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Comparatıve study of the effects of radıatıon and salt stresses on the actıvıty of oxaloacetate dexarboxylase ın leaves of common bean

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Abstract

The activity of oxaloacetate decarboxylase (OAD, EC 4.1.1.3) was studied comparatively in leaves of the common bean (*Phaselus vulgaris* L.) plants grown under radiation (1,5,10,50,100,200 Qy) and salt (1,5,10,50,100 mM NaCl) stress conditions. The OAD enzyme was found to be more sensitive to stress in plants grown under various doses of radiation compared with those grown under salinity. The activity of OAD, the major enzyme of the pyruvate biosynthesis, increased during the initial period of the plant development and decreased under stress. However, as adaptive processes occur gradually under salt stress, the plant is more tolerant to stress factors. In both cases, occupying one of the central positions in the energy space, OAD plays a key role in the coordination of interconnected metabolic processes and the energy balance of the organism.

Keywords: *Phaselus vulgaris* L., OAD, radiation, NaCl, aktivity, stress, adaptation

Introduction

The productivity of crops decreases by ~30-35% due to abiotic stress factors such as salinity, soil drought, radiation, etc. [Peters *et al*., 2004] [21]. To resolve the food problem, expected because of the salinization of ~ 20% of the world soils [Kafi, 2003] [7], and on the other hand, continuous population growth (~10 billion till 2025) [Dyson, 1999] [15], the selection of stress-tolerant, highly-productive plant varieties is of great importance. This problem is more actual because of the dependence of plant stress tolerance and productivity on activities of the enzymatic systems of energy exchange [Miflin, 2000] [20].

Components of the energy system localized in the chloroplasts, cytosol, and mitochondria of the leaf cells of higher plants ensure the maintenance of the physiological stability of the organism by acting coordinated in metabolism. From this point of view, complex biochemical processes such as photosynthesis, glycolysis, and respiration, involved in the energetic system can play an important role in the plant adaptation to stress at functional and energetic levels.

The metabolism of pyruvate, formed as a result of the anaerobic oxidation of glucose, one of the final products of photosynthesis, is known to be multilateral. Thus, pyruvate is synthesized in living organisms from carbohydrates, lipids, and proteins, and organic acids such as malate and oxaloacetate (OA) and facilitating the synthesis of OA, lactate, alanine, acetyl CoA, ethyl alcohol, acetate aldehyde, malate, glucose, etc. it participates in the process of energy generation [Lehninger *et al*., 2008] [18]. Under aerobic conditions, pyruvate with acetyl-CoA is included in the Krebs cycle and continues its metabolism. As a result of the anaplerotic reaction, it is converted into OA, one of the intermediate metabolites of the Krebs cycle, which is used to re-synthesize glucose by gluconeogenesis [Lehninger *et al*., 2008] [18]. Decarboxylation of pyruvate is known to lead to the formation of acetyl-CoA, when pyruvate is carboxylated OA is formed, as a result of transamination of pyruvate alanine is formed and its reduction results in the formation of lactate [Lehninger *et al*., 2008] [18]. The role of pyruvate is irreplaceable as an intermediate metabolite in plants exposed to stress.

It was found that the NADP-ME activity in etiolated leaves of pea and barley plants increased in the first days of the lighting and then decreased [Karpilov, 1977] [6]. The NADP-ME activity decline in the ontogenesis of C3 plants is accompanied by the OAD activity increase. The activity of one enzyme is replaced by the activity of another enzyme without any contradiction.

Most OA transported to chloroplasts was decarboxylated in the reaction catalyzed by OAD and used in the pyruvate synthesis [He, Hou, 2014]. Pyruvate dehydrogenase multi-enzyme complex realizing pyruvate metabolism was revealed in C3 plant chloroplasts [Camp *et al*., 1988] [12]. The enzymes such as OAD, NAD-malate dehydrogenase, MADP- malate dehydrogenase, NADP-malic enzyme, carboanhydrase, PEP-carboxylase, RBP-carboxylase, etc. create a normal biochemical environment for pyruvate metabolism and maintain order in the organism. Malate and OA, intermediate metabolites of the Krebs cycle, can be considered as a direct source of pyruvate used in chloroplasts. Therefore, the carbon dioxide released as a result of the decarboxylation reaction of OA can be considered as a substrate of RBPC [Ivanishev, 1990] [1].

