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## THE INFLUENCE OF SALT STRESS ON THE CONTENT OF PHOTOSYNTHETIC PIGMENTS IN THE PEA, THE SEED OF WHICH EXPOSED TO GAMMA IRRADIATION BEFORE SEEDING

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**Abstract:** The presented work reflects the results on the functioning of photosynthetic apparatus of the pea plant under the conditions of salt stress at its different concentrations. Herewith, the influence of salt on the process of photosynthesis was evaluated based on the quantitative change in the chlorophyll content. Considering the fact that the ionizing radiation can activate the antioxidant protection system of the plant as a result of which its adaptive ability to high salinity condition may increase, the seeds of the plant were irradiated with gamma-rays at different doses before sowing.

Our results gave grounds to assume that the synthesis of green pigments shows sensitivity both to the effect of salt stress and to the irradiation dose.

It has been established that presowing irradiation of seeds at doses up to 50 Gy can't exert a negative influence on the development of pea seedlings under salt conditions within the concentrations 1-10 mM and a normal synthesis of chlorophylls occurs in the leaves of the plant. However, the irradiation of seeds at doses above 50 Gy in the same salt concentrations leads to inhibition of synthesis of green pigments.

It is interesting that the dependence of chlorophyll synthesis on irradiation dose in salt concentrations above 10 mM has a different character. So, if the salt stress in these concentrations has a negative influence on chlorophyll synthesis at low irradiation doses (up to 10 Gy), then at high doses stimulates the synthesis of these pigments.

The fact of the increased synthesis of chlorophylls even at high salt concentrations in plant seedlings, the seeds of which were irradiated at high doses before sowing, is of specific interest. It is most probably associated with an increase in the antioxidant protection system of the seeds under high dose radiation. As a result, seedlings grow normally and normal synthesis of chlorophylls occurs under strong salt stress.

**Key words:** Pisum L. (pea), presowing irradiation of seeds,  $\gamma$ -irradiation, salt stress, chlorophylls and b.

### 1. Introduction

In nature, plants are sometimes compelled to grow under conditions of increased salinity. Calculations show that to date, 30% of all suitable agricultural lands are subjected to high salinity [1].

Herewith, the attempts in using salted lands through intensive irrigation works tend to lead to violation of their water balance. And this, in its turn, creates problems in regulation of ion exchange, which plays an important role in the life of plant [2].

Undoubtedly, the primary reaction of plants to salinization of soil (to salt stress) should be manifested in the process of photosynthesis, as well [3]. In this case, the effect of salt stress can be direct or indirect. It is assumed that in the case of direct effect of salt stress there may be a delay in CO<sub>2</sub> assimilation through stomata and mesophyll due to the diffusion [4] or occur changes in metabolism of photosynthesis itself [5]. Indirect effect of salt stress is associated with the occurrence of oxidative stress, which is quite acceptable in conditions of joint effect of salt

stress with environmental factors [6].

It should be noted that the response of plants to environmental factors at the level of photosynthesis is rather complex. This process involves some parallel occurring physiological, cellular and molecular processes occurring in plants at a high rate. In this case, the metabolic machine must be fully mobilized to ensure the adaptation of plants to unfavorable environmental factors. For this reason, greater accuracy is required for studying the effect of stress factors on plant metabolism, in particular, on the process of photosynthesis.

The experiments conducted on the influence of ionizing radiation on the process of photosynthesis, show high stability of this process to radiation [7]. We should mention that there are plants for which even small doses of ionizing radiation lead to a change in the intensity of photosynthesis. For example, irradiation of cotton at doses 5, 20, 100 Gy leads to a decrease in the intensity of photosynthesis in 10 days [8].

Considering that the ionizing radiation can activate the antioxidant protection system and, as a result, the adaptation ability of plants to salinity may increase, it has been carried out presowing irradiation of pea seeds. Further, the irradiated seeds of this plant were grown in salt conditions at different concentrations. The effect of salt stress on the process of photosynthesis was evaluated on the basis of the changes in the amount of chlorophylls.

Let's assume that the conducted researches in this direction will make it possible to clarify the adaptation mechanism of plants to stressful conditions which is very important for conserving the biodiversity of wild plants and increasing the fertility of agricultural plants.

