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ROLE OF LOW-MOLECULAR ANTIOXIDANTS IN THE REACTION OF PEA PLANT TO THE SEPERATE AND COMBINED EFFECTS OF SALT AND RADIATION STRESS

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Abstract: Herein, the reaction of the pea plant, processed by γ-rays before sowing, to the effects of salt stress has been studied. In this case, the reaction of the plant to salt and radiation stresses was evaluated based on the changes in the amount of low molecular antioxidants such as proline, carotenoid, anthocyanin, and flavonoids. It has been found that the plant's reaction to salt and radiation causes certain changes in the amount of mentioned antioxidants. Strengthening of individual stress factors leads to the increase in the amount of proline and carotenoids, and to the decrease in that of anthocyanin and flavonoids. Under the combined effects of two stress factors, along with the decrease in the number of antioxidants, in some cases stimulation of their synthesis may also occur.

Key words: peas, seed processed by γ-rays, salt stress, proline, carotenoids, anthocyanins, flavonoids.

As it is known, plants are considered to be under stress when changes in the environment can adversely affect their growth and development by disrupting metabolic processes. In this case, stressed plants react to such effect by either making different changes in their morphological structures or by restoring stress-induced disorders at submolecular, molecular and intracellular levels.

The acceleration of oxidation processes accompanying the formation of active forms of oxygen in plant cells under stress conditions has been confirmed in numerous studies [3,5,9,16]. These forms of oxygen, which are the real sources of danger for the cell, are neutralized by a system called the antioxidant protection system (AOPS) [1,14]. This unique system is binary, composed of antioxidant enzymes and low-molecular antioxidants. The main antioxidant enzymes are superoxide dismutase (SOD), peroxidase (PO), ascorbate peroxidase (APO) catalase (CAT), and low-molecular antioxidants are polyphenols such as ascorbic acid, glutathione, proline, polyamines, tocopherol, ubiquitin, carotenoids, flavonoids. [1,14].

The results of the research show that the antioxidant protection system that protects cells from the harmful effects of stress, results in detoxification of the active forms of oxygen, and high-molecular components (antioxidant enzymes) play a key role in this process [1].

The ability of enzymes of active forms of oxygen, produced under oxidative stress, to inactivate themselves and in this case, the need for time to their *de novo* synthesis demand the low-molecular antioxidants, which take on protective functions.

It should be noted that, despite numerous studies, the role of low-molecular components of the antioxidant protection system under stress conditions is still unclear [1,2,6,10,11]. For this reason, the study of the protective role of these biologically active substances under stress conditions is of particular scientific and practical importance.

The present study explores the activity of the antioxidant protection system in the context of radiation and salt stress based on changes in the amount of low-molecular antioxidants such as carotenoids, flavonoids, anthocyanins, and proline.

The main objective was to study the effect of the processing of seeds by γ-rays before sowing on the growth of the plant under salt stress.

1. Materials and methods

The object of study. The pea plant (*Cicer arietinum* L.) was selected as the object of the study. Seeds of this plant were tested in three directions. Initially, the dry seeds were irradiated at a "*RUXUND*" radiation device having ^{60}Co source at the doses of 1, 5, 10, 50, 100, 200, 300 Gy. The irradiated seeds were first grown in ordinary water (only radiation stress conditions) and the second case, in NaCl solutions with concentrations of 1, 5, 10, 50, 100, 200, 300 mM (combined radiation and salt stress conditions). The third option was the cultivation of non-irradiated seeds in the above-concentrated salt solutions (only salt stress conditions).

Low-molecular antioxidants, such as anthocyanins, flavonoids, carotenoids, and free proline, have been identified using a green mass of 2-week plant seeds. The results obtained for each of the three variants were compared with the results corresponding to the control sample.

Equipment. "RUXUND" installation with a ^{60}Co γ-radiation source, spectrophotometer "JENWEY - 6_7 Series (United Kingdom)", centrifuge "HIMAC-CT 15 RE (United Kingdom)", dielectric separator "SDL - 1", grain moisture meter "Fauna - M", thermostat, chamber (phytotron) for growing seedlings.

