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ORIGINAL ARTICLE

The Effect of Seed Treatment with Gamma Irradiation on Biometric Indices and Chlorophyll Synthesis in the Maize (*Zea mays*) Plant Grown under Salt Stress

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The presented research is about the effect of seed treatment with γ-radiation on the development of the maize plant and on the chlorophyll synthesis under salt stress. It has been revealed that separately high doses of radiation and low or high NaCl concentrations inhibit the plant growth as well as chlorophyll synthesis in the leaves, while pre-sowing irradiation of the seeds with a dose of 50 Gy stimulates the growth of the plant and chlorophyll synthesis even at high concentrations of NaCl.

Key words: biometric indices, chlorophyll, maize (Zea mays), presowing seed irradiation, salt stress

Recent research suggests that the technology of treating plant seeds with different doses of γ-rays may increase their tolerance to various stress factors, and attempts are being made to reduce the negative effects of stressors using this method (Dehpour *et al*. 2011; Mohammed *et al*., 2012). Thus, it was found that seed treatment with a 50 Gy dose of γ-rays could stimulate the development of the barley plant under salt stress by weakening oxidative stress. Reducing oxidative stress manifested itself in a decrease in the MDA amount, which is the product of lipid peroxidation, a decrease in the H_2O_2 amount, and activation of the antioxidant defense system (Wang *et al*., 2018). According to the authors, pre-sowing treatment of seeds with γ-rays reduced damage caused by salt stress in the ultrastructure of chloroplasts, and the expression of genes related to salt stress increased (Wang *et al*., 2018). Pre-sowing irradiation of seeds was found to increase significantly the number of buds, stem length, and chlorophyll b content in the *Gannouch* population of the *Medicago sativa* (L.) Gabe's plant, grown under salt stress (Rejili *et al*., 2008).

Another study showed that irradiated protoplasts were able to grow even at the highest NaCl concentration (8000 mg/l) (Helaly and Hanan El-Hosieny, 2011). It was noted that radiation could also increase the amount of osmoregulators such as carotenoids, proline, total soluble phenols, glycine betaine while preventing the negative effects of high salinity on total chlorophyll, total sugar, total soluble protein (Helaly and Hanan El-Hosieny, 2011). It was also found that the effects of radiation increased the activity of antioxidant enzymes, which, according to the authors, ensured cell protection from oxidative stress caused by high salinity (Helaly and Hanan El-Hosieny, 2011).

According to Macovei et al. (2014), the treatment of seeds with γ-rays stimulated the development of the rice plant under conditions of salt stress because of the activation of the antioxidant defense system.

The article deals with the effect of pre-sowing irradiation of the maize plant seeds with γ-rays on the development of the plant and on the chlorophyll synthesis under salt stress.

MATERIALS AND METHODS

Object of the study. The maize (*Zea mays*) plant was chosen as the study object. Maize seeds were exposed to radiation doses of 1, 5, 10, 50, 100, 200, and 300 Gy (dose rate was 0.048 Gy/sec in all cases) using a ^{60}Co radiation source. The irradiated seeds, together with the control samples, germinated in the dark (in a thermostat, using Petri dishes), under conditions identical to the underground. After 4 days, the seedlings were transferred to special containers of 0.5 L with 0, 1, 5, 10, 50, 100, and 200 mM NaCl solutions and grown in a special chamber (phytatron). In this case, the conditions were also the same as for cultivation in the soil. A photoperiod of 12 h/12 h (day/night) was applied in the chamber, with the help of incandescent lamps (220 V, 150 W), the temperature was 23 ± 1ºC in the daytime and 15 ± 1ºC at night, and using fluorescent lamps the lighting condition was 37.6 W/m^2. The chamber had a relative humidity of 55% during the day and 70% at night.

