Isoenzyme Spectrum, Localization and Some Physicochemical Properties of NAD-Malate Dehydrogenase in Amaranth Leaves Under Drought

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Malate dehydrogenase (MDH) plays a crucial role in energy and cell metabolism. Activity, subcellular distribution, isoenzyme spectrum and kinetic properties of cytosolic (cMDH) and mitochondrial malatdehydrogenases (mMDH) have been studied in the phases of pre-anthesis, anthesis and grain ripening. Subcellular localization, isoenzyme spectrum and kinetic properties of NAD-malate dehydrogenase (l-malate-NAD-oxidoreductase, NAD-MDH, EC 1.1.1.37) were studied in subcellular fractions (SCFs) of assimilating tissues of *Amaranthus cruentus* L. leaves during pre-anthesis, anthesis and grain ripening phases. NAD-MDH was found to have a wide isoenzyme spectrum in amaranth leaves, which changed depending on the number of isoenzymes, type of tissues and SCFs. Main part of the enzyme general activity was localized in cytosolic and mitochondrial fractions of both tissues. V_{max} (OAA) value of the reaction catalyzed by cNAD-MDH in mesophyll cells (MC) were 2-times higher compared with V_{max} (OAA) of the reaction catalyzed by mNAD-MDH. Whereas, in bundle sheath cells (BSC) V_{max} (OAA) for mNAD-MDH was 3-4-times higher than the same parameter of the enzyme in MC. The enzyme isoforms had a wide range of pH optimum and were tolerant to temperature. Depending on SCFs the enzyme had higher sensitivity to the substrate oxloacetate (OAA) and lower sensitivity to malate. The kinetics of the enzyme follows Michaelis-Menten plot and this enzyme is not allosteric. The enzyme activity is strongly regulated by substrates, mono and bivalent ions. Inhibitory effect of ATP to the all isoforms even more increased under drought.

Keywords: *Amaranth; drought; NAD-MDH; isoforms; localization; kinetics; tolerance*

INTRODUCTION

NAD-malate dehydrogenase (MDH; EC 1.1.1.37) catalyzes the reversible reaction of OAA conversion into malate. This reaction occurs due to the oxidation and reduction of NAD coenzyme (Minarik et al., 2002). Most eukaryotes have cytosolic and mitochondrial types of MDH (Sasaki et al., 2014). Citric acid cycle is a central oxidative pathway in aerobic procaryotes and eucaryotes. In this cycle the reversible reaction of malate conversion into OAA is catalyzed by NAD and NADP-MDH (Takahashi-Íñiguez et al., 2016). The enzymes of plant MDH system are considered as dynamic balance of proteins capable of responding to the organism requirements, physiological state, and environmental changes (Pinheiro et al., 1991) .

MDH occurs in all living organisms including archaebacteria and mammalians and in all subcellular organoids (motochondria, glyoxisomes, peroxisomes, chloroplasts etc.) (Selinski et al., 2014)

All MDHs are multimeric enzymes consisting of 2 and 4 identical subunits (Minarik et al., 2002; Yashvant et al., 2013). Plant mNAD-MDH is assumed to function in several directions. It oxidizes malate to OAA-the final product of the classical Krebs cycle. mNAD-MDH participates not only in the oxidation of NADH in the Krebs cycle, but also in the metabolism of reducing equivalents among metabolic pathways in the various cell compartments (Minarik et al., 2002; Tomaz et al., 2010). According to some authors, MDH isoforms are related not only to respiration (Tomaz et al., 2010), but also to the ß-oxidation of fatty acids, grain germination and stress tolerance (Pracharoenwattana et al., 2007; Cousins et al., 2007; Wang et al., 2014).

Thus, having an important and complex role, NAD-MDH participates in carbon and energy distribution in higher plants, which arises new ideas on molecular mechanisms connecting respiration, photosynthesis and photorespiration (Cousins et al. 2007; Tomaz et al., 2010).

The main purpose of our research was to study the activity, isoenzyme spectrum, localization and some physicochemical and kinetic properties of NAD-MDH in SCFs (cytoplasm, mitochondria) of assimilating tissues (AT) of amaranth leaves during active phases of plant development under drought.

MATERIALS AND METHODS

NAD-ME type C_4 plant *Amaranthus cruentus* L.cultivated under natural conditions was used as

the research object. Soil drought was imposed on the 30th day of the development by ceasing watering, while control plants were watered till the end of the vegetation.

To study subcellular distribution of the enzyme, MC and BSC were isolated using the mechanical method, purified and SCFs were derived. For this purpose the method developed by Edwards and Gardestrom (Gardestrom, Edwards, 1983) for the maize leaves was modified according to the studied object (Guliev et al., 2003).

Enzyme preparation: Leaves were detached from stems, washed with distilled water, dried on filter paper, cut into small pieces and homogenized for 2 min at +4°C in 100 mM Tris-HCl buffer (pH 8.0), containing 20 mM $MgCl_2 \cdot 6H_2O$, 1 mM EDTA, 5 mM DTT, 1% Triton X-100 and 0.5% PVP-25 (1:5, m/V). The obtained homogenate was filtered through 2-fold capron cloth and centrifuged for 5 min at 1000g and then for 15 min at 5000g to remove nucleus and tissue residues. After removing the precipitate, supernatant was used for the determination of the enzyme activity.

