International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 28-05-2020; Accepted: 16-06-2020: Published: 30-06-2020
www.botanyjournals.com
Volume 5; Issue 4; 2020; Page No. 23-28

Non-fermentative response to salt stress of the maize plant, which seeds were exposed to pre-sowing gamma-irradiation

ES Jafarov[1], MZ Velijanova[2]
[1, 2] Institute of Radiation Problems of Azerbaijan National Academy of Sciences Baku, Azerbaijan

Abstract
For the first time, the effect of pre-sowing treatment of seeds with different doses of γ-rays on the development of maize was studied under salt stress. The response of the plant to such conditions was evaluated based on changes in the amount of antioxidants such as anthocyanins, carotenoids, and flavonoids. The treatment of seeds with γ-rays in the dose range of 1-5 Gy was found to stimulate the growth of plants exposed to salt stress. In this case, carotenoids play a key role in protecting the plant from salt stress. An increase in the concentration of salt leads to a significant increase in the amount of yellow pigments. In this process, along with carotenoids, anthocyanins also play a certain role.

Keywords: maize (Zea mays L.), salt stress, pre-sowing γ-irradiation treatment of seeds, antioxidants, anthocyanins, carotenoids, flavonoids

1. Introduction

Plants are known to accumulate small-molecule organic antioxidants (phenolic compounds such as proline, polyamines, anthocyanins, flavonoids, carotenoids, etc.) as one of the mechanisms of protection against oxidative stress [1, 2]. The accumulation of the mentioned metabolites in individual cell compartments under the influence of stress factors is thought to lead to a decrease in the intensity of oxidative stress and an increase in the tolerance of plants to stress factors [3].

It should be noted that despite the availability of some results in this area, a generalized opinion on the stimulation mechanism of some physiological and biochemical processes under stress has not given yet. The results on the role of small-molecule non-enzymatic antioxidants under stress are few and in some cases even contradictory [4].

In the present study, based on the fact that ionizing radiation can stimulate the growth and development of plants in relatively small doses [5, 6], for the first time, the development of maize plants, which seeds were exposed to pre-sowing γ-irradiation treatment, was studied under salt stress. The response of the plant to such conditions was evaluated based on changes in the amount of antioxidants such as anthocyanins, carotenoids, and flavonoids.

We believe that research in this area will not only shed some light on the mechanism of adaptation of plants to stress but also identify ways to use ionizing radiation for growing plants under extreme conditions.

Materials and Methods

The maize (Zea mays L. - Zagatala variety) plant from the family of cereals was taken as the study object. Seeds with a moisture content of 16-17% were used for the experiments. The seeds were separated by an electric separator, their humidity was determined using a dielecometer and irradiated with a ^{60}Co radiation source at doses of 1, 5, 10, 50, 100, 200, and 300 Gy (absorption dose rate was 0.048 Gy / sec in all cases). Both irradiated and non-irradiated (control) seeds were germinated in Petri plates.

In the next stage (after 4 days), the seedlings exposed to various irradiation doses were transferred to special containers with 1, 5, and 10 mM NaCl solutions, along with the control sample. The containers were then placed in a special chamber (phytotron) under a 12/12 (light/dark) photoperiod. The day (23 ± 1°C) and night (15 ± 1°C) temperatures were provided by incandescent lamps (220 V, 150 W) and 37.6 W / m^2 illumination was created by luminescent lamps.

Relative humidity of 55% during the day and 70% at night was created by placing special containers filled with water in the chamber.

The amounts of carotenoids, flavonoids, and anthocyanins were determined spectrophotometrically using the "Ultrospec 3300 pro Amersham Bio-sciences" spectrophotometer.

The total amount of anthocyanins and carotenoids in plant samples was calculated using the formula proposed by Sims and Gamon [7]. Flavonoids were determined using the method developed by S.S. Lombayeva *et al.* [8].

Determination of the total anthocyanin's content.

