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**COMPARATIVE STUDY OF THE EFFECTS OF
RADIATION AND SALT STRESSES ON ENERGY
METABOLISM ENZYMES IN BEAN PLANTS**

Specialty: **2418.01 – Radiobiology**

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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

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INTRODUCTION

Relevance of the topic: Environmental problems caused by factors such as drought and salinity are relevant all over the world including our country. Research shows that the area of saline soils in the Republic increased to 661.9 thousand hectares in 2007, which is ~ 46.6% of the total area. At present, more than 50.0% of our lands are uncultivated¹.

According to the estimates, the world's population will reach ~ 10 billion by 2025. One of the main tasks before us for preventing the impending threat on a global scale is to turn arid and saline lands into highly economically viable agricultural facilities at the expense of salt-tolerant and drought-tolerant plants². Therefore, the biochemical mechanisms of adaptation need to be studied at different levels, from the population to the molecules³.

Although the seed is an organ in which the metabolism of substances and energy are at rest, the genetic development program stored in them becomes active when the conditions are right, causing the seed to develop. Ecologically clean, safe, and inexpensive physical means are preferred to chemical and biological means to stimulate the development of seeds. γ -radiation in small doses has advantages such as preventing damage to seeds during processing, high neutralization of planting material, absence of induced mutations, stimulating effect, and reduction of energy consumption.

Although seed pre-sowing γ -irradiation treatment at stimulating doses does not change the genetic development program, its energy may be sufficient to affect regulatory systems.

¹ Məmmədov Q. Torpaqşünaslıq və torpaqların coğrafiyasının əsasları. Bakı: Elm, 2007, 664 s.

² Dyson, H.I.T. Population and food. Global trends and future prospects // European Journal of Population, - 1999, 15, - p. 203-204.

³ Bais, A.F. Environmental effects of ozone depletion, V radiation and interactions with climate change / A.F.Bais, R.M.Lucas, J.F. Bornman, [et al.] // Photochemical and Photobiological Sciences, - 2018, 12, - p. 127-179.

This, in turn, accelerates the passage of the initial phases of ontogenesis by accelerating the implementation of the development program, shortens the ripening period of the plant, and can improve its quality indices by increasing productivity under favorable conditions.

Stress factors are known to produce reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxide anion (OH^-), which disrupt cell structure. ROS cause lipid peroxidation (LPO) in cell membranes, breaks down proteins, nucleic acids, and disrupts cell metabolism by inhibiting enzymes⁴.

The activities of the catalase enzyme (CAT), pyruvate kinase (PK) playing an important role in the metabolism of C4 acids, oxaloacetate decarboxylase (OAD), NAD-malate dehydrogenase (NAD-MDH) were studied in the leaves of bean plants and the activity of the H^+ -pumps was determined in the roots under various radiation doses and salt concentrations.

The purpose and tasks of the research. The main purpose of the study was to clarify the role of metabolic enzymes in adaptive processes based on the study of small and large-molecule antioxidants in the leaves of plants obtained from the cultivation of bean seeds treated with γ -rays in stimulating doses before sowing under salt stress. In order to achieve the goal, the following tasks were set:

1. Study of morpho-biometric variability in bean seedlings grown under separate and combined effects of salt and radiation.
2. Study of the amount of photosynthetic pigments and MDA in the leaves of bean plants grown under separate and combined effects of salt and radiation.
3. Study of the CAT activity in the leaves of bean plants grown under separate and combined effects of salt and radiation.

⁴ Noreen, Z. Salt induced regulation of some key antioxidant enzymes and physio-biochemical phenomena in five diverse cultivars of turnip (*Brassica rapa* L.) / M.Ashraf, N.A.Akram, // J. Agron. and Crop Sci., - 2010, 196, № 4, - p. 273-285.

4. Study of the PK activity in the leaves of bean plants grown under separate and combined effects of salt and radiation.
5. Study of the activities of the enzymes NAD-MDH and OAD in the leaves of bean plants grown under separate and combined effects of salt and radiation.
6. Study of the amounts of adenine nucleotides and nicotinic coenzymes in the leaf tissues of bean plants grown under separate and combined effects of salt and radiation.
7. Comparative study of the activity of H^+ -pumps in root cells and enzymes of metabolic pathways in the leaves of bean plants grown under separate and combined effects of salt and radiation.
8. Determination of optimal radiation doses and salt concentrations, which determine the high activity of energy metabolism enzymes in the leaves of bean plants grown from irradiated seeds under salt stress.

Scientific novelty of the research. A comparative analysis of biometric indicators of the plant along with adaptive reactions due to changes in the amounts of intermediate metabolites, adenine nucleotides, nicotinic coenzymes, and photosynthetic pigments in the leaves, the antioxidant defense system, and adaptive responses to changes in the activity of metabolic pathway enzymes and H^+ -pump in root systems was performed for the first time in the early stages of vegetation, under the single and double stress (combined effect of radiation and salt).

For the first time, based on the dynamics of changes in the activity of the antioxidant system, and some enzymes of the metabolic pathways in the early stages of ontogenesis of the bean plant, it was suggested that the intensity of energy processes such as photosynthesis and respiration changes under the influence of double stress.

For the first time, a correlation was found under salt stress between the activity of H^+ -pumps localized in the membranes of root cells and the dynamics of changes in the activity of the enzymes of metabolic pathways in leaves.

Theoretical and practical significance of the research. The obtained results can be used as a database in the study of mechanisms of plant adaptation to stress. The results obtained can be used in practical work, such as stimulating the growth and development of plants under salt stress using pre-sowing gamma-irradiation treatment. The obtained results can be used as a valuable theoretical source in the creation of productive plant varieties tolerant to various environmental stressors. The results can be used as a source in the teaching of biochemistry and physiology courses at universities.

Main points presented to the defense of the dissertation.

1. According to changes in the biometric parameters of the bean plant, which depend on the radiation dose, salt concentration, and combined effect of radiation and salt, there is a certain correlation between dose limits and radiation-salt regimes at the level of the whole plant and vegetative organs.
2. In the early stages of ontogenesis, the enzymes of the energy metabolism pathways work in coordination with the antioxidant defense system in the adaptation of the bean plant to radiation and salt stresses.
3. In various combinations of radiation and salt, there is an adequate regularity between the mineral nutrition in the roots of the bean plant and the physiological and biochemical processes occurring in the leaves.
4. The stimulating effect caused by a combination of radiation and salt (50 Gy radiation + 10-50 mM NaCl) creates inductive mechanisms in the bean plant at the level of photosynthetic pigments, adenine nucleotides, nicotinic coenzymes, MDA, protein content, and activities of CAT, PK, NAD-MDH, OAD, H⁺-pumps.
5. Changes in the intensity of photosynthesis in plants grown under a high salt concentration from seeds exposed to pre-sowing gamma-irradiation treatment are associated with a decrease in the number of chloroplasts, an increase in the hydrolytic activity of chlorophyllase, and the breakdown of the chlorophyll-protein complex.

