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THE ROLE OF ENZYMES IN PLANT RESPONSES TO STRESS FACTORS

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Abstract. In the face of an escalating ecological crisis, environmental stress factors exert a significant influence on plant vegetation. This study explores the adaptation mechanisms of plants to new conditions while maintaining their development in the presence of stress factors. Under unfavorable environmental conditions, plants experience stress that may either lead to their complete destruction or to their adaptation to these conditions. Stress in plants results in a weakening of their normal life activity. Thus, the process that begins with a weakening of biosynthesis at the cellular level extends to tissues and organs, eventually disrupting the plant's normal life processes and potentially leading to death. The present research examines ezyme changes in the green leaves of bean plants (*Phaselus vulgaris* L.), whose seeds were exposed to gamma radiation at doses ranging from 1-300 Gy prior to sowing. To analyze the response of the bean plant, the activities of two enzymes, Pyruvate kinase (PK) and Oxalate Decarboxylase (OAD) were investigated. It was observed that the activity of PK and OAD varies depending on both the developmental stage of the plants (depending on time) and the radiation dose, compared to the control sample. Thus, while the development of plants is accelerated at a certain level of radiation dose, the growth of plants starts to slow down as the dose increases.

Keywords: *Environment, plant, Pyruvate kinase (PK), Oxalate Decarboxylase (OAD), gamma radiation, Phaselus vulgaris L.*

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1. Introduction

The ongoing ecological changes have reached a crisis level. Due to the impacts of drought, salinity, radiation, heat and other abiotic stressors, plant biodiversity is significantly threatened, leading to the extinction of numerous species. It is known that plants play a critical role in supporting human life, as they provide oxygen through photosynthesis and form the basis of human nutrition. Therefore, the degradation of plant life could severely disrupt the nutritional balance of human populations, leading to severe food shortages in the future (Boyer, 1982; Geraskin *et al*., 2015; Kafi *et al*., 2003).

All living organisms are constantly exposed to various biotic and abiotic environmental factors throughout life cycles. Plants, in particular, face challanges posed by the environmental factors. The responses of plants to the effects of abiotic factors play an important role in their lives. It is known that various biochemical and physiological changes occur in plants under unfavorable environmental conditions. These changes cause a number of reactions at the cellular level. The duration of stress, the development

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phase of the plant, etc. play a vital role in the course of the processes. The responses of plants to environmental stress factors are often adaptive in nature (Calabrese, 2013; Rogozhin, 2013; Filippov & Troeva, 2016).

Plant metabolism is a process influenced by abiotic factors in the external environment, which ensures stability in the reactions occurring within plants. Scientists employ various methods to develop plant varieties that are more resistant to environmental stress factors, one of which is radiation technology. The use of radiation as a physical method has gained widespread adoption in recent years for several reasons (Borzouei *et al*., 2010; He & Hou, 2014). First and foremost, it allows for the creation of new, stress-resistant varieties without causing harm to the environment. To date, numerous experiments have been conducted on a variety of plants using radiation technologies, resulting in shortened ontogenetic periods, increased productivity and enhanced levels of proteins, fats, vitamins, etc. (Bais *et al*., 2018; Romanova, 1980).

Gamma radiation treatment of seeds has been shown to have a stimulating effect on plant development, even under the influence of various stress factors. By applying ionizing radiation in agricultural plants, it is possible to sterilize them and at the same time to create a stimulating effect and to obtain beneficial mutations.

It is well known that seeds, as generative organs, typically experience metabolic dormancy. However, under favorable conditions, development is initiated based on genetic information within seeds. When seeds are irradiated prior to sowing, they exhibit a stimulating effect, which in turn enhances their resistance to environmental stress factors throughout their subsequent development (Kafi *et al*., 2003).

The most critical biochemical processes in plants are photosynthesis and energy metabolism. This study examines the activity of enzymes, Pyruvate kinase and Oxaloacetate decarboxylase, which are the key enzymes of energy metabolism (Shinozaki & Yamaguchi-Shinozaki, 2007).

