**Advanced Studies in Biology, Vol. 12, 2020, no. 1, 1 – 7**
**HIKARI Ltd, www.m-hikari.com**
**https://doi.org/10.12988/asb.2020.91212**

# Comparative Study of Catalase, Pyruvate Kinase, Oxaloacetate Decarboxylase, NAD-Malate Dehydrogenase Activities in Leaves and Activity of H+ Pumps ın Roots of Common Bean Plants Exposed to Salt Stress

**N. R. Guliyeva[1], E. S. Jafarov[1,*] and H. G. Babayev[2]**

[1] Institute of Radiation Problems
Azerbaijan National Academy of Sciences Baku, Azerbaijan
[*]Corresponding author

[2]Institute of Molecular Biology and Biotechnologies
Azerbaijan National Academy of Sciences, Baku, Azerbaijan



**Abstract**

Activities of NAD-malate dehydrogenase (NAD-MDH, EC 1.1.1.37), oxaloacetate decarboxylase (OAD, EC 4.1.1.3), pyruvate kinase (PK, EC 2.7.1.40), catalase (CAT, EC 1.11.1.6) involved in the metabolism of dicarbon acids such as malate and oxaloacetate (OA) in leaves of common bean (Phaseolus vulgaris L.) plants exposed to salt stress and activity of H+ pumps related to the mineral nutrition in the root cell system have been studied comparatively. It has been established that these enzymes acting in concert play an important role in maintaining the constant energy balance and creating stress tolerance of the organism by the regulation of the metabolic processes at various concentrations of NaCl.

**Keywords:** Phaseolus vulgaris L.-CAT-PK-NAD-MDH-OAD-$H^+$-pump-activity-NaCl-stress

## Introductıon

About 1/15 of the Earth is known to be saline soils. Excessive absorption of salts in the soil causes osmotic shock in plant cells, resulting in disturbance of water balance, reduction in turgor pressure, stomatal closure, reduced intensity of photosynthesis, and destruction of the cell membrane system, which eventually leads to cell death [1]. High salt concentrations that increase the amounts of reactive oxygen species (ROS), such as singlet oxygen-$^1O_2$, superoxide anion radical - $O_2^{\cdot-}$, hydrogen peroxide $H_2O_2$, and hydroxyl radical - $OH^{\cdot}$ cause oxidative stress [2]. ROS oxidizing the cell structure, proteins, and DNA can cause "damage" to a various degree [3]. It is known that only at high concentrations of $H_2O_2$, the enzyme catalase (CAT) catalyzes its decomposition to water and oxygen [4].

Ashraf and Harris observed a higher synthesis rate of low-molecular (25-33 kDa) weight, specific proteins, involved in the regulation of osmotic and turgor pressure, in salt-tolerant varieties exposed to salt stress [5].

Pyruvate is a final product of the anaerobic oxidation ensuring the energy balance of the organism. Being a universal substrate it plays an important role in a number of vital processes. Therefore, the study of biosynthesis and conversion reactions of pyruvate under salt stress is considered to be relevant. The enzymes OAD, NAD-MDH, NADP-MDH, NADP-ME, CA, PEPC, RBPC, etc. localized in the chloroplasts of $C_3$ plants are directly or indirectly involved in pyruvate metabolism. NADP-MDH and MAD-ME are localized in the stroma, whereas PEPC, OAD, and CA in the membranes of chloroplasts and the similarity of the functions of these enzymes in many cases suggests that there is a strong biochemical environment around pyruvate. According to He and Hou, OA transported to chloroplasts causes not only malate reduction, but it is also decarboxylated resulting in the pyruvate synthesis and RBP-carboxylase (RBPC) reduces ribulose-1,5-bisphosphate (RBP) to triose phosphate at the expense of the final product of the reaction - $CO_2$. Calvin cycle of photosynthetic $CO_2$ assimilation starting with the involvement of RBPC as a catalyst provides the cell with intermediate metabolites and plastic substances [6].