Approbation of the work. The main results of the dissertation were discussed at many international, national, and regional conferences and symposiums: At the conference "Innovations in Biology and Agriculture to Solve Global Challenges" dedicated to the 90th anniversary of Academician J.A. Aliyev (Baku, 2018); International Scientific and Practical Conference "Environmental, Industrial and Energy Safety" (Sevastopol, 2019); The VIII International Congress of Radiobiology (Moscow 2021); the Scientific-Practical Conference "Modern Information, Measurement And Control Systems: Problems And Perspectives" (Baku, 2020), and in the scientific seminars held in departments of the Institute of Radiation Problems, and Institute of Genetic Resources, ANAS.

Publications. Twelve scientific works covering the results of the dissertation were published in local and international journals.

Structure and volume of the dissertation. The dissertation consists of 178 printed pages, including an introduction, 3 chapters, results, conclusions, recommendations, a list of used literature, lists of appendices, and abbreviations. There are 3 diagrams, 7 figures, 4 graphs, 17 tables, and 11 diagrams. In the analysis of the obtained results, 289 sources were cited, including 8 Azerbaijani, 74 Russian, and 207 foreign literature sources.

CONTENT OF THE WORK

In the **introduction**, the topic, relevance, and main points of the dissertation presented to the defense were discussed, the goals and tasks of the research, scientific novelty, and practical significance of the obtained results were defined.

In the **literature review**, the literature information on the topic of the work was critically analyzed. The modern level of adaptation to the effects of radiation and salt in higher plants, regulation, dynamics of changes in ontogenesis, physiological functions, physicochemical and kinetic properties of antioxidant and energy metabolism enzymes were discussed on the basis of literature data. At the end of the literature review, the topic of the dissertation and the main directions of research were identified.

Materials and methods

Common bean (*Phaseolus vulgaris* L.), the species belonging to the *Phaseolus* genus (*Phaseolus* L.) of the Fabaceae (*Fabaceae* L.) family was chosen as the study object. Leaf surface area was calculated according to the formula $S = 0.66 \cdot l \cdot d$ (l -leaf length, d - leaf width), and seed germination percentage was calculated by the formula $A = n/m \cdot 100\%$ (A -germination %, n -number of germinated seeds, m -total number of seeds).

The activity of H^+ -pumps was measured by the pH-metric method. Activities of the enzymes NAD-MDH⁵, CAT⁶, PK⁷, OAD⁸, amounts of photosynthetic pigments⁹, proline¹⁰, MDA¹¹, H_2O_2 ¹², protein¹³, adenine nucleotides, and nicotic coenzymes were determined using the spectrophotometric method.

The average mathematical errors and deviations ($M \pm m$)

⁵ Scheibe, R. Comparison of NADP-MDH activation, OA reduction and O_2 evolution in spinach leaves / M.Stitt, // *Plant Physiol. Biochem.*, - 1988, 26, - p. 473-481.

⁶ Kumar, C. Changes in lipid peroxidation and lipolytic and free-radical scavenging enzyme during aging and sprouting of potato (*Solanum tuberosum* L.) seed-tubers / N.Knowles, // *Plant Physiol.*, - 1993, 102, - p. 115-124.

⁷ Романова, А. Биохим. мет. изучения автотрофии у мик-ов / -М.: Наука, - 1980, - 160 с.

⁸ Иванишев, В. Биол. знач. мет-ма ОА в хлп C_3 -раст. // *Физ. раст.*, - 1997, 44, с. - 462.

⁹ Sims, D. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages / J.Gamon, // *Remote Sensing of Environment*, - 2002, 81, - p. 337-354.

¹⁰ Bates, L. et al. Rapid det-tion of free proline for water-stress studies // *Plant and Soil*, - 1973, 39, - p. 205-207.

¹¹ Hodges, D.M. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds / J.M.DeLong, C.F.Forney, [et al.] // *Planta*, - 1999, 207, - p. 604-611.

¹² Velikova, V. et al. Oxidative stress and some antioxidant sistems in acid rain-treated Bean plants: Protective role of exogenous polyamines // *Plant Science*, - 2000, 151, - p. 59-66.

¹³ Sedmak, J.J. A rapid, sensitive, and versatile assay for protein using coomassie Brilliant Blue G-250 / S.E. Grossberg, // *Annals of Biochem.*, - 1977, 79, - p. 544-552.

were taken into account in the analysis of the results, the values of $P < 0.05$ were considered statistically significant.

RESULTS AND THEIR ANALYSIS

3.1. The effects of radiation and salt stresses on the biometric parameters of bean plants. Seed germination percentage in 1-3-day-old bean seedlings is ~ 1.6-fold higher than in control at all radiation doses. In 5-day-old seedlings, the germination percentage is almost independent of the radiation dose and is approximately the same in both variants. Pre-sowing treatment of bean seeds with γ -rays generated by the radioisotope Co-60 was shown to stimulate plant growth and development in small doses (1-50 Gy) and inhibit in high doses (100-300 Gy), depending on the stage of plant development. In 5-day-old plants, a radiation dose of 100 Gy significantly slows plant growth, this delay further increases at doses of 200 Gy, and 300 Gy seeds do not almost germinate

It was found that at higher doses (100 Gy) the leaf length is reduced by ~30% compared to the control, and at a dose of 200 Gy by ~70%. In 10-day-old seedlings, plant growth and development are accelerated at a radiation dose of 1-50 Gy, and in 15-day-old seedlings, growth is intensified at a radiation dose of 5 Gy. Apparently, this radiation dose increases biochemical parameters and biological productivity due to accelerating metabolism by activating the genes responsible for growth and development. Thus, the weight of leaves at 5 Gy increases by ~20% in 10-day-old seedlings and by ~30% in 15-day-old seedlings compared to the control and decreases at doses of 100-200 Gy.

The results show that irradiation of plant seeds at selected optimal doses can accelerate their growth and development, increase productivity, improve product quality, and shorten the vegetation period. According to the effects of radiation, 200, 300 mM of salt slows down the growth and development of plants, while 1, 5, 10 mM of NaCl accelerates the growth and development of leaves and stems, and slows down the development of roots.

Whole-plant growth increased by ~ 1.32-fold, root growth by ~ 1.15-fold, and leaf growth by ~ 1.22-fold on the 10th day of vegetation compared to the 5th day, and on the 15th day of vegetation by ~ 1.3-fold, ~ 1.64-fold, and ~ 1.49-fold compared to the 5th day, respectively.

The results show that although root and leaf growth accelerated during the first 10 days of development, it weakened by ~ 15% during the next 5 days of development.

It was found that in the 5-day-old seedlings the L_l / L_r ratio was 1.16 in the control and at 50 mM NaCl, this ratio increased by 2.84-fold, indicating that the leaf length growth was more intensive at 50 mM NaCl than the root length growth.

Modifications in plant metabolism under stress are manifested, above all, in the dynamics of changes in the morpho-biometric parameters.

3.2. Study of the protein amount in bean leaves under radiation and salt stresses.

Depending on the radiation doses and salt concentrations in the bean leaves, the total amount of protein gradually increased in the first 5 days of plant growth at doses of 50-100 Gy. The increase accelerated at radiation doses of 10Gy and 50Gy in 10-day-old plants, and at 1-5 Gy in 15-day-old plants. The results show that there is an inverse relationship between plant age and radiation doses. Thus, in older plants, protein synthesis is intensified at low radiation doses. Similar results were obtained under various salt concentrations.