## 2. Experimental

As a research object it was selected pea seeds (*Pisum L.*). The amount of chlorophylls was determined spectrophotometrically using a spectrophotometer "*Ultrospec 3300 pro Amersham Bio-sciences*".

### Creating the conditions for growth

1. Pea seeds were irradiated using  $^{60}\text{Co}$  at doses of 1, 5, 10, 50 and 100 Gy (irradiation dose rate was 0.048 Gy/s in all cases).
2. Irradiated seeds were germinated under conditions, identical to natural, in the dark (in a thermostat - in Petri-dishes).
3. At the next stage (after 4 days), the seedlings together with the control samples were planted in special dishes with a volume of 0.5 liters. The seedlings grew in a special chamber (phytotron) in NaCl solution with concentrations of 1, 5, 10, 50, 100 mM. And, in this case the conditions corresponding to growing in the soil have been created. In the chamber, it was created the conditions of day and night with 12-hour interval. Temperature created by incandescent lamp (220 V, 150 W) in the afternoon was  $23 \pm 1^\circ\text{C}$ , but at night  $15 \pm 1^\circ\text{C}$ . Lighting conditions, equal to  $37,6 \text{ W/m}^2$ , were created by Luminescent lamps. In the afternoon, relative humidity in the chamber was 55%, but at night 70%.

### Determination of the amount of chlorophyll *a* and *b* in the samples of plant leaves.

The equations of Sims and Gamon were used to define the concentration of chlorophyll both *a* and *b* [9]. Thus, the optical density of the extracts was established by the spectrometric method at 537 nm, 647 nm and 663 nm wavelengths and the amount (concentration) of green pigments was determined by the formulas below in  $\mu\text{mol} \cdot \text{ml}^{-1}$ :

$$K_{xl,a} = 0.01373 \cdot A_{663} - 0.000897 \cdot A_{537} - 0.003046 \cdot A_{647}$$
$$K_{xl,b} = 0.02405 \cdot A_{647} - 0.004305 \cdot A_{537} - 0.005507 \cdot A_{663}$$

### 3. Results and discussion

Results of determination of amount of chlorophylls in pea leaves, of which seeds have undergone the presowing irradiation and which are grown under the condition of salt stress, are reflected in figures 1-5.

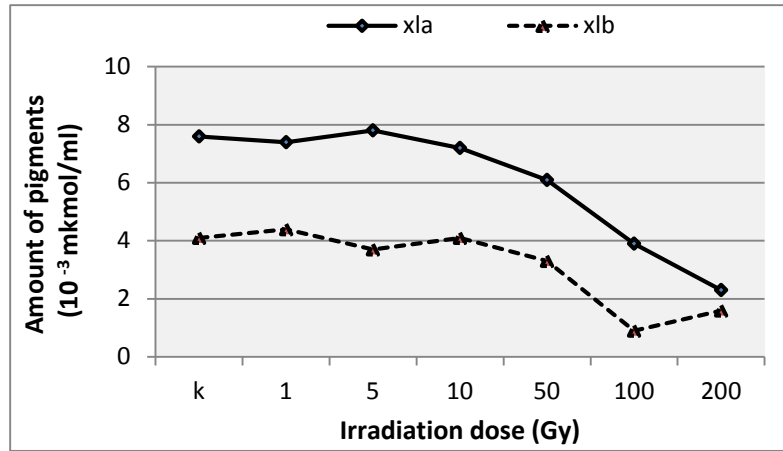


Fig. 1. Change of the amount of pigments depending on irradiation dose in NaCl solution with a concentration of 1 mM

