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Changes of nicotinamide coenzymes and adenylate energy charge in leaves of hybrid and parental tomato forms in an *in vitro* culture

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Abstract

Interrelation between dry matter in leaves and catabolic and anabolic activity in parental forms and F_1-hybrids of *Lycopersicon esculentum* Mill was investigated under *in vitro* culture. Heterosis in dry matter was observed in hybrids and similar values of adenylate energy charge (AEC) and the redox charge (RC), displaying balance between the systems generating energy and the processes associated with its consumption, were revealed. A higher recovery degree of NADP-NADPH system in comparison with NAD-NADH one, showing inutilization of recovery equivalents in the NADPH form in biosynthetic processes, was detected in the initial cultivars. The content of adenylate nucleotides, nicotinamide coenzymes and their changes might play a role in regulatory mechanisms of biomass accumulation in F_1-tomato hybrids and their parents.

Key words: heterosis, *Lycopersicon esculentum*, metabolic control, nucleotides.

Introduction

Nicotinamide coenzymes and adenyl nucleotides are the key components of the basic systems of energy generation - photosynthesis, electron transport chain of mitochondria, glycolysis and pentose phosphate pathway. They are also of importance in controlling almost all oxidative processes of plant metabolism. A general feature of adenyl and nicotinamide systems is that they display quite completely activity, stress and direction of energy metabolism (Khotyleva *et al.* 1991).

The functions of two systems NAD-NADH and NADP-NADPH are different in metabolism control. Taking into consideration the key role of each of them in ensuring functioning of catabolic and anabolic pathways, Andersen and Meyenburg

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Abbreviations: AEC - adenylate energy charge; AN - adenyle nucleotides; ARC - anabolic reduction charge; CRC - catabolic reduction charge; PN - pyridine nucleotides; RC - redox charge.

(1977) proposed to call the ratio [NADH]/[NAD]+[NADH] the catabolic reduction charge (CRC) and the ratio [NADPH]/[NADP]+[NADPH] the anabolic reduction charge (ARC) and together with adenylate energy charge (AEC) - [ATP]+0.5[ADP]/[ATP]+[ADP]+[AMP] (Atkinson and Walton 1967) to consider them as regulatory parameters of cell metabolism. For quantitative expression of energy ratio of oxidized and reduced forms of nicotinamide coenzymes was introduced one more parameter - the redox charge (RC) - [NADH]+[NADPH]/([NAD]+[NADH])+([NADP]+[NADPH]) (Quebedeaux 1981), defining the total degree of cell system reduction.

Many researchers attach particular importance to a comparative study on the activity of the most important metabolic systems, directed to energy provision of growth, for ascertaining physiological and biochemical mechanisms of heterosis and developing tests of its prediction. However, information on characteristic properties of energy metabolism in connection with heterosis manifestation is scanty and in most cases is limited by establishment of facts about enzyme activity, the content and age dynamics of macroergic compounds (Mino and Inoue 1980, Shigemi *et al.* 1990, Khotyleva and Titok 1994). As a rule, regulation of general metabolic activity of the processes responsible for bioenergetic and plastic exchange in F_1-hybrids and initial forms of plants is not taken into account. Since the degree of heterosis effect manifestation depends on growth conditions of plant organism it was expedient to use plant propagation in an *in vitro* culture for producing a sufficient amount of physiologically and genetically homogeneous material. The results of the comparative analysis of the adenyl and pyridine nucleotide contents and their charge values as well as estimation of energy exchange efficiency and its role in controlling activity of growth processes in leaves of tomato parental cultivars and their F_1-hybrids in an *in vitro* culture are presented in this paper.

Materials and methods

Plant material: Tomato (*Lycopersicon esculentum* Mill) plants of three cultivars, Tropson, Premier TM, Son TM C F_1 were used. Hybrids were produced by the following crossing combinations: Tropson × Son, Premier × Son, and Tropson × Premier.