3.3. Study of the amount of pigments in bean leaves under radiation and salt stresses. It was found that the total chlorophyll in bean leaves on the 10th day of development, under 50 mM NaCl increased by 2.73-fold, on the 15th day by 2.9-fold compared to the 5th day, and decreased under 100 mM NaCl. The chlorophyll a/b ratio was 1.06, 1.77, and 1.65 on the 5th, 10th, and 15th days of the development of the control variant, respectively. The fact that this ratio is greater than one in the first 5 days of development at concentrations of 1-100 mM NaCl indicates that the amount of chl a in bean leaves is higher than chl b. The highest value of the ratio

was observed at 10-50 mM NaCl. In the variants with high values of Chl a/b, the rate of photosynthesis and activities of the energetic enzymes were also high. In our experiments, the decrease in the amount of chl a relative to chl b under the influence of a stressor can be explained by the accumulation of chl b, and this can be related to the pigment composition of PS II. It is known that chl b is a part of the light-harvesting complex II, which transmits the absorbed light energy to the reaction center of PS II. A small part of chl b is involved in the complex that forms the reaction centers of PS I and PS II. The complex consisting of Chl b and the II light-harvesting complex plays a significant role in the regulation of light energy flow entering the reaction centers of PS I, PSII and in the adaptation of photosynthetic apparatus to external conditions¹⁴.

Increases in salt concentration to 100 mM and stress duration to 10-15 days result in the enhanced amount of Car, while the MDA amount decreases.

The decrease in the chl (a + b)/car ratio was due to an increase in the amount of carotenoids, and in this case, the plant is tolerant to stress.

Based on the results, changes in the molecular structure of chlorophylls under the influence of radiation in the leaves of control and experimental plants, and the breakdown of chloroplasts in the leaves due to osmotic shock under salt stress lead to certain differences in photosynthesis and respiration processes.

According to our results and literature data, the weakening of photosynthesis in the salt medium is due to a decrease in the amount of green pigments, an increase in the activity of chlorophyllase, and the breakdown of the chlorophyll-protein complex.

¹⁴ Bassi, R. Chlorophyll binding proteins with antenna function in higher plants and green algae / R.Bassi, F.Rigoni, G.M.Giacometti // Photochem. Photobiol. - 1990, 52, - p. 1187-1206.

3.4. Selection of optimal conditions to study the enzyme activities in bean leaves.

In enzymology, the study of each enzyme begins, first of all, with the selection of optimal conditions for determining its activity. From this point of view, as shown in Figure 3.1, NAD-MDH activity was highest in the leaves during the flowering phase of plant development, and the leaves, together with the roots, were able to maintain some of this activity until the end of the vegetation (Fig. 3.1 D).

NAD-MDH has optimal activity at pH 7.5-8.0 (Fig. 3.1A), when the air temperature is 45-50°C (Fig. 3.1 B), at around 12.⁰⁰-14.⁰⁰, in the afternoon (Fig. 3.1C). For other enzymes, these indices are given in Table 3.1. As seen in the table, the enzymes we have studied consume different amounts of the substrate (K_m) to implement the catalysis process. Although they use different amounts of the substrate, their V_{max} values are approximately close to each other, except for PK. Since OA, the product of the NAD-MDH reaction, is the substrate of the OAD reaction, the activities of both enzymes vary in parallel.

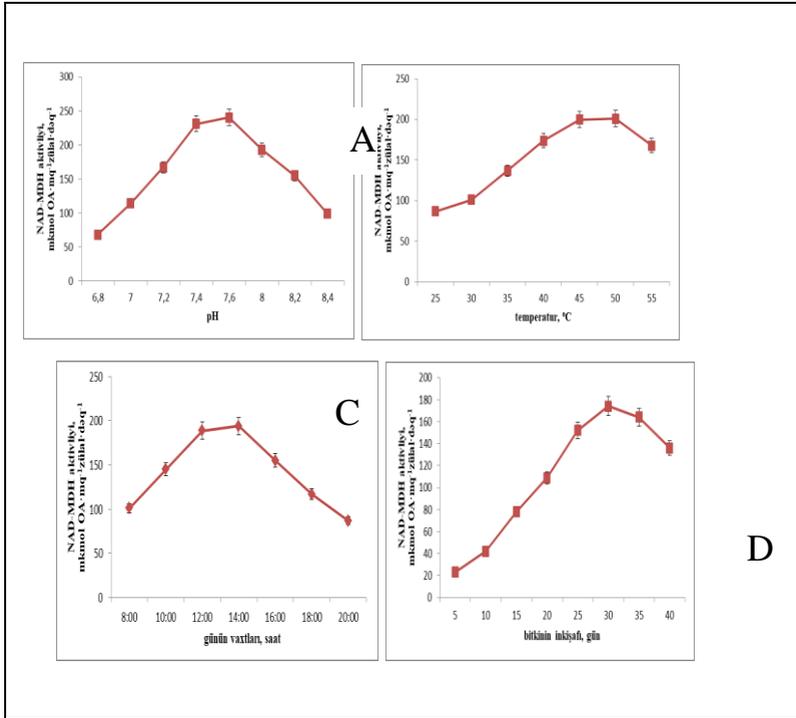


Figure 3.1. Dependence of NAD-MDH activity on pH (A) of the homogenization medium, air temperature (B), time of the day (C), and plant development phases (D) in bean leaves

PV, DTT, and mercaptoethanol were added to the homogenization medium to protect the enzyme from the oxidizing effect of phenolic compounds, 0.5% Triton X-100 was added for the separation of membrane-bound proteins, and 1 mM EDTA to prevent the inactivating effect of heavy metal ions on the enzyme.

Table 3.1. Optimum activity indices of the enzymes CAT, PK, NAD-MDH, and OAD in bean leaves, K_m and V_{max-l} parameters of the reactions catalyzed by the enzymes

Enzymes	Period, days	Time of the day	pH	T, °C	Kinetic parameters	
					K_m	V_{max}
CAT	25-30	11 ⁰⁰ -17 ⁰⁰	7.0-7.2	35-45	7.2	2.5
PK	20-30	15 ⁰⁰	7.4-7.6	30-40	3.3	0.5
NAD -MDH	25-35	13 ⁰⁰ -14 ⁰⁰	7.5-8.0	45-50	22.0	2.0
OAD	30-35	14 ⁰⁰ -16 ⁰⁰	7.0-7.2	35-45	4.0	1.9

Note: K_m - Michaelis-Menten coefficient -mM, V_{max} -maximum rate-EU/mg

3.5. Effect of radiation and salt stresses on the CAT activity in bean leaves. In the next stage, the CAT activity was studied comparatively in leaves of bean plants obtained from seeds irradiated with 0 (control), 1, 5, 10, 50, 100, 200, and 300 Qy doses and unirradiated seeds, which were planted at 0 (control), 1, 5, 10, 50, 100 and 200 mM concentrations of NaCl at the beginning of vegetation (Fig. 3.2).

