARANTH LEAVES UNDER DROUGHT STRESS

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In the present study the activity of NADP-malate dehydrogenase (NADP-MDH, EC 1.1.1.82) was found to be localized in chloroplasts - 80-90% and cytosolic fraction -10-20% of mesophyll (MC) and bundle sheath cells (BSC). Unlike to other MDH system enzymes NADP-MDH has no broad isoenzyme spectrum. A new 90 kDa isoform of NADP-MDH appears in MC under drought stress that does not occur in BSC.

Keywords: *Amaranthus cruentus*, NADP-MDH, isoform, activity, localization, drought

Introduction

C4 pathway of photosynthetic carbon assimilation is an improved form of C3 pathway. Because of the better adaptation ability to high light intensity, hot temperature and water deficiency C4 plants dominate in tropic and subtropic zones with hot climate conditions (Edwards et al., 2010).

The diversity of decarboxylation ways of C4 dicarbon acids in C4 plants is related to their enzyme content and facilitates plant stress protection (Furbank, 2011; Wang et al., 2014).

Enzymes of malate dehydrogenase (MDH) system are found in all living organisms from archaeobacteria to mammals (Selinski et al., 2014). They have complex molecular forms manifesting certain dynamic balance.

Several molecular forms of MDHs differing in their subcellular localization and cofactor specificity were found involved in several metabolic processes in higher plants (Goward, Nicholls, 1994).

NADP-MDH was isolated from spinach leaves for the first time and it was found to be localized in chloroplasts (Rocha et al., 1968). In higher plants the enzyme is mainly localized in chloroplasts and plays an important role in the equilibration of reducing equivalents between cytosol and stroma (Scheibe and Stitt, 1998).

NADP-MDH catalyses the reversible reaction of oxaloacetate (OA) conversion into malate using NADPH as cofactor (Wang et al., 2014).

NADP-MDH was isolated from leaves of NADP-malic enzyme type C4 plant maize (Jacquot et al., 1981) and C3 plant chick-pea (Fickenscher and Scheibe, 1988) and it was established to be localized only in chloroplasts of MC. In MC of C4 plants malate formed in the NADP-MDH reaction is transported to chloroplasts of BSC and participates in providing CO₂ for the Carbon concentrating mechanism in carboxylation centers (Byrt et al., 2011).

The presented paper is devoted to the study of plant responses to drought stress in leaves of NAD-malic type C4 plant Amaranth.

Materials and methods

Amaranthus cruentus L. was used as the investigation object. Watering was ceased on the 30th day of the plant growth and soil drought conditions were created. Whereas control plants were watered till the end of the vegetation. Purification of MC, BSC and subcellular fractions from Amaranth leaves were conducted by mechanical means (Guliev et al., 2003). The activity of NADP-MDH was measured according to (Scheibe and Stitt, 1988), native gel electrophoresis was performed using the method of Davis (1964) and tetrazolium blue was used for specific revelation of gells (Fieldes, 1992). Electrophoresis was performed to determine the enzyme molecular mass (Laemmly, 1970), and the Lowry method was used for total protein quantification (Lowry et al., 1951).

Results and discussion

The activity of NADP-malate dehydrogenase (NADP-MDH, EC 1.1.1.82) was found to be localized in chloroplasts (80-90%) and cytosolic fraction (10-20%) of mesophyll (MC) and bundle

sheath cells (BSC) (fig.1). On the other hand mitochondrial fractions of MC and BSC have no NADP-MDH activity. Contrary to the other enzymes of MDH system the activity of NADP-MDH decreased in all phases under drought.