To preserve the unique genetic features and to exclude the influence of growth conditions on plant material we used a method for tomato propagation in an *in vitro* tissue culture of a meristematic type (Podlisskikh and Yermishin 1990). This cultivation produces a sufficient amount of genetically and physiologically homogeneous material. Plants were grown in 500 cm^3 Erlenmeyer flasks (2 apex per flask) containing 75 cm^3 of a complete MS (Murashige and Skoog 1962) containing 8 % agar medium without hormonal addition under irradiance of 100 W m^{-2} ("white light" fluorescent tubes). The photoperiod was 16 h, day/night temperature 25/18 °C and air relative humidity 70 ± 5 %. Biochemical analysis was carried out in 36 - 40-d-old plants with five leaves (Titok *et al.* 1994).

Extraction of adenyl nucleotides, nicotinamide coenzymes: The frozen tissue (150 - 200 mg) was ground in a mortar cooled by liquid N_2, transferred into *Potter-Elvehjem* glass homogenizer after weighing and treated in extragent solution. Adenyl nucleotides, nucleosides, bases and oxidized forms of nicotinamide coenzymes [adenin (Ade), adenosine (Ado), AMP, ADP, ATP, NAD^+, $NADP^+$] were extracted by ice-cold 6 % $HClO_4$. Recovered forms of nicotinamide coenzymes (NADH, NADPH) were isolated with 2.8 % KOH in 50 % C_2H_5OH followed by 1 min water bath heating at 70 °C and immediate cooling (Maciejewska and Kasperska 1987). After homogenization the suspension was incubated in ice for 10 min and centrifuged (0 °C, 15 min, 14 000 *g*), the pellet being removed and the supernatant being neutralized by supplement of 5 M K_2CO_3 and 2 M HCl, respectively.

Chromatographic procedure: The reaction of adenine and its analogues with bromoacetaldehyde to form the highly fluorescent 1,N^6-etheno derivates has been described and utilized in an analytical procedure (Preston 1983, Perrett 1985). Derivatization was carried out by mixing of nucleotide solutions, acetate buffer (1.0 M, pH 4.5) and bromoacetaldehyde (2 M) of 10:10:1 and placing them in water bath (80 °C for 15 min) (Yoshioka *et al.* 1984). The mixture was cooled, filtered through a 0.22 μk filter (*Millipore*, Bedford, USA) and subjected to reversed-phase ion-pair high-performance liquid chromatography (HPLC). HPLC analysis was carried out on microcolumn apparatus *CL-1311* (*Optron*, Minsk, Belarus) equipped with fluorimetric detector (flowing cuvette 0.1 cm^3, extinction - 232 nm, emission > 389 nm) (Perrett 1985). The investigated components were performed using glass column packed with 5 μm *Separon SGX C18* (150 × 1 mm) (*Tessek*, Prague, Czech Republic); the buffers were 20 mM $(NH_4)H_2PO_4$, 2 mM tetrabutyl ammonium phosphate of pH 7.0 (*A*) and the same buffer with 14 % acetonitrile (v/v) (*B*). The elution programme was as follows: 3 min *A* only; 3 - 10 min linear increase in *B* from 0 to 90 %; 10 - 20 min linear increase in *B* from 90 - 100 %; 20 - 35 min *B* only; 35 - 45 min *A* only. The flow rate was 0.04 cm^3 min^{-1} and the volume of the introduced sample - 0.25 mm^3. Peak identities were confirmed by coelution with standards (*Sigma Chemical Co.*, USA). The peak area was determined so that the amount of nucleotide present could be calculated using a computing integrator *C1-100 A* (*Laboratorní přístroje*, Praha, Czech Republic).

Statistics: The means and the mean errors were calculated from 8 - 9 experiments. Student's *t*-tests for independent random samples were used for the statistical analysis (Significance levels: * - $P < 0.05$; ** - $P < 0.01$).

Results and discussion

Formation and accumulation of dry substances are a generalized index of activity and interaction between physiological and biochemical processes of a plant organism. Our results on accumulation of dry mass in leaves did not show the presence of genotypic variability both among the initial parental forms and among hybrid combinations (Fig. 1). However, significant differences (89.6 and 241.6 mg,

respectively) were revealed between mean dry mass in parents and F_1-hybrids. A high percentage of heterosis in the dry mass content in leaves (on the average 138 %) was observed in all hybrid genotypes.