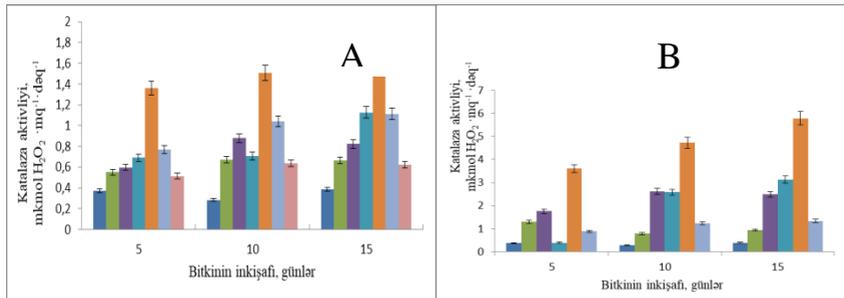


Figure 3.2. Effect of radiation (A) and salt (B) on the dynamics of changes in the CAT activity in the early stages of bean plant development.

■ -C, ■ -1 Qy, ■ -5 Qy, ■ -10 Qy, ■ -50 Qy, ■ -100 Qy, ■ -200 Qy
 ■ -C, ■ -1 mM, ■ -5 mM, ■ -10 mM, ■ -50 mM, ■ -100 mM

It was found that over time, the amount of MDA in the leaves of control plants decreased by 10% on the 10th day of development compared to the 5th day, and by 20% on the 15th day compared to the 5th day. In this case, the amount of protein increased by ~ 2% on the 10th day of plant development compared to the 5th day but decreased by ~ 2% on the 15th day compared to the 5th day, and ~ 4% compared to the 10th day.

In this case, although the amount of H₂O₂ remained relatively stable on the 10th day of plant development, it increased by ~ 7% on the 15th day compared to the 5th day. Under these conditions, on the 15th day of development, the CAT had the highest activity at a radiation dose of 50 Gy (Fig. 3.2A) compared to the 5th and 10th days, at a concentration of 200 mM NaCl, as shown in Figure 3.2B.

3.6. The effect of radiation and salt stresses on the activity of metabolic pathway enzymes in the bean plant leaves. Pyruvate, the end product of anaerobic oxidation was found to play an important role in the regulation of the energy balance in the organism. The direct and indirect involvement of malate metabolism enzymes such as OAD, PK, NAD-MDH, etc. in the metabolism of pyruvate and the fact that the functions of these enzymes are, in many cases, similar, indicate that there is a serious biochemical environment around pyruvate.

3.7. Study of the PK activity in bean leaves. It was established that the main part of pyruvate, which is responsible for energy production in plant tissues, is synthesized from PEP in the presence of PK.

As seen in Figure 3.3A, the PK activity gradually increased with increasing radiation dose in the leaves of 5-day-old plants and was ~ 1.6-fold higher than control at a radiation dose of 200 Gy (Fig. 3.3A). On the 10th day of vegetation, the kinetics of changes in the PK activity depending on radiation doses are similar to a sigmoidal curve characterized by 2 peaks. One of these peaks corresponds to the PK activity at a radiation dose of 5 Gy and the other to the enzyme activity at a radiation dose of 100 Gy.

On the 15th day of plant development, the total spectrum of the PK activity corresponded to the spectrum of the PK activity in

the leaves of 10-day-old seedlings with a little difference (Fig. 3.3A).

Thus, in the early stages of ontogenesis of seedlings obtained from seeds exposed to pre-sowing gamma-irradiation treatment with different doses of radiation, PK activity is characterized by hyperbola in the leaves of 5-day-old plants, by a sigmoidal curve with 2 peaks in 10-day-old plant leaves, and by a parabolic curve with 1 peak in the leaves of 15-day-old plants.

As a result of radiation stimulating effect on the PK activity in the first seedlings at all radiation doses, the PK activity was quite high at 5-10 Gy and 100-200 Gy radiation doses in 10-day-old plants, and in the later stages of development, there was a synchronous decrease, which can be explained by genetic damage to the embryo.

As seen in Figure 3.3B, although the PK activity was highest and relatively equal on the first day of the experiment at 1, 10, and 50 mM NaCl, at other concentrations of salt, a slight decrease was observed.

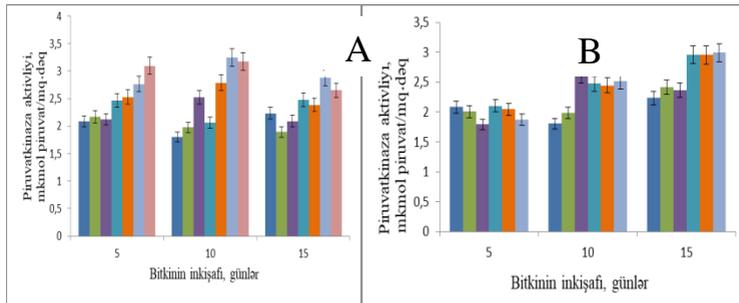


Figure 3.3. Effect of radiation (A) and salt (B) on the dynamics of changes in the PK activity in the early stages of bean plant development

■ - C, ■ -1 Qy, ■ -5 Qy, ■ - 10 Qy, ■ -50 Qy, ■ -100 Qy, ■ -200 Qy
 ■ -C, ■ -1 mM, ■ -5 mM, ■ - 10 mM, ■ -50 mM, ■ -100 mM

The figure shows that the PK activity gradually increased in plants exposed to salt for 10 days, was highest at 5 mM concentration of

salt, and remained relatively equal with a slight decrease during subsequent increases in salt concentrations. In 15-day salt exposures, the PK activity increased 0.6-fold in line with the increase in salt concentration. Under these conditions, the spectrum of changes in the enzyme activity is characterized by a sigmoidal curve in the leaves of 5-day-old plants, by a parabola with one peak in the leaf cells of 10-day-old plants, and by a hyperbola in the leaves of 15-day-old plants, which shows that different concentrations of salt have different effects on the embryo of bean seeds.

3.8. Study of the NAD-MDH activity in bean leaves. As we know, the formation and conversion of OA are directly related to the activity of the enzymes OAD and NAD-MDH. As seen in Figure 3.4, NAD-MDH activity changed differently during the first 15 days of bean plant development under conditions of changing radiation doses (A). The NAD-MDH activity was found to change similarly at all radiation doses and slightly decreased only at 200 Gy, on the 10th and 15th days. Similar results were obtained under salt stress. As seen in Figure 3.4 B, on days 5, 10, and 15 of plant development, the activity of the enzyme gradually increased with increasing concentrations of salt..

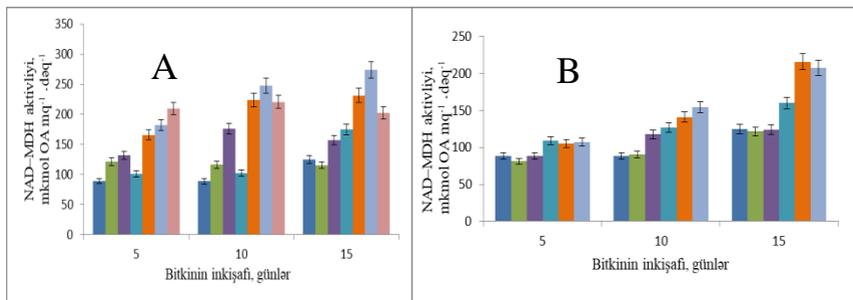


Figure 3.4. Effect of radiation (A) and salt (B) on the dynamics of changes in the NAD-MDH activity in the early stages of bean plant development

■ - C, ■ -1 Gy, ■ -5 Gy, ■ -10 Gy, ■ -50 Gy, ■ -100 Gy, ■ -200 Gy
 ■ -C, ■ -1 mM, ■ -5 mM, ■ -10 mM, ■ -50 mM, ■ -100 mM

Over time, the NAD-MDH activity increased by ~ 30% on the 15th day compared to the 10th day and by 43% compared to the 5th day. The obtained results can be related to the catalytic functions of the enzyme

3.9. Study of the OAD activity in bean leaves. Pyruvate is known to be involved in the process of energy generation by facilitating the synthesis of lipids, carbohydrates, proteins, organic acids such as malate and OA, and also acetyl-CoA, glucose, and lactate in the organism. Pyruvate forms acetyl-CoA during decarboxylation, OA during carboxylation, alanine during transamination, and lactate during reduction¹⁵.