The amount of chlorophyll a and chlorophyll b in plant samples was determined spectrometrically using the Ultrospec 3300 pro Amersham Bio-sciences spectrophotometer. The Sims and Gamon (2002) equations were used for this purpose. More precisely, the optical densities of plant extracts at wavelengths of 537 nm, 647 nm, and 663 nm were determined and the concentrations of green pigments (in *µmol ml*$^{-1}$) were calculated using the formulas:

$$\boldsymbol{C}_{\boldsymbol{chl.a}} = 0.01373 \cdot A_{663} - 0.000897 \cdot A_{537} - 0.003046 \cdot A_{647}$$

$$\boldsymbol{C}_{\boldsymbol{chl.b}} = 0.02405 \cdot A_{647} - 0.004305 \cdot A_{537} - 0.005507 \cdot A_{663}$$

The experiments were performed in 3 biological and 3 analytical replicates, which allowed us to obtain results with an error of ± 20-25%.

The results were statistically processed in the *Sigma Plot* program using standard methods of the statistics of variation. The arithmetic mean values of the measured quantities are shown in the figures.

We evaluated the difference in the results obtained from the control and experimental samples based on the

Student's t-test (Lakin, 1990). Differences are considered statistically significant at | t | > 2 (p<0.05).

RESULTS AND DISCUSSION

The study of the effect of pre-sowing γ-irradiation of seeds on the growth and development of the maize plants.

Seedlings grown in three different ways were used to clarify the separate and combined effects of radiation and salt stresses. Seedlings were grown in a normal aqueous medium from seeds irradiated with different doses, from non-irradiated seeds at different concentrations of NaCl solution, and from irradiated seeds at different concentrations of NaCl solution.

Figure 1 shows 2-week-old maize seedlings grown in an aquatic medium from irradiated seeds. The figure presents the dependence of biometric parameters of the maize plant on irradiation doses. Or more precisely, a radiation dose of 50 Gy stimulated, while 100 Gy and more doses inhibited plant development.

To study the effect of salt stress separately on the growth and development of the maize plant, non-irradiated seeds of the plant were used.

Figure 2 shows 2-week-old maize seedlings grown at various salt concentrations. As seen in the figure, salt in all concentrations had a retarding effect on the growth and development of maize, and the intensification of salt stress further increased this effect. The retarding effect was also evident in the root system of the plant (Figure 4).

Interestingly, maize seeds can develop at high salt concentrations (50-200 mM), although weakly, as in the case of high doses (200-300 Gy) of irradiation. Thus, maize seeds can show high resistance to ionizing radiation, as well as high tolerance to salt stress.

Pre-sowing irradiation of seeds is known to stimulate the development of some plants under salt stress (Beyaz, 2019; Geng *et al*., 2019; Kumar *et al*., 2017; Qi *et al*., 2014; Sansenya *et al*., 2019). To test whether a similar effect was true for the maize plant, we considered it expedient to study the growth and development of the maize plant grown at different concentrations of NaCl solution from irradiated seeds with a dose of 50 Gy before sowing. The radiation dose of 50 Gy was chosen since it is in the stimulating dose range for maize.

Figure 3 shows 2-week-old maize seedlings grown in a NaCl solution from seeds irradiated with a dose of 50 Gy.

We have already mentioned that radiation inhibits the growth of maize at high doses (200-300 Gy) and salt manifests its retarding effect at high concentrations (100-200 mM). However, under conditions of dual stress, we are witnessing a different picture. Thus, it is clear that pre-sowing irradiation of the maize seeds with a dose of 50 Gy can stimulate growth, even at high salt concentrations.

In the case of irradiation of seeds, no significant differences in the number and length of roots depending on the salt concentration were observed (Figure 4).

The results suggest that the use of γ-ray treatment technology of seeds for growing maize in saline soils can lead to the desired results.

Study of the effect of seed pre-sowing treatment with γ-rays on the amount of photosynthetic pigments in leaves of maize grown under salt stress.

According to international organizations, up to 30% of the world's agricultural land is exposed to high salinity (Unesco Water Portal. 2007). In this case, under real conditions, plants sometimes have to grow under the influence of high salinity. Attempts are indeed made to use saline soils through intensive irrigation, but this results in disturbances in the water balance of the soil creating problems in the regulation of ion exchange, which plays an important role in plant life (Passioura, 2007).

