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INFLUENCE OF THE PROCESSING OF SEEDS WITH GAMMA RAYS BEFORE SOWING ON THE ACTIVITY OF CO_2 METABOLISM ENZYMES IN SOME PLANTS

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Abstract: In natural medium, it has been comparatively studied the activities of the enzymes of carboanhydrase (CA, CF 4.2.1.1), phosphoenolpyruvate carboxylase (PEPC, CF 4.1.1.31) and ribulose-1.5-bisphosphate carboxylase (RBPC, CF 4.1.1.39) in the leaves during active growth of ontogenesis of plants of ordinary bean (*Phaseolus vulgaris* L.), eggplant (*Solanum melongena* L.), cucumber (*Cucumis sativus* L.) and tomato (*Solanum licopersicum* L.), of which seeds irradiated at 1, 5, 10, 50, 100, 200, 300 and 400 Gy doses (irradiation rate was *p=0.19 Gy/sec*). It has been established that CA and RBPC enzymes in the studied plants exhibit similar activity under radiation exposure. The enzyme PEPC has a low catalytic activity due to the photosynthesis mechanism of the plants.

Keywords: the processing of seeds with γ-rays before sowing, beans, eggplant, cucumber, tomato, ribulose-1.5-bisphosphate carboxylase, phosphoenolpyruvate carboxylase, carboanhydrase

1. Introduction

As it is known, biochemical reactions that form the basis of metabolism in living organisms cannot occur without the presence of enzymes. It has been established that the activity of enzymes depends on the influence of extreme factors [6]. Therefore, the study of enzymes of energy metabolism systems, which are dependent on the influence of abiotic stress factors, such as radiation, drought, salinity, high light intensity, etc. and have an irreplaceable role in the formation of adaptive reactions by responding adequately to this effect, is of great scientific and practical importance. It is worth noting that the carboanhydrase (CA) enzyme, which performs the diffusion of CO_2 from the atmosphere and the transfer of that to carboxylation centers through stomata in the photosynthesis process, is very important. Thus, CA creates the basis for the reaction of ribulose-1.5-bisphosphate carboxylase (RBPC) by repeatedly increasing CO_2 concentration in photosynthesis reaction centers in leaves. In higher plants having C_3 - photosynthesis mechanism, the RBPC enzyme continues the metabolism of carbon by carboxylation of ribulose-1.5-bisphosphate (RBP) due to diffused CO_2 [3].

But, in higher plants having C_4 - photosynthesis mechanism, phosphoenolpyruvate carboxylase (PEPC) plays the role of the main carboxylate enzyme. This enzyme, which plays an important role in nitrogen and carbon metabolism in higher plants, forms the basis for subsequent stages of metabolism by converting phosphoenolpyruvate (PEP) into oxaloacetate [7].

As it is known, the influence of stress factors on metabolic processes eventually leads to a decrease in plant productivity and eventually to premature aging. In this regard, radioactive radiation, as a stress factor, has a large weight ratio [4]. Considering these it should be noted that despite the fact that the biochemical composition of plants is genetically formed during the evolution process, its quantitatively and qualitatively change is possible under the influence of environmental factors.

It is thought that although the processing of seeds with γ-rays before sowing at low doses (in the stimulating dose region) cannot change the genetic development program, the energy of this irradiation is enough to influence regulatory systems. And as it accelerates to pass the initial phases of ontogenesis by accelerating the implementation of the plant development program, it can lead to the shortening of its growth period, to the increase of productivity under favorable conditions and to the improvement of their quality indicators [2].

Herein, the change dynamics of the activities of CA, RBPC, PEPC, which are considered enzymes of CO_2 metabolism of the processing of seeds with γ-rays at different doses before sowing, have been investigated.

2. Materials and methods

Sprouts obtained in the natural medium in the active growth period of the ordinary bean (*Phaseolus vulgaris* L.), eggplant (*Solanum melongena* L.), cucumber (*Cucumis sativus* L.) and tomato (*Solanum Licopersicum* L.) plants, of which seeds irradiated at 1, 5, 10, 50, 100, 200, 300 and 400 Gy doses (irradiation rate was *p=0.19 Gy/sec*) were used as research objects. Irradiated seeds were sown together with control samples in the experimental area of the Scientific Research Institute of Vegetable Growing. Irrigation was carried out normal in all plant samples until the end of the growing. The leaf samples for the experiment were taken after the illumination under the sun's rays for 3 hours (for the activation of enzymes), that is, at ~11.00 and placed in liquid nitrogen, then delivered to the laboratory.

Plants that were taken for the experiment are characterized by many positive qualities. For example, bean plants are rich in protein, vitamins, microelements, as well as carbohydrates, amino acids, B group vitamins, vitamin C and also sodium, potassium, and calcium. The instant sate feeling that comes during nutrition with beans is due to the high amount of protein in it. As beans have a positive effect on the nervous system, people who regularly eat it are mild and kind. Beans do not lose their beneficial properties when canning. In this case, it contains up to 70% vitamins and up to 80% minerals.

In Eastern countries, eggplant is called a "plant of longevity". As eggplant is rich in potassium salts, meals made from it have a positive effect on the regulation of salt balance in the elderly for bettering the digestive system, and eliminating cardiovascular and gastrointestinal diseases. It also reduces blood pressure and eliminates thirst.

Cucumber, as a vegetable also plays an important role in the nutrition of people. It also has a diuretic property like other vegetables and prevents the formation of kidney stones. When we keep a clove in cucumber water for a day and then drink this mixture with honey syrup for a certain period, it will rejuvenate and refresh our facial skin. Cucumber also eliminates the headaches and heart palpitations. Putting cucumber on the skin can help relieve itching and will soften the skin. Cucumber seeds are very good for liver cancer and chest diseases.

Today, tomatoes are used in many countries around the world as food. Tomato contains 95% water, 6% sugar, 1.7% organic acids (mainly citric and apple acids), 0.3% pectin, 0.8% cellulose, 0.33% fat, 1.2% carotene, as well as phytoncide enzymes, vitamins A, C, B_1, B_2, B_3, B_6, B_9, H, E, PP, high amount of iron, cobalt, zinc microelements, potassium and manganese salts and so on. Tomato slows down the development of cancer cells.

In order to obtain the enzyme extract, Tris-HCl buffer (5 mM DTT, 1 mM EDTA) with 7 ml 100 mM, pH 8.0 was added to a 1 g leaf sample taken separately from each plant and homogenized with quartz sand in mortar and pestle. Sediment was obtained after the homogenate was filtered out of 4-fold Capron and then the supernatant was precipitated in the centrifuge at

3000g for 5 minutes in order to be released from low-molecular compounds. As the sediment is thrown away, sedimentary liquid (leaf extract) was then used to measure the enzyme activity.

The activity of CA enzyme was determined by the electrometric method, that is, according to the initial change in the number of hydrogen ions formed due to $CO_2 + H_2O \rightarrow H^+ + HCO_3^-$ reaction [8]. CA activity was calculated with conventional units according to the formula $U=10(T_o/T-1)$ (here T_o- is the time spent for the change of pH in the control reaction, T - the time spent for the change of pH in the experiment reaction (sec)) [5]. The activity of 1 mg protein was considered as the enzyme's specific activity.

The RBPC activity was based on the quantitative determination of 3-FQT in the reaction that occurred with the presence of phosphoglyceratkinase and glyceraldehyde phosphate dehydrogenase enzymes, by the spectrophotometric method [1].

At this time, a reaction medium consisting of 0.05 M Tris-HCl buffer (pH 7.8), 0.05 M $NaHCO_3$, 0.01 M $MgCl_2$, 0.05 M ditiotreytol, 0.01 M ATF, 0.25 mM NADH, 0.3 mM RBF, 10V phosphoglyceratkinase, 10V glyceraldehydesphosphate-dehydrogenase and 0.2-0.4 mg protein has been formed. The enzyme activity was measured on the basis of a decrease in the optical density at a 340 nm wavelength at the temperature of 30ºC.

All the required components but NADH were included in the reaction medium in the control version. Enzyme activity was calculated based on the formula $A = \Delta OS \frac{V}{\varepsilon \cdot b}$ (here, V- bathtub volume (3 ml), ΔOS-optical density change, ε-extinction constant (6.62 mM^{-1} cm^{-1}), b – the volume of enzyme extract (300 mkl). RBPC activity was expressed with CO_2/ml·sec.

PEPC activity was determined by the spectrophotometric method [1]. The reaction medium was formed with the introduction of 50 mM Tris-HCl buffer (pH 8.0), 10 mM $NaHCO_3$, 0.5 mM $MgCl_2$, 0.01 M ATF, 0.25 mM NADH, 10V malatdehydrogenase, 0.4-0.8 mg. protein and 5 mM PEP. In this case, all the components but NADH were included in the control version. PEPC activity was measured based on a change in optical density at the wavelength of 340 nm and calculated using the formula $A = \Delta OS \frac{V}{\varepsilon \cdot b}$ (here V- bathtub volume (3 ml), ΔOS-optical density change, ε-extinction constant (6.62 mM^{-1} cm^{-1}), b- the volume of enzyme extract (300 mkl) The enzyme activity is expressed with HCO_3^-/ml·sec.

3. Obtained results and their discussion

Changes in the activity of enzymes depending on the radiation dose of seeds are the main focus of this work. The figures show the results of the experiments on the leaves of four different plants, such as beans, eggplant, cucumbers, and tomatoes. All four plants have a C_3 photosynthesis mechanism and have high photosynthesis intensity.

Table 1 presents the results of the dependence of CA activity on the dose of seed irradiation. As can be seen from the figure, CA, which acts as a catalytic function in the leaves of these plants, has similar and distinct features depending on the radiation dose and the type of plant. Therefore, the studied plants can be divided into two groups according to the dependence of CA activity on the irradiation dose in leaves. Of these, beans and eggplant plants have similar CA activity, and their CA activity ranges between 4-7 mg/protein. At the same time, the CA activity in eggplant leaves was ~15-30% higher than in the bean leaves depending on the irradiation dose. As it is shown in the figure, although the enzyme has the highest activity at 50-100 Gy doses in eggplant leaves and at 5-10 Gy in the bean leaves, their activity at 200- 400 Gy doses have decreased by ~2 times compared with 1-100Gy doses and control samples.

Although the CA activity changed with a similar tendency in cucumber and tomato plants, it sharply differed from beans and eggplant plants in terms of activity level. Thus, the

change in CA activity in the leaves of these plants varied in the range of 11-14 mg/protein. CA activity in the cucumber plant at all but 10 Gy radiation doses and in control samples was lower than the tomato plant. In control samples of cucumber and tomato plants, CA activity got the highest value and as the radiation dose increased, the enzyme activity gradually decreased and was lower than all doses at 400 Gy radiation dose.

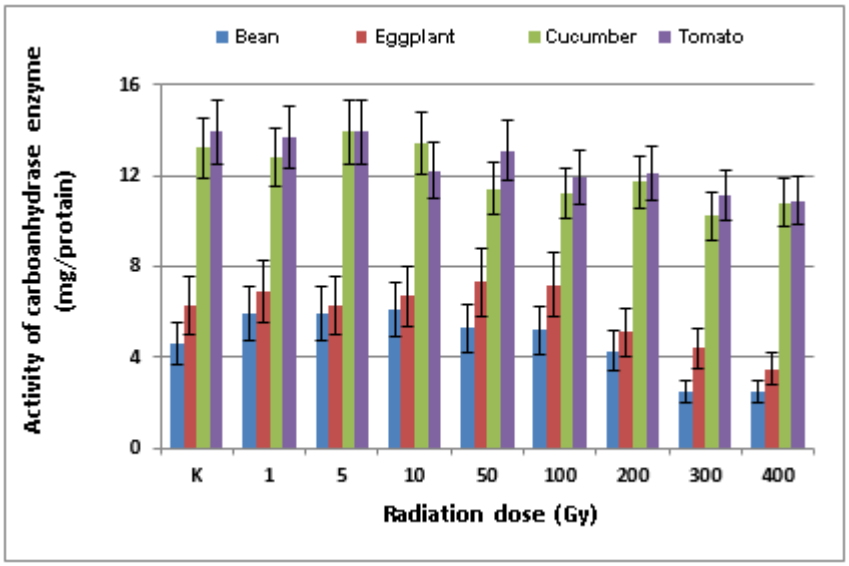

Fig. 1. Dependence of the activity of carbonic anhydrase enzyme on the radiation dose of some higher plant seeds

The results for the change dynamics of RBPC activity depending on the radiation dose are shown in Figure 2.

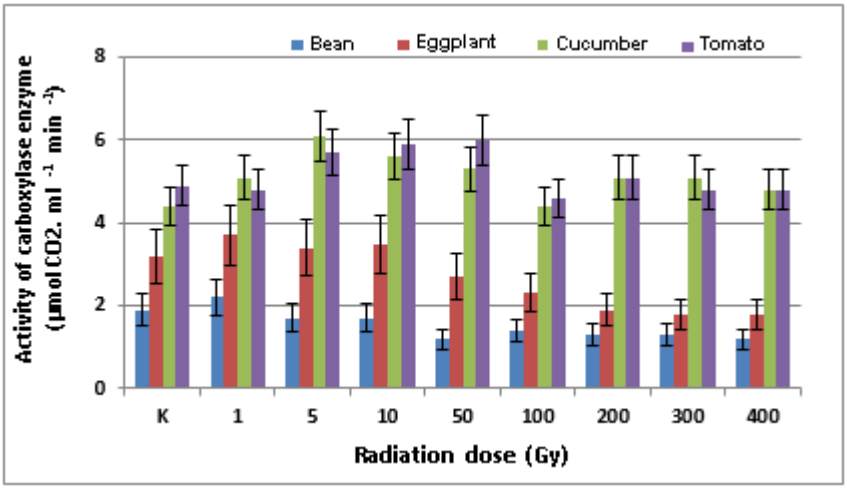

Fig. 2. Dependence of the RBPC activity on the radiation dose of some higher plant seeds

As it is seen from the figure, RBPC has different activity in the control and experiment samples of each of the studied plants. Here, RBPC activity in bean and eggplant plants is ~2-2.5 times lower than in cucumber and tomato plants. The change in the activity in beans and eggplant plants is characterized by a decreasing tendency, but that in cucumber and tomato plants by a parabolic curve. That is, the enzyme activity increased gradually at 1-50 Gy doses, but there is observed a gradually slow decrease at 200-400 Gy, except at 100 Gy.

The dependence of PEPC activity on the radiation dose of seeds was given in figure 3. As can be seen from the figure, the tomato plant has the highest, the cucumber plant - relatively lower, the eggplant - lower than that, and the beans - the lowest PEPC activity at all irradiation doses. Analysis of the results shows that PEPC has ~2 times lower activity in the bean plant compared with the eggplant plant, ~4 times lower than the cucumber plant and ~4 – 4.5 times lower than the tomato plant. These indicators were relatively lower in the 300-400 Gy dose range.

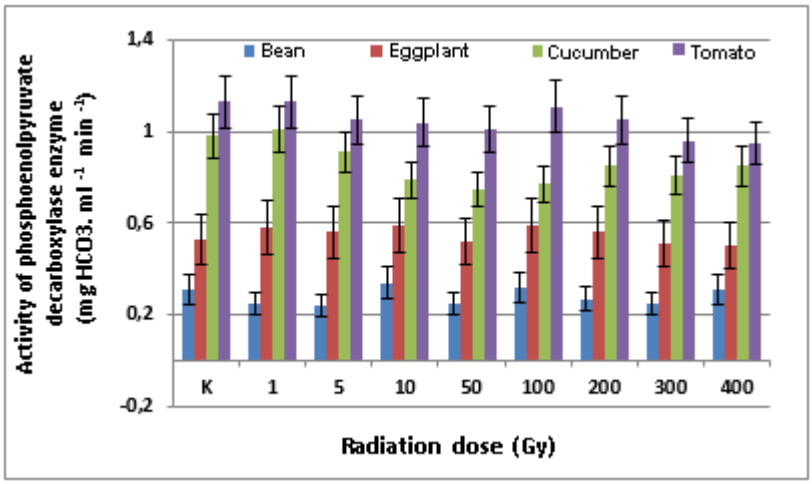

Fig. 3. Dependence of the activity of phosphoenolpyruvate carboxylase enzyme on the radiation dose of seeds

Finally, results on the change in the activity of CA, RBPC, PEPC enzymes, which play an important role in energy metabolism in higher plants, as the seed's response to γ-rays show that the activity of all three enzymes was high in cucumber and tomato plants, it was relatively lower in eggplant and much lower in beans. One interesting fact is that the activities of CA and RBPC enzymes change with a similar tendency in the leaves of cucumber and tomato plants. But the dependence of the activity of the PEPC enzyme on the radiation dose has different features for the abovementioned plants. Thus, the reason why the activity of this enzyme is much lower in the shown plant samples than that of CA and RBPC enzymes can be explained by the fact that these plants have a C_3 photosynthesis mechanism. A similar parallel change in the activity of CA and RBPC enzymes in the taken samples and the increase in this change up to the certain dose level of radiation can be explained by the proximity of their functional activities and adaptive properties.

That is, if the activity of the CA enzyme, which started to act as the initiator of the process of photosynthesis in higher plants, is higher, then the amount of CO_2 absorbed by the digestive cells in the leaves will be higher, too and the concentration of CO_2 in carboxylation areas in chloroplasts will also be higher, which is of great importance for the RBPC enzyme. In this case, CA will transfer further metabolism of CO_2 to the RBPC enzyme, which will provide the high intensity of photosynthesis even if in the high stress condition by incorporating them in the chain and cyclic metabolism (Calvin cycle) carboxylation RBP with CO_2

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ВИЯНИЕ ПРЕДПОСЕВНОГО ОБЛУЧЕНИЯ СЕМЯН НЕКОТОРЫХ РАСТЕНИЙ НА АКТИВНОСТИ ФЕРМЕНТОВ МЕТАБОЛИЗМА CO_2

К.Г. Караева

Резюме: Исследованы активности ферментов карбоангидразы (КА, KF 4.2.1.1), фосфоенолпируваткарбоксилазу (FEPK, CF 4.1.1.31) и рибулозо-1,5-бисфосфатный карбоксилазы (RBFK, KF 4.1.1.39) в листьях фасоли (*Phaseolus vulgaris* L.), баклажана (*Solanum melongena* L.), огурца (*Cucumis sativus* L.) и томата (*Solanum licopersicum* L.), произрастающих в обычной среде с дозами облучения 1,5, 10, 50, 100, 200, 400 и 400 Гр (мощность дозы облучения - р = 0,19 Гр/с) семян.

Показано, что в условиях радиационного стресса для исследованных растений ферменты КА и RBFK проявляют аналогичную активность. Однако фермент FEPK имеет низкую каталитическую активность, связанной, по всей видимости, с механизмом фотосинтеза этих растений.

Ключевые слова: предпосевное γ-облучение семян, фасоль, баклажан, огурец, томат, карбоангидраза, фосфоенолпируваткарбоксилаза, рибулозо-1,5-бисфосфатный карбоксилаза.

TOXUMLARIN SƏPİNDƏN ƏVVƏL QAMMA ŞÜALARLA İŞLƏNMƏSİNİN BƏZİ BİTKİLƏRDƏ CO_2 METABOLIZMI FERMENTLƏRİNİN AKTİVLİKLƏRİNƏ TƏSİRİ

K.G. Qarayeva

Xülasə: Təbii mühitdə, toxumları 1,5,10,50,100,200,300 və 400 Qr dozalarda (şüalanmanın gücü *p=0,19 Qr/san*) şüalandırılmış adi lobya (*Phaseolus vulgaris* L.), badımcan (*Solanum melongena*L.), xiyar (*Cucumis sativus* L.) və pomidor (*Solanum licopersicum* L.) bitkilərinin ontogenezinin aktiv inkişaf dövründə yarpaqlarında karboanhidraza (KA, KF 4.2.1.1), fosfoenolpiruvatkarboksilaza (FEPK, KF 4.1.1.31) və ribuloza-1,5-bifosfat karboksilaza (RBFK, KF 4.1.1.39) fermentlərinin aktivlikləri müqayisəli şəkildə tədqiq edilmişdir. Müəyyən olunmuşdur ki, tədqiq olunan bitkilərdə KA və RBFK

fermentləri radiasiyanın təsiri şəraitində oxşar formada aktivlik nümayiş etdirir. FEPK fermenti isə götürülən bitkilərin fotosintez mexanizmi ilə əlaqədar olaraq aşağı katalitik aktivliyə malik olur.

Açar sözlər: toxumların səpindən əvvəl γ-şüalarla işlənməsi, lobya, badımcan, xiyar, pomidor, ribuloza-1,5-bifosfat karboksilaza, fosfoenolpiruvat karboksilaza, karboanhidraza