The data on the activity changes of OAD, which plays an important role in pyruvate metabolism, are given in Figure 3.5. As in the figure, in the control and experimental variants, OAD showed the highest activity in the first 5 days of plant development at a dose of 200 Gy. The OAD activity decreased by 80-90% on the 10-day-old plants compared to 5-day-old plants with a further decrease on the 15th day.

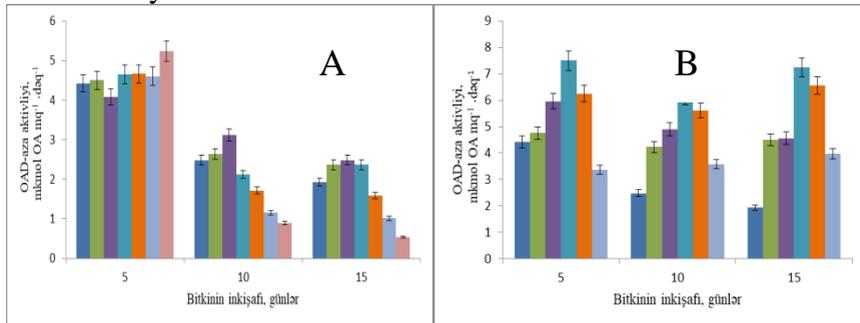


Figure 3.5. Effect of radiation (A) and salt (B) on the dynamics of changes in the OAD activity in the early stages of bean plant development

■ -C, ■ -1 Qy, ■ -5 Qy, ■ -10 Qy, ■ -50 Qy, ■ -100 Qy, ■ -200 Qy
 ■ -C, ■ -1mM, ■ -5mM, ■ -10mM, ■ -50mM, ■ -100 mM

¹⁵ Lehninger, A. Principles of Biochemistry / -New York, NY: W.H. Freeman and Company. - 2008, - p. 528.

On the 15th day of plant development, the decrease in the enzyme activity was similar in all variants.

As seen in Figure 3.5B, changes in the OAD activity occur similarly for variants depending on the salt concentration and the stress duration. The highest activity occurred at 5, 10, and 50 mM salt concentrations, while at 10 mM concentration, this activity remained almost unchanged until the end.

NaCl begins to affect the OAD activity from the first days of plant development. Unlike the effects of radiation, OAD is highly active at all stages of development during salt stress, which can be explained by the need for pyruvate as a universal substrate in energy processes.

Finally, it should be noted that OAD is more sensitive to the effects of radiation. In plants grown under different concentrations of NaCl, adaptive processes are gradual, which makes the plant more tolerant to stress factors. In both cases, OAD ensures the relationship of interdependent metabolic processes by occupying one of the central positions in the energy space and plays a key role in coordinating the energy balance of the organism.

3.10. The effects of radiation and salt stresses on the amount of adenine nucleotides and nicotinic coenzymes in the leaves of bean plants. Based on the results showing the importance of adenine nucleotides and nicotinic coenzymes in the regulation of energy processes, the amount of ATP in control variants and at radiation doses of 1-100 Gy gradually increased over time. In contrast to ATP, the amount of ADP in all cases decreased in control variants and under radiation doses of 1-50 Gy. At 100 Gy, the ADP amount was relatively equal in 5, 10, and 15-day-old plants, and at 200 Gy, it increased slightly. Besides, under radiation and salt stress, the amount of nicotinic coenzymes in bean leaves was reduced compared to the amount of ATP. Therefore, there are similarities in changes in the amounts of ADP, NAD^+ , and NADP^+ under stress. The results can be attributed to the inhibition of glycolysis under radiation and salt stress leading to a weakening synthesis of ATP in the Krebs cycle, which reduces the energy supply to tissues (Table 3.2).

In contrast to the effects of radiation, the amount of ATP in the leaves of 5, 10, and 15-day-old plants, in the control variant and at 1-50 mM NaCl concentrations gradually increased, while at 100 mM NaCl concentration, remained almost constant. In this case, in contrast to the effect of radiation, the amount of ADP increased in the control and 100 mM NaCl variants and at salt concentrations of 1-50 mM, first, it increased and then decreased.

Table 3.2. Effect of radiation and salt stress on the amounts of adenine nucleotides and nicotinic coenzymes in the leaves of bean plants

Variants	Amount of adenine nucleotide, μg						Amount of nicotinic coenzymes, μg					
	5 days		10 days		15 days		5 days		10 days		15 days	
	ATP	ADP	ATP	ADP	ATP	ADP	NAD ⁺	NADP	NAD ⁺	NADP	NAD ⁺	NADP
Radiation, Qy												
C	58	30	66.5	19.5	76.0	14.0	182	106	180	104	149	76
1	65	29	75.0	25.0	83.0	24.0	183	102	182	101	152	69
5	66	36	67.0	33.0	79.0	26.0	185	103	182	100	152	67
10	64	31	65.0	30.0	67.0	28.0	185	102	184	98	154	64
50	58	17	64.0	15.0	71.0	10.0	200	101	189	96	156	62
100	50	16	57.0	18.0	59.0	13.0	201	89	188	86	156	59
200	40	10	50.0	8.0	50.0	12.0	177	88	173	85	147	58
NaCl, mM												
C	43	15	44.0	16.0	50.0	20.6	154	88	144	84	133	78
1	35	12	45.0	17.0	51.0	17.0	140	85	139	83	131	72
5	39	13	40.0	18.0	48.0	14.0	140	84	136	80	126	69
10	35	8	36.0	17.0	39.0	12.0	144	82	143	78	126	67
50	28	3	29.0	18.0	38.0	12.0	146	83	144	76	125	65
100	26	3	28.0	11.0	33.0	10.0	143	80	142	74	123	62

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

The results show that the quantitative changes in ATP, ADP, NAD^+ , NADPH, which are important for energy processes, occur similarly. NAD^+ releases energy during oxidation and absorbs energy during reduction. The resulting NADH is then involved in energy processes. The molar amounts of NAD^+ and NADP^+ increase significantly under stress, indicating that they are oxidized under stress and NADH and NADPH are reduced, indicating mutual consistency between actions of adenine nucleotides and nicotinic coenzymes.

Based on the results, adenine nucleotides and nicotinic coenzymes, together with pigments, can participate in the regulation of energy metabolism and adaptation in the cell by strictly regulating the activity of OAD, which implements the biosynthesis of pyruvate under radiation and salt stresses.