It is believed that the initial reaction of plants to salinity (salt stress) should manifest itself in the process of photosynthesis (Munns *et al*., 2006). According to the literature data, in this case, the effect of salt stress on the process of photosynthesis can be direct or indirect. It is noted that in the case of direct effect, there may be a delay in the absorption of CO_2 due to diffusion through the stomata and mesophyll (Flexas *et al*., 2007) or a change in the metabolism of photosynthesis (Lawlor, Cornic, 2002). The indirect effect of salt stress is associated with the formation of oxidative stress, which is quite probable under the influence of other environmental factors (Chaves, Oliveira, 2004).

In general, the response of plants to environmental factors at the level of photosynthesis should involve fairly complex processes. Thus, the response of plants to stressors must be accompanied by a number of physiological, cellular, and molecular processes that occur rapidly and simultaneously. It is clear that in this case, the metabolic machine must be fully mobilized for plants to adapt to unfavorable environmental conditions. For this reason, the study of the effects of stress on the metabolism of plants as a whole and, in particular, on the process of photosynthesis requires considerable accuracy, and any results in this area can contribute to the clarification of the listed and quite complex processes.

Separate exposure to radiation and salt stresses. As in the study of biometric parameters, the separate and combined effects of radiation and salt stress on photosynthetic pigments were evaluated. The results obtained for separate effects of radiation and salt stresses are presented in Figure 5, and the results for the combined effects are in Figure 6.

According to the results, when seeds were exposed to a low radiation dose, a small increase was observed in the amount of chlorophyll in the leaves of maize grown in an ordinary aquatic medium, and when seeds were irradiated with low doses, a large decrease in the pigment amount occurred. More precisely, in the case of irradiation of seeds with a dose of 5 Gy, the amount of the chl a and chl b pigments was 1.3 and 1.4-fold higher, respectively, compared to the control sample, and 4.4 and 2.6-fold lower when seeds were exposed to 200 Gy dose.

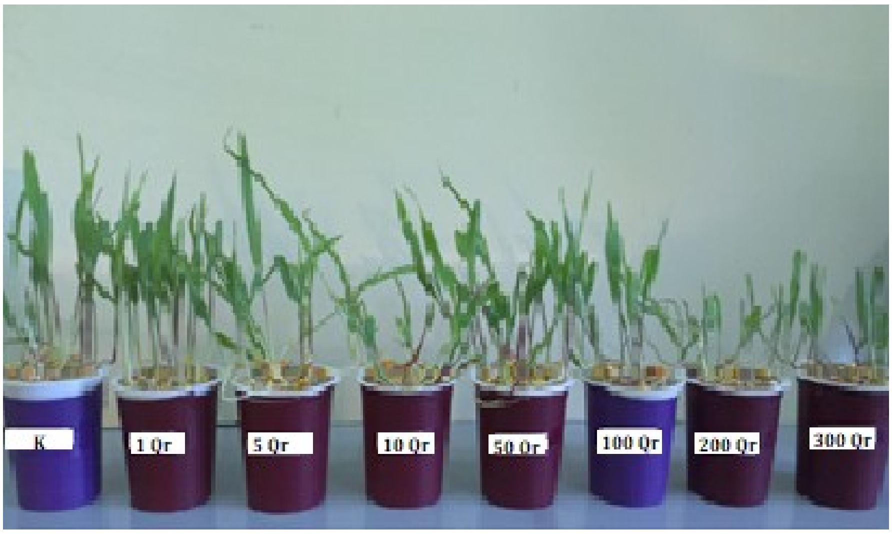

Figure 1. Two-week-old maize seedlings grown in an aquatic medium from irradiated seeds.