Total anthocyanin (its concentration in μmol/ml) was determined spectrophotometrically at wavelengths λ = 537 nm, 647 nm and 663 nm according to the formula presented in [7]:

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

(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 anthocyanin and chlorophylls *a*, *b* according to the formula [7]:

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

(where $K_{ant.}$, $K_{xl.a}$ *and* $K_{xl.b}$ – the concentration of anthocyanin’s, 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 in [7]:

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

Determination of the flavonoids content.

For determining of the flavonoids content in plant samples were used a method developed by S.S. Lombayeva *et al* [8]. 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).

The experiments were performed in two biological and three analytical replicates. The results were statistically processed using standard methods of variation statistics. The difference in the results obtained in the control and experimental variants was assessed based on the Student's t-test [9]. Statistically significant difference must meet the condition $| t |> 2$ ($p<0.05$)

Results and Discussion

The effect of the pre-sowing γ-irradiation treatment of seeds on the amount of small-molecule antioxidants.

The results on the amount of antioxidants in 15-day-old seedlings of maize, which seeds were exposed to pre-sowing γ-irradiation and grown in the aquatic environment are shown in Fig. 1.

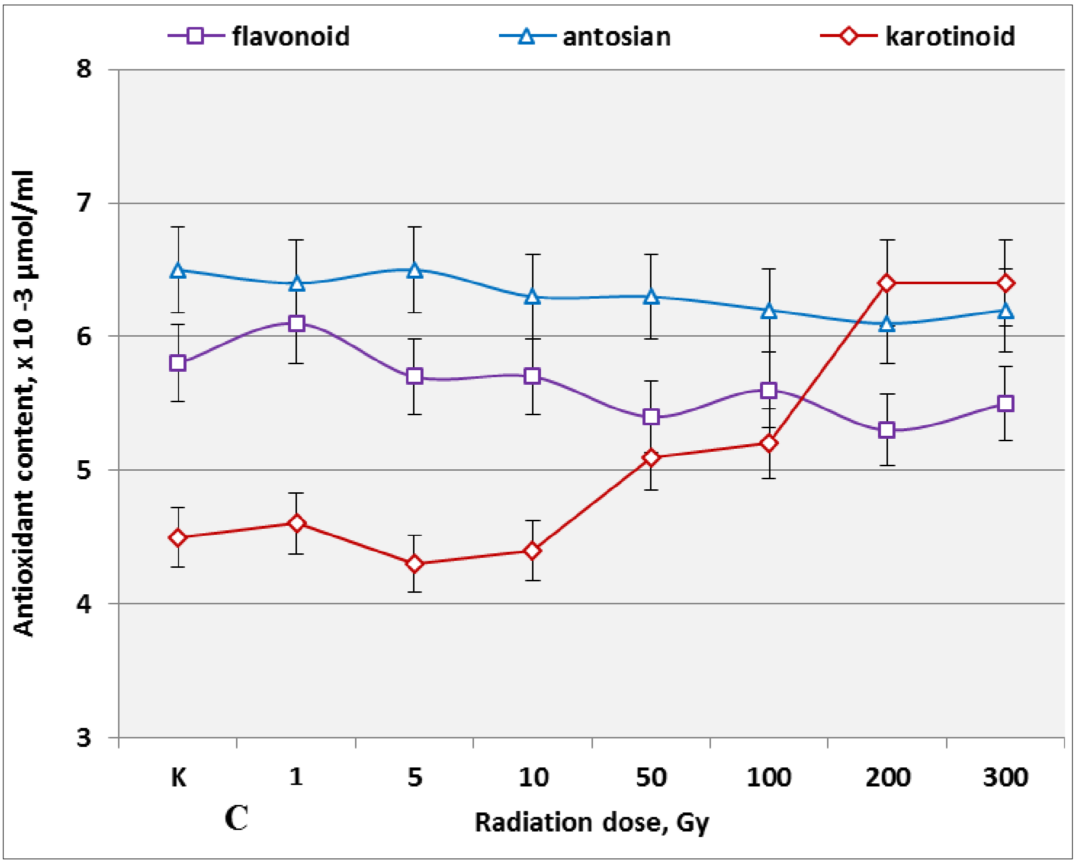

Fig 1: Dependence of the content of antioxidants on the radiation dose of maize seeds

As seen in the figure, the pre-sowing exposure of the seeds to different doses of irradiation activates the antioxidant defense system of the cell. This activation manifests itself mainly at high radiation doses, and carotenoids play a key role in protecting the plant from the harmful effects of stress. Thus, at high radiation doses, the amount of carotenoids is ~ 1.4 times higher than in control. The results showed a high content of flavonoids and anthocyanins and a relatively small amount of carotenoids in the control sample.