NAD-MDH activity: NAD-MDH activity was determined spectrophotometrically (Ultrospec 3300 pro, Amersham, USA) (Scheibe, Stitt, 1998). The reaction medium for the OAA reduction was Tris-HCl buffer (pH 8.0), containing 1mM OAA, 10 mg/ml BSA, 10 mM $MgCl_2 \cdot 6H_2O$, 0.15 mM NAD·H and 5-10 µl enzymatic preparation. The reaction started by adding 1 mM OAA into the reaction medium. The reaction medium for the malate oxidation was 100 mM Tris-HCl buffer (pH 9.0), containing 30 mM malate and 0.2 mM NAD. The enzyme activity was determined spectrophotometrically based on the decline in optic density due to the NAD·H expenditure for 1 min. Extinction coefficient for NADH and NADPH was 6.22 $mM \cdot cm^{-1}$ at 340 nm.

Native gel electrophoresis was performed on the polyacrylamide gel (PAAG) (Sigma, USA, Mo 63178, Model E-4266) at +4ºC (Davis, 1964).

Specific detection of NAD-MDH: detection of NAD-MDH isoforms on the electrophoretic gels was performed in a specific medium using the tetrazole method (Fieldes, 1992). To detect NAD-MDH isoforms using gel-electrophoresis, gels were incubated in a fresh reaction mixture (100 ml 0.1 M Tris-HCl buffer, pH 8.0, containing 0.05 M malate, 0.02 M NAD^+, 0.01 M nitrotetrazolium blue, 0.01M phenasine metasulfate) prior to fixation at 37°C for 35-40 min.

Molecular weight of NAD-MDH was determined using gel-electrophoresis (Laemmli, 1970). Protein markers such as β-galactosidase (116 kDa), bovine serum albumin (BSA) (66.2 kDa), ovalbumin (45 kDa), lactate dehydrogenase (35 kDa), restriction endonuclease BSP981 (25 kDa), α-lactate globulin (18.4 kDa) and lysozyme (14.4 kDa) were used for the determination.

Total protein was determined according to the Sedmak, Grossberg method. BSA was used for the construction of the calibration curve. (Sedmak and Grossberg, 1997).

Statistical analysis: the data presented in the tables and figures are the mean values of at least three biological and mathematical replicates.

RESULTS AND DISCUSSION

C_4 plants are known to be more tolerant to extreme environmental factors compared to C_3 plants. Activity, localization and isoenzyme content of NAD-MDH in amaranth leaves were studied to clarify the role of the enzyme in the creation of biochemical mechanisms of plant tolerance. The obtained results are presented in Figures 1-2 and in Table 1.

Table 1. Subcellular distribution of NAD-MDH activity in amaranth leaves under soil drought conditions during anthesis (A - specific activity, U/mg protein)

Object, Variant		NAD-MDH activity			
		Cytosol		Mitochondria	
		A	%	A	%
Anthesis					
MC	C	56.6±1.68	28.0	125.5±3.2	61.6
	D	46.3±1.43	23.6	123.4±4.1	63.1
BSC	K	79.5±1.71	31.8	152.0±4.4	60.6
	D	51.1±0.99	25.1	140.0±3.8	69.0

Note: MC - mesophyll cells, BSC - bundle sheath cells, C - control, D – drought.

As seen in Figure 1 in the pre-anthesis phase NAD-MDH activities were similar in drought and control variants in MC and BSC of cytosolic fraction at the beginning of drought. However, in the anthesis and grain ripening phases the enzyme activity increased in both variants and this increase was more in drought-exposed plants than in control plants.

The activity of mesophyllic enzyme in MC and BSC (Figure 1B) was higher in all phases compared with cytosolic enzyme (Figure 1A). The enzyme activity increased in the drought-exposed plants and decreased only at the end of the vegetation, though this decrease occurred slower. Thus, the enzyme activity in the mitochondrial fraction was ~2.5 times higher in the pre-anthesis phase and ~2 times higher in the anthesis phase compared with the cytosolic fraction.

Electrophoresis revealed that NAD-MDH of amaranth leaves had a wide range of isoenzyme spectrum and isoform changes occurred in the isoenzyme spectrum under drought. Thus, five

isoforms of the enzyme with molecular weights of 58, 63, 68, 72 and 77 kDa were detected in SCFs of amaranth leaves, in the initial period of stress during active pre-anthesis phase of ontogenesis (Figure 2A). The distribution of these isoforms in SCFs was as follows: 58, 63, 68 and 77 kDa isoforms were localized in MC, 63 and 72 kDa isoforms in cytosol of BSC, 63 kDa isoform in chloroplasts of MC and BSC, 63, 68 and 77 kDa isoforms in mitochondrial fractions of MC and BSC.

No change was observed in cNAD-MDH of MC during the pre-anthesis and anthesis phases in control plants. However, 63 and 68 kDa isoforms disappeared under drought. During the phase of grain ripening 63, 68 and 77 kDa (with the exception of 58kDa isoform) isoforms of cNAD-MDH disappeared in MC of control plants. An inductive, 72 kDa isoform emerged under drought in addition to 58 and 77 kDa isoforms. All the isoforms disappeared at the end of vegetation and only 58 kDa isoform remained in cytosolic fraction of MC of control plants.

Different isoenzyme change patterns were observed in MC and BSC. As seen in the Figure in the cytosolic fraction of BSC of both control and drought variants 2 constitutive isoforms of the enzyme (63 and 72 kDa) were present in the pre-anthesis phase. Isoenzyme spectrum changed in the anthesis phase, 58 and 77 kDa isoforms emerged in control, while 58 kDa inductive isoform emerged in the drought-exposed variant. However, in the grain ripening phase only 58kDa isoform emerged in control, while 68 kDa inductive isoform emerged along with 58 kDa isoform in plants exposed to drought. The intensity of this isoform sharply declined to the end of vegetation (Figure 2).

