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ORIGINAL ARTICLE

Study of radioprotective properties of potassium humate in gamma irradiated wheat seedlings

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The radioprotective properties of potassium humate were studied under model conditions. The growth dynamics of wheat seedlings treated with 0.01%, 0.001% and 0.001% solutions of potassium humate both before and after γ-irradiation at a dose of 200 Gy was studied. A positive effect of potassium humate solutions on the activity of catalase, the amount of malondialdehyde, chlorophyll pigments and carotenoids, fluorescent characteristics (the maximum quantum yield of PSII (photosystem II) in seedlings obtained from gamma-irradiated wheat seeds was revealed.

Key words: wheat seedling, γ-irradiation, lipid peroxidation, potassium humate, malondialdehyde, seed germination

One of the main problems today is chemical pollution of the environment. Humic-based substances are mainly used in crop production as microfertilizers. With their systematic use, the structure of the soil improves, as well as its buffer and ion exchange properties, soil microorganisms become more active. It is known that ionizing radiation has a damaging effect on biological systems. Damage initiated by free radicals is enhanced by reactive oxygen species (ROS), which cause oxidative modification of macromolecules, disruption of the integrity of cellular structures. The stimulating effect of humic compounds on the growth and development of plants, increasing their resistance to adverse environmental factors. Soluble forms of humates in small concentrations significantly stimulate the growth and development of plants, increase the supply of nutrients to plants, activate protein and carbohydrate metabolism, and increase crop yields (Costa *et al*., 2002, Zenkov, 1993). The effect of humic substances on plants is especially noticeable when external conditions deviate from the norm: at elevated temperatures, oxygen deficiency and pollution of the environment. However, their radioprotective function has not been sufficiently studied. It is known that the free form of the toxicant has the maximum activity. The bound substance loses its toxicity. On this basis, humic acids can be considered as natural detoxifiers. There are a lot of scientific works on the use of humic substances in the rehabilitation of contaminated soils (Van Stempvoort *et al*., 2005, Beyer *et al*., 1999, Holman *et al*., 2002, MacCarthy *et al*., 1990, Stevenson 1982). Salts of humic acids with potassium, ammonium and iron protect plants under conditions of radiation and salt stress (Muslimova *et al*., 2013, 2014). Thus, obtaining and studying the biological activity and radioprotective properties of humic substances is a very relevant research.

MATERIALS AND METHODS

To obtain humate K, the feedstock - peat - was treated with a 3% KOH solution with constant stirring at a temperature of 35-40 degrees for 5 hours. After that, the dissolved potassium humate was separated in a centrifuge. The objects of research were wheat seeds of the “Barakatli 95” variety. To study the radioprotective properties of humates, wheat seeds were treated with solutions of potassium humate for 15 hours. Then the seeds were irradiated with a dose of 200 gray using the URİ (K-25) device. In experiments to study the effect of potassium humate on the dynamics of growth and development of seedlings were used 0,01% 0,001% and 0,0001% solutions. Experiments to study the effect of potassium humate on the dynamics of growth and development of seedlings were also carried out after irradiation of seeds. Experiments were carried out in 5 repetitions.

To determine the product of lipid peroxidation, malonicdialdehyde, 1 g of fresh weight of seedlings was homogenized in a porcelain mortar with a small amount of the reaction mixture consisting of a 0,25% solution of thiobarbituric acid (TBA). The homogenate was transferred into a glass tube in small portions of the reaction mixture. The samples were mixed and placed in a water bath heated to 950 C for 30 min. Then the contents of the samples were transferred into centrifuge tubes and centrifuged for 10 min at 10,000 g. Optical density was measured on a spectrophotometer.

The amount of chlorophyll a (662 nm), chlorophyll b (644 nm), carotenoids (440 nm), malondialdehyde (532 nm) pigments, and catalase activity was determined spectrophotometrically (Multiscan Go, Germany). The maximum fluorescence quantum yield of wheat seedlings was measured on a MINI-PAM fluorometer (Germany).

Statistical processing of materials was carried out using the statistical tools of MS Office Excel.