3.11. The effects of salt and radiation stresses on the activity of CAT and some enzymes of the metabolic pathways in the leaves and ion exchange in the root of bean plants. As seen in Table 3.3, there is an inverse relationship between the CAT activity and H_2O_2 levels at 5, 10, and 50 mM NaCl concentrations at the beginning of vegetation. At 100 mM NaCl, the amounts of H_2O_2 and MDA increased due to the decrease in the CAT activity. It was found that in the control variants there was a positive correlation between the biological parameters of the above-ground organs of the bean plant and the ion exchange processes in their root systems. A different picture was obtained in the experimental variants.

In contrast to CAT, the activities of NAD-MDH and PK enzymes, as shown in the table, started from control and gradually increased to a maximum with increasing NaCl concentrations from 1mM to 100 mM. Contrary to them, the OAD activity gradually decreased as the salt concentration increased in the direction of the 100 mM NaCl concentration starting from control in the leaves of 5-, 10-, and 15-day-old plants.

As seen in Table 3.4, the amount of MDA increased as the plant's growth cycle lengthens at high radiation doses, while the H_2O_2 amount decreased over time at the control and all radiation doses, the CAT activity increased, with some exceptions, NAD-

MDH activity increased more at high radiation doses, the PK activity was higher on the 15th day of plant development than on days 5 and 10, at all radiation doses (Table 3.4).

Table 3.3. The effect of various NaCl concentrations on the activities of CAT, NAD-MDH, PK, and OAD in the leaves and H⁺-pumps in the roots of bean plants

NaCl, mM	MDA	H ₂ O ₂	CAT	NAD-MDH	PK	OAD	C _H ⁺
5-day-old seedlings							
C	132.0±14.3	199.0	0.371±0.14	88.8±9.44	2.1±1.20	4.4±0.65	0.14·10 ⁻⁶
1	129.6±12.7	198.0	1.29±0.37	91.1±11.3	2.5±0.89	4.7±0.81	0.4·10 ⁻⁵
5	147.0±20.8	184.5	1.75±0.81	98.1±14.2	2.8±0.78	5.9±1.16	0.71·10 ⁻⁵
10	162.0±16.7	143.5	2.38±0.16	109.2±15.1	2.1±1.22	7.5±3.39	0.76·10 ⁻⁵
50	172.0±19.1	142.0	3.59±1.01	115.4±18.6	2.1±1.11	6.3±2.43	0.86·10 ⁻⁵
100	177.0±17.8	172.0	0.88±0.92	136.8±20.3	1.9±0.65	3.4±1.77	0.43·10 ⁻⁵
10-day-old seedlings							
C	130.0±10.3	00.0	0.38±0.07	98.6±14.7	1.8±0.99	2.5±0.92	0.12·10 ⁻⁵
1	132.0±9.82	190.0	1.78±0.28	99.6±15.1	1.9±0.87	4.2±1.18	0.23·10 ⁻⁴
5	134.0±8.78	172.8	2.62±1.08	117.6±21.7	2.6±0.11	4.9±1.15	0.55·10 ⁻⁴
10	146.0±8.98	132.5	2.58±0.98	127.1±20.2	2.5±0.39	6.1±1.47	0.66·10 ⁻⁴
50	151.0±9.76	102.3	4.72±1.76	141.0±23.0	2.5±0.76	5.6±2.41	0.56·10 ⁻⁴
100	139.8±12.9	163.5	1.23±0.11	154.5±19.8	2.5±1.31	3.6±1.23	0.46·10 ⁻⁴
15-day-old seedlings							
C	129.0±12.4	12.0	0.48±0.31	125.0±17.1	2.2±1.00	1.9±0.59	0.24·10 ⁻⁵
1	148.0±9.32	193.5	1.94±0.32	121.5±14.3	2.4±0.34	4.5±2.11	0.62·10 ⁻³
5	123.0±8.73	158.5	2.89±1.22	123.9±12.9	2.36±1.03	4.6±2.34	0.65·10 ⁻³
10	105.0±9.11	161.0	3.13±1.12	159.9±26.5	2.9±1.12	7.2±2.25	0.79·10 ⁻³
50	105.0±9.13	189.6	5.78±2.43	215.8±25.9	2.9±2.11	6.6±2.24	0.89·10 ⁻³
100	98.5±11.92	154.7	1.34±0.86	247.7±22.2	2.9±0.92	3.9±1.45	0.78·10 ⁻³

Note: CAT - μmol H₂O₂·mg⁻¹protein·min⁻¹; NAD-MDH - μmol OA·mg⁻¹protein·min⁻¹; PK - μmol pyruvate·mg⁻¹protein·min⁻¹; OAD - μmol OA mg⁻¹protein·min⁻¹; C_H⁺ - μekv/hour, MDA - mM/ml; H₂O₂-μM/ml; C – control

In contrast, the OAD activity decreased over time at all radiation doses compared to day 5. Due to the above, the rhizosphere is acidified as a result of the activation of H⁺-pumps in the roots. Here, 10-100 Gy can be considered stimulating doses of radiation. The activity of H⁺-pumps in bean root cells increased significantly over time at 10-50 mM concentrations of NaCl

compared to other variants (Table 3.3). Based on all these indicators, there is a positive correlation between the enzymes involved in the metabolism of pyruvate in the leaves of bean plants and the biological parameters of vegetative organs and ion exchange processes in the roots (Table 3.3; 3.4).

Table 3.4. The effect of various radiation doses on the activities of CAT, NAD-MDH, PK, and OAD in the leaves and H⁺- pumps in the roots of bean plants.

Radiation, Qy	MDA	H ₂ O ₂	CAT	NAD-MDH	PK	OAD	C _H ⁺
5-day-old seedlings							
C	0.36	109.0	0.37	88.8	2.08	4.43	0,14·10 ⁻⁶
1	0.12	88.5	0.55	121.3	2.17	4.50	0,20·10 ⁻⁵
5	0.11	88.5	0.59	131.9	2.12	4.08	0,32·10 ⁻⁵
10	0.13	72.0	0.69	101.2	2.46	4.65	0,65·10 ⁻⁵
50	0.18	67.5	1.36	165.7	2.53	4.66	0,98·10 ⁻⁵
100	0.14	67.5	0.77	181.8	2.77	4.60	0,44·10 ⁻⁵
200	0.17	65.6	0.51	209.7	3.09	5.24	0,35·10 ⁻⁵
10-day-old seedlings							
C	0.33	87.5	0.28	88.6	1.80	2.48	0,12·10 ⁻⁵
1	0.15	75.5	0.67	116.0	1.98	2.64	0,19·10 ⁻⁴
5	0.17	74.5	0.88	176.5	2.52	3.12	0,38·10 ⁻⁴
10	0.54	72.7	0.71	102.5	2.06	2.12	0,48·10 ⁻⁴
50	0.79	66.0	1.51	223.8	2.79	1.72	0,60·10 ⁻⁴
100	0.27	63.0	1.04	247.3	3.25	1.15	0,18·10 ⁻⁴
200	0.19	59.0	0.64	220.8	3.18	0.89	0,12·10 ⁻⁴
15-day-old seedlings							
C	0,29	41.0	0.39	124.9	2.23	1.93	0,24·10 ⁻⁵
1	0,44	41.0	0.66	115.4	2.19	2.36	0,30·10 ⁻³
5	0,69	53.0	0.82	157.1	2.09	2.49	0,35·10 ⁻³
10	1,41	51.0	1.13	174.7	2.48	2.36	0,39·10 ⁻³
50	1,15	52.5	1.67	231.4	3.39	1.58	0,43·10 ⁻³
100	1,18	53.0	1.11	274.1	3.88	1.01	0,20·10 ⁻³
200	1.03	54.0	0.63	202.1	2.65	0.54	0,17·10 ⁻³

Note: Accuracy index is less than 3%

The obtained results show that at increasing NaCl concentrations, vegetative organs by acting “coordinated” at the level of photosynthesis, respiration, glycolysis processes, some

enzymes of the antioxidant defense system, intermediate metabolites, proteins, photosynthetic pigments, and mineral nutrients cause tolerance to stress in bean plants.

