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FEATURES OF METABOLIC ADAPTABILITY IN AMARANTH PLANTS IN THE CONDITIONS OF HYPOBARIC HYPOXIA

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S u m m a r y

On 24-day seedlings of two species of amaranth: *Amaranthus hypochondriacus*L. and *A. tricolor*, the authors shown, that the Valentina variety in the conditions of 16-hour experimental hypobaric hypoxia in assimilating tissues activates the oxidative pentose-phosphate pathway and reduces amaranthine content, having antioxidant properties. At the same time the Kizlyarets amaranth variety during deficiency in oxygen deeply raising the activity of alcohol dehydrogenase processing an ethanol. The different mechanisms of biochemical adaptability to deficiency in oxygen in plants of Amaranthus genus are discussed.

Key words: *Amaranthus*, hypobaric hypoxia, respiratory metabolism, adaptation.

Anoxia and hypoxia occur at permanent or temporary waterlogging of soil, during the formation of ice crust, construction of city pavements, in high mountains and, finally, in spaceships. Plants' ability to survive in such conditions is provided by unique adaptive mechanisms, such as ability to transform metabolism (1-5).

The effect of oxygen deficiency in root system of plants is the most studied issue at present time. The structure and function of photosynthetic apparatus under root hypoxia haven't been studied enough, as well as metabolic adaptations of green plants to oxygen deficiency. Pressure chambers make possible the experimental simulation of hypobaric hypoxia in a whole plant by simultaneous impact of variable atmospheric pressure and deficit of oxygen and carbon dioxide.

In both resistant and sustainable plants, root hypoxia and anoxia leads to changes in assimilative organs expressed as decrease in number of active reaction centers of photosystems. The more prolonged root anoxia results in increased number of osmiophilic globules in chloroplasts of leaves owing to a partial destruction of thylakoid membranes (6). The study of hypobaric hypoxia effects in a whole plant revealed in green leaves the compensatory interaction between photosynthesis and respiration processes, as well as activation of anaplerotic mechanisms - accumulation of low molecular weight metabolites and formation of energy equivalents (7). In this context, physiology of stress in plants pays great importance to nuclear-cytoplasmic relationship, when the normal ontogeny of plastids is a prerequisite for correct expression of nuclear stress-reactive genes. It has been shown the transduction of stressor signal with participation of endogenous components - ethylene, jasmonic acid, carbohydrates, polyamines and various antioxidants including amaranthine. According to modern concepts, it plays an important role in photosynthetic, metabolic and protective reactions of plants (8).

The genus Amaranthus has high content of amaranthine, especially *Amaranthus tricolor* L. (the culivar Valentina). There's the literature data about reactions to stress in different *Amaranthus* species, particularly, to hypoxia (9). It has been also reported about their ability to rapid recovery of leaves' turgor and photosynthesis rate after the action of water stress (10). These facts suggest the search of endogenous metabolites providing resistance of plants even within a single genus or species as the promising direction of stress physiology.

Amaranth is the introduced crop from the family *Amaranthaceae*, the order *Caryophyllaceae* Juss. of the class *Dicotiledones*. This is a highly-productive plant with a great adaptive potential. Amaranth is a herbaceous annual colored purple-green or green (with different shades), height up to 2,5-4,0 m, mature panicles reach over 50 cm length and 15 cm diameter. Amaranth seeds and green phytomass are widely used as food, fodder and technical raw. Quality indicators of this crop (contents of protein, amino acids, vitamins, macro- and micronutrients, bioactive substances and oils) exceed basic traditional food and fodder crops (8, 11). Like maize, millet and sorghum, amaranth has C_4-type of photosynthesis, so it belongs to aspartate C_4 plants having rich content of lysine in the biomass, high rate of carbon dioxide fixation per leaf surface unit and high productivity under the conditions of intense insolation, sufficient heat and moisture supply.

The purpose of this work - to study the effect of hypobaric hypoxia on the content of betatcyanine amaranthine in assimilating tissues of two *Amaranthus* species and to assess their metabolism by the activity of some dehydrogenases at oxygen deficiency.

Technique. The objects of study were 24-days-old seedlings of two amaranth species - *Amaranthus hypochondriacus* L. (the cultivar Kizlyarets) and *A. tricolor* L. (the cultivar Valentina by VNIISSOK selection); the latter is known to have an increased content of betacyanine amaranthine (8). Plants were grown on soddy-meadow soil under fluorescent lamps (40 W/m^2) with 12 h photoperiod at 25 °C. To create anaerobic conditions, the samples were placed in a pressure chamber with low partial pressure of oxygen (P = 8 kPa, PO_2 = 2 kPa) for 16 h in darkness (to prevent photosynthesis). Control plants we placed in the dark under normal aeration and atmospheric pressure (P = 101 kPa, PO_2 = 21 kPa).