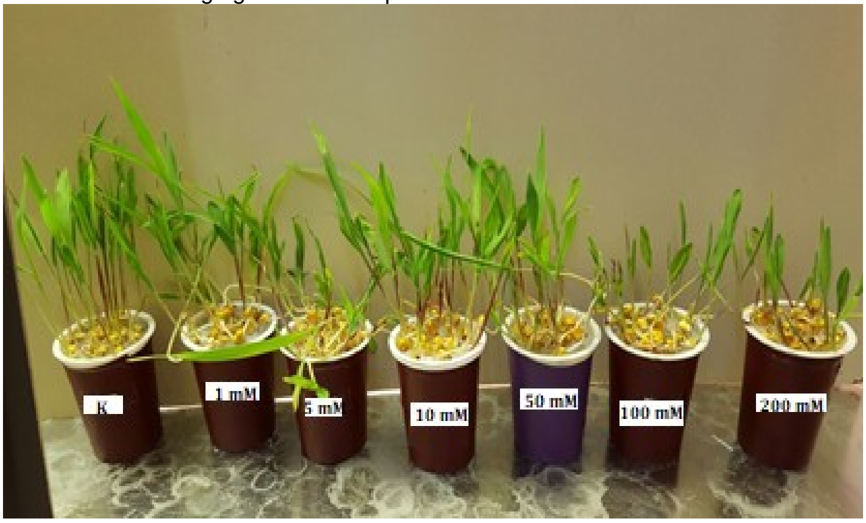

Figure 2. Two-week-old maize seedlings grown from non-irrigated seeds at various salt concentrations.

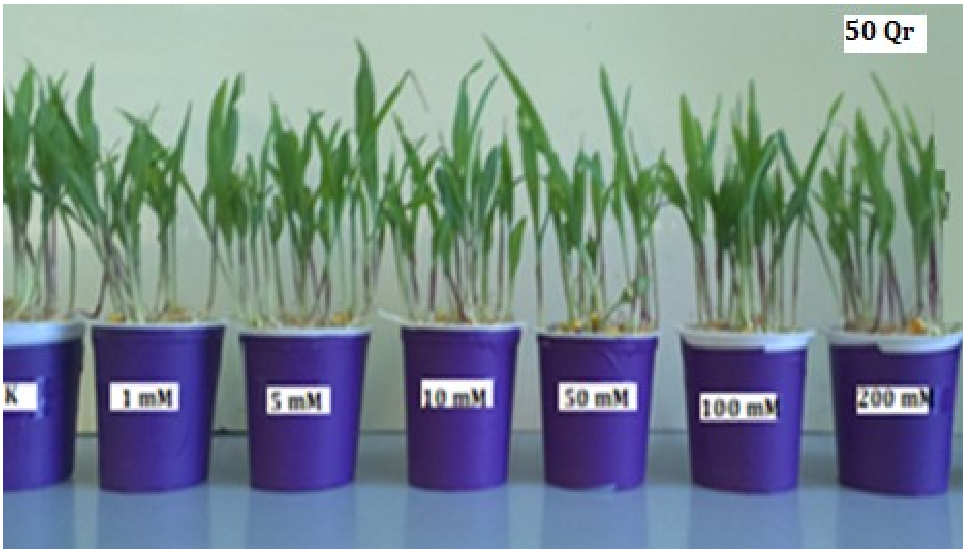

Figure 3. Two-week-old maize seedlings grown in a NaCl solution from seeds irradiated with a dose of 50 Gy.