The main part of pyruvate is synthesized in the conversion of PEP in the reaction catalyzed by pyruvate kinase (PK), which generates adenosine triphosphate (ATP). Ivanishev observed an increase in the activities of OAD and NAD-MDH enzymes in cotton plants exposed to drought [7], which is in accordance with the intensifying metabolism of $C_4$-dicarbon acids and biosynthesis of amino acids [8]. According to Hatch, an increase in the OA concentration up to 50-100 μM is one of the main reasons for high pyruvate concentrations in cell organoids [9]. Camp et al. revealed pyruvate dehydrogenase complex implementing pyruvate metabolism in $C_3$ plant chloroplasts [10].

The main purpose of the presented paper was a comparative study of the effects of various NaCl concentrations on the activity of energetic enzymes of the synthesis and metabolism of pyruvate, playing a key role of a substrate in

anaerobic and aerobic oxidation, gluconeogenesis, synthesis of carbohydrates, lipids and some amino acids in the leaves and the activity of $H^+$ pumps having an important role in mineral nutrition of root cells of common bean plants. The performed research is of great scientific and practical importance.

## Materıals and Methods

The object of the study was the common bean *(Phaseolus vulgaris)* plant. The seeds of the plant were disinfected in 3% $H_2O_2$ solution for 15min, washed 2–3 times with distilled water and placed into thermostat in Petri dishes for germination. Seedlings were transferred to vegetation vessels containing water or 1- 100 mM NaCl solutions and kept in an artificial environment with a temperature of 25-28ºC, photoperiod-14 h, relative air humidity-60-70%, light intensity-15-20 lux. The leaf samples were taken after 2 hours of illumination of the plants.

To obtain the enzyme preparation, the leaves were washed with distilled water, dried on the filter paper, and homogenized using a mortar and pestle at a temperature of +4ºC, adding 7 ml of the homogenization solution per 1 g of leaves. The obtained homogenate was passed through 4-fold cheesecloth, the pulp was removed, and the filtrate was centrifuged first at 300g for 5 min and then at 5,000g for 10 min. After removing the pallet, the supernatant was used for the assay.

Activities of NAD-MDH [11], PK [12], CAT [13], and OAD [14] were determined using spectrophotometric methods.

The MDA content was determined by the Hodges method [15], $H_2O_2$ according to the Velikova method [16], total protein by the Lowry method [17], the activity of $H^+$ pumps was found by the pH-metric method [18].

When analyzing the results of the study, mean mathematical errors and deviations (M±m) were taken into account. Static processing of the results was performed by the "*Excel*" program.

## Results and Discussıon

When creating standard conditions in our experiments, we considered that at the level of energy metabolism, plant tolerance to salt stress depends on the age of the plant, species, developmental stage, the level of stress factors and the duration of the stress.

It is known that the first organ exposed to increased salt concentrations is the root and the first physiological process is the ion pumping in the root cell membranes. Ion pumps have the potential to severely affect physiological and biochemical and other processes in other organs by creating physiological links between root cells and the rhizosphere. Therefore, we attempted to study the enzymes playing a certain role in the biosynthesis and metabolism of pyruvate under stress, in relation to the functions of $H^+$ - pumps.

As seen in the table, the amounts of MDA, which is a product of LPO gradually decreased in the control variant and this decrease increased over time. First, the amount of $H_2O_2$ remained relatively stable in the control variant and then slightly increased. The MDA content markedly decreased at 1-100 mM NaCl. This decrease was 10% on the 10th day of the development compared with the 5th day and 20% on the 10th and 15th days compared with the 5th day. Contrary to MDA, the amount of $H_2O_2$ decreased at 5-50 mM NaCl over time and increased at 100 mM NaCl. At 1mM NaCl this decrease was slight and at 100 mM NaCl the $H_2O_2$ amount increased. This parameter was more pronounced at 10-50 mM concentrations of NaCl. At these concentrations, CAT catalyzed the reaction of the $H_2O_2$ decomposition with the maximum rate. The dynamics of the MDA and $H_2O_2$ content in the control and experimental variants correlates with the CAT activity dynamics, which depends on the salt concentration and plant developmental stages (Table).

The spectrum of the change in total protein in leaves differed from the spectra of MDA and $H_2O_2$. Thus, protein amounts increased on the 10th day of the plant development by ~2% compared with the 5th day, whereas on the 15th day this parameter decreased by ~2% compared with the 5th day and ~4% compared with the 10th day (data not shown).