Summarizing the results about small-molecule antioxidants, it can be noted that radiation stress mobilizes the internal potential of the plant, and the initial reaction of the plant to the stress factor leads to the acceleration of the synthesis of antioxidants such as carotenoids. In other words, yellow pigments play a key role in protecting the plant against stress, while anthocyanins and flavonoids cannot play an important role in this process.

The effect of salt stress on the amount of small-molecule antioxidants.

The results on the amount of antioxidants in 15-day-old seedlings of the maize plant grown at different concentrations of salt are shown in Fig. 2. In this case, the seeds were not exposed to pre-sowing γ-irradiation. Based on the results, more significant changes occur in the amount of antioxidants depending on the NaCl concentration. An interesting fact is that in the case of salt stress, similar to radiation stress, increased stress leads to an increase in the amount of carotenoids. But in this case, there are large-scale changes in the amount of carotenoids. For example, at a concentration of 10 mM, the amount of yellow pigments is ~ 1.6 times higher than in the control. Whereas, at a radiation dose of 100 Gy, this increase was ~ 1.4 times.

Another interesting finding is that salt stress, in contrast to radiation stress, can cause significant changes in both anthocyanin and flavonoid levels. Thus, even in the case of the smallest salt concentrations, their amount is significantly reduced. For example, at 5 mM NaCl, the amounts of anthocyanins and flavonoids are reduced by ~ 1.3 and~ 1.2 times, respectively, compared with the control. It should be noted that the amount of these pigments in the case of radiation stress, except for small deviations, almost did not change.