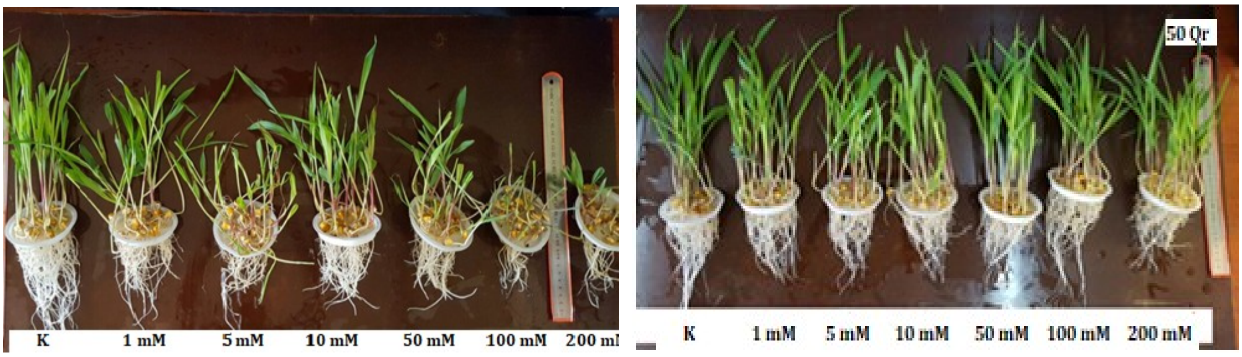

Figure 4. Dependence of root formation in 2-week-old seedlings of maize, grown from irradiated seeds with a dose of 50 Gy, on the concentration of NaCl.

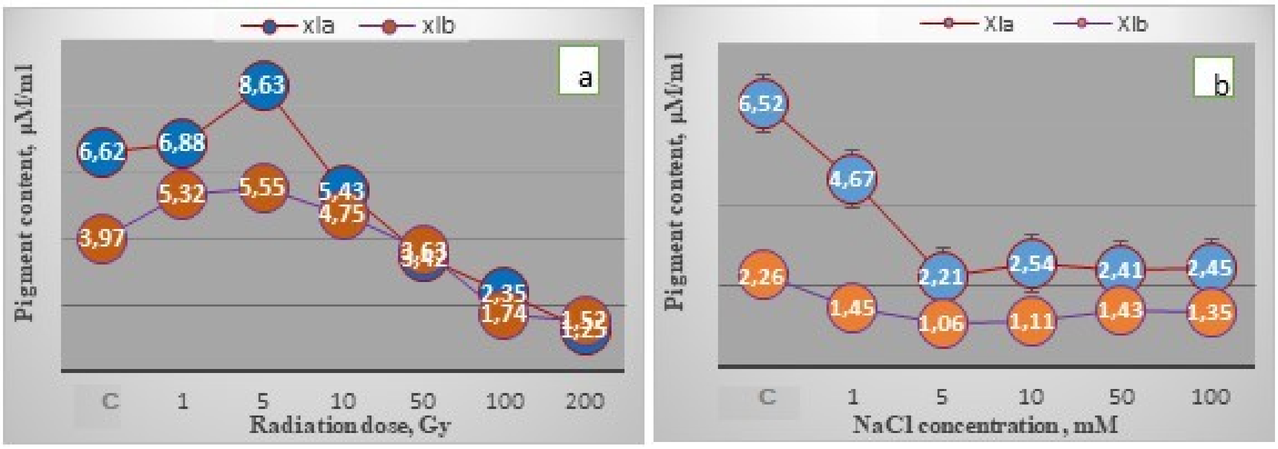

Figure 5. Effects of radiation (a) and salt stress (b) separately on the amount of chlorophyll in 2-week-old maize leaves.