Pyruvate kinase is the basis of carbon and glycolysis metabolism in plants. This enzyme catalyzes the reaction of pyruvate and ATP synthesis. This reaction is a direct reaction. Due to the high energy consumption, the reaction is nearly irreversible (Ambasht & Kayastha, 2002). The substrate phosphoenolpyruvate, formed during the PK reaction, is one of the high-energy (ΔG0=58 kC) intermediate metabolites (ΔG0 ATP =29 kC). Given these considerations, the PK reaction is highly favorable for ATP synthesis. Pyruvate itself also plays a crucial role in numerous metabolic pathways within the cell (Ambasht & Kayastha, 2002).

During seed development, PK plays an important role in the synthesis of fatty acids and carbohydrates (Borzouei *et al*., 2010). Inhibition of glycolysis enzymes can result in the failure of seed germination (Munoz & Ponce, 2013).

The enzyme oxaloacetate decarboxylase (OAD; EC 4.1.1.3) is another critical enzyme involved in the metabolism of OA. The reaction of formation and accumulation of pyruvate from OA is catalyzed by the enzyme OAD, which is a C4-dicarboxylic acid. It has been established that the formation of pyruvate occurs under the catalysis of the enzymes, such as succinate dehydrogenase, MDH and OAD. In the mitochondria, these enzymes sequentially convert succinate to malate, malate to OA and OA to pyruvate (Paul *et al*., 2018; Ivanishchev, 1991).

2. Material and Methods

In the presented work, studies were conducted on green mass of bean plants that were treated with gamma rays prior to sowing.

Figure 1.

For this purpose, 20 dry seeds of the common bean plant, which was taken as the object of research, were used for each irradiation dose. The seeds were irradiated using the RUXUND 20,000 irradiation device (Co60 source) at doses of 1, 5, 10, 50, 100, 200 and 300 Gy. Dry seeds of both irradiated and non-irradiated bean plant were disinfected in a 3% H_2O_2 solution for 15 minutes, washed with distilled water and placed in petri dishes and kept in a thermostat for germination. The resulting seedlings were watered as needed. For optimal plant development, the seedlings were transferred to an artificial environment created under hydroponic conditions at a temperature of 25-28ºC. The cultivation conditions of the plants were the same in all variants.

Leaf samples taken for the determination of the activity of the intended enzymes were washed with distilled water, dried with filter paper. The samples were then homogenized in a buffer solution appropriate for each enzyme. The composition of the homogenization solutions for each enzyme was as follows:

For OAD: 10 mM NaCl, 1 mM EDTA-Na, 1 mM Na-ascorbate, 0.4 M sucrose, 0.1% polyethylene glycol (PEG), 50 mM, Na-acetate-acetic acid buffer (pH 6.8-7.0).

For PK: 100 mM Na_2HPO_4 - NaH_2PO_4 buffer containing 1 mM EDTA, 20 mM $MgCl_2$, 0.5% PVP, 0.5% Triton X100, 5 mM DTT (pH 7.4).

Homogenization was carried out at +4°C.

The PK enzyme catalyzes the conversion of PEP to pyruvate with ATP formation. This reaction is essentially irreversible. For this purpose, the leaf samples and buffer solution were taken in a 1:7 ratio. The homogenate was filtered through double-layered capron, followed by centrifugation at 300 g for 5 minutes and at 5000 g for 10 minutes to remove the nucleus and non-decomposed tissue particles. The supernatant was used to measure the enzyme activities. The enzyme activity was determined in a reaction medium containing 0.1 M Tris-HCl buffer (pH 7.6), 50 mM $MgSO_4$ or 0.2 M KCl, 10 mM FEP, 94 mM ADF, 12 mM NADH, 450 mg LDH (50 µl of 35 mg/ml was taken). The reaction was initiated by adding 60-80 µl of enzyme extract to the reaction mixture. Enzyme activity was calculated based on the change in optical density over 3 minutes at 25°C and a wavelength of 340 nm, measured using a spectrophotometer (Muscolo *et al*., 2001; Baud *et al*., 2007).

Enzymatic analyses were performed spectrophotometrically in 1 ml cuvettes. OAD activity was measured by monitoring the decrease in absorbance at a wavelength of 280

nm under standard conditions (50 mM, pH 6.8-7.0, Na-acetate-acetic acid buffer, supplemented with -10 mM $MnCl_2$, 1 mM OA, 1 mM Na-ascorbate, 0.4 M sucrose, 0.1% polyethylene glycol (PEG) and 5-10 µl enzyme extract). To fully activate the enzyme, divalent metal ions (Mn^{2+} or Mg^{2+}) were added to the reaction mixture. The optimal pH of OAD was determined in the range of pH 6.0-8.0 in 50 mM Na-acetate-acetic acid buffer. The amount of pyruvate, the product of the decarboxylation reaction of OA catalyzed by OAD, was analyzed by this reaction.

For this, an OA decarboxylation test was first performed under standard conditions for one minute. The reaction mixture was then adjusted to a final concentration of 200 mM NaCl, 0.2 mM NADH, pH 7.0. The pyruvate concentration was calculated based on the decrease in NADH absorbance at 340 nm after adding 5 U ml-1 LDH, pH 7.0. The enzyme activity unit was defined as the amount of enzyme that catalyzed the decarboxylation reaction of 1 µM OA in 1 min at 30°C under all conditions. The specific activity of the enzyme was calculated per mg protein.

3. Results

The activity of Pyruvate Kinase (PK) enzyme in bean leaves was examined comparatively under various radiation doses (1-200 Gy). The results revealed that while PK enzyme activity increased during the first 5 and 10 days of the experiment, it gradually declined in both variants compared to the control sample. It was observed that oxaloacetate (OA), transported to the chloroplasts, is utilized not only for malate reduction but also for pyruvate synthesis through decarboxylation (Geraskin *et al*., 2015) With the catalysis of PK, a significant portion of pyruvate is synthesized from PEP. The study focused on 5, 10 and 15-day-old plants, showing that increasing radiation doses significantly enhanced PK activity. At a radiation dose of 200 Gy, it was observed to be approximately 1.6 times higher than the control sample. On the 10th day of sprout development, the general tendency of increasing PK activity continued, depending on radiation doses. In 10-day-old plants, enzyme activity at various radiation doses surpassed the activity seen in the control variant (Guliyeva *et al*., 2020).

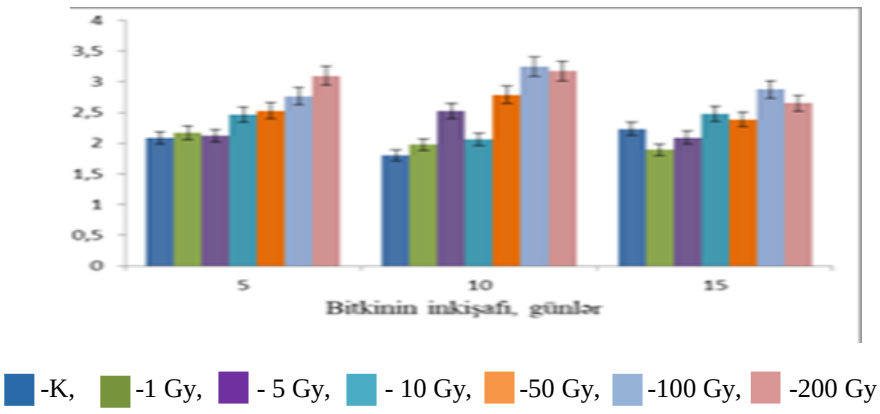

Figure 2. The effect of radiation on the dynamics of changes in PK activity in the early stages of bean plant development

As illustrated in the Figure 2, one of these peaks in PK activity corresponds to a 5 Gy radiation dose, while the second peak corresponds to a 100 Gy dose. On the 15th day, PK activity continued to increase in relation to the radiation dose. In the 15-day-old

control sample, PK activity was higher than that in the leaves of the 5- and 10-day-old control plants. Notably, this enzyme also demonstrates the highest activity in the leaves of 15-day-old plants exposed to a radiation dose of 100 Gy. However, despite the preservation of growth dynamics, the activity of the enzyme in 15-day-old plants was lower than the activity in the leaves of 10-day-old plants. These results suggest that during the early stages of ontogenesis in bean sprouts treated with γ- radiation at various doses, the PK activity increased as the radiation dose increased. Although this increase was sustained, overall plant growth declined over time. The highest PK enzyme activity occured in the leaf cells of 10-day-old plants. Although gamma radiation initially has a stimulating effect on the activity of the PK enzyme at all radiation doses, over time, the effect of radiation on the activity of this enzyme leads to different results at different doses. Thus, PK activity was significantly higher in 10-day-old plants treated with 5-10 Gy and 100-200 Gy doses and a synchronous decrease in enzyme activity was observed in later developmental stages.

The presented results show that the OAD enzyme exhibits higher activity during the first 5 days of plant development compared to the following days both in the control sample and in all radiation dose-exposed variants. In the variant treated with a 200 Gy radiation dose, this enzyme demonstrated slightly higher activity.

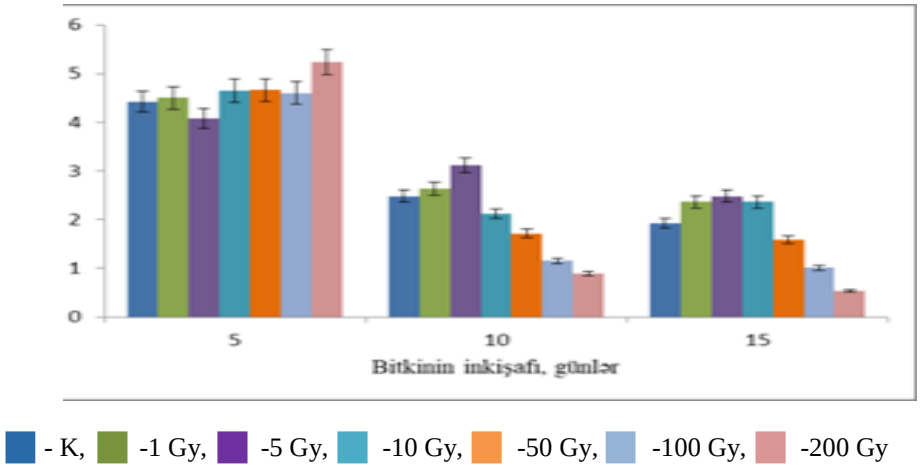

Figure 3. The effect of radiation on the dynamics of changes in OAD activity during the early stages of bean plant development

In the later stages of plant development, sharp differences appeared in the activity of this enzyme, depending on both developmental time and radiation dose. OAD activity in the leaves of 10-day-old plants was 80-90% lower compared to 5-day-old plants and in 15-day-old plants it was even lower than that of 10-day-old plants. In 10-day-old plants, the intensification of radiation stress at lower radiation doses gradually increased the enzyme activity, reaching its peak at a 10 Gy radiation dose. Then, an increase in the radiation dose led to a significant reduction in the enzyme activity. In the variant treated with 200 Gy dose, OAD activity was at its lowest level, which is approximately five times lower than the levels in 5-day-old plants. The decrease in the activity of the OAD enzyme on the 15th day of plant development continued in all variants following a similar trend. These results highlight that the OAD enzyme in plants grown under radiation exposure at various doses is particularly sensitive to the stress. While the activity of OAD, the main enzyme in the biosynthesis of pyruvate, a central substrate, is accelerated under the

influence of stress during the early days of plant development, it rapidly declines over time (Guliyeva *et al*., 2020).

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