Given the role of pyruvate in multilateral physiological and biochemical processes in plant tissues, the comparative study of the regulation of the activity of OAD, involved in its biosynthesis, under radiation and salt stresses is of great scientific and practical importance.

Materıals and Methods

The leaves of common bean (*Phaselus vulgaris* L.) were used for the study. Common bean seeds exposed to radiation of 1, 5, 10, 50, 100 and 200 Qy were disinfected in 3% H_2O_2

for 15 min, then washed with distilled water 2-3 times, and placed in Petri plates covered with filter paper. The plates were kept in the thermostat, in the darkness.

Seedlings obtained from irradiated seeds were transferred to ordinary spring water, and seedlings from non-irradiated seeds were put to 400 ml vegetation containers with 1, 5, 10, 50 and 100 mM NaCl solution. The containers were placed into an artificial climate chamber with a light intensity of 5-10 klx, a temperature of 25-28ºC, a photoperiod of 14 hours, humidity 60-70%. Samples for the experiment were taken every 5 days after the plants were illuminated for 2-3 hours.

To obtain the enzyme preparation, the leaves were washed with distilled water and dried with filter paper. 10ml of 20 mM Tris-HCl buffer (pH 7.8) containing 10 mM NaCl, 1 mM EDTA-Na, 1 mM Na-ascorbate, 0.4 M sucrose and 0.1% polyethylene glycol (PEG) was added to 1g of leaves and homogenization was performed by the Polytron disintegrator at +4ºC. The obtained homogenate was passed through 4 layers of gauze, the pellet was removed and the filtrate was centrifuged at 300g, for 5 min and then at 5000g, for 10 min. The supernatant was used for biochemical analyses.

The spectrophotometric method (Ultrospec 3300 pro, USA) was used for the determination of the OAD activity [Dimroth, Thomer, 1986] [14]. One ml of the reaction mixture consists of 20 mM Tris-HCl buffer (pH 7.8) containing 0.1 mM OA, 0.1 mM NADH, 1 EU LDH and 30 µl of the enzyme extract. The OAD activity was determined based on changes in the amount of NADH at 340 nm. NADH content decreased in the reaction of the formation of lactate from pyruvate due to OA decarboxylation. $\varepsilon=6.22$ $mM^{-1}\cdot cm^{-1}$.

Adenine nucleotides [Romanova, 1980] [9], and nicotine coenzymes were determined spectrophotometrically [Wahl, Kozaloff, 1962] [255]. The content of pigments was determined by the Sims and Gamon [Sims, Gomon, 2002] [24] method and the Lowry protein assay was applied to determine total protein [Lowry *et al*., 1951] [19].

The values given in the tables and figures are the mathematical mean of 3 independent experiments performed in 3 biological repetitions and reflect the standard deviation. Static processing of the results was performed in the Exel program.

Results and Dıscussıon

Weakening plant development due to stress factors depends on the distribution of photosynthetic products between different organs, as well as the balance between photosynthesis and respiration [Flexas *et al*., 2006] [16]. Leaves adapt to the changing conditions of the environment, playing an important role in the production and storage of energy based on the assimilation of carbon in the plant kingdom. Leaves play a crucial role in the accumulation of energy of photosynthetic pigments. Therefore, quantification of the pigments can provide valuable information when characterizing the state of plants exposed to stress.

The results of the quantitative analysis of pigments in the leaves of common bean plants are presented in Table 1. The Chl a / b and Car / Chl (a+b) ratios are known to provide more information and are frequently used in assessing the effects of abiotic factors on plants [Smolnikova *et al*. 2011]. Although carotenoids have mainly tolerance properties, they participate in photosynthesis together with chlorophylls. An increase in the ratio of these two parameters leads to an increase in the intensity of photosynthesis and, accordingly, the tolerance of plants to the effects of stress factors.