RESULTS AND DISCUSSION

At the beginning of the experiments were studied the effects of potassium humate solutions on the germination of seeds irradiated at a dose of 200 Gy.The germination rate of seeds that have not been treated with potassium humate solution has dropped to 58% due to radiation. As a result of irradiation, the germination rate of seeds was reduced by 40% compared to control. The germination rate of wheat seeds irradiated at a dose of 200 Gray was - 78% in the variant treated with 0.01% potassium humate solution, 82% in the variant treated with 0.001% potassium humate solution and 76% in the variant treated with 0.0001% potassium humate solution.

The study of the dynamics of growth and development of seedlings obtained from gamma-irradiated wheat seeds was carried out for 4 weeks. Seedling growth was measured every 4-5 days. The study of the effect of potassium humate on the growth and development of seedlings was carried out before and after seed irradiation. Figure 1 shows the results of experiments on the study of the growth and development of seedlings treated with solutions of potassium humates before irradiation.

The figure 1 clearly shows the effect of potassium humate solutions on the growth of seedlings obtained from gamma irradiated seeds. It can be seen that at the initial stages of development, the greatest development is observed in the variant when the seeds were treated with 0.01% and 0.001% solutions of potassium humate before irradiation. But at further stages of development, the seedlings gradually equal each other.

Figure 2 shows the dynamics of the development of wheat seedlings, the seeds of which were treated with solutions of potassium humate after irradiation. The figure clearly shows that in all stages of development, the greatest development is observed in the variant when the seeds were treated with 0.001% solutions of potassium humate.

It is known that gamma irradiation of seeds negatively affects the processes of photosynthesis in plants: the synthesis of pigments is disturbed, the photochemical activity of chloroplasts decreases. Studies show that sodium and potassium humic salts have a positive effect on the process of photosynthesis under stressful conditions. Studies show that seed treatment with 0.005% sodium humate increases the concentration of chlorophyll pigments in plants. Humates have also been found to accelerate the release of CO_2 from the leaves. (Maslova & Nyutin 1973, Khristeva, 1951).

Experiments to determine the amount of photosynthetic pigments showed that the amount of chlorophyll pigments and carotenoids in seedlings obtained from seeds irradiated with 200 Gray decreased by an average of 55% compared with the control. In seedlings obtained from wheat seeds treated with a potassium humate solution before irradiation, a weaker decrease in the amount of chlorophyll pigments and carotenoids is observed than in the control. 0.001% potassium humate solution used in the experiments significantly reduces the effect of radiation on the photosynthetic apparatus in wheat seedlings used as model plants.

In the following experiments, the effect of potassium humate solutions on lipid peroxidation was studied. The free radicals that appeared after irradiation interact with the lipids of cell membranes. The result is lipid peroxidation. The lipid peroxidation reaction causes the formation of several end products. One of them is malondialdehyde. The amount of formation of this product determined the degree of cell damage (Montiller & Cacas, 2004). Lipid peroxidation (LPO) is an indicator reaction of damage to cell membranes. Experiments to determine the effect of potassium humate solutions on lipid peroxidation were carried out at the beginning of the development of wheat seedlings in the first three weeks. High doses of ionizing radiation reduced seed germination and stimulated lipid peroxidation in week-old seedlings. After two and three weeks, a decrease in the rate of lipid peroxidation was observed in seedlings treated with potassium humate solutions. With further development of seedlings, the differences in growth and activity of the antioxidant system leveled out. Humate K solutions reduced the damaging effect of ionizing radiation on plants at all stages of plant development.

On fig. 4 clearly shows dynamic of the change of amount of malondialdehyde, a product of lipid peroxidation, under the action of gamma radiation. Thus, in the irradiated variant, the amount of malondialdehyde, a product of lipid peroxidation (malonic dialdehyde), increased by 30-35% compared to the non-irradiated control. In samples treated with solutions of potassium humate, the amount of lipid peroxidation product - malonic dialdehyde - decreased. It can be assumed that a noticeable increase in the amount of lipid peroxidation products due to irradiation at a dose of 200 gray in the first week is the initial reaction of plant tissue to irradiation. In the third week, a significant decrease in the amount of lipid peroxidation product malonic dialdehyde was observed in the