The table shows the dynamics of some energy metabolism enzymes and the activity indices of $H^+$ pumps in the roots depending on the time and salt concentrations. As seen in the table, there is a negative correlation between the activity of the CAT enzyme and the amount of its substrate, $H_2O_2$ accumulated in the cell. The observed correlation was more pronounced on the 5th, 10th and 15th days of the common bean plant development, at 5, 10 and 50 mM concentrations of NaCl. As the CAT activity increased slowly at 100mM NaCl, the amount of $H_2O_2$ increased.

Various enzymes are involved in the metabolism of a C4 acid –OA. NAD-MDH and OAD are the most important among them. As seen in the table, the activity of NAD-MDH that catalyzes the reduction reaction of OA to malate increased in 5-, 10-and 15 day-old-seedlings exposed to stress. This increase was more pronounced at 50-100mM concentrations of NaCl. The OAD enzyme showed higher activity at 5-50mM concentrations of NaCl. First, the OAD activity decreased and then increased surpassing the previous level. The OAD activity probably depends not only on the salt amount but also on the duration of its effect. Moreover, the activity of PK, which catalyzes the synthesis of posphoenol pyruvate at the final stage of glycolysis, increased more sharply at 10-100mM NaCl. There were reports confirming these results. Lui et al. established that 50 mM NaCl decreased the MDA and ROS content in leaves and roots of the *Theiiungiella halophila* plant, whereas 200-400 mM NaCl increased MDA and ROS amounts as well as activities of PK and CAT [19].

**Table.** The effect of various NaCl concentrations on the dynamics of activities of the CAT, NAD-MDH, PK, OAD enzymes in the leaves and $H^+$ pump in the root system cells of the common bean plant

| NaCl, mM | MDA | $H_2O_2$ | CAT | NAD-MDH | PK | OAD | $C_{H^+}$ |
|---|---|---|---|---|---|---|---|
| The 5th day | | | | | | | |
| C | 132.0±14.3 | 199.0 | 0.371±0.14 | 88.81±9.44 | 2.08±1.20 | 4.43±0.65 | $0.14 \cdot 10^{-6}$ |
| 1 | 129.6±12.7 | 198.0 | 1.291±0.37 | 91.14±11.3 | 2.51±0.89 | 4.75±0.81 | $0.4 \cdot 10^{-5}$ |
| 5 | 162.0±20.8 | 184.5 | 1.753±0.81 | 98.14±14.2 | 2.79±0.78 | 5.96±1.16 | $0.71 \cdot 10^{-5}$ |
| 10 | 144.0±16.7 | 143.5 | 2.387±0.16 | 109.2±15.1 | 2.11±1.22 | 7.49±3.39 | $0.76 \cdot 10^{-5}$ |
| 50 | 162.0±19.1 | 142.0 | 3.599±1.01 | 115.4±18.6 | 2.05±1.11 | 6.25±2.43 | $0.86 \cdot 10^{-5}$ |
| 100 | 157.0±17.8 | 172.0 | 0.879±0.92 | 136.8±20.3 | 1.87±0.65 | 3.37±1.77 | $0.43 \cdot 10^{-5}$ |
| The 10th day | | | | | | | |
| C | 134.0±10.3 | 200.0 | 0.383±0.07 | 98.63±14.7 | 1.81±0.99 | 2.48±0.92 | $0.12 \cdot 10^{-5}$ |
| 1 | 132.0±9.82 | 190.0 | 1.781±0.28 | 99.63±15.1 | 1.99±0.87 | 4.23±1.18 | $0.23 \cdot 10^{-4}$ |
| 5 | 114.0±8.78 | 172.8 | 2.620±1.08 | 117.6±21.7 | 2.62±0.11 | 4.90±1.15 | $0.55 \cdot 10^{-4}$ |
| 10 | 126.0±8.98 | 132.5 | 2.580±0.98 | 127.1±20.2 | 2.48±0.39 | 6.12±1.47 | $0.66 \cdot 10^{-4}$ |
| 50 | 141.0±9.76 | 102.3 | 4.720±1.76 | 141.0±23.0 | 2.45±0.76 | 5.60±2.41 | $0.56 \cdot 10^{-4}$ |
| 100 | 139.8±12.9 | 163.5 | 1.230±0.11 | 154.5±19.8 | 2.51±1.31 | 3.58±1.23 | $0.46 \cdot 10^{-4}$ |
| The 15th day | | | | | | | |
| C | 129.0±12.4 | 212.0 | 0.486±0.31 | 125.0±17.1 | 2.23±1.00 | 1.93±0.59 | $0.24 \cdot 10^{-5}$ |
| 1 | 128.0±9.32 | 193.5 | 1.939±0.32 | 121.5±14.3 | 2.41±0.34 | 4.49±2.11 | $0.62 \cdot 10^{-3}$ |
| 5 | 123.0±8.73 | 158.5 | 2.887±1.22 | 123.9±12.9 | 2.36±1.03 | 4.56±2.34 | $0.65 \cdot 10^{-3}$ |
| 10 | 105.0±9.11 | 111.0 | 3.123±1.12 | 159.9±26.5 | 2.96±1.12 | 7.23±2.25 | $0.79 \cdot 10^{-3}$ |
| 50 | 105.0±9.13 | 89.6 | 5.782±2.43 | 215.8±25.9 | 2.95±2.11 | 6.56±2.24 | $0.89 \cdot 10^{-3}$ |
| 100 | 98.5±11.92 | 154.7 | 1.335±0.86 | 247.7±22.2 | 2.99±0.92 | 3.98±1.45 | $0.78 \cdot 10^{-3}$ |