Table 1: The effect of radiation and salt stresses on dynamics of photosynthetic pigments in common bean leaves

Radiation, Qy	**Pigment parameters**			**Salt, mM**	**Pigment parameters**		
	chl (a/b)	**car**	**car/chl (a+b)**		**chl (a/b)**	**car**	**car/chl (a+b)**
5-day-old seedlings							
C	1.06	1.72±0.61	4.06	C	1.06	2.72±0.74	4.06
1	1.25	1.92±0.33	4.41	1	2.35	2.80±0.42	4.99
5	1.48	2.77±0.42	6.27	5	2.37	2.01±0.56	5.17
10	1.85	2.82±0.23	4.22	10	2.12	3.71±0.49	5.55
50	1.41	3.59±0.08	8.03	50	1.44	3.88±0.23	10.02
100	0.94	3.96±0.09	6.47	100	1.14	3.45±0.11	9.39
200	0.47	2.84±0.07	5.61	-	-	-	-
10-day-old seedlings							
C	0.77	1.61±0.22	5.26	C	0.77	2.61±0.33	6.26
1	0.87	2.03±0.36	6.38	1	0.64	2.01±0.19	8.22
5	0.62	2.58±0.44	6.54	5	0.75	3.42±0.37	7.11
10	0.63	3.37±0.51	5.22	10	0.87	4.61±0.45	8.05
50	0.67	3.02±0.29	7.86	50	0.52	3.01±0.24	4.32
100	0.49	3.11±0.31	6.27	100	0.28	2.31±0.08	7.38
200	0.30	2.42±0.34	7.24	-	-	-	-
15-day-old seedlings							
C	0.65	1.67±0.07	8.44	C	0.65	2.67±0.11	8.44
1	0.64	2.45±0.06	9.84	1	0.63	3.12±0.31	9.70
5	0.32	2.53±0.08	8.89	5	0.78	3.45±0.30	8.06
10	0.58	2.88±0.21	7.71	10	0.66	4.16±0.26	10.04
50	0.44	3.14±0.51	9.68	50	0.78	4.33±0.32	10.69
100	0.29	3.68±0.12	8.55	100	0.22	3.29±0.06	8.45
200	0.20	2.33±0.43	9.58	-	-	-	-

Note: C – control, amounts of carotenoids are expressed in mmol/ml

As seen in Table 1, in leaves of the 5-, 10- and 15-day-old seedlings, the chl (a/b) ratio and amounts of carotenoids are lower in the control compared with all experimental variants. An increase in these parameters began upon imposing stress and increased with intensifying stress effects. A similar tendency was observed for the car/chl (a+b) ratio. As seen in Table 1, this ratio increased in all variants.

Based on the results, changes in the molecular structure of chlorophylls under radiation in the leaves of control and experimental plants, and the decomposition of chloroplasts in leaves under osmotic shock caused by salt stress result in certain differences in the intensity of photosynthesis and respiration. It was found that in the variants with a high content of photosynthetic pigments and large values of the chl a / b ratio, the rate of photosynthesis and the activity of energy metabolism enzymes are also high. In the case of 100 mM NaCl, the low chl (a / b) ratio also weakened the energy metabolism processes.

Some authors suggest that the accumulation of Na^{+} and Cl^{-} ions in chloroplasts during salt stress damages the thylakoid membranes, which in turn leads to the breakdown of chlorophyll and its reduction. Salt stress in sunflower (Heliantus annus) plants was found to reduce the amount of chlorophyll in chloroplasts by destroying the synthesis of its precursor δ-aminolevulinic acid [Akram, Ashraf, 2011] [11].

It should be noted that the pigment apparatus of different plant varieties show different sensitivity to the effects of abiotic stress factors, such as salt and drought. Moreover, in many drought-tolerant varieties of wheat, the amount of pigments does not change or changes less than in susceptible varieties [Nikolayeva *et al*., 2010] [4].

Many researchers showed that pyruvate can be transported by diffusion to the chloroplasts of C3-plants, providing up to 4-5% of the total amount of lipid synthesized there [Roughan *et al*., 1979] [22]. According to subsequent studies, up to ~ 95% of the pyruvate used in lipid biosynthesis is synthesized from OA in chloroplasts in the reaction catalyzed by the OAD enzyme [Ivanishev, 2002] [2].