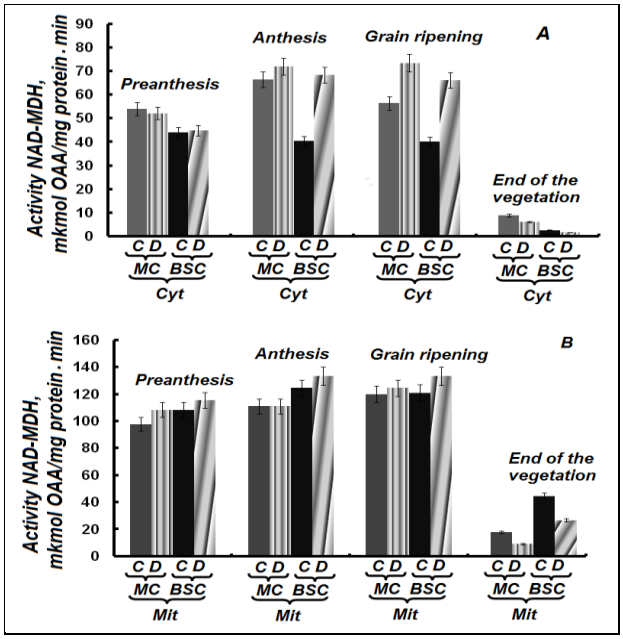

Figure 1. Dynamics of changes in NAD-MDH activity in subcellular fractions of MC and BSC in amaranth leaves under drought. A-cytosol, B-mitochondria, MC-mesophyll cells, BSC-bundle sheath cells, C-control, D-drought, OAA-oxaloacetate.

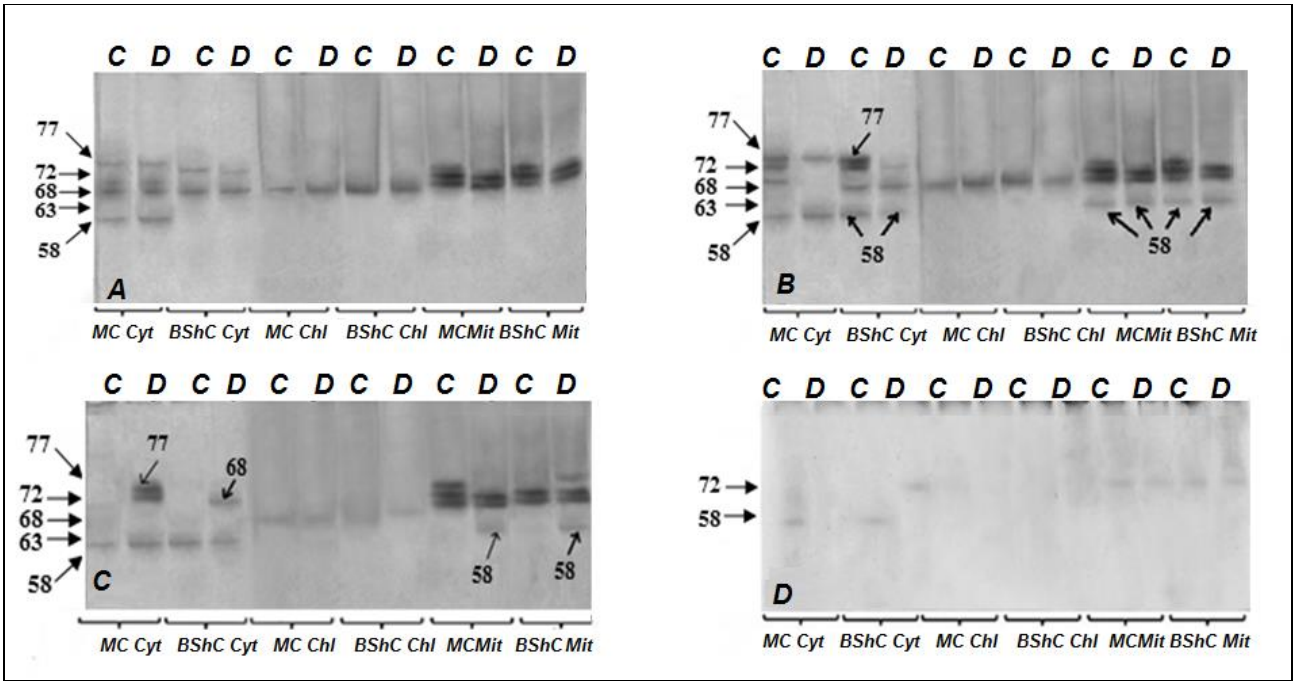

Figure 2. Determination of NAD-MDH isoforms in subcellular fractions of MC and BSC in drought-exposed amaranth leaves using gel-electrophoresis. A-pre-anthesis, B-anthesis, C-grain ripening and D-the end vegetation stages. Cyt-sitoplasma, Chl-chlorophyll, Mit-mitochondria, C-control, D-drought.

Table 2. Some kinetic parameters of the reaction catalyzed by NAD-MDH in subcellular fractions of MC and BSC of the leaves of amaranth exposed to soil drought in the anthesis phase.

Object,		Vari-ant	OAA (0.1-10 mM)		Malate (1-50 mM)		$MgCl_2$ (1-50 mM)		ATP (0.1-3 mM)	
			K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_i	V_{max}
Anthesis										
MC	Cyt	C	0.70	23.6	0.06	71.4	3.9	47.6	1.50	100.0
		D	0.53	43.4	0.05	333.0	3.9	100.0	2.10	20.0
	Mit	C	0.40	10.0	0.72	50.0	2.8	192.0	1.30	125.0
		D	0.30	50.0	0.40	111.0	4.0	240.0	1.20	100.0
BSC	Cyt	C	0.50	40.0	0.05	100.0	4.6	45.5	1.00	83.3
		D	0.63	100.0	0.04	250.0	5.0	100.0	2.30	33.3
	Mit	C	0.65	25.0	0.19	50.0	4.9	10.0	1.20	100.0
		D	0.51	50.0	0.23	100.0	6.0	25.0	1.03	66.7

Note: MC - mesophyll cells, BSC - bundle sheath cells, C - control, D - drought, Cyt - cytosol, Mit - mitochondria, OAA - oxaloacetate, V_{max} - mM/mg protein.