*Note: CAT- μmol $H_2O_2 \cdot mg^{-1}protein \cdot min^{-1}$; NAD-MDH-μmol oxaloacetate· $mg^{-1}protein \cdot min^{-1}$; PK - μmol pyruvate· $mg^{-1}protein \cdot min^{-1}$; OAD-μmol OA $mg^{-1}protein \cdot min^{-1}$; $C_{H^+}$ - μequiv/hour, MDA - mM/ml; $H_2O_2$-μM/ml; C - control.*

OAD and NADP-ME decarboxylate OA forming pyruvate under NADPH deficiency, however, an excessive amount of NADPH leads to the reduction of OA with the formation of malate. It suggests that the increase in the $CO_2$ concentration due to the decarboxylation in the chloroplast stroma is probably one of the main factors in the evolution of $C_4$ plants [7]. In root system cells of common bean plants, the activity of H+ pumps was higher at 5, 10 and 50 mM concentration of NaCl compared with the control plants. When comparing all these indices in control variants, a positive correlation was detected between the enzymes involved in pyruvate metabolism, biological characteristics of vegetative organs (data not shown) and ion –exchange processes due to ATPase reactions in roots.

Some authors reported that a considerable increase in the MDA content causes a quite decrease in the chlorophyll amount and this change correlates with reduced APO and CAT activities [2, 20]. Tester and Davenport showed that the antioxidant system operates more effectively in roots than in leaves, therefore, salt stress does not cause an enhanced MDA accumulation and ROS generation in roots. A system that neutralizes sodium more quickly in the roots either directs $Na^+$ ions to vacuole or exports them out of the cell to the environment [21].

The obtained results and theoretical data suggest that a wide spectrum of adaptive signs emerged, in the $C_3$ plant common bean against the increasing NaCl concentrations at various levels, including the levels of the development of

vegetative organs, photosynthesis - the basis of energy exchange, some enzymes of respiration and glycolysis, intermediate metabolites, proteins, photosynthetic pigments, and mineral nutrition, create the defense ability of the plant due to their "coordinated" action.

## References

[1] P.M. Hasegawa, R.A. Bressan, J.K. Zhu, H.J. Bohnert. Plant cellular and molecular responses to high salinity, *Plant Physiol.*, **51** (2000), 463-499. https://doi.org/10.1146/annurev.arplant.51.1.463

[2] O.G. Polesskaya, E.I. Kashirina, N.D. Alexina. The effect of salt stress on the plant antioxidant system depending on the nitrogen nutrition conditions, *Plant Physiol.*, **53** (2) (2006), 207-214 (in Russian). https://doi.org/10.1134/s1021443706020063

[3] R. Mitteler, Oxidative stress, antioxidants and stress tolerance, *Trends Plant Sci.*, **7** (2002), 405-409. https://doi.org/10.1016/s1360-1385(02)02312-9

[4] O.G. Polesskaya, *Plant cell and reactive oxygen species*, M.: KDU, 2007, p.140 (in Russian).