3.12. Dynamics of changes in the enzyme activities in bean leaves under combined effect of various radiation doses and NaCl concentrations. As seen in Table 3.5, three optimal radiation doses (5, 10, 50 Gy) were chosen and seeds exposed to each dose were planted separately at 1, 5, 10, and 50 mM NaCl concentrations. It was found that in the early stages of vegetation in the resulting seedlings, the total amount of protein in the control samples was highest in the 50 Gy+salt combinations and decreased as the duration of stress increased. In the 5 Gy and 10 Gy+salt combinations, the total amount of protein increased over time. The activity of H⁺ -pumps was highest in the combinations of 5 Gy and 10 Gy+salt. In general, the activity of H⁺ -pumps increased ~ 25-fold on the 10th day of development compared to the 5th day, and ~ 30-fold on the 15th day compared to the 5th day. Whereas, in 50 Gy+ salt combinations, it decreased by about 10-fold compared to 5 Gy and 10 Gy + salt combinations.

As seen, in the 50 Gy+ 50 mM salt combination, the H⁺ -pump became more active, the total amount of proline and synthesized protein increased, and MDA decreased even more. These signs suggest that the activation of the pump increases the amount of protective proteins by accelerating ion exchange in the roots, the weakening of LPO, and the increase in the amount of proline optimize other metabolic reactions in the plant under dual stress.

In the 50 Gy + 5 mM NaCl variant, the amount of carotenoids in the leaves increases over time compared to other variants. In these variants, as seen in the table, the chl (a + b) /car ratio was highest on the 5th day (3.37) and lowest on the 15th day (0.3). This occurred due to a gradual decrease in chl (a + b) and an increase in the amount of carotenoids. Here, the combination of 50 Gy + 5-50 mM also played an optimizing role.

Although the amount of ATP and ADP in 50 Gy+salt

combinations is highest in 15-day-old plants, the amounts of NAD⁺ and NADPH in all combinations decrease over time. These show that plants try to maintain optimal levels of NAD⁺ and NADPH,

Variant	5 günlük bitki			10 günlük bitki				15 günlük bitki				
	H ⁺ -ATF- aza	C zülal, MDA	Prolin	H ⁺ - ATF- aza	C zülal, MDA	Prolin	H ⁺ - ATF- aza	C zülal	MDA	Prolin		
Kontrol	0.19 · 10 ⁻⁶	14.78	0.298	19.6	0.32 · 10 ⁻⁵	15.56	0.25	14.2	0.31 · 10 ⁻⁵	16.9	0.27	14.9
5 Qr+1 mM	0.09 · 10 ⁻⁵	13.03	0.095	21.3	0.35 · 10 ⁻⁴	14.58	0.13	20.2	0.1 · 10 ⁻³	15.2	0.17	21.3
5 Qr+5 mM	0.22 · 10 ⁻⁵	13.58	0.143	21.6	0.43 · 10 ⁻⁴	12.12	0.24	23.7	0.22 · 10 ⁻³	15.8	0.23	24.8
5 Qr+10 mM	0.62 · 10 ⁻⁵	13.12	0.166	22.0	0.28 · 10 ⁻⁴	12.09	0.17	26.9	0.19 · 10 ⁻³	14.9	0.17	28.1
5 Qr+50 mM	0.71 · 10 ⁻⁵	14.51	0.169	22.0	0.14 · 10 ⁻⁴	14.92	0.17	26.8	0.1 · 10 ⁻³	15.1	0.17	28.8
Kontrol	0.19 · 10 ⁻⁶	14.78	0.298	19.6	0.32 · 10 ⁻⁵	15.56	0.25	14.2	0.31 · 10 ⁻⁵	16.9	0.27	14.9
10 Qr+1 mM	0.65 · 10 ⁻⁵	14.21	0.127	17.0	0.86 · 10 ⁻⁵	15.41	0.16	16.8	0.38 · 10 ⁻³	16.2	0.13	18.2
10 Qr+5 mM	0.71 · 10 ⁻⁵	14.21	0.162	16.5	0.23 · 10 ⁻⁵	15.62	0.17	17.1	0.27 · 10 ⁻³	15.5	0.15	18.1
10 Qr+10 mM	0.86 · 10 ⁻⁵	14.44	0.177	16.0	0.55 · 10 ⁻⁴	14.93	0.2	17.6	0.41 · 10 ⁻³	15.1	0.13	18.8
10 Qr+50 mM	0.88 · 10 ⁻⁵	14.51	0.181	16.6	0.48 · 10 ⁻⁴	14.90	0.18	17.6	0.07 · 10 ⁻³	14.9	0.16	19.4
Kontrol	0.19 · 10 ⁻⁶	14.78	0.298	19.6	0.32 · 10 ⁻⁵	15.56	0.25	14.2	0.31 · 10 ⁻⁵	16.9	0.27	14.9
50 Qr+1 mM	0.91 · 10 ⁻⁶	17.22	0.223	19.11	0.6 · 10 ⁻⁴	16.11	0.28	19.6	0.39 · 10 ⁻³	14.5	0.23	20.7
50 Qr+5 mM	0.81 · 10 ⁻⁶	17.1	0.247	19.12	0.66 · 10 ⁻⁴	15.53	0.24	18.7	0.08 · 10 ⁻³	14.1	0.19	19.9
50 Qr+10 mM	0.86 · 10 ⁻⁶	16.83	0.279	18.61	0.78 · 10 ⁻⁴	15.04	0.23	18.4	0.08 · 10 ⁻³	13.9	0.19	21.7
50 Qr+50 mM	0.86 · 10 ⁻⁶	16.47	0.296	19.2	0.66 · 10 ⁻⁴	16.72	0.24	19.2	0.89 · 10 ⁻³	16.2	0.13	22.1

which play an important role in energy generation to combat stress.