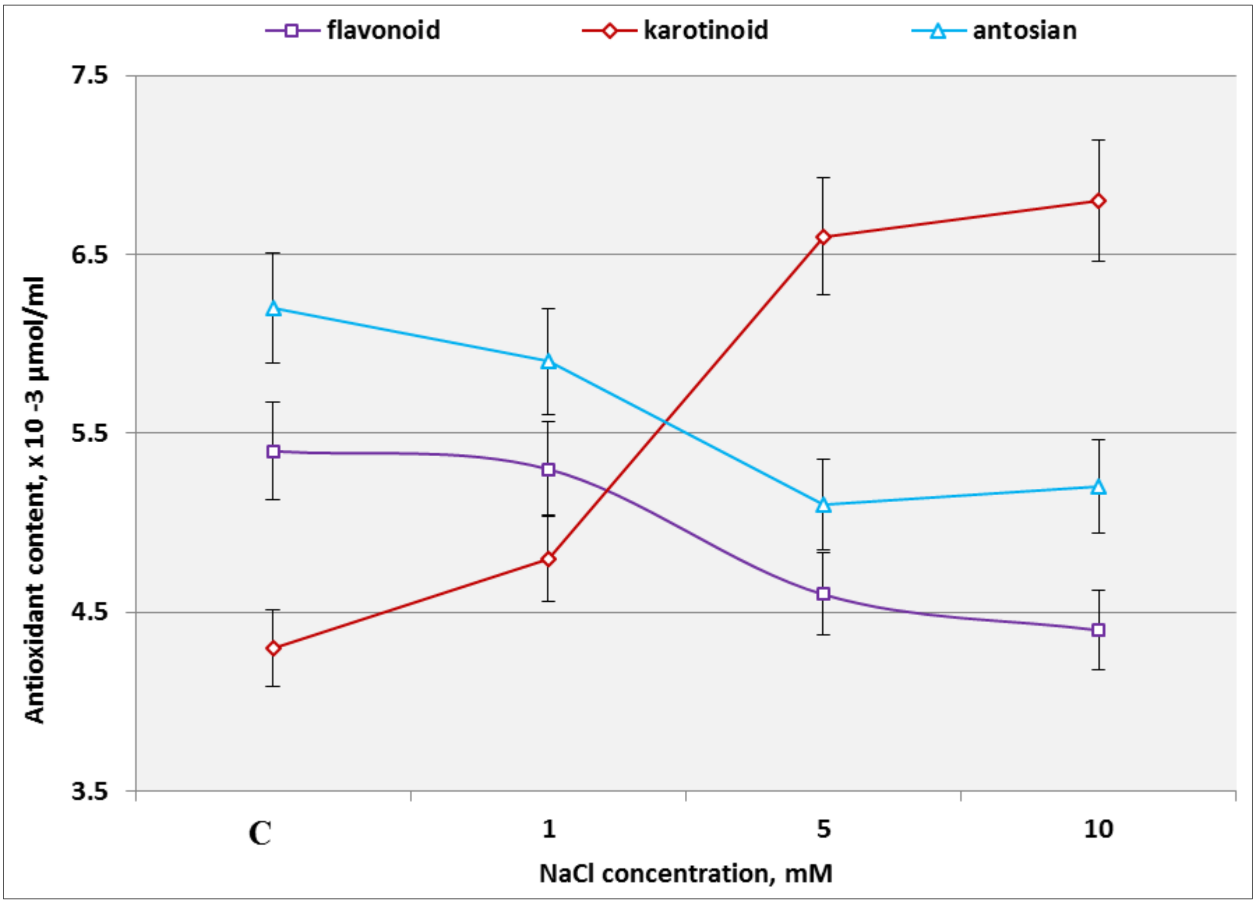

Fig 2: Dependence of antioxidant content on NaCl concentration

Summarizing our results on the effects of stressors of two different natures, it can be concluded that the mentioned stress factors have a similar role in the synthesis of carotenoids. Based on our findings on phenolic compounds, it can be assumed that while radioactive radiation cannot stimulate the synthesis of anthocyanins and flavonoids, salt stress has an inhibitory effect on this process. In this case, anthocyanins and flavonoids themselves can be exposed to the destructive effects of salt stress.

The effect of salt stress on the amount of small-molecule antioxidants in maize plants which seeds were exposed to pre-sowing γ-irradiation

The antioxidant content in 15-day-old seedlings of maize plants, which seeds were exposed to pre-sowing γ-irradiation and grown at different concentrations of NaCl, are shown in Figures 3, 4, and 5.