Given the high biological qualities of pyruvate, the study of the activity of OAD, one of the enzymes involved in its synthesis, is of great scientific importance. OAD and NADP-ME decarboxylate OA to pyruvate in the absence of NADPH. It was suggested that the increase in the concentration of CO2 in the stroma of chloroplasts due to CO2 formed as a result of decarboxylation may be one of the key factors in the evolution of C4-plants [Ivanishev, 1997] [3].

The results of the study of OAD are presented in Figures 1 and 2.

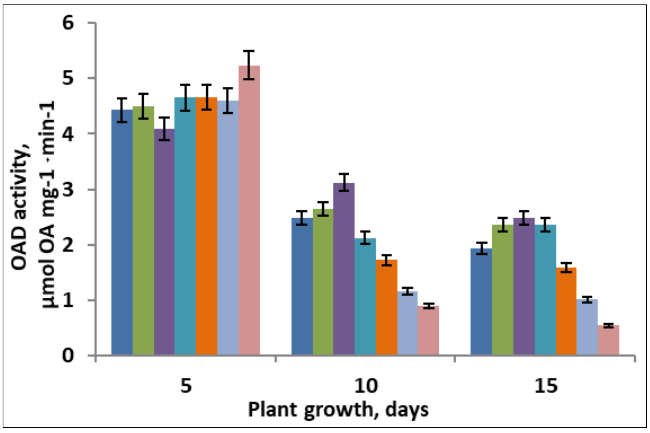

Fig 1: Time-dependent dynamics of the OAD activity in common bean leaves under various radiation doses.

🟦-C, 🟩-1 Qy, 🟪-5 Qy, 🟦-10 Qy, 🟧50 Qy, 🟦100 Qy, 🟥200 Qy

According to the presented data, there is no marked dependence between the OAD activity and radiation dose (except for small deviations) in the initial 5 days of the plant growth. A slight increase occurred in the enzyme activity under 200Qy radiation. Under all other doses, in the initial 5 days of the plant growth, the OAD activity was very high and close to the activity in the control plants.

At a subsequent stage of the plant growth (in 10-day-old seedlings), a sharp dependence of the activity of this enzyme on both time and radiation dose was observed. More precisely, in the control sample of 10-day-old seedlings, the activity of the enzyme was about 2 times lower than that of 5-day-old seedlings. The OAD activity was also significantly lower in 10-day-old seedlings grown from irradiated seeds compared with 5-day-old seedlings.

However, the dependence of the OAD activity on the radiation dose was different. Thus, an increase in the radiation dose in the range of 1-10 Gy resulted in a gradual increase in the activity of the enzyme, and at a radiation dose of 10 Gy, the enzyme showed the highest activity.On the contrary, an increase in the radiation dose in the range of 10 - 200 Gy, led to a pronounced decrease in the activity of the enzyme. At a radiation dose of 200 Gy, the activity of the enzyme was about 2.5 times less than in the control.

Changes in the enzyme activity on the 15th day of the plant growth did not differ from activity in 10-day-old seedlings.

Figure 2 shows the dynamics in the activity of OAD in the chloroplasts of common bean leaves depending on the salt concentration.

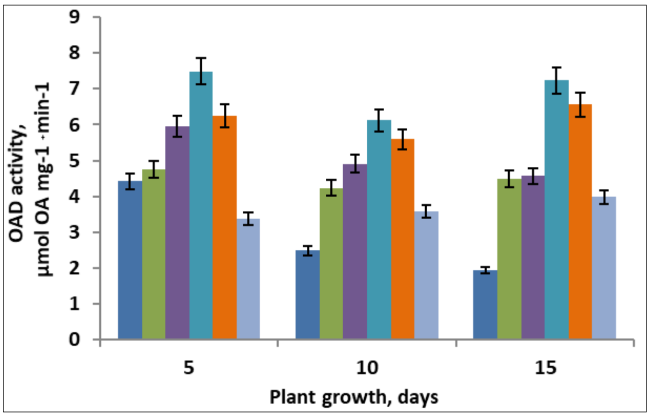

Fig 2: Time-dependent dynamics of the OAD activity in the chloroplasts of common bean leaves depending on various NaCl concentrations