Table 3.5. Study of the dynamics of changes in the amounts of adenine and nicotinic coenzymes in the early stages of the bean plant ontogenesis under the combined effect of different doses of radiation and different concentrations of NaCl

Note: C_H^+ - $\mu\text{ekv}/\text{hour}$, H_2O_2 - $\mu\text{M}/\text{ml}$; C – control, MDA-malondialdehyde- mM/ml ;. Accuracy is less than 3%

Table 3.6 . Study of the dynamics of changes in the activities of CAT, PK, OAD, and NAD-MDH enzymes in leaves of bean plants in the early stages of ontogenesis under the combined effect of different doses of radiation and different concentrations of NaCl

Variants	5-day-old plant				10-day-old plant				15-day-old plant			
	CAT	PK	OAD	NAD-MDH	CAT	PK	OAD	NAD-MDH	CAT	PK	OA D	NAD- MDH
Control	0.50	2.69	5.63	1.01	0.43	3.02	4.16	0.63	0.59	2.54	2.89	1.16
5 Qy+1 mM	1.28	3.53	8.65	2.32	1.14	3.26	5.83	1.74	1.51	3.08	4.71	1.65
5 Qy+5 mM	2.26	3.66	8.90	2.28	2.31	3.52	7.07	2.60	2.80	3.33	5.16	1.66
5 Qy+10 mM	2.39	3.96	9.50	2.59	2.59	3.84	8.42	2.92	3.31	3.64	7.41	1.94
5 Qy+50 mM	6.42	6.07	14.8	4.26	5.40	5.0	11.63	3.58	6.56	3.87	7.49	2.08
Control	0.50	2.69	5.63	1.01	0.43	3.02	4.16	0.63	0.59	2.54	2.89	1.16
10 Qy+1 mM	1.62	3.76	8.98	1.52	1.33	3.67	3.76	1.56	1.40	3.31	2.97	1.56
10 Qy+5 mM	3.90	4.12	9.23	1.67	3.02	3.58	3.73	1.66	3.20	3.54	3.30	1.83
10 Qy+10 mM	2.69	4.19	9.23	1.78	3.37	3.91	7.49	1.48	3.41	3.87	3.50	2.06
10 Qy+50 mM	5.64	4.29	9.23	1.90	5.84	3.98	7.60	2.15	6.22	3.78	3.56	2.55
Control	0.50	2.69	5.63	1.01	0.43	3.02	4.16	0.63	0.59	2.54	2.89	1.16
50 Qy+1 mM	1.76	2.71	7.18	1.95	1.99	3.40	4.83	2.48	2.47	3.60	4.59	3.18
50 Qy+5 mM	2.56	2.91	8.44	2.12	2.93	3.68	5.0	2.76	3.31	3.78	4.79	3.61
50 Qy+10 mM	2.91	3.15	8.62	2.35	3.42	3.91	6.19	3.13	3.9	4.18	6.06	4.22
50 Qy+50 mM	5.81	3.58	8.93	2.58	6.96	4.07	6.33	3.58	7.15	3.75	5.44	4.44

Note: CAT - $\mu\text{mol H}_2\text{O}_2 \cdot \text{mg}^{-1} \text{protein} \cdot \text{min}^{-1}$; NAD-MDH - $\mu\text{mol OA} \cdot \text{mg}^{-1} \text{protein} \cdot \text{min}^{-1}$; PK-ase - $\mu\text{mol pyruvate} \cdot \text{mg}^{-1} \text{protein} \cdot \text{min}^{-1}$;

OAD-ase - $\mu\text{mol OA mg}^{-1}\text{protein}\cdot\text{min}^{-1}$; Accuracy is less than 3%

Time-related changes in the activities of CAT, PK, NAD-MDH, and OAD were also studied (Table 3.6). As seen in the table, the CAT activity decreased at the beginning of the dual stress effect in the 50 Gy+50 mM salt combinations, but then increased. Whereas, the PK activity decreased over time in the 5Gy and 10 Gy + salt combinations, and increased in the 50 Gy + salt variants. The OAD activity in all radiation + salt combinations gradually decreased as the duration of stress increased, while the NAD-MDH activity increased in 50 Gy + salt combinations over time.

Based on the obtained results, the combined action of radiation and salt 50 Gy+10-50 mM forms inductive mechanisms in bean plants by stimulating effect at the level of amounts of intermediate metabolites, proteins, pigments, adenine nucleotides and nicotinic coenzymes, and activities of CAT, PK, NAD-MDH, OAD, and H^+ -pumps. Thus, by combining functions at the intersection of 5 metabolic pathways, and linking them at the level of substrates and intermediate metabolites signs of temporary or permanent tolerance are created.

CONCLUSIONS

1. In the ontogenesis of bean leaves, a homology was found between the dynamics of changes in the amount of photosynthetic pigments, γ -radiation doses, and salt concentrations. During the first 10 days of bean plant development, high chlorophyll content was detected at relatively small radiation doses, and salt concentrations (1, 5, 10, 50 Gy and 1, 5, 10, 50 mM NaCl), while at high radiation doses and salt concentrations (100, 200 Gy and 100, 200 mM), the content of carotenoids was high, which is related to the protective function of these pigments under these conditions.
2. For the first time, it was found that the increase in the PK activity at high radiation doses (100-200 Gy) and salt concentrations (10-100 mM) depending on the duration of exposure to stress was related to meeting the plant's need for pyruvate under stress.

3. High radiation doses (50-200 Gy) and high concentrations of NaCl (50-100 mM) were found to have a more stimulating effect on the activity of the enzyme NAD-MDH, depending on the duration of stress.
4. Although the OAD activity in bean leaves was higher at the beginning of vegetation, it was found to weaken over time as a result of the breakdown of chloroplasts under the influence of γ -radiation. Gradual exposure to salt ensures high levels of the OAD activity for a long time.
5. Correlation between an increase in the PK activity over time at high radiation doses (100-200 Gy) and salt concentrations (10-100 mM NaCl), a greater increase in the MDH activity at the radiation doses of 50-200 Gy and 50-100 mM NaCl, maintaining high levels of the OAD activity at the beginning of vegetation under radiation, and for a long time under the effect of various salt concentrations and the activity of H⁺-pumps in the roots promotes the development of signs of stress tolerance of higher plants at the antioxidant system level.
6. Under normal and stress conditions, activities of the energy metabolism enzymes increase similarly and in parallel to the activity of nicotinic coenzymes (NAD⁺, NADPH). The decrease in ATP and the increase in ADP in the regulation of energy balance prove that adenine nucleotides have an effective role under high radiation and salt concentrations.
7. For the first time, it has been found that the combined effect of radiation and salt (50 Gy + 10-50 mM NaCl) has a stimulating effect on the bean plant, which forms inductive mechanisms at the level of the amounts of intermediate metabolites, proteins, pigments, adenine nucleotides, and nicotinic coenzymes, activities of CAT, PK, NAD-MDH, OAD, and H⁺-pumps.

RECOMMENDATIONS

1. The use of the results obtained in relation to the dynamics of changes in the activity of energy metabolism pathways and antioxidant defense system enzymes in the selection of plant varieties tolerant to radiation and salt can lead to positive results.
2. Experiments on plants formed from seedlings obtained under the combined effect of radiation and salt (50 Gy + 50 mM NaCl) would be more effective to increase the tolerance and productivity of higher cultivated plants.
3. Breeders should consider the following aspects when characterizing a variety:
 - Multiple increases in the amount of carotenoids in the leaves of the new variety and multiple decreases in the chl (a + b)/car ratio,
 - Ten-fold increase in the activity of the H⁺ -pump at the beginning of vegetation with subsequent stabilization,
 - An increase in the total protein content at the combination of 50 Gy+salt,
 - A decrease in the amount of MDA depending on the intensity and duration of stress, and an increase in proline,
 - A significant increase in the CAT activity under double stress conditions (50 Gy + 50 mM) over time, a more marked increase in the PK activity, a higher OAD activity at the beginning of vegetation that decreases over time, and a gradually increasing NAD-MDH activity over time.

List of published scientific works on the topic of the dissertation

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