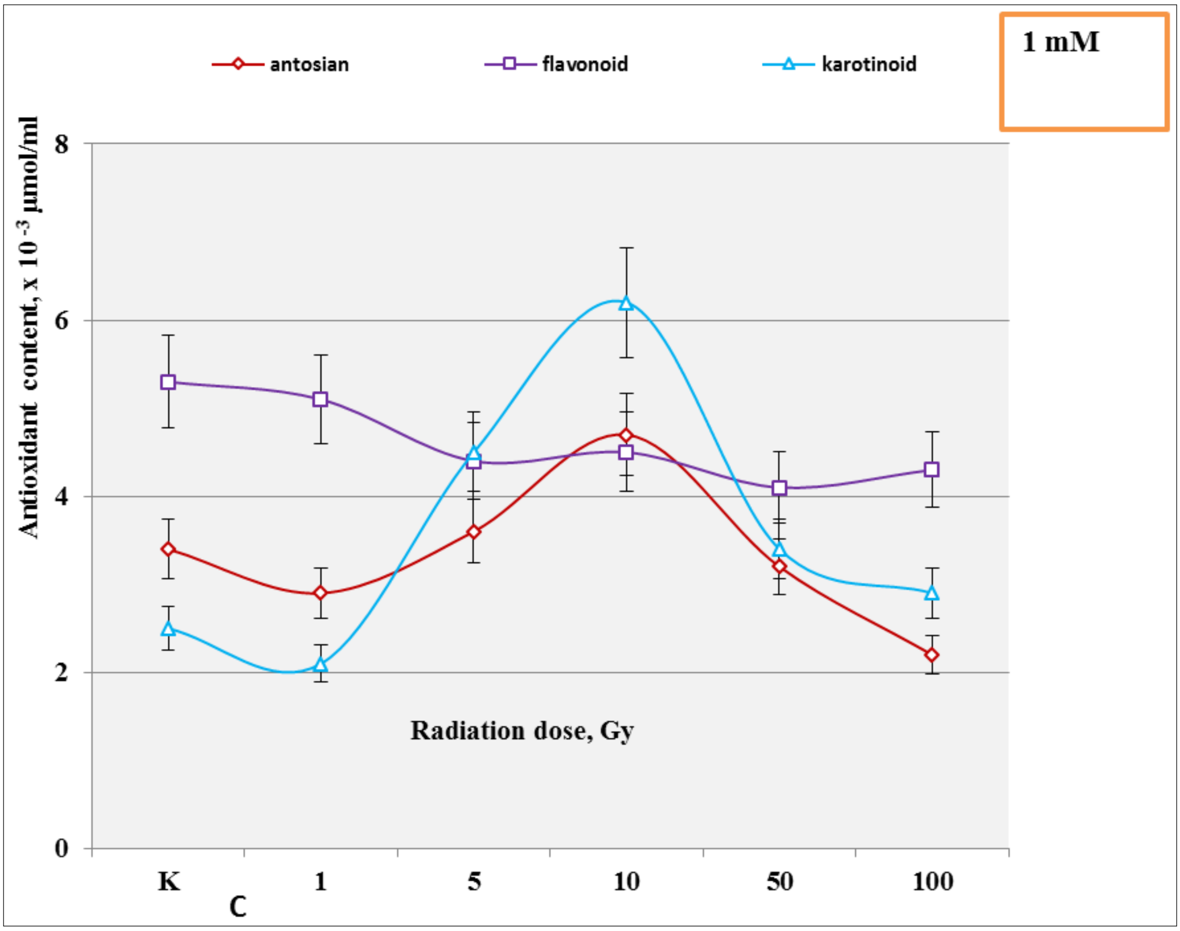

Fig 3: Dose dependence of antioxidant content at 1 mM NaCl.