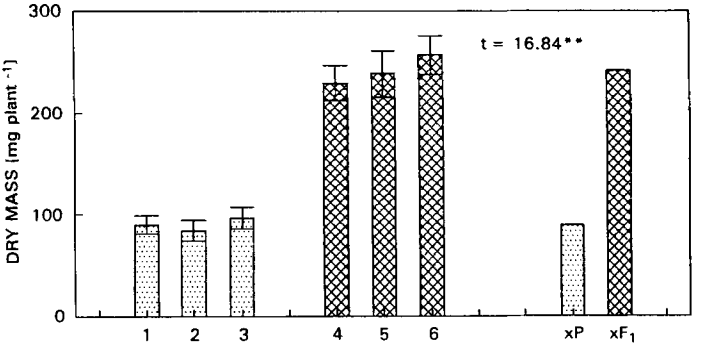

Fig. 1. Dry matter of leaves of cultivars: *1* - Tropson, *2* - Premier, *3* - Son and F_1 hybrids of tomato: *4* - Tropson × Son, *5* - Premier × Son, *6* - Tropson × Premier. *xP* - mean of parents, xF_1 - mean of hybrids. Mean of five replications ± S.E. The difference between parents and F_1-hybrids was significant at $P < 0.01$.

The content of some adenyl nucleotides in leaves of the analyzed tomato forms varied in a wide range (Table 1). Among the initial genotypes the cv. Premier was characterized by a low level of all adenyl nucleotides and by their total content that may indicate low efficiency and power of energy exchange. The excess of the total content of all adenyl nucleotides over the initial cv. Premier (on the average above 3.5-fold). On the whole, a significant advantage of F_1-hybrids over the parents in the content of macroergic compounds (ADP and ATP) and the sum of adenyl nucleotides in tomato leaves indicate more effective functioning of energy generating systems.

Table 1. Content in specific and total adenine nucleotides (AN) in leaves of parents and F_1-hybrids of tomato [nmol g^{-1}(f.m.)], mean of five replications ± SE. Difference between parents and F_1-hybrids significant at * - $P< 0.05$; ** - $P< 0.01$.

	AMP	ADP	ATP	AN
Tropson	35.14± 6.08	74.96±13.13	73.51±13.28	157.46±41.53
Premier	27.15± 2.96	47.94± 5.24	48.93± 7.79	123.01±13.18
Son	41.76± 4.45	78.40± 8.49	45.09± 3.88	189.76±22.93
Tropson × Son	22.78± 1.49	69.89± 9.62	95.24±12.36	187.85±21.64
Premier × Son	58.26±10.09	90.28±10.33	89.97±17.13	238.53±27.17
Tropson × Premier	30.65± 5.65	99.31±17.25	112.44±15.12	245.11±35.31
Mean of parents	34.30	65.81	62.84	154.98
Mean of F_1-hybrids	33.02	85.74*	101.05**	220.90**

Further on, the interrelation between the content of nicotinamide coenzymes and the level of their reduction, and the revealed differences in the degree of biomass accumulation between hybrid and the initial forms of tomato were studied. The

comparative analysis of pyridine nucleotides did not show any significant genotypic differences between the initial and hybrid forms (Table 2). Concentrations of pyridine nucleotides seem to be saturated for a number of key enzymes of energy metabolism (*e.g.* as isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase) that indicates the presence of more perfect systems of controlling redox processes in cells of hybrid plants with respect to the parental ones. It should be noted that NADP was revealed in relatively low concentrations as compared to other components of nicotinamide coenzymes (Ogren and Kroggmann 1965, Zhao *et al.* 1987), more than half of NADP total cellular pool being reduced. In plants under *in vitro* conditions NADPH oxidation by cytochrome system seems to be of minor importance, in this case reduction reactions function more intensively and hydrogen accumulation is directed at biosynthetic processes and not at maintaining the rate of metabolic reactions under variable growth conditions in the field (Bonzon *et al.* 1983).

Table 2. Content of specific and total pyridine nucleotides (PN) in leaves of parent and F_1-hybrids of tomato [nmol g^{-1}(f.m.)], mean of five replications ± SE. Difference between parents and F_1-hybrids significant at * - $P< 0.05$; ** - $P< 0.01$.

	NAD	NADP	NADH	NADPH	PN
Tropson	24.15± 1.35	8.01±0.49	47.29± 7.18	47.53± 9.64	126.98
Premier	18.21± 4.08	4.84±1.39	39.96± 7.22	17.20± 4.22	76.64
Son	26.86± 4.69	5.29±0.41	48.82±10.21	27.20± 8.01	98.51
Tropson × Son	11.00± 2.19	5.38±1.58	34.86± 5.73	31.31±12.55	67.08
Premier × Son	44.76±10.11	25.82±9.11	51.36± 9.35	45.55± 3.72	167.52
Tropson × Premier	17.81± 2.96	9.06±2.25	34.03± 3.16	16.57± 2.68	79.46
Mean of parents	23.21	5.92	44.43	26.68	100.71
Mean of F_1-hybrids	20.47	10.94	37.83	28.26	104.69

The course of metabolic processes in cell depends not so much on absolute content of some forms of NA-coenzymes as on the molar concentration of every component in the whole system of pyridine nucleotides (Williamson *et al.* 1967), *i.e.* from reduction charges. In leaves of parents significant excess of ARC over CRC (1.2-fold) was observed (Fig. 2) that can be evidence of higher reduction rate of NADP-NADPH system in comparison with NAD-NADH and indicate substantial formation of reduction equivalents in the NADPH form not utilized in biosynthetic processes (Fig. 1). The lesser difference in the values of all charges tested was detected in leaves of hybrid plants (Fig. 2). Practically similar values of CRC and ARC in hybrids Tropson × Son and Premier × Son displayed equilibrium course of energy generating and energy consuming processes.

Calculated charges of nicotinamide coenzymes and AEC display specific features of energy metabolism in leaves of hybrid and parental forms of tomato in an *in vitro* culture. The lesser value of adenylate energy charge in comparison with redox charge was observed in parental genotypes that can be indicative of energy disbalance state between the systems generating energy and the processes associated with its consumption. In hybrids approximately equal values of AEC and RC show

equilibrium between intensity of functioning adenyl- and pyridine nucleotide systems.

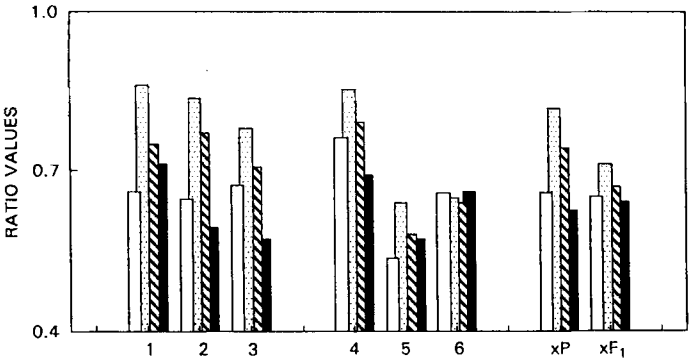

Fig. 2. Anabolic reduction charge (*empty columns*), catabolic reduction charge (*dotted columns*), redox charge (*hatched columns*) and adenylate energy charge (*full columns*) in leaves of tomato cultivars and F_1 hybrids. For symbols see Fig. 1.

Thus, the systems, related with energy generation and formation of reduction equivalents in tomato hybrids, function more effectively than in parental forms. Oxidation and reduction of nicotinamide coenzymes in leaves of hybrids seem to be in equilibrium with the ratio of adenyl nucleotides and such a state is typical for the energy consuming systems. Probably during hybridization an optimum level of metabolic activity is created that may be a real precondition for heterosis in biomass accumulation. In parental forms the ratios of charges of nicotinamide coenzymes and adenyl nucleotides are more characteristic of the energy accumulating system that can be caused by dissociation of energy production - and energy consumption processes.

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