Experiments were performed 4-fold in 3-4 biological replications. Adult leaves were taken from the penultimate tier of 24-days-old seedlings, homogenized on ice in 8 ml chilled extraction medium (Tris-HCl buffer, pH 7,8 - 0,05 M, sodium ascorbate - 5 mM, cysteine - 3 mM, $MgCl_2$ - 1 mM , dithiothreitol - 5 mM) (12). The homogenate was squeezed through 4 layers of caprone and centrifuged in cold at 10 000 rpm for 20 min (the centrifuge K-24, Germany). Origin of reagents - "Sigma"(USA) and "Reachim" (Russia).

Enzymatic activity of alcohol dehydrogenase (K.F.1.1.1.1, NAD-ADH and NADH-ADH) was determined in the redox transformations of NAD+ and NADH+ in reaction mediums containing Tris-HCl buffer, pH 7,5 - 0,2 m , NADH - 2 mM, acetaldehyde - 50 mM (or NAD - 15 mM) and ethanol - 50 mM (13); the activity of NAD-malate-dehydrogenase (EC 1.1.1.37, NAD-MDH) – by reduction of NAD at presence of malate (Tris-HCl, pH 9,1 - 0,1 M, malate Na - 1,93 M and NAD - 11 mM) (12); NADP-glucose-6-

phosphate dehydrogenase (EC 1.1.1.49; NADP-GPDH) - in the medium containing Tris-HCl, pH 7,4 – 0,03 M, sodium glucose-6-phosphate – 0,12 mM, $MgCl_2‡6H_2O$ - 0,25 M and NADP - 11 mM (14). Enzyme activity was assessed spectrophotometrically (Shimadzu UV-2100, "Shimadzu Corp.", Japan) by the change in optical absorption at λ=340 nm per 1 mg protein and 1 g wet weight. The content of soluble protein was determined according to Bradford (15), the content of amaranthine - by absorption at λ=537 nm (16, 17).

Results were processed statistically, differences between compared averages were calculated at $p < 0,05$ (18).

Results. Hypobaric environment is characterized by simultaneous decrease in partial pressure of gases, including the most important for plants O_2 and CO_2. In contrast to other types of hypo- and anoxia (flooding, displacement of air with inert gases, etc.), the effects of hypobaric hypoxia can be studied in autotrophic tissues which directly interact with air of rarefied oxygen-depleted atmosphere.

The authors compared the content of betacyanine amaranthine in leaves of 24-days old amaranth seedlings, which confirmed the literature data on its higher content in cv Valentina (8). The impact of 16-hour hypoxia didn't affect amaranthine content in leaves of cv Kizlyarets, while it significantly decreased in cv Valentina (Table 1).

1. The content of betacyanine amaranthine in leaves of 24-days-old seedlings of two amaranth varieties under hypobaric hypoxia

Cultivar	Variant	Amarantine content	
		ug/g wet weight	%
Kizlyarets	Control	115,6±9,3	100
	Experiment (hypoxia)	99,7±9,8	87
Valentina	Control	375,02±6,43	100
	Experiment (hypoxia)	288,16±27,20*	76

Note: Conditions of hypobaric hypoxia — P = 8 kPa, PO_2 = 2 kPa, 16 h
* Differences between control and experiment are reliable at $p < 0,01$.

Considering the protective function of amaranthine, such quantitative changes indicate metabolic differences of these plants in conditions of hypobaric hypoxia.

2. Alcohol dehydrogenase (ADH) activity in leaves of 24-days-old seedlings of two amaranth varieties at normal aeration and under hypobaric hypoxia

Medium, enzyme	Variant	Enzyme activity	
		mU/mg protein	mU/g wet weight
	Cv. Kizlyarets		
Acetaldehyde, NADH-ADG	Control	27,58±3,60	61,38±5,40
	Experiment (hypoxia)	21,71±2,77	46,47±4,08
Ethanol, NAD-ADG	Control	6,28±0,84	16,58±1,71
	Experiment (hypoxia)	9,30±1,09	22,06±2,48
	Cv. Valentina		
Acetaldehyde, NADH-ADG	Control	36,67±2,51	66,86±5,94
	Experiment (hypoxia)	87,63±8,85	138,26±22,84
Ethanol, NAD-ADG	Control	11,39±1,19	25,82±2,44
	Experiment (hypoxia)	6,38±0,98	17,20±0,90

Note: all differences between control and experiment are reliable at $p < 0,01$.
Conditions of normal aeration — P = 101 kPa, PO_2 = 2 kPa, hypobaric hypoxia — P = 8 kPa, PO_2 = 2 kPa, 16 h.

The activity of NADH-dependent alcohol dehydrogenase – reductant of acetaldehyde - was determined, which revealed differences in enzyme functioning in the studied varieties (Table 2). After the 16-hour hypoxia, the activity of NADH-ADH in leaves of cv Kizlyarets decreased, in cv Valentina – it sharply raised (possibly, this indirectly indicates accumulation of ethanol). NAD-ADH showed an opposite activity trend - it increased in cv Kizlyarets and declined - in cv Valentina.