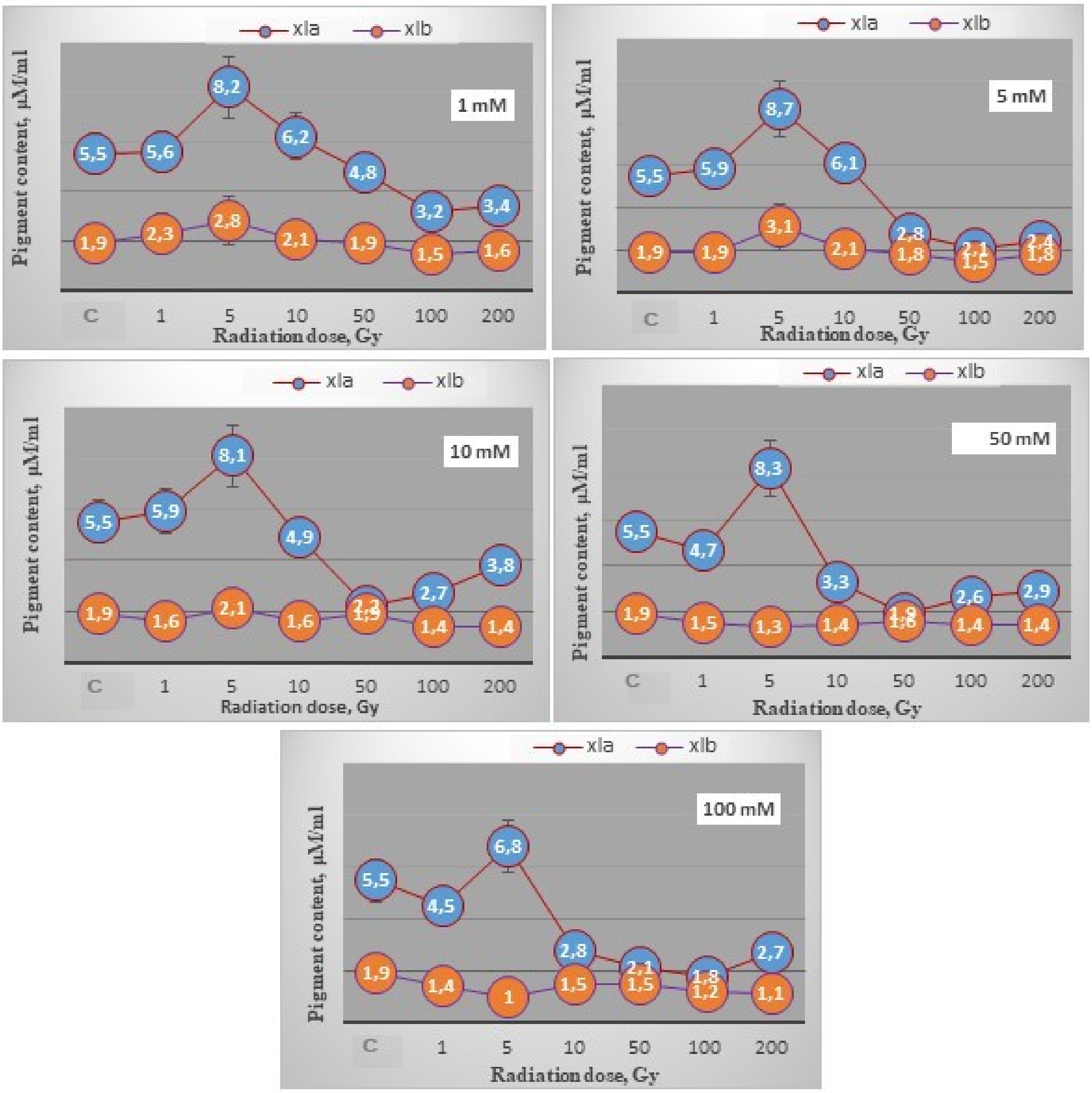

Figure 6. Dependence of chlorophyll content on radiation dose in 2-week-old maize leaves grown at different concentrations of NaCl.

The results obtained on the dependence of the amount of chlorophylls on the concentration of NaCl in the leaves of maize grown in saline solution from non-irradiated seeds differ from the results on the dependence of the amounts of green pigments on radiation doses. Thus, increasing NaCl concentration resulted in a regular decrease in photosynthetic pigment amounts, which reached a minimum value at 5 mM NaCl (decreased approximately by 2.9 and 2.1 times) and remained almost stable.

It can be assumed that salt stress can inhibit the synthesis of chlorophyll in maize plants even at small concentrations of NaCl, and this process continues to a certain extent (up to 5 mM). Further increase in the concentration of NaCl does not change the amount of green pigments.

The results of studies conducted in different years show that the data on the effect of ionizing radiation on chlorophyll synthesis do not always coincide. Although some authors claimed that small doses of ionizing radiation have a stimulating effect on chlorophyll biosynthesis (Antonov *et al*., 2002; Kulikov *et al*., 1991), others observed inhibition of chlorophyll synthesis in irradiated plants (Nazirov, 1964; Saakov, 2002).

The stimulating effect of low-dose ionizing radiation on the process of photosynthesis in plants was observed in maize (Antonov *et al*., 2002), rosemary, water lettuce, basil plants (Koseki *et al*., 2002) grown from seeds exposed to pre-sowing γ-irradiation.