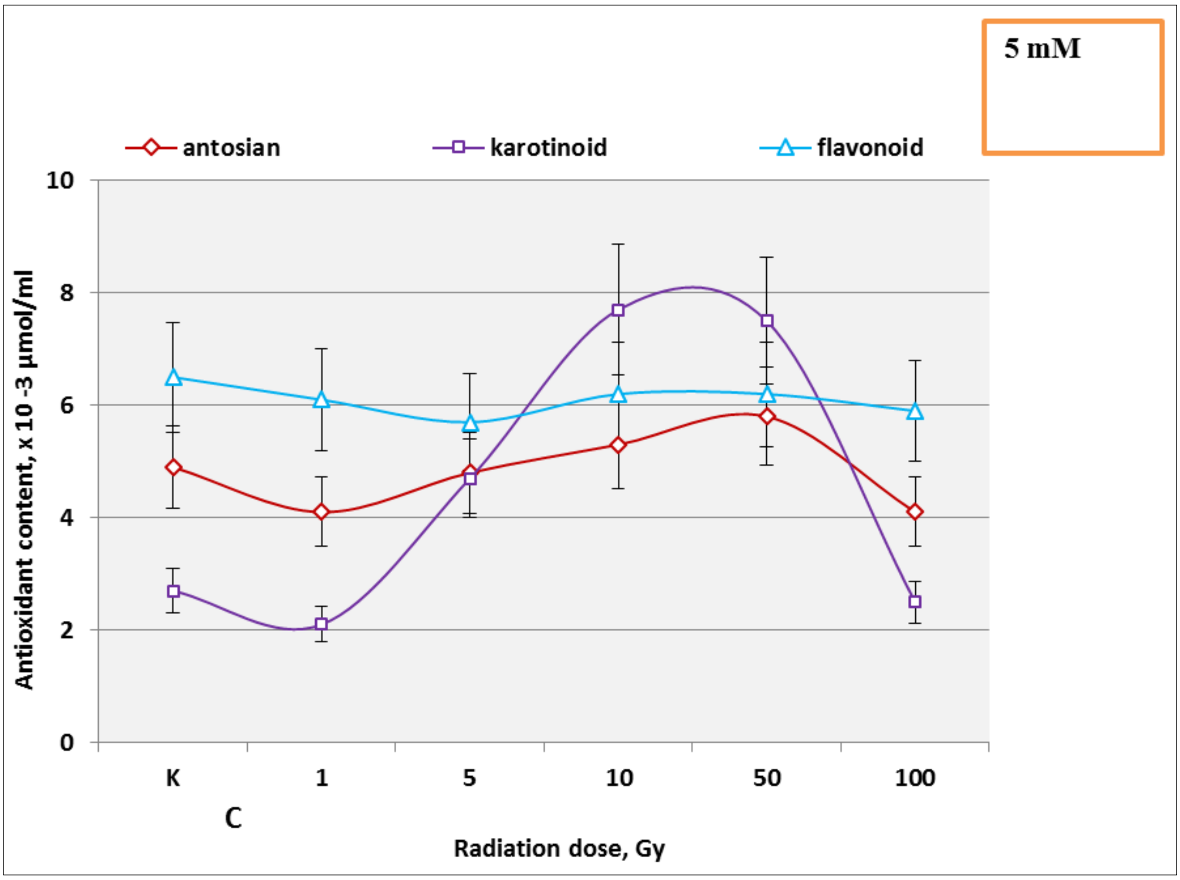

Fig 4: Dose dependence of antioxidant content at 5 mM NaCl

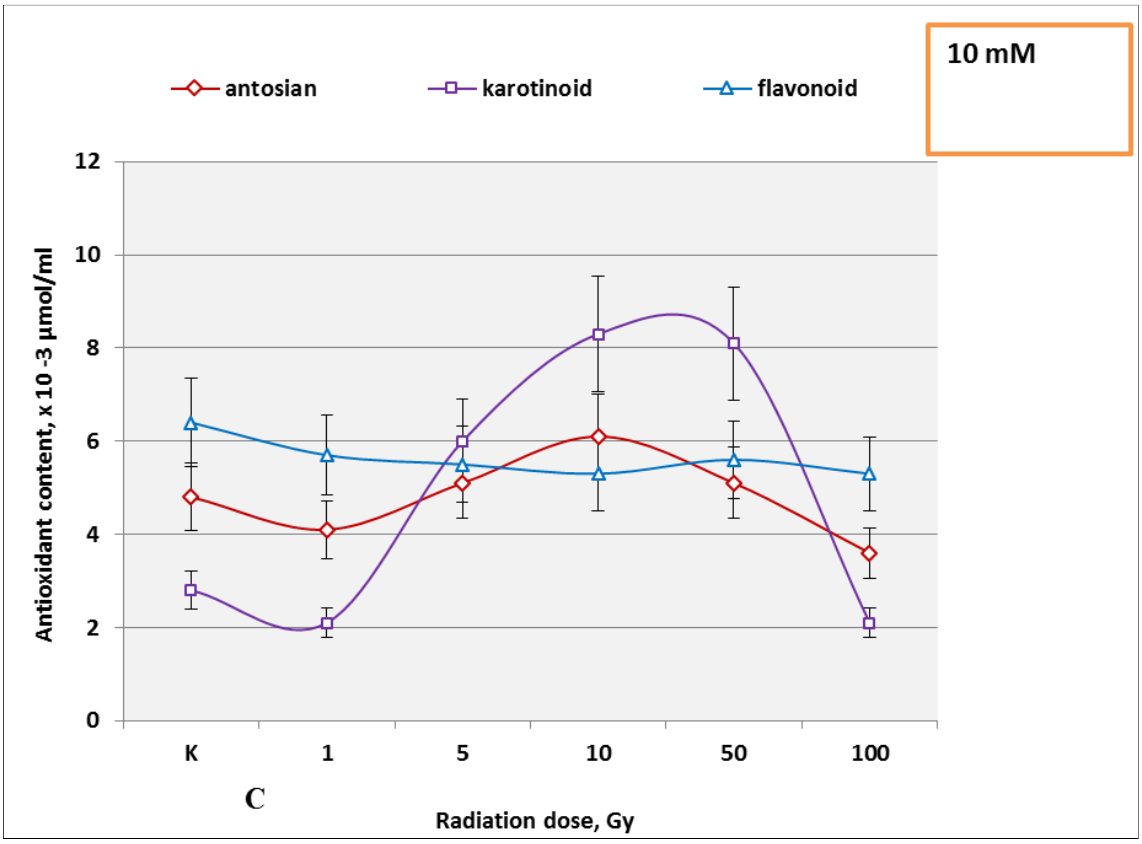

Fig 5: Dose dependence of antioxidant content at 10 mM NaCl

The first thing that draws attention from the images is the lack of a sharp dependence between the salt concentration and the amount of substances studied. Thus, at all three concentrations of NaCl (1 mM, 5 mM, 10 mM) antioxidants exhibit similar dynamics of change depending on the radiation dose. More precisely, in all three cases, changes in the radiation dose in the range of 1-10 Gy lead to an increase in the amount of anthocyanins and carotenoids, while the radiation dose over 10 Gy results in a decrease in these pigments.

Similar changes occurred in the amount of flavonoids. Thus, in this case, there is a similar dependence on the radiation dose at all three salt concentrations. More precisely, in all three cases, changes in the radiation dose in the range of 1-100 Gy can not cause significant changes, except for small deviations in the amount of flavonoids.

Summarizing the information on small-molecule antioxidants, the following can be concluded:

- Control samples of this plant have high anthocyanin and flavonoid content, relatively small carotenoid content;
- Pre-sowing γ-irradiation treatment of seeds in the dose range of 1-5 Gy can stimulate the development of the plant under salt stress. Along with carotenoids, anthocyanins also play a role in this process;
- Pre-sowing treatment of seeds with γ-rays in the dose range of 1-300 Gy does not cause large-scale changes in the amount of small-molecule antioxidants
- (excluding carotenoids). Only a small increase in the amount of yellow pigments is observed at high radiation doses;