NAD-malate dehydrogenase operates at one of final stages of Krebs cycle. 16-h hypoxia suppressed its activity in leaves of both studied species (Table 3): by 22% - in cv Kizlyarets and by 41% - in cv Valentina, therefore, reactions in the citric acid cycle slowed down. At the same time, the activity of NADP-glucose-6-phosphate dehydrogenase (conversion of glucose in the oxidative pentose-phosphate pathway) in cv Valentina increased by 33% compared with control, in cv Kizlyarets – remained almost unchanged (in terms of both 1 g wet weight and 1 mg protein) (Table 3).

3. The activity of NAD-malate-dehydrogenase and NADP-glucose-6-phosphate dehydrogenase in leaves of 24-days-old seedlings of two amaranth varieties under hypobaric hypoxia

Cultivar	Variant	NAD-malate-dehydrogenase		NADP-glucose-6-phosphate dehydrogenase	
		U/g wet weight	mU/mg protein	U/g wet weight	mU/mg protein
Kizlyarets	Control	2,93±0,27	501,80±35,48	34,17±1,43	5,85±0,26
	Experiment (hypoxia)	2,87±0,28	390,00±39,83*	29,53±1,50*	5,78±0,18
Valentina	Control	3,28±0,25	503,30±27,25	51,74±2,34	7,66±0,24
	Experiment (hypoxia)	2,34±0,16*	296,80±18,30*	92,21±3,75*	10,19±0,43*

Note: Conditions of hypobaric hypoxia — P = 8 kPa, PO_2 = 2 kPa, 16 h
* Differences between control and experiment are reliable at $p < 0,01$.

The above-mentioned patterns suggest the following transformation mechanisms of metabolism in amaranth assimilative organs under the conditions of hypobaric hypoxia in the dark. In this variant of the experiment, amaranthine content decreased only in cv Valentina keeping initially high content of this betacyanine - 300-500 mg/g wet weight (vs. 90-130 mg/g wet weight in cv Kizlyarets). The activity of NAD-ADH indicates that oxygen deficiency enhances the function of this enzyme in cv Kizlyarets; consequently, ethanol processing occurs, and, possibly, switching of biochemical processes from ethanol synthesis into formation of other compounds, as well as to oxidation of reduced coenzymes necessary for maintenance of high reaction rate in the glycolytic pathway. At the same time, cv Kizlyarets demonstrated the decrease in activity of NAD-malate dehydrogenase, which process occurred without activation of oxidative pentose-phosphate pathway, as it was indicated by the activity level of NADP-glucose-6-phosphate dehydrogenase per 1 mg protein.

The variety Valentina exhibited a distinct nature of respiratory metabolism under the short-term hypoxia. The decline in enzymatic activity of the Krebs cycle and increase in ADH activity (reduction of acetaldehyde into ethanol) were accompanied by enhancing of oxidative pentose-phosphate pathway. These mechanisms along with the peculiar high content of bioactive antioxidant amaranthine can ensure the resistance of this genus to oxygen deficiency.

Oxidative pentose-phosphate pathway provides NADPH, which is used as the reducing agent in biosynthesis when there's no formation of NADPH in photosynthesis. This pathway is especially important in non-photosynthesizing tissues, germinating seeds, as well as in the dark period. During this process, oxidation of carbohydrates results in ribose-5-phosphate required for synthesis of nucleotides and nucleic acids. In hypoxic conditions, the content of amaranthine decreased only in cv Valentina, but, apparently, this loss can be refilled by the products formed in oxidative pentose-phosphate pathway. It has been shown, that amaranthine synthesis occurs through the formation of shikimic acid by condensation of erythrose-4-phosphate (the product of pentose-phosphate pathway) and phosphoenolpyruvate (the product of glycolytic pathway) (19).

The ability of betacyanine amaranthine to form complexes with variable-valence ions of iron, copper, zinc - regulators and catalysts of free-radical processes (8) proceeding in plants at hypoxic stress (3, 20) - leads to consumption of amaranthine and provides the resistance to hypoxia in cv Valentina. Such activation of oxidative pentose-phosphate pathway is typical for plants adapted to oxygen deficiency (3).

Thus, two species of two different *Amaranthus* varieties with unequal amaranthine content were subjected to hypobaric hypoxia in the dark, which allowed to reveal peculiarities of respiratory processes in assimilating tissues. Amaranthine is the antioxidant providing protective responses of plants, such as resistance of cv Valentina to oxygen deficit. Amaranthine decreases the intensity of free-radical processes in cells, and simultaneous intensifying of glucose utilization in oxidative pentose-phosphate pathway allows to supply its reserves and reduction of coenzymes. In conditions of oxygen deficit, cv Kizlyarets demonstrated constant, but lower content of amaranthine, and stress-resistance provided by enhanced oxidation of fermentation products, which process was activated by alcohol dehydrogenase utilizing ethanol as a substrate.

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