Three NAD-MDH isoforms with molecular weights of 63, 68 and 77 kDa were detected in mitochodrial fractions of both tissues in pre-anthesis and anthesis periods in control variants. In the pre-anthesis phase 77 kDa isoform disappeared in both tissues under drought conditions and 58 kDa inductive isoform emerged in both tissues of both variants in the anthesis phase.

In the grain ripening phase of control plants 58 kDa inductive isoform disappeared in MC and BSC of the mitochondrial fraction, while 58 kDa isoform remained and 72 kDa isoform emerged in BSC of the mitochondrial fraction under drought (Figure 2, Table 2) and only traces of 68 kDa isoform were detected. The differences observed in the NAD-MDH isoenzyme spectra of SCFs of assimilating tissues under drought are possibly related to metabolic properties of these tissues. Thus, one of the alternative ways of the plant protection against CO_2 deficiency due to the stomatal closure under water stress is the acceleration of synthesis rate of C_4 acids at the expense of MDHs. An increase in mNAD-MDH activity enhances the synthesis of C_4 acids. An enhancement in the synthesis of C_4 acids activates carbon concentrating mechanism upon stomatal closure and reduction of gas-exchange, and as a result Calvin cycle is provided with CO_2 (Wang et al., 2014).

The obtained results confirmed that complex anatomic structure of C_4 plant leaves obtained through the course of evolution resulted in the formation of photosynthesizing tissues and complexity in the function of the enzymatic systems and in the isoenzyme content due to stress.

Being very mobile NAD-MDH isoforms in SCFs of amaranth leaves can change depending on the phases of plant development and drought effects, and an isoform constitutive for one fraction may be inductive for another under stress. cNAD-MDH is less studied than mNAD-MDH. cNAD-MDH participates in some shuttle mechanisms and in the metabolism of substrates and reducing equivalents between cytoplasm and other cell organoids (Yu, Qing-Hu, 2004). Temperature sensitivity is one of the important properties of the enzymes. Temperature plays an important role in the regulation of activities of enzyme systems, biochemical conversions, formation of the balance between the enzyme molecule and enzyme-substrate complex (Cook, Cleland, 2007; Cornish-Bowden, 2012). Temperature influences on the enzyme stability, the rate of the destruction of the enzyme-substrate complex, enzyme-substrate affinity, enzyme affinity for activator and inhibitor (Cornish-Bowden, 2012).

The results obtained for SCFs of assimilating tissues showed that gradual rise in temperature till 45^{o}C resulted in the increase of the enzyme activity, maximum rate of the reaction was gained at 45-55°C, and at 70-80°C the enzyme activity was minimum, due to the denaturation of the enzyme structure. Optimum temperature range for NAD-MDH from the leaves of monocot C_3-plant was 45-50°C. According to the reports optimum temperature range for MDHs from various resources was 30-60^{o}C (Pinheiro et al., 1991).

The dependence between the rate of the direct and reverse reactions catalyzed by NAD-MDH and concentrations of substrates - OAA and malate was studied and the results are presented in Figures 3- 4 and Table 2. The isoforms localized in SCFs of amaranth assimilating tissues catalyzed the reaction rate stronger at 1.0 mM concentration of OAA under normal conditions. The NAD-MDH activity at 1.0 mM concentration of OAA increased by 20% under drought. As seen in Table and Figures, K_m(OAA) and V_{max} (OAA) values for sNAD-MDH and mNAD-MDH of mesophyll cells increased similarly in control and drought-exposed plants.

K_m (OAA) of cNAD-MDH in BSC decreased under drought, whereas V_{max} (OAA) increased. However, for mNAD-MDH of BSC the values of both K_m (OAA) and V_{max} (OAA) increased under drought (Figure 3, Table 2).