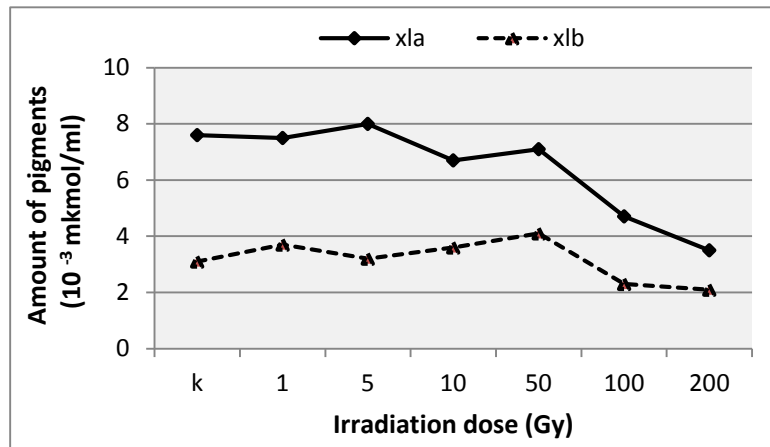


Fig. 2. Change of the amount of pigments depending on irradiation dose in NaCl solution with a concentration of 5 mM

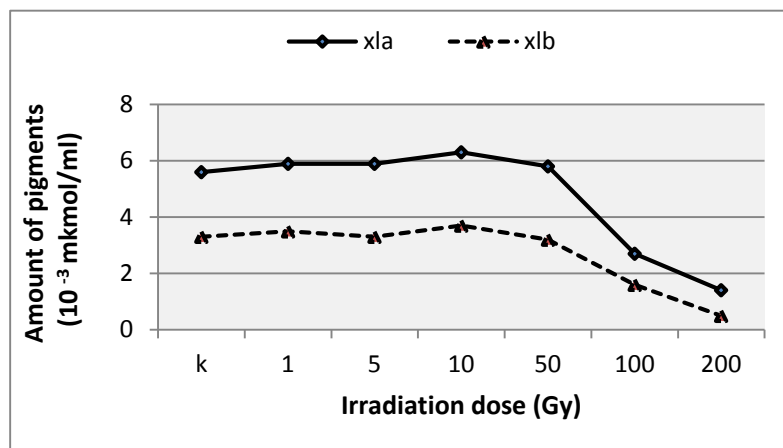


Fig. 3. Change of the amount of pigments depending on irradiation dose in NaCl solution with a concentration of 10 mM

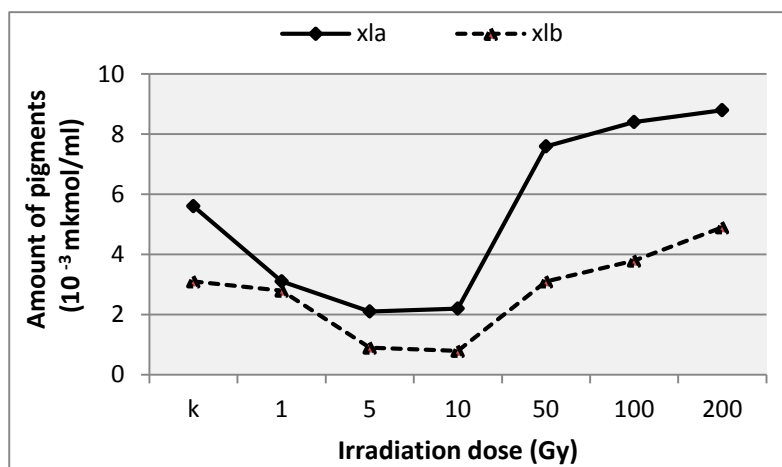


Fig. 4. Change of the amount of pigments depending on irradiation dose in NaCl solution with a concentration of 50 mM

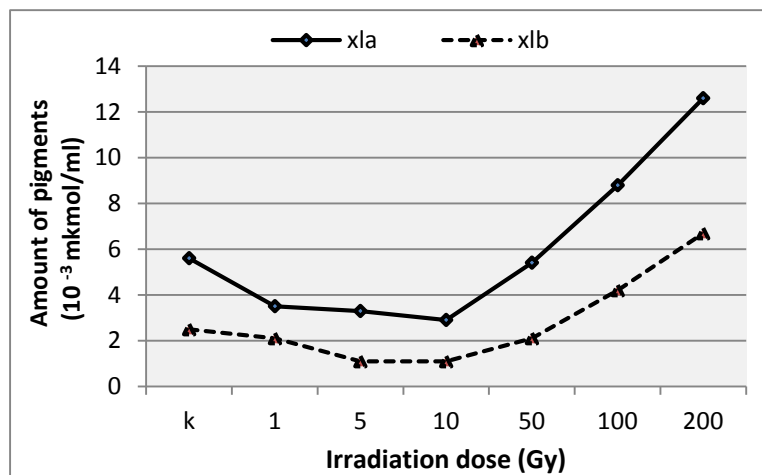


Fig. 5. Change of the amount of pigments depending on irradiation dose in NaCl solution with a concentration of 100 mM