[5] M. Ashraf, P.J.C. Harris, Potential biochemical indicators of salinity tolerance in plants, *Plant Sci.*, **166** (2004), 3-16. https://doi.org/10.1016/j.plantsci.2003.10.024

[6] W. He, H.J.M. Hou. Chemistry of Biomimetic mixed valence oxomanganese-based materials mimicking photosynthetic water splitting (Edited by Allakhverdiev S.I., Rubin A.B., Shuvalov V.A.) *Contemporary Problems of Photosynthesis*, Moscow, **1** (2014), 381-405.

[7] V.V. Ivanishev, Biological significance of the metabolism of oxaloacetate in chloroplasts of $C_3$ plants, *Plant Physiol*., **44** (3) 1997), 462-470 (in Russian).

[8] K.D. Sharma, K.S. Datta, S.K. Varma, Effect of chloride and sulfate type of salinity on some metabolic drifts in Chickpea, *Cicer arietinum*. *Ind. J. Exp. Biol*., **28** (9) (1990), 890-892.

[9] M.D. Hatch, Mechanism of $C_4$-photosynthesis in *Chloris gayana*: Pool sizes and kinetics of $^{14}CO_2$ incorporation into 4-carbon and 3-carbon intermediates, *Arch. Biochem. Biophys*. **194** (4) (1979), 117-127. https://doi.org/10.1016/0003-9861(79)90601-5

[10] P.J. Camp, J.A. Miernyk, D.D. Randall, Some kinetic and regulatory properties of the pea chloroplasts dehydrogenase complex, BBA: *Bioenergetic*, **933** (2) (1988), 269-275. https://doi.org/10.1016/0005-2728(88)90034-5

[11] R. Scheibe, M. Stitt, Comparison of NADP-malate dehydrogenase activation, QA reduction and $O_2$ evolution in spinach leaves, *Plant Physiol. Biochem*., **26** (1988), 473-481.

[12] А.К. Romanova. *Biochemical methods for the study of autotrophy in microorganisms*, M.: Nauka, 1980, p.160 (in Russian).

[13] C.N. Kumar, N. Knowles, Changes in lipid peroxidation and lipolytic and free-radical scavenging enzyme during aging and sprouting of potato (*Solanum tuberosum* L.) seed-tubers, *Plant Physiol.,* **102** (1993), 115-124. https://doi.org/10.1104/pp.102.1.115

[14] P. Dimroth, A. Thomer, Kinetic analysis of the reaction mechanism of oxaloacetate decarboxylase from *Klebsiella aerogenez*, *Eur. J. Biochem*., **156** (1) (1986), 157-162. https://doi.org/10.1111/j.1432-1033.1986.tb09561.x

[15] D.M. Hodges, J.M. DeLong, C.F. Forney, *et al.*, Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds, *Planta*, **207** (1999), 604-611. https://doi.org/10.1007/s004250050524

[16] V.Velikova, I. Yordanov, A.. Edreva, Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Protective role of exogenous polyamines, *Plant Sci*., **151** (2000), 59-66. https://doi.org/10.1016/s0168-9452(99)00197-1

[17] O.H. Lowry, N.J. Roserbrough, A.L. Farr, R.L. Kandell, Protein measurement with the Folin phenol reagent, *J. Biol. Chem.,* **193** (1) (1951), 256-266.

[18] V.V. Polevoy, T.S. Salamatova, Ptoton pumps and their functional role// Протонные насосы и их функциональная роль. *Results of science and technology, physiol. sci. series*. (M., VINITI), **4** (1980), 78-125 (in Russian).

[19] A.R. Lui, Y.B. Zhang, D.K. Chen, Y. Zhiwu, *Bull. Bot. Res.,* **26** (2) (2006), 216-221 (in Chinese).

[20] M.S. Akram, M. Ashraf, Exogenous application of potassium dihydrogen phosphate can alleviate the adverse effects of salt stress on sunflower, *J. Plant Nutr.,* **34** (2011), 1041-1057. https://doi.org/10.1080/01904167.2011.555585

[21] M. Tester, R. Davenport, $Na^+$ tolerance and $Na^+$ transport in higher plants, *Ann. Bot*., (London), **91** (2003), 503-527. https://doi.org/10.1093/aob/mcg058

**Received: February 1, 2020; Published: February 20, 2020**