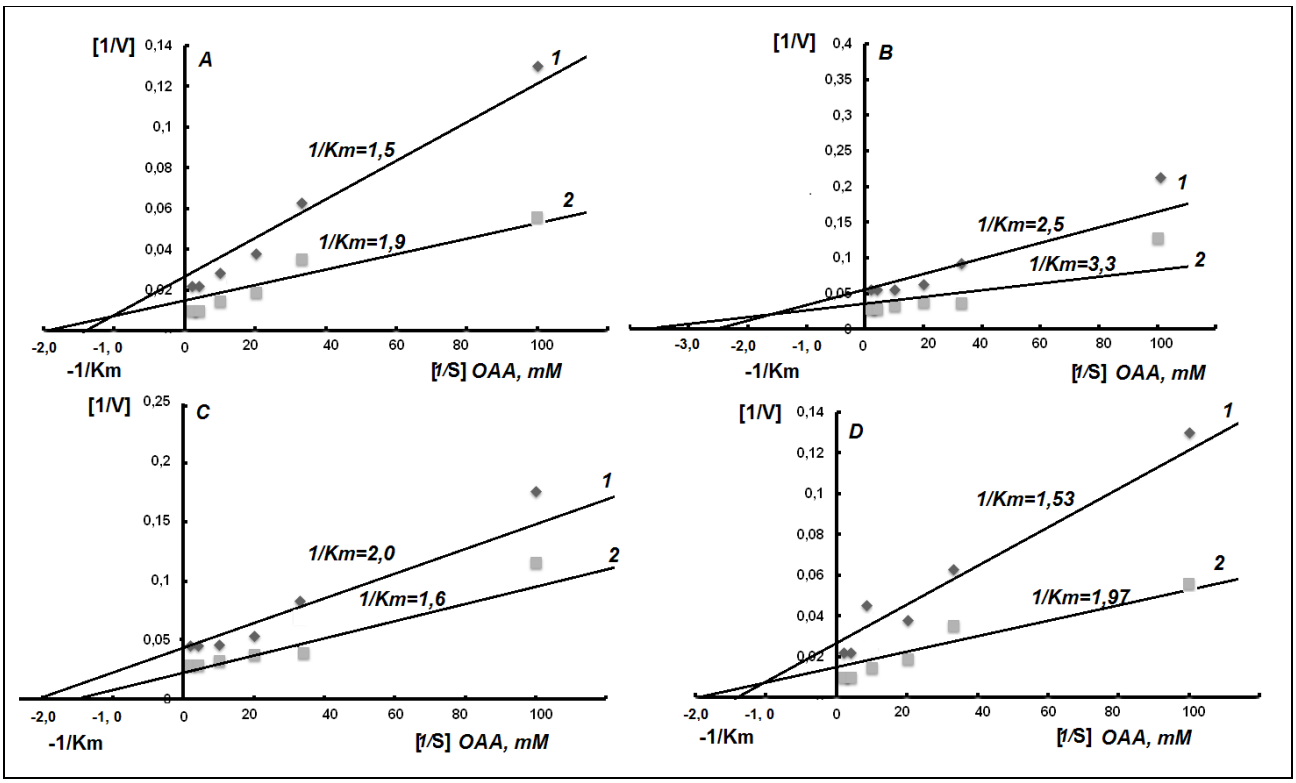

Figure 3. Kinetics of the changes of OAA reduction reaction depending on the substrate concentrations in various subcellular fractions of MC and BSC during the anthesis phase.
1 - Control, 2 - Drought. A - MC, cytosol, B - BSC, mitochondria, C - BSC, cytosol, D - BSC, mitochondria.

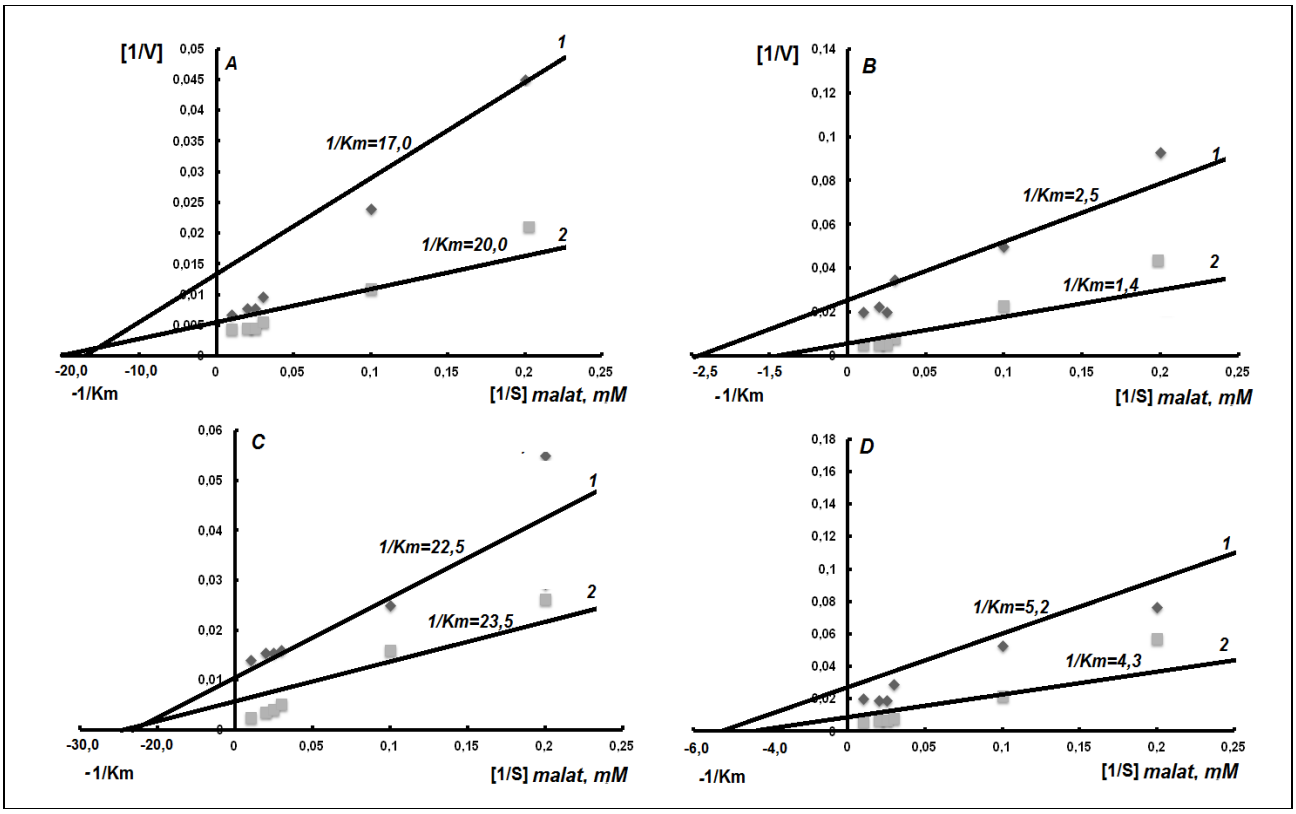

Figure 4. Kinetics of the changes of the reaction of malate reduction depending on the substrate concentrations in various subcellular fractions of MC and BSC during the anthesis phase.
1 - Control, 2 - Drought. A - MC, cytosol, B - BSC, mitochondria, C - BSC, cytosol, D - BSC, mitochondria.