From the results it becomes clear that, irradiation of pea seeds at 1-50 Gy doses in the growing their seedlings in a saline solution with a concentration of 1 mM does not lead to special deviations in the amount of chlorophylls. In contrast, a significant decrease of the amount of green pigments has been observed in seedlings, of which seeds have undergone presowing irradiation at 50-200 Gy doses.

A similar situation is observed in cases of solutions with concentrations of 5 and 10 mM. In such concentrations, salt stress does not influence on the synthesis of chlorophylls.

Generalizing the experimental data obtained by us, it is possible to come to the following conclusions:

- presowing irradiation of pea seeds at doses up to 50 Gy, does not negatively influence on the development of seedlings in saline solution with concentrations of 1-10 mM, and normal chlorophyll synthesis occurs in the plant leaves;
- presowing irradiation of pea seeds over 50 Gy, leads to inhibition of chlorophylls synthesis at the above concentrations.

Based on these results, it can be confidently assumed that, not the salt stress play the major role in reducing the amount of chlorophylls, but higher doses of irradiation.

A completely different picture is observed in the case of 50 mM concentration of NaCl. In other words, salt stress at lower irradiation doses (1-5 Gy) leads to an insignificant delay in the synthesis of chlorophylls, but at high doses (10-100 Gy), it is accompanied with an increase of the rate of synthesis of green pigments.

A similar situation in the synthesis of chlorophylls also occurs in salt concentration of 100 mM, and only in this case, at high doses of irradiation, the rate of synthesis of chlorophylls becomes more large-scale.

The fact of more intensive synthesis of chlorophylls in seedlings, growing in saline solutions with high concentrations and irradiated at high doses is of particular interest. Most likely, this is due to the fact that irradiation of seeds at high doses activates the antioxidant system of plant protection, and this leads to normal plant development even at high salt concentrations. Consequently, there is a normal synthesis of chlorophylls in the leaves.

The results obtained by us, on the dependence of the amount of chlorophylls on the irradiation dose and NaCl concentration suggest that the amount of chlorophylls is sensitive to both the irradiation dose and the influence of salt stress.

Another fact that attracts attention is that in all studied cases, the amount of chlorophyll *a* was more compared with chlorophyll *b*, and both chlorophylls show a similar dynamics of changes in the dependence on irradiation dose and on salt concentration.

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## ВЛИЯНИЕ СОЛЕВОГО СТРЕССА НА СОДЕРЖАНИЕ ФОТОСИНТЕТИЧЕСКИХ ПИГМЕНТОВ У ГОРОХА, СЕМЕНА КОТОРОГО ПЕРЕД ПОСЕВОМ ПОДВЕРГЛИСЬ ГАММА ОБЛУЧЕНИЮ

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**Резюме:** В представленной работе отражены результаты по функционированию фотосинтетического аппарата растения горох, в условиях солевого стресса при ее разных концентрациях. При этом влияние соли на процесс фотосинтеза было оценено на основе количественного изменения содержания хлорофилла. Учитывая тот факт, что ионизирующее излучение может активировать антиоксидантную систему защиты растения, в результате которого может повыситься ее адаптационная способность к условию высокой засоленности, семена растения перед посевом были облучены гамма-лучами в разных дозах.

Полученные нами результаты дали основания предположить, что синтез зеленых пигментов проявляет чувствительность, как к действию солевого стресса, так и к дозе облучения.

Было определено, что предпосевное облучение семян в дозах до 50 Гр, не может оказать негативного влияния на развитие проростков гороха в солевых условиях при концентрациях 1 – 10 мМ и происходит нормальный синтез хлорофиллов в листьях растения. Однако, облучение семян в дозах свыше 50 Грв этих же солевых концентрациях приводит к ингибированию синтеза зеленых пигментов.