Stobart and Griffiths (1985) believe that chlorophyll synthesis, as the primary target, is exposed to the toxic effects of ionizing radiation at the initial stage of the development of seedlings when active chlorophyll formation occurs. In other words, in the early stages of plant development, ionizing radiation inhibits the biosynthesis of chlorophyll by affecting the photosynthetic apparatus.

Thus, in some cases, the data on the stimulating effect of ionizing radiation in small doses may be non-replicable and even contradictory.

It should be noted that the non-replicability of the stimulatory effect, in other words, the fact that radiation can have a stimulating or inhibitory effect on chlorophyll synthesis, depending on the conditions, has been observed in preliminary studies in radiobiology. The emergence of radiation effects is attributed to the ability of plants to use their internal potential, along with the activity of the antioxidant defense system playing an important role under stress conditions. In other words, the importance of how plants use their potential under stress conditions is emphasized (Kuzin, 1986; Mokronosov, 1981).

Therefore, extensive research is needed for the unequivocal assessment of the effect of ionizing radiation on the process of photosynthesis, which is closely related to the growth and development of plants, specifically on the synthesis of photosynthetic pigments.

Photosynthesis is known to be one of the vital physiological processes that salinity can affect and is closely related to the growth and development of plants. It is a complex process that depends on gas exchange processes, photosynthetic pigments, photosystems, components of the electron transport system, and the activities of various enzymes involved in carbon metabolism. Therefore, "damage" to any of these components has a negative effect on photosynthesis (Ashraf, Harris, 2013).

It is believed that the decrease in the rate of photosynthesis under salinity is primarily related to osmotic stress, which causes the stomatal closure, and secondly, to the high accumulation levels of Na^+ and Cl^- ions, which can damage the thylakoid membranes of chloroplasts (Hasanuzzaman *et al*., 2013; Mishra *et al*., 2013).

It should be noted that we cannot establish exactly which of these processes is affected by salt stress based on the results obtained. However, considering the inhibitory effect of salt stress on the growth and development of the maize plant, it can be assumed that the decrease in the rate of photosynthesis under salinity conditions primarily occurs due to osmotic stress, which causes the stomatal closure.

Most likely, under salt stress, the water balance is disturbed, the amount of intracellular water is reduced, and cell elongation and division are delayed. Excess salt should also lead to the stomatal closure, a decrease in leaf surface area, and a delay in photosynthesis and growth (Hasanuzzaman *et al*., 2013).

High accumulation levels of Na^+ and Cl^- ions, which can damage the thylakoid membranes of chloroplasts, may also be the factor reducing the intensity of photosynthesis. It should be noted that such an idea was supported by Hasanuzzaman et al. (2013) and Mishra et al. (2013).

The results we have obtained are in line with these ideas.

Combined effects of radiation and salt stresses. The study of the effect of salt stress on photosynthetic pigment amounts in maize leaves grown from irradiated seeds showed that although even low doses of radiation inhibited chlorophyll synthesis when the plant was exposed to salinity (all applied concentrations of NaCl), irradiation with 5Gy dose led to the acceleration of chlorophyll *a* synthesis (Figure 6).

The effect of salt stress on the synthesis of chlorophyll *b* in maize leaves grown from irradiated seeds was different. Thus, at a radiation dose of 5 Gy, a slight increase in the amount of chlorophyll *b* was observed only at low salt concentrations (1 and 5 mM NaCl), and at high salt concentrations (10 and 50 mM NaCl) the amount of this pigment remained almost unchanged.

Interestingly, the dependence of the amount of chlorophyll on the radiation dose of seeds at all salt concentrations we use is characterized by a similar curve, except for small deviations. More precisely, in the case of irradiation of seeds with a dose of 5 Gy, the synthesis of chlorophyll *a* at all salt concentrations accelerated, and at higher irradiation doses there was a gradual delay. For chlorophyll b, a similar dependence was observed at a radiation dose of 5 Gy in NaCl solutions with concentrations of 1, 5, and 10 mM. At doses over 5 Gy, the amount of chlorophyll at all salt concentrations remained approximately unchanged, as in the case of individual salt stress.