The analysis of the results shows that K_m (OAA) values for the reaction catalyzed by NAD-MDH in amaranth leaves (1.2-2.1 mM) were less than those obtained for the same values in flag leaves of wheat genotypes. V_{max} values in BSC of amaranth leaves are 3-5 times higher compared with V_{max} of the same reaction catalyzed in SCFs of mesophyll cells of wheat flag leaves. In amaranth V_{max} was always higher in BSC than in MC and this difference increased under drought. In the cytosolic fraction of MC V_{max} was~2 times higher than in the mitochondrial fraction. Although K_m (OAA) and V_{max}(OAA) values were almost the same in the control and drought variants of cytosolic fractions of MC and BSC, V_{max} (OA) in the mitochondrial fraction of BSC was ~3-4 times higher compared with that in the mitochondrial fraction of MC.

The obtained results show that MDH is more sensitive to OAA, than to malate and these data are consistent with the results of other researchers (Figure 3, 4; Table 2). It was reported that low sensitivity of the enzyme to malate is related to the conformational changes of malate during its catalytic conversion (Сатар и др., 2010) The comparison of K_m values shows that cNAD-MDH is less sensitive to malate than to OAA. K_m values depended on plant species, type of the metabolism, the function performed in the cell and growth conditions (Figure 3, 4; Table 2). Analysis of the enzyme kinetic properties according to Lineweaver-Burk plots showed that during the enzyme inhibition K_m remained unchanged, while V_{max} decreased. As seen in the figure in SCFs of amaranth leaves 20-40 mM concentrations of malate caused an increase in the enzyme activity (Figure 3, 4; Table 2).

In direct and reverse reactions NAD-MDH exists in two conformation states differing in kinetic parameters. As malate oxidation is accompanied by overcoming additional energetic barrier and significant conformation changes in the structure of molecule, the reaction does not proceed with a stable rate. The OAA conversion reaction is not related to conformational changes occurring in the enzyme structure.

The differences in the kinetics of direct and reverse reactions may be due to the physiological functions, which NAD-MDH performs in SCFs of amaranth leaf cells.

Activators and inhibitors play am important role in the regulation of the enzyme activity. Activators facilitate substrate binding in the active center, formation of the enzyme-substrate complex, formation and stabilization of three-dimensional structure. Table 3 shows kinetics of the activating effects of Mg^{2+} ions on NAD-MDH isoforms in SCFs of amaranth leaves. As seen in the Table and Figure low concentrations of Mg^{2+} ions stabilize, while high concentrations inhibit MDH isoforms (Figure 5, Table 3).

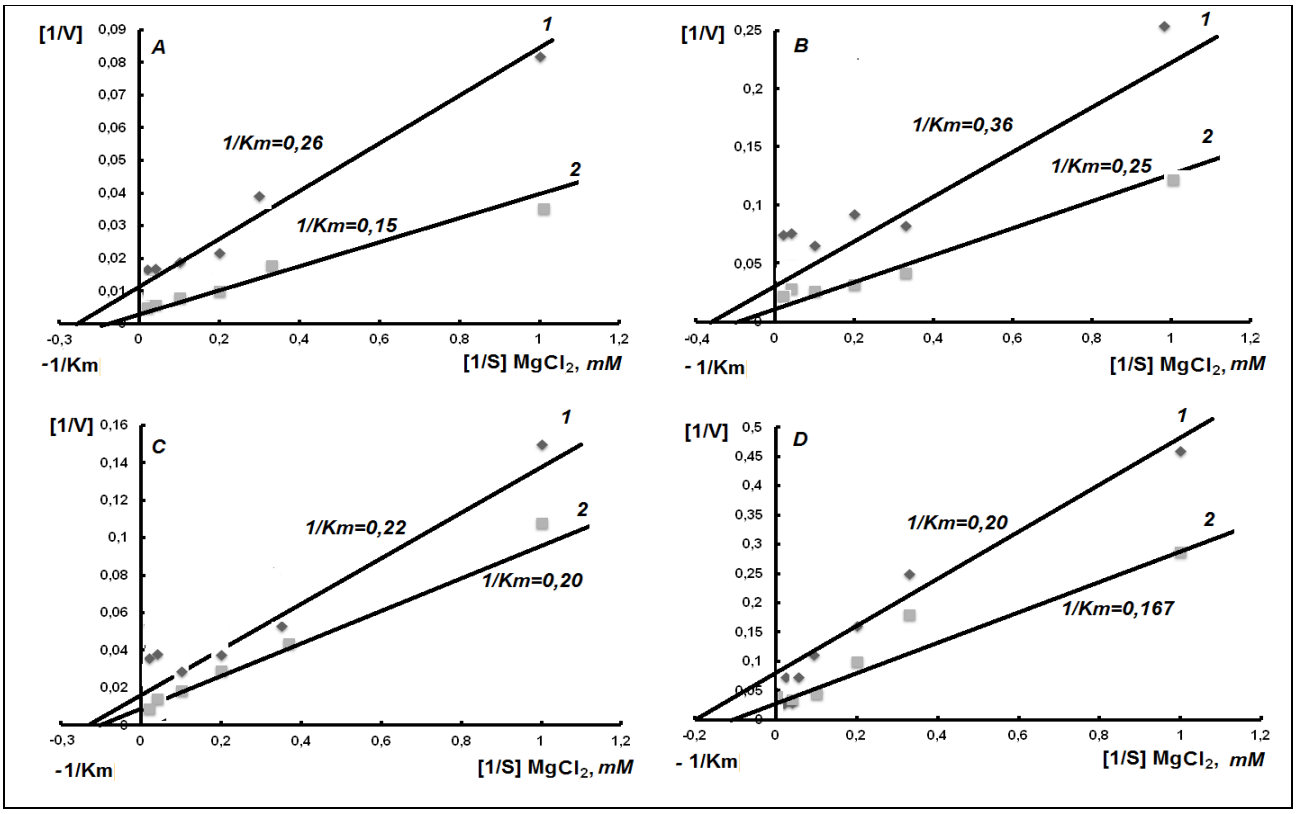

Figure 5. Kinetics of the NAD-MDH activation by Mg^{2+} in anthesis phase of amaranth plants exposed to soil-drought. 1 – control, 2 - drought, A - cytosol, B – mitochondria.