Интересно, что зависимость синтеза хлорофиллов от дозы облучения в концентрациях соли свыше 10 мМ имеет иной характер. Так как если солевой стресс при этих концентрациях оказывает негативное влияние на синтез хлорофилла в случаях низких дозах облучения (до 10 Гр), то при высоких дозах стимулирует синтез этих пигментов.

Факт повышенного синтеза хлорофиллов даже в условиях высоких концентраций соли у проростков растения, семена которых перед посевом подверглись облучению при высоких дозах, вызывает определенный интерес. По всей вероятности, это связано с повышением антиоксидантной системы защиты семян под воздействием высоких доз радиации. В результате этого нормально растут проростки, и происходит нормальный синтез хлорофилла в условиях сильного солевого стресса.

**Ключевые слова:** *Pisum L.* (горох), предпосевное облучение семян,  $\gamma$ -облучение, солевой стресс, хлорофиллы *a* и *b*.

## DUZ STRESİNİN TOXUMLARI SƏPİNDƏN ƏVVƏL MÜXTƏLİF DOZALARDA GAMMA ŞÜALARIN TƏSİRİNƏ MƏRUZ QALMIŞ NOXUD BİTKİSİNDƏ FOTOSİNTETİK PİQMENTLƏRİN MİQDARINA TƏSİRİ

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**Xülasə:** Təqdim olunan işdə müxtəlif konsentrasiyalı duz stresi şəraitində noxud bitkisinin fotosintetik aparatının fəaliyyətinə dair nəticələr öz əksini tapmışdır. Duz stresinin fotosintez prosesinə təsiri xlorofillərin miqdar dəyişmələri əsasında qiymətləndirilmişdir. İonlaşdırıcı şüalanmanın bitkinin antioksidant müdafiə sistemini “fəallaşdırma” biləcəyini və onun yüksək şoranlıq şəraitinə adaptasiya qabiliyyətini artırma bəcəyini nəzərə alaraq, onun toxumları səpindən əvvəl müxtəlif dozalarda gamma şüalarla şüalandırılmışdır.

Aldığımız nəticələr yaşıl piqmentlərin sintezinin həm şüalanma dozasının, həm də duz stresinin təsirinə müəyyən qədər həssaslıq göstərə bilməsi fikrini söyləməyə əsas vermişdir.

Müəyyən edilmişdir ki, səpindən əvvəl toxumların 50 Qr şüalanma dozasına qədər  $\gamma$ -şüaların təsirinə məruz qalması noxud bitkisi cücərtilərini 1-10 mМ konsentrasiyalı NaCl məhlulunda inkişafına

mənfi təsir göstərə bilmir və bitki yarpaqlarında normal xlorofil sintezi baş verir. Toxumların 50 Qr - dən yuxarı dozalarda şüalandırılması isə həmin duz konsentrasiyalarında xlorofil sintezinin ingibirləşməsi ilə nəticələnir.

10 mM - dan yuxarı duz konsentrasiyalarında xlorofil sintezinin şüalanma dozasından asılılığı tamamilə fərqlənir. Belə ki, bu konsentrasiyalarda duz stresi aşağı şüalanma dozalarında (10 Qr -ə qədər) xlorofil sintezinə mənfi təsir göstərdiyi halda, yuxarı dozalarda piqmentlərin sintezinə stimullaşdırıcı təsir göstərir.

Toxumları daha böyük dozalarda şüalanmaya məruz qalmış bitki cücərtilərinin hətta yüksək konsentrasiyalı duz məhlulunda belə daha yaxşı xlorofil sintez edə bilməsi faktı kifayət qədər maraqlıdır. Çox yəqin ki, bunun səbəbi yüksək dozalı şüalanmanın noxud toxumlarının antioksidant mühafizə sistemini fəallaşdırmasıdır ki, nəticədə bitki cücərtiləri duz stresi şəraitində belə normal inkişaf edir və normal xlorofil sintez edir.

**Açar sözlər:** noxud bitkisi, səpindən əvvəl toxumların şüalanması,  $\gamma$ -radiasiya, duz stresi, xlorofil *avə b*.