Our results suggest that "treatment of seeds with certain doses of γ-rays before sowing increases the tolerance of the maize plant to salt stress."

Based on other reports, gamma radiation may reduce the effects of both drought and high salinity to some extent (Beyaz, 2019; Mohammed *et al*., 2012; Song *et al*., 2012).

Treatment of seeds with gamma rays of 40 and 80 Gy doses was found to increase the resistance of *Ambrosia maritima* L. to salt stress, which was expressed by the increase in plant height, fresh and dry mass, the amount of photosynthetic pigments compared to the control plant (Hanafy Ahmed *et al.,* 2011).

Given that soil salinity is a serious factor limiting plant growth and physiological reactions, El-Beltagi et al. (2013) tried to reduce the negative effects of soil salinity using γ-radiation during the growth of *Vigna sinensis*. According to the results of the study, pre-sowing treatment of seeds with γ-rays significantly improved the growth of plants cultivated under saline conditions and increased the amounts of photosynthetic pigments, total phenol, proline, and total free amino acids. Gamma radiation was also shown to reduce lipid peroxidation. The authors believe that this occurs due to the activation of processes involved in reducing the harmful effects of salt.

Studies on the effect of γ-radiation on the tolerance of plants to salt stress were also carried out by Qi et al. (2014). The results of the study showed that the response of *Arabidopsis* seedlings, grown from seeds irradiated with small doses (50 Gy) to salt stress increased germination index and root length. In this case, the amounts of H_2O_2 and MDA in irradiated plants were smaller compared to non-irradiated plants, and the activity of antioxidant enzymes and proline, on the contrary, was higher.

Kumar et. al. (2017) also attempted to elucidate the mechanism of physiological and biochemical changes caused by salt stress in plants whose seeds were irradiated. It was found that γ-radiation in small doses could stimulate salt tolerance of plants due to the maintenance of gas exchange, the activity of antioxidant enzymes, membrane stability index (ratio of K^+ to Na^+), and amounts of proline and glycine betaine.

According to Beyaz (2019), gamma radiation treatment of seeds with small doses can stimulate the tolerance of fodder peas (*Vicia sativa* L.) to salt stress and, further, stop the increase in the activity of antioxidant enzymes such as CAT, SOD, and APX.

Another study revealed that seedlings grown from irradiated seeds in a NaCl medium with a concentration of less than 20 mM had higher levels of proline and 2-acetyl-1-pyrroline than seedlings grown from irradiated seeds under normal conditions (Sansenya *et al*., 2019).

It is known that the high sensitivity to salinity severely limits the distribution of decorative *Osmanthus fragrans* (Thunb.) Lour plant, having flower fragrant, in the landscape. Geng et al. used a new approach, i.e. the technology of pre-sowing treatment of seeds with γ-rays to increase the tolerance of the plant to high salinity (Geng *et al*., 2019). The authors conclude that the technology of γ-radiation treatment of seeds can change the antioxidant activity of the plant and the balance of reactive oxygen species, which can increase the tolerance of plants to salt stress.

Thus, it can be concluded that pre-sowing irradiation of seeds with γ-rays leading to the regulation of the balance of reactive oxygen species in cells is very important for the plant adaptation to salt stress and this approach is a potential tool for increasing plant tolerance to salt stress.

CONCLUSIONS

Thus, separately high doses of radiation and low or high NaCl concentrations inhibit the plant growth as well as chlorophyll synthesis in the leaves, while pre-sowing irradiation of the seeds with a dose of 50 Gy stimulates the growth of the plant and chlorophyll synthesis even at high concentrations of NaCl. Our results suggest that "treatment of seeds with certain doses of γ-rays before sowing increases the tolerance of the maize plant to salt stress."

It can be concluded that pre-sowing irradiation of seeds with γ-rays leading to the regulation of the balance of reactive oxygen species in cells is very important for the plant adaptation to salt stress and this approach is a potential tool for increasing plant tolerance to salt stress.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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