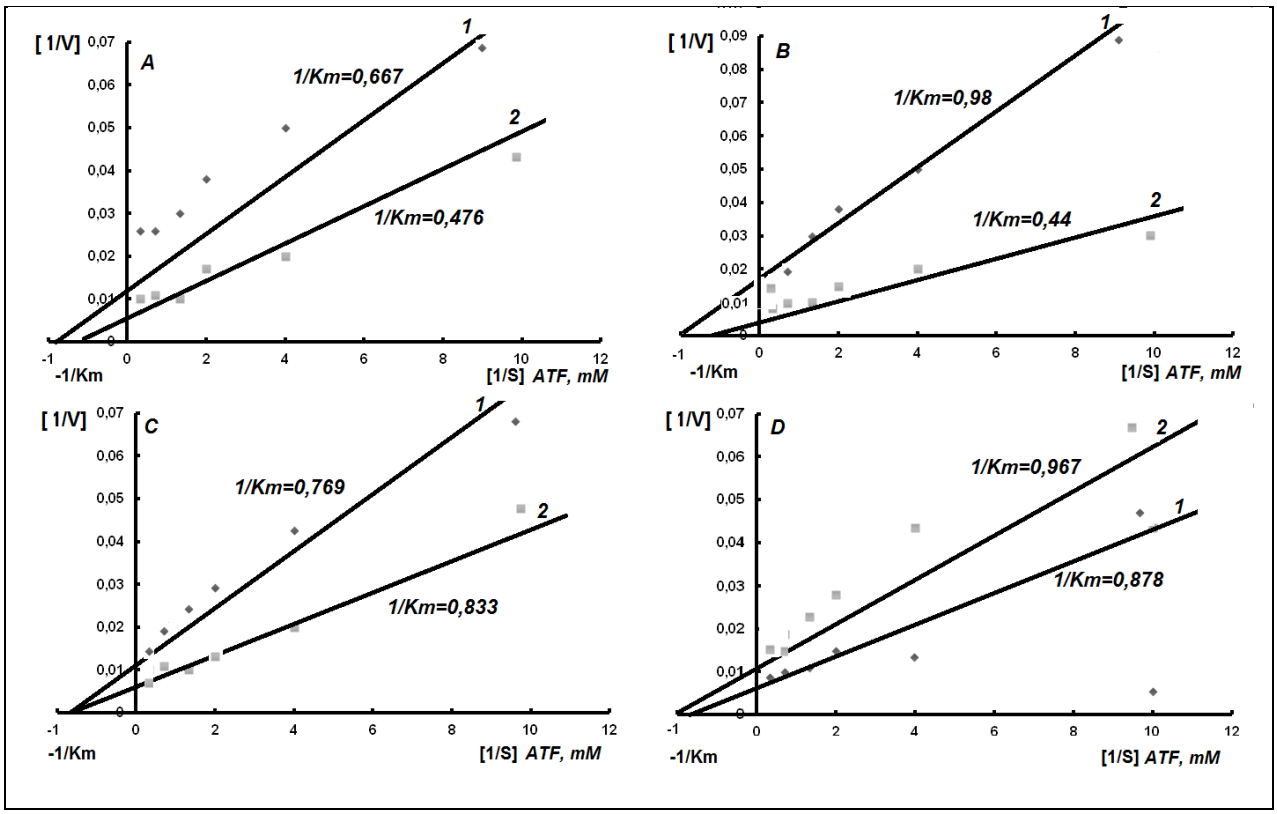

Figure 6. Kinetics of the NAD-MDH inhibition by ATP in anthesis phase of amaranth plants exposed to soil-drought. 1 – control, 2 - drought, A - cytosol, B – mitochondria.

According to some authors Mg^{2+} ions act as a competitive inhibitor of the MDH molecule [11]. Activating effect of Mg^{2+} ions on MDH isoenzymes can be related to the interaction of substrates with metal ions, which results in stabilization. K_m and V_{max} values obtained in the experiments performed with subcellular fractions from leaves of well-watered and stressed plants revealed that this ion was not a specific cofactor for the MDH isoforms.

ATP was found to be the efficient inhibitor of the enzymes of plant and animal origin which contradicts the previous reports. Results of the experiments showed that among MDH isoforms mMDH was more sensitive to ATP effects.

MDH is suggested to be one of the crucial links in the concerted action of metabolic pathways such as catabolism (Krebs cycle) and anabolism (gluconeogenesis). This feature is related to the fact that, mMDH is one of the main elements of the regulatory site between malate and citrate in the Krebs cycle. The mNAD-MDH activity is strongly controlled by the NAD^+/NADH and ATP/ADP ratios (Amthor, 2010).

Inhibitory effect of various ATP concentrations is presented in Figure 6 and Table 2. It was found that NAD-MDH activity of amaranth leaves sharply declined at 3 mM concentration of ATP.

As seen in the Table and Figure maximum rate of the reaction catalyzed by NAD-MDH (V_{max}) in MC decreased 5 and 0.25 times in cytosolic and mitochondrial fractions of amaranth leaves under drought, while 2.5 and 1.5 –fold decreases were detected in BSC, respectively. The obtained results confirmed that ATP inhibited cMDH localized in MC more than the enzyme localized in BSC, probably due to the subcellular localization of cMDH.