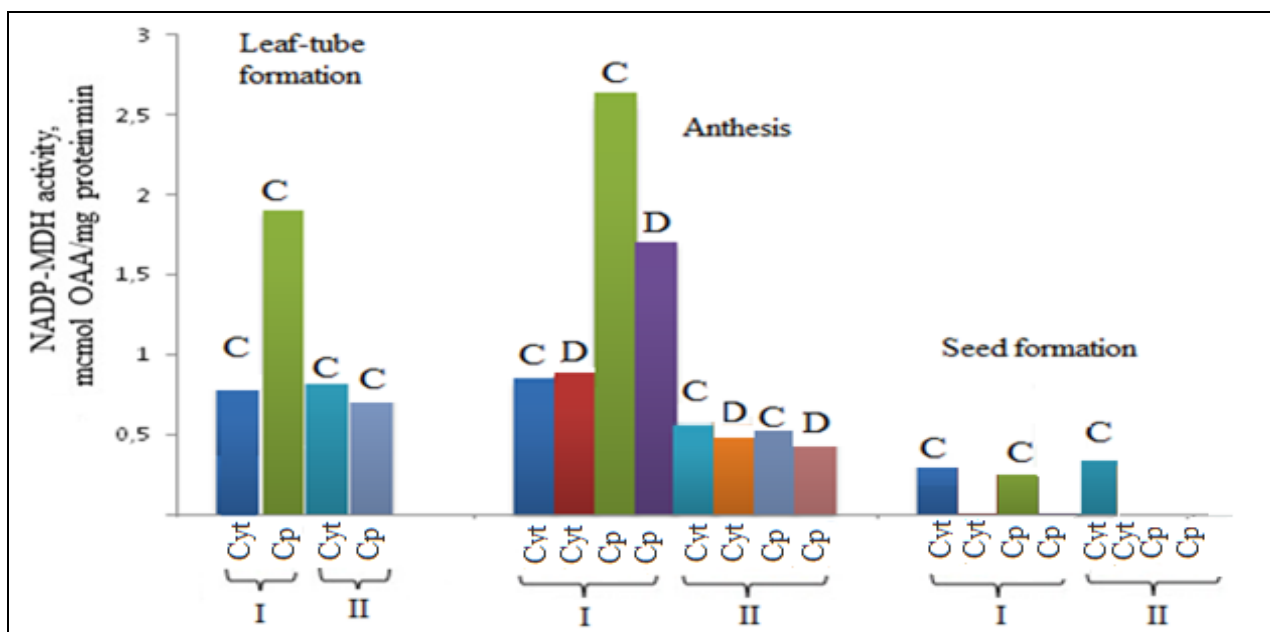


Fig. 1. Dynamics of changes of NADP-MDH activity in cytosolic and chloroplast fractions of MC and BSC in leaves during ontogenesis of amaranth under drought. Cyt-cytosol, Cp-chloroplast; C-control, D-drought; I-MC, II-BSC; OAA-oxaloacetic acid

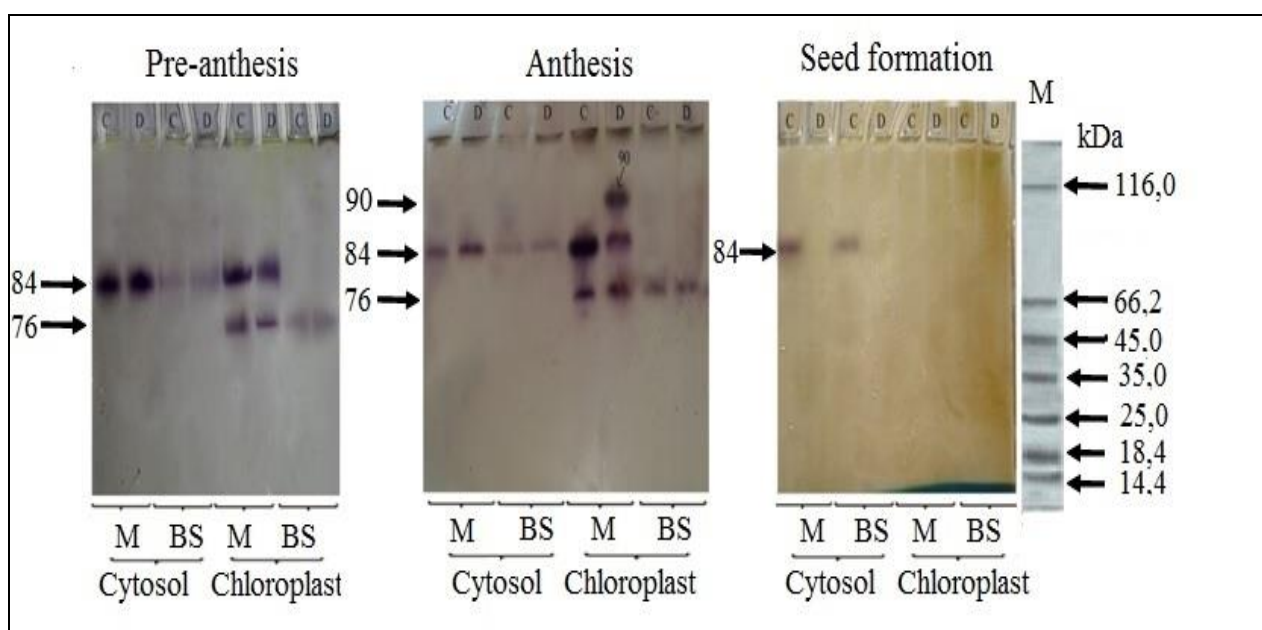


Fig. 2. Determination of NADP-MDH isoforms by gel-electrophoresis in cytosol and chloroplasts of MC and BSC at separate stages of ontogenesis in amaranth leaves. C-control, D-drought, M-marker proteins