- −carotenoids play a key role in protecting plants against salt stress, even at the lowest salt concentration. An increase in the concentration of salt leads to a sharp increase in the amount of yellow pigments.

References

1. Radyukina NL, Shashukova AV, Shevyakova NI, Kuznetsov Vl. B. Participation of proline in the antioxidant defense system in sage under the action of NaCl and paraquat // Plant Physiology. 2008; 55:721-730.
2. Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: A review // Annals of Botany. 2003; 91:179-194.
3. Merzlyak MN, Melo TB, Naqvi KR. Effect of anthocyanins, carotenoids, and flavonols on chlorophyll fluorescence excitation spectra in apple fruit: signature analysis, assessment, modelling, and relevance to photoprotection // J. Exp. Bot. 2008; 59:349-359.
4. Ramel F, Mialoundama AS, Havaux M. Nonenzymic carotenoid oxidation and photooxidative stress signalling in plants // J. Exp. Bot. 2013; 64:799-805
5. Kumar S, Kumar S, Singh B. Influence of gamma irradiation of seed on growth, sex-expression and yield in sponge gourd (*Luffa cylindrica* L.) // Plant Arch. 2002; 2(2):225-227.

6. Honda I, Kikuchi K, Saito H. Hormesis of heavy-ion irradiation to lettuce // RIKEN Accel. Progr. Rept. 2003; 37:149.
7. Sims DA, Gamon JA. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages // Remote Sensing of Environment. 2002; 81(2-3):337-354.
8. Lomboeva SS, Tankhaeva LM, Olennikov DN. Method for the quantitative determination of the total flavonoid content in the terrestrial part of Ortilia one-sided (*Orthilia Secunda* L.) House) // Chemistry of plant materials. 2008; (2):65-68.
9. Lakin GF Biometrics M. Science, 1990, 352.