CONCLUSION

It was concluded that isoenzyme spectrum of amaranth NAD-MDH changed depending on the type of assimilating tissues, subcellular fraction and effects of drought stress. This change was more observed in cytosolic and mitochondrial fractions.

The disappearance of some constitutive isoforms of NAD-MDH and emerging of some inductive isoforms can be considered as a component of adaptive mechanism of plants against drought, which neutralizes stress effects and protects photosynthesis and CO_2 metabolism. This is also confirmed by the synchronous changes in the enzyme activity and some kinetic parameters. Thus, qualitative and quantitative changes occurring in the activity, isoenzyme spectrum, physicochemical and kinetic properties of the enzyme in assimilating tissues of amaranth leaves under the influence of drought and climatic factors show that NAD-MDH is a labile and adaptive enzyme.

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Quraqlıqda Amarant Yarpaqları NAD-Malatdehidrogenazasının İzoferment Spektri, Lokalizasiyası və Bəzi Fiziki-Kimyəvi Xassələrinin Tədqiqi

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Malatdehidrogenaza (MDH) enerji və hüceyrə metabolizmində mühüm rol oynayır. Bu məqalədə amarant yarpaqlarında sitozol (sMDH) və mitoxondri (mMDH) malatdehidrogenazalarının aktivliyi, subhüceyrə paylanması, izoferment spektri və kinetik xassələrinin quraqlığın təsirindən dəyişməsi bitkinin inkişafının çiçəkləmə önü, çiçəkləmə, toxum yetişmə fazalarında tədqiq olunmuşdur. *Amaranthus cruentus* L. yarpaqlarının assimilyasiyaedici toxumalarının subhüceyrə fraksiyalarında (SHF) bitkinin inkişafının çiçəkləməönü, çiçəkləmə, toxum yetişmə fazalarında quraqlığın təsirindən NAD-malatdehidrogenaza (l-malat-NAD-oksidreduktaza, NAD-MDH, EC 1.1.1.37) fermentinin aktivliyinin subhüceyrə paylanması, izoferment spektri və kinetik xassələri tədqiq olunmuşdur. Müəyyən olunmuşdur ki, amarant yarpaqlarında NAD-MDH geniş izoferment spektrinə malikdir və izoformaların sayı stres, toxuma növü və SHF-dan asılı olaraq dəyişir. Fermentin ümumi aktivliyinin əsas hissəsi hər 2 toxumanın sitozol və mitoxondri fraksiyalarına lokalizə olunmuşdur. MH-nin sNAD-MDH-sının kataliz etdiyi reaksiyanın V_{max} (OA) qiyməti mNAD-MDH-ya nisbətən 2 dəfə, ÖTH-nin mNAD-MDH-sının V_{max} (OA) qiyməti isə MH-nin mNAD-MDH müvafiq göstəricisindən 3-4 dəfə yüksək olmuşdur. Fermentin izoformaları geniş pH optimuna malik olub temperatura qarşı davamlıdırlar. Ferment SHF-dan asılı olaraq öz substratı olan oksalasetata (OA) qarşı yüksək, malata qarşı isə aşağı həssaslığa malikdir. NAD-MDH reaksiyası ümumilikdə Mixaelis-Menten qanunauyğunluğuna tabe olub substrata qarşı heç bir allosteriklik göstərmir. Fermentin aktivliyi aralıq substratlar, bir və iki valentli ionlarla ciddi tənzim olunur. ATF-in fermentin bütün izoformalarına inhibirləşdirici təsiri quraqlığın təsirindən daha da artır.

Açar sözlər: *Amarant, quraqlıq, NAD-MDH, izoforma, lokalizasiya, kinetika, tolerantlıq*

Изоферментный Спектр, Локализация и Некоторые Физико-Химические Свойства NAD-Малатдегидрогеназы в Листьях Амаранта При Засухе

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Малат дегидрогеназа (MDH) играет основную роль в энергетическом и клеточном метаболизме. Активность, субклеточное распределение, изоферментный спектр и кинетические свойства цитозольной (цMDH) и митохондриальной малатдегидрогеназы (mMDH) изучались перед цветением, в фазах цветения и созревания зерна. Субклеточная локализация, изоферментный спектр и кинетические свойства НАД-малатдегидрогеназы (l-малат-НАД-оксидоредуктаза, НАД-МДГ, КФ 1.1.1.37) изучались в субклеточных фракциях (СКФ) ассимилирующих тканей листьев *Amaranthus cruentus* L. перед цветением, в фазах цветения и созревания зерна. Было обнаружено, что НАД-МДГ имеет широкий изоферментный спектр в листьях амаранта, который меняется в зависимости от количества изоферментов, типа тканей и СКФ. Основная часть общей активности фермента была локализована в цитозольных и митохондриальных фракциях обеих тканей. Значение Vmax (ОА) реакции, катализируемой цитозольным ферментом в мезофильных клетках (МК), в 2 раза превышает значение Vmax (ОА) реакции, катализируемой митохондриальным ферментом. Однако, в клетках обкладки (КО) Vmax (ОА) для мНАД-МДГ в 3-4 раза превышает, Vmax (ОА) в МК. Изоформы фермента обладают широким диапазоном оптимальных значений pH и устойчивы к температуре. В зависимости от СКФ фермент обладал высокой чувствительностью к оксоацетату (ОАА) и низкой чувствительностью к малату. НАД-МДГ подчиняется кинетике Михаэлиса-Ментена, и не является аллостеричным. Активность фермента строго регулируется субстратами, одно- и двухвалентными ионами. Ингибирующее действие АТФ на все изоформы увеличивается при засухе.

Ключевые слова: *Амарант, засуха, НАД-МДГ, изоформы, локализация, кинетика, толерантность*