Based on electrophoretic mobility and molecular masses the isoenzyme spectrum of NADP-MDH in MC and BSC was studied in Amaranth leaves (fig.2). It was established that NADP-MDH did not possess broad isoenzyme spectrum contrary to other MDHs. As seen in fig.2 in the control variant 76 kDa isoform of NADP-MDH was localized in cytosol of both tissues, 76 and 84 kDa isoforms in chloroplasts of both tissues.

A new isoform (inductive) of 90 kDa was formed in chloroplasts of MC at anthesis. At the stage of seed formation due to destruction of chloroplasts the enzyme localized in chloroplasts lost its activity, while a slight activity was observed in cytosol of MC and BSC.

The obtained data show that due to stomata closure during drought CO₂ uptake decreased causing weakening in photosynthesis and amounts of C₄ dicarbon acids such as malate and aspartate and also CO₂ increased in intercellular spaces. More malate was synthesized from OA due to the increase in activities of decarboxylating enzymes (Long et al., 1994) and CpNADP-MDH. Easily penetrating through membranes malate participates in the Calvin cycle reactions as CO₂ source. The formation of inductive isoform of CpNADP-MDH under drought is probably needed for concentrating CO₂ in carboxylation centers to protect plants from stress. According to recent reports due to the high sensitivity to stress factors MDH enzymes play an important role in the formation of adaptive reactions (Furbank, 2011; Wang et al., 2014).

Thus, the comparative study of CO₂ metabolism enzymes in amaranth leaves shows that the key enzyme of C₄ photosynthesis NADP-MDH is more labile under drought unlike to the Calvin cycle enzymes, which could be considered as a factor contributing to plant drought tolerance.

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QURAQLIĞIN TƏSİRİ ZAMANI AMARANT YARPAQLARINDA NADP-MALATDEHİDROGENAZA FERMENTİNİN TƏDQIQI

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Aparılan tədqiqatlar zamanı amarant (*Amaranthus cruentus* L.) yarpaqlarının mezofil (MH) və örtük topa hüceyrələrinin (ÖTH) xloroplastlarında NADP-malatdehidrogenaza (NADP-MDH, EC 1.1.1.82) fermentinin fəallığının 80-90%, həmin toxumaların sitozol fraksiyalarında isə 10-20% lokalizasiya olunduğu müəyyən olunmuşdur. MDH sisteminin digər fermentlərindən fərqli olaraq, NADP-MDH geniş izoenzim spektrinə malik deyildir. Quraqlığın təsirindən MH-nin xloroplastlarında NADP-MDH-nin molekulyar kütləsi 90 kDa olan yeni bir izoformasına əmələ gəlsə də, ÖTH-nin xloroplastlarında belə bir effekt müşahidə olunmur.

Açar sözlər: *Amaranthus cruentus*, NADP-MDH, izoforma, fəallıq, lokalizasiya, quraqlıq

ИЗУЧЕНИЕ ФЕРМЕНТА НАДФ-МАЛАТДЕГИДРОГЕНАЗЫ В ЛИСТЬЯХ АМАРАНТА ПОД ДЕЙСТВИЕМ ЗАСУХИ

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В результате проведенных исследований установлено, что 80-90% активности фермента НАДФ-зависимой малатдегидрогеназы (НАДФ-МДГ, КФ 1.1.1.82) локализовано в хлоропластах мезофильных клеток (МК) и клеток обкладки (КО) и 10-20% - в цитозольной фракции этих же клеток. В отличие от других ферментов системы МДГ, этот фермент не обладает широким изоферментным спектром. Появляется новая изоформа с молекулярной массой 90 кДа в МК при засухе, однако в клетках обкладки такой эффект не наблюдается.

Ключевые слова: *Amaranthus cruentus*, НАДФ-МДГ, изоформа, активность, локализация, засуха