Determination of the proline content. The content of proline was determined by the classical method of Bates et al. [8]. For this purpose, 0.3 g of plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid. The homogenate filtered through 2 filter paper and was precipitated in a centrifuge for 15 minutes at 1000 g. 2 ml of filtrate was reacted with 2 ml acid - ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 hour at 100^oC, and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed vigorously with a test tube stirrer for 15-20 sec.

The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm using toluene for a blank.

The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

[(μg proline/ml · ml toluene)/115,5 μg/μmole]/[(g sample)/5] = μmoles proline/g of fresh weight material.

Determination of the total anthocyanins content. Total anthocyanins (its concentration in μmol/ml) were determined spectrophotometrically at wavelengths λ = 537 nm, 647 nm and 663 nm according to the formula presented in [15]:

$$\mathbf{K_{ant.}} = 0.08173 \cdot A_{537} - 0.00697 \cdot A_{647} - 0.002228 \cdot A_{663}$$

(where A_{537}, A_{647} and A_{663} - optical density at wavelengths of *537, 647,* and *663 nm*, respectively).

Determination of the total carotenoids content. The concentration of carotenoids was determined based on the content of anthocyanins and chlorophylls *a, b* according to the formula [15]:

$$\mathbf{K_{karot.}} = \{A_{470} - [17.1 \cdot (K_{xl.a} + K_{xl.b}) - 9.479 \cdot K_{ant.}]\} / 119.26$$

(where $K_{ant.}$, $K_{xl.a}$ and $K_{xl.b}$ − the concentration of anthocyanins, chlorophyll *a*, and chlorophyll *b* in μmol/ml, respectively).

Wherein the concentrations of chlorophylls *a* and *b* (in μmol/ml) were determined by the formulas [15]:

$$\mathbf{K_{xl.a}} = 0.01373\ A_{663} - 0.000897\ A_{537} - 0.003046\ A_{647}$$

$$\mathbf{K_{xl.b}} = 0.02405\ A_{647} - 0.004305\ A_{537} - 0.005507\ A_{663}.$$

Determination of the flavonoids content. For determining of the flavonoids content in plant samples were used a method developed S.S. Lambayeva [13]. Using differential spectrophotometry at a wavelength λ = 414 nm the total flavonoids content was determined in percentages to rediscount at rutin and dry raw material by the formula:

$$K_{flav} = \frac{D \cdot K^V}{m} \cdot \frac{m_s}{D_s \cdot K_S^V} \cdot \frac{100}{100 - W} \cdot 100$$

(where D - optical density of the test solution, D_s – optical density of the solution GSO rutin, m – weight in grams of the starting material, m_s - weight in grams GSO rutin, K^V- the dilution coefficient of the test solution (K^V=1250), K_S^V - the dilution coefficient of the GSO rutin solution (K_S^V = 2500), W - weight loss in percent on drying of the starting material).

Experiments were carried out in double biological and triple analytical replicates, which gave results with an error of 0 to 20%. The figures show the mean values of the measured values. Statistical processing was performed by standard methods of variation statistics. The significance of differences of control and experimental results was assessed using Student's t – distribution of [12]. The differences were significant at $|t| > 2$ ($p < 0.05$).

2. Results and its discussion

As is know, plants are often affected by several stresses, not just one. Some of these effects may be permanent and some may be short-term. Recent research has shown that the adaptation of plants to dual stress conditions is different from their adaptation to single stress conditions. From this point of view, such scientific research works are of particular interest, but also of great relevance. It is believed that when more than one stress factor is involved, mechanisms called cross adaptation in plant organisms are introduced [4, 7, 18, 17], and there is an urgent need to study the mechanism of this process. Thus, research in this area allows for purposeful intervention in these processes.

1) Change dynamics of low-molecular antioxidants dependent on NaCl concentration

The results we obtained for the 2-week sprouts of pea plants grown under salt stress with different concentrations are shown in figure 1.

It is clear from the results that the increase in salt stress has led to a sharp decrease in the number of anthocyanins and flavonoids and a sharp increase in the carotenoids and a significant increase in the amount of proline. Thus, in pea sprouts grown under salt stress with 5 and 10 mM concentration, the amount of anthocyanins in the pea plants is ~ 7 times lower, and the amount of flavonoids is ~ 3 times lower than that of the control. There is ~ 2-time increase at the concentrations of 1mM and 5 mM, and ~ 4-time increase at the concentration of 10 mM in the number of carotenoids.

Although the amount of proline was approximately the same in the samples grown in a salt solution with a concentration of 1 and 5 mM and the control sample, there was a ~ 1.5-time increase in the amount of this pigment at 10 mM concentration.

As the plant does not grow in the medium where the salt concentration is higher than 10 mM, no results corresponding to antioxidants at concentrations of 50, 100, 200, and 300 mM.

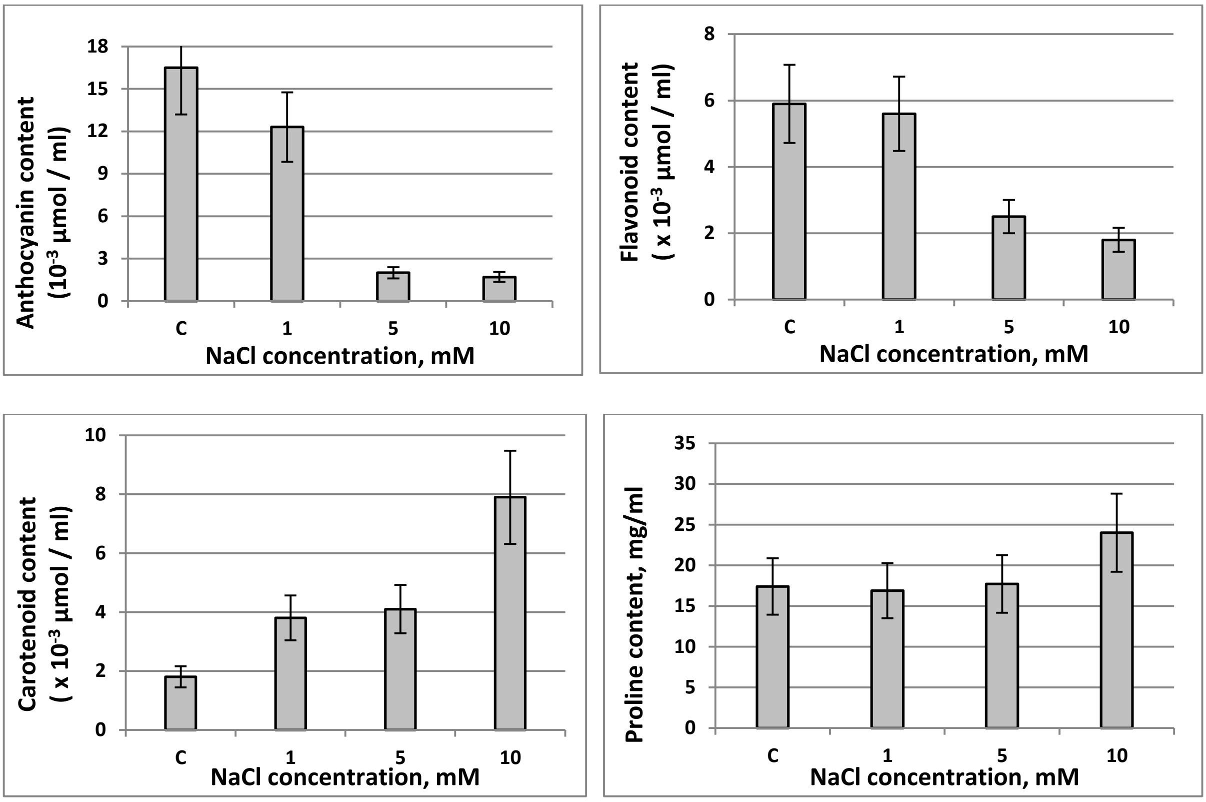

Fig. 1. Change dynamics of the amount of low-molecular antioxidants depending on NaCl concentration.

2) Change dynamics of the amount of low-molecular antioxidants depending on the dose of seeds irradiation

Our results on the amount of low-molecular antioxidants in the 2-week sprouts of pea plants exposed to different doses of γ-rays before seed sowing are illustrated in figure 2.

The first thing that draws attention from the figure is that the increase in stress leads to the increase in the number of carotenoids and proline and to the decrease in that of anthocyanins and flavonoids as in the case of salt stress

An analysis of the results reveals that changes in the number of carotenoids in the dose range of 1 - 10 Gy irradiation are within the error in the experiment. In other words, the amount of pigment in this case, does not almost differ from that of the control sample. An increase in the radiation dose in the 10-100 Gy range causes large-scale (~ 2.5 times) changes in the number of yellow pigments, but subsequent dosa does not cause significant changes.

The number of proline increases in proportion with the increased dose of radiation at relatively high doses.

There are no large-scale changes in the number of anthocyanins and flavonoids depending on the radiation dose. Simply the increase in the radiation dose shows a slight tendency to decrease in the amount of these pigments, except for minor deviations. For example, at doses higher than 100 Gy, the amount of both anthocyanins and flavonoids is ~ 25% lower than the control.

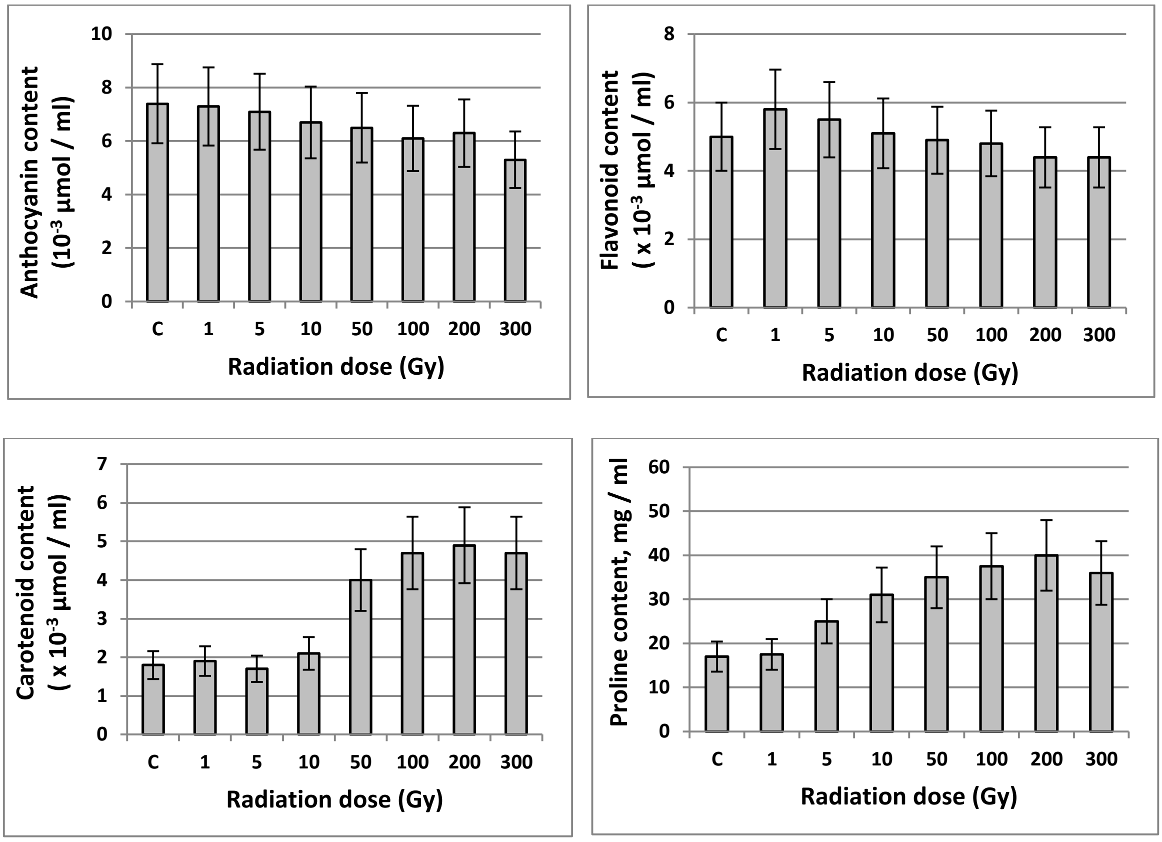

Fig. 2. Change dynamics of low-molecular antioxidants depending on the dose of seeds irradiation.

3) Change dynamics of low-molecular antioxidants under the combined effects of salt and radiation stress

A part of the results of our research relates to pea sprouts whose seeds were pre-treated by γ-rays and grown in the salt solution with different concentrations. The main purpose of these studies is to clarify the possible impact of seed processing by γ-rays on plant resistance to salt stress.

Results on the number of individual antioxidants are illustrated in figures 3, 4. 5 and 6.

From our results on the amount of proline (figure 3), it is clear that an increase in the radiation dose at 1 and 5 mM salt concentration cannot lead to significant changes in the proline amount. At a concentration of 10 mM, the amount of proline is higher only at the radiation dose of 10 Gy, whereas at other radiation doses, it is approximately the same as the control sample.

Based on the results, it can be assumed that proline is not required in all but the combined effects of 10 mM NaCl and 10 Gy radiation dose cases. In other words, the processing with γ-rays can protect the plant from the harmful effects of salt stress. Most likely, in these cases, other antioxidants protect the plant from the stress, as result there is less demand for proline.

Under the combined effects of 10 mM NaCl and 10 Gy radiation dose, it is likely that, along with the antioxidant enzymes, there is also a need for low-molecular antioxidant proline and its synthesis is accelerated.

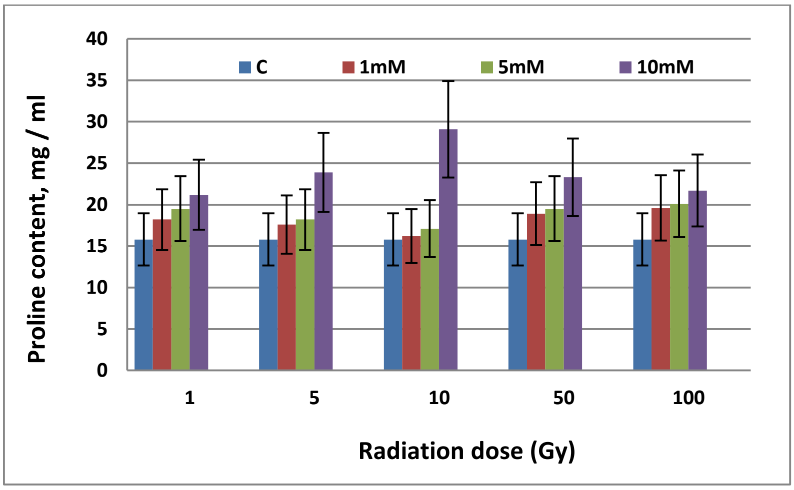

Fig. 3. Change dynamics of the proline amount under dual stress condition.

The results for the number of carotenoids under dual stress conditions are presented in figure 4. The first thing that draws the attention is that the change dynamics of the number of carotenoids under the conditions of dual stress are different from the change dynamics of the proline amount. Thus, at all salt concentrations, the increase in the radiation dose in the range of 1 – 10 Gy leads to a gradual increase in the amount of these pigments, and but the increase in 10 – 100 Gy range to a gradual decrease (excluding minor deviations). Based on the results, it can be considered that carotenoids play a major role in protecting the plants from the harmful effects of salt stress at low radiation doses at all salt concentrations, however, their role is not significant at high doses.

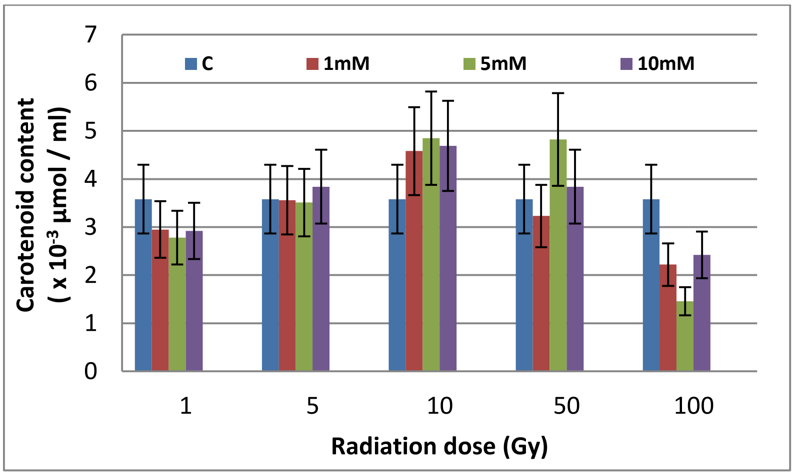

Fig. 4. Change dynamics of the carotenoids amount under dual stress condition.

The results on the number of anthocyanins in dual stress condition (figure 5) show that this antioxidant also plays some role in protecting pea seeds from salt stress.

The results presented in the figure 5 show that an increase in salt concentrations at 5, 10, and 50 Gy radiation doses result in a significant increase in the number of anthocyanins. The fact that seeds are exposed to very low doses (1 Gy) and very high doses (100 Gy) does not produce large-scale changes in its amount.

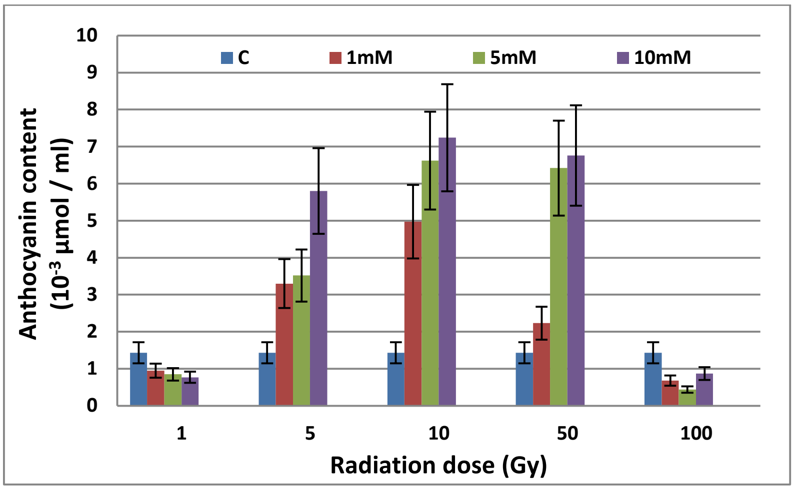

Fig. 5. Change dynamics of the anthocyanin amount under dual stress condition.

Consequently, the anthocyanins perform their protection function under certain conditions. Interestingly, carotenoids also function similarly. It can be assumed that the anthocyanins and the carotenoids function relationally.

Figure 6 shows the results for the number of flavonoids under dual stress conditions.

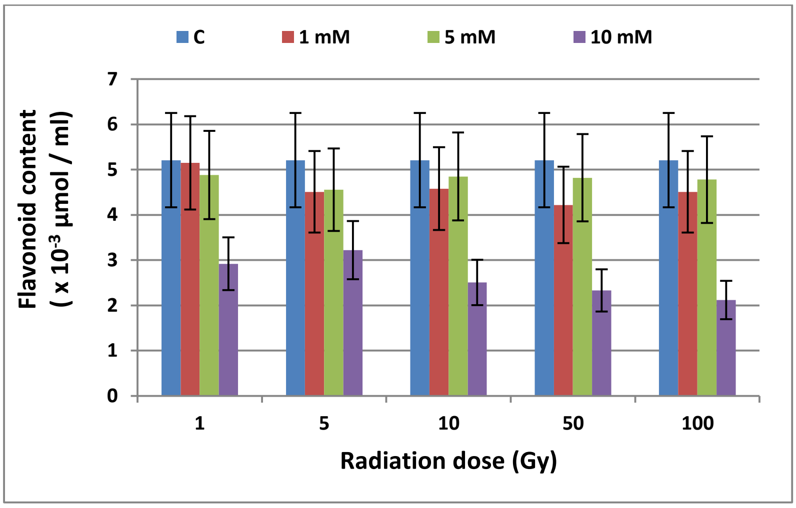

Fig. 6. Change dynamics of the number of flavonoids in dual stress conditions.

The results show that the number of flavonoids is changing dual stress conditions. Increased salt concentration during seed irradiation at 1 Gy indicates a decrease in flavonoids amount. This case shows itself at a concentration of more than 10 mM. Even at the 5, 10, 50, and 100 Gy radiation doses, the number of flavonoids is slightly differed from (~ 2 times) the control samples in salt concentrations of 1 and 5 mM, it is significantly lower in a salt solution with 10 mM concentration.

The reason why the number of flavonoids is significantly lower in the dual stress condition is that they are most likely exposed to structural destruction under the influence of salt and radiation.

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РОЛЬ НИЗКОМОЛЕКУЛЯРНЫХ АНТИОКСИДАНТОВ В РЕАКЦИИ РАЗДЕЛЬНОГО И СОВМЕСТНОГО ВОЗДЕЙСТВИЯ РАДИАЦИИ И СОЛИ У РАСТЕНИИ ГОРОХА

М.З. Велиджанова

Резюме: В работе изучена реакция гороха, семена которого перед посевом подверглись воздействию γ-лучей, на воздействию солевого стресса. При этом реакция растения оценена на основе изменения содержания низкомолекулярных антиоксидантов, таких как пролин, каротиноиды, антоцианы и флавоноиды. Показано, что реакция растения на радиационный и солевой стресс вызывает количественное изменение содержания указанных антиоксидантов. Усиление стрессового воздействия в отдельности как радиационного, так и солевого приводит к увеличению содержания пролина и каротиноидов. При этом этот процесс сопровождается уменьшением количества антоцианов и флавоноидов. Однако в условиях двойного стресса имеет места уменьшение содержания всех антиоксидантов, а некоторых отдельных случаях происходит даже стимулирования их биосинтеза.

Ключевые слова: горох, предпосевное γ-облучение семян, солевой стресс, пролин, каротиноиды, антоцианы, флавоноиды.

NOXUD BİTKİSİNİN DUZ VƏ RADİASİYA STRESLƏRİNİN AYRILIQDA VƏ BİRGƏ TƏSİRİNƏ REAKSİYASINDA KİÇİK MOLEKULLU ANTİOKSİDANTLARIN ROLU

M.Z.Velijanova

Xülasə**:** İşdə toxumları səpindən əvvəl γ-şüalarla işlənmiş noxud bitkisinin duz stresinin təsirinə reaksiyasi öyrənilmişdir. Bu halda bitkinin duz və radiasiya streslərinə reaksiyası prolin, karotinoid, antosian və flavonoidlər kimi kiçik molekullu antioksidantlarin miqdar dəyişmələrinə əsasən qiymətləndirilmişdir. Müəyyən edilmişdir ki, bitkinin duz və radiasiyanın təsirinə reaksiyası adı çəkilən antioksidantların miqdarında müəyyən dəyişikliklər yaradır. Ayrılıqda stress amillərin güclənməsi prolin və karotinoidlərin miqdarının artmasına, antosian və flavonoidlərin miqdarının isə azalmasına səbəb olur. İki stress amilinin birgə təsiri şəraitində isə antioksidantların miqdarının azalması ilə yanaşı, bəzi hallarda onların sintezinin stimullaşması da baş verə bilir.

Açar sözlər**:** noxud, toxumların γ-şüalarla işlənməsi, duz stresi, prolin, karotinoidlər, antosianlar, flavonoidlər.