🟦-C, 🟩-1 mM, 🟪- 5 mM, 🟦-10 mM, 🟧-50 mM, 🟦-100 mM, 🟥-200 mM

As seen in the figure, there was a certain similarity in the activity of OAD in 5-, 10- and 15-day-old seedlings depending on the salt concentration and the duration of stress. Thus, an increase in salt concentrations in the range of 1 - 10 mM led to an increase in enzyme activity in all stages of the plant growth, whereas, at concentrations above 10 mM, an increase in salt stress resulted in a decrease in enzyme activity.

According to the results on the ROD activity under stress conditions such as salt and radiation, the enzyme activity was not similar under various stresses.

Although radiation did not significantly affect the activity of OAD in the leaves of 5-day-old seedlings formed from irradiated seeds, NaCl began to affect the activity of this enzyme from the first days of the plant growth. Contrary to radiation stress, salt stress had a stimulating effect on the OAD activity in all stages of the plant growth at certain concentrations (5, 10, 50 mM), which can be attributed to the need for pyruvate as a universal substrate in energy processes.

Ivanishev found an increase in the activity of OAD and NAD-MDH enzymes in cotton plants during drought stress, which [Ivanishev, 1997] [1] is consistent with the intensification of metabolism of C4-dicarboxylic acids and amino acid biosynthesis [Sharma *et al*., 1990] [23].

Quantitative analysis of ATP and ADP nucleotides in the cell is also of great importance in the study of the regulation of glycolysis and respiratory processes. From this point of view, quantitative analysis of adenine nucleotides (ATP, ADP) and nicotine coenzymes (NAD +, NADP) in common bean leaves under both radiation and salt stresses was carried out and the results are presented in Table 2.

Table 2: Time-dependent dynamics of the amounts of adenine nucleotides and nicotine coenzymes in common bean leaves exposed to various radiation doses and NaCl concentrations

Variants	**Adenine nucleotides, µg**						**Nicotine coenzymes, µg**					
	5th day		**10th day**		**15th day**		**5th day**		**10th day**		**15th day**	
	ATP	**ADP**	**ATP**	**ADP**	**ATP**	**ADP**	**NAD^+**	**NADP**	**NAD^+**	**NADP**	**NAD^+**	**NADP**
Radiation, Qy												
C	58.0	30.0	66.5	19.5	76.0	14.0	181.8	106.1	179.6	103.7	149.1	76.4
1	65.0	29.0	75.0	25.0	83.0	24.0	183.0	102.3	182.3	100.9	151.6	69.3
5	66.0	36.0	67.0	33.0	79.0	26.0	184.7	103.1	181.8	100.0	152.1	66.6
10	64.0	31.0	65.0	30.0	67.0	28.0	185.5	102.0	184.4	98.14	153.7	64.4
50	58.0	17.0	64.0	15.0	71.0	10.0	199.7	10.6	188.5	95.7	155.8	61.6
100	51.0	16.0	57.0	8.0	59.0	13.0	187.9	88.9	188.7	86.2	156.1	59.5
200	40.0	10.0	50.0	8.0	50.0	14.0	176.8	87.7	172.9	84.6	147.3	57.7
NaCl, mM												
C	43.3	15.0	44.0	16.0	50.0	20.6	154.4	88.3	143.6	83.9	132.6	77.8
1	35.0	12.0	45.0	17.0	51.0	17.0	140.3	85.5	138.9	82.5	130.7	71.5
5	39.0	13.0	40.0	18.0	48.0	14.0	139.8	84.3	136.0	79.7	125.6	68.7
10	35.0	8.0	36.0	17.0	39.0	12.0	144.3	82.2	142.7	78.5	126.4	66.9
50	28.0	3.0	29.0	18.0	38.0	12.0	145.7	81.7	144.0	75.8	125.1	64.7
100	26.0	3.0	31.0	9.0	33.0	14.0	142.7	80.1	141.9	73.9	123.2	61.7

Note: ATP - adenosine triphosphate;; ADP- adenosine diphosphate; NAD- nicotinamide adenine dinucleotide; NADP – nicotinamide adenine dinucleotide phosphate. The accuracy is less than 3%.

According to the results, in all control and experimental variants, a gradual increase in the ATP amount occurred as the plant grew. However, an increase in both the radiation dose and the salt concentration led to a decrease in the ATP amount.

In contrast, in all variants, throughout the plant growth, the ADP amount decreased both in the control variant and in plants exposed to 1-50 Gy radiation doses. Approximate equalization was observed under 100 Gy radiation dose, and a slight increase occurred under 200 Gr radiation dose.

However, it should be noted that the ATP amount in common bean leaves exposed to radiation and salt stress increased over time, while the amount of both nicotine coenzymes decreased. The change in the ADP amount was found to be similar to the changes in the amounts of NAD^+, $NADP^+$ under stress. All this can be explained by the fact that the inhibition of glycolysis under radiation and salt stress weakens the synthesis of ATP in the Krebs cycle, thereby reduces the energy supply to tissues. Kuhn and his colleagues show that in peas, under high concentrations of NaCl, an alternative pathway of respiration that is not related to the synthesis of ATP is activated. The synthesis of ATP also weakens like weakening the cytochrome pathway of respiration [Kuhn *et al*., 2001].

Contrary to the effects of radiation, the amounts of ATP and ADP at different concentrations of NaCl were different compared to the control. The amount of ATP gradually increased in leaves of 5, 10 and 15-day-old seedlings, both in control variants and in plants exposed to 1-50 mM NaCl concentrations, but remained approximately constant at 100 mM NaCl concentrations. Unlike the radiation effect, salt stress increased the ADP amount in the control variant and at 100 mM NaCl. Whereas, in seedlings exposed to 1-50 mM concentrations of NaCl, the ADP amount, first, increased with increasing salt concentrations and then decreased.

As seen in Table 2, the NAD^+ and $NADP^+$ amounts decreased in all variants under radiation and salt stresses over time. Under both stress conditions, the largest amounts of NAD^+ and $NADP^+$ were found in the control variants.

Based on the obtained results, the quantitative changes in ATP and ADP, which are the main indicators of the energy exchange, occurred synchronously with quantitative changes in NAD^+ and $NADP^+$. Oxidation of NAD^+ was accompanied by energy release, and reduction was accompanied by energy absorption. The resulting NADH was then involved in the energy generation process. Molar amounts of NAD^+ and $NADP^+$ significantly increased, indicating their oxidation and reduction of NADH and NADPH under stress. This confirms the interrelated action of adenine phosphates and nicotine coenzymes.

Radiation and salt stress are known to cause significant changes in the energy metabolism of higher plants. These stresses drastically reduce the energy efficiency of respiration, and destroy the connection between oxidation and phosphorylation. As a result, as the synthesis of ATP in the cell becomes difficult, the normal course of metabolic reactions is disrupted. The penetration of Na + ions into the cytosol under high salinity disrupts the process of absorption of photons, as well as the electronic transport circuit, which results in the formation of ATP and NADPH molecules in PSII, and has a negative effect on the rate of the Calvin cycle reactions. In this case, the synthesis of proteins is completely disturbed due to the disruption of the biosynthesis of ATP in the cell. As a result, the ATP amount in the mitochondria decreases [Kasumov, 2012] [5].

The low values of the ratio between reduced and oxidized forms of natural nucleotides in chloroplasts create a favorable thermodynamic environment for malate reduction absorbed by chloroplasts, the formation of OA and utilization of OA in the reaction catalyzed by OAD. The released pyruvate can be used by the pyruvate dehydrogenase multienzyme complex acting in the chloroplasts of C3 plants [Camp, Randall, 1985] [13].

Based on the results, it can be concluded that the activity of the OAD enzyme, which carries out the biosynthesis of pyruvate by decarboxylation of OA, together with pigments, adenine nucleotides, and nicotine coenzymes, may be involved in the regulation of cellular energy metabolism and the emergence of adaptability signs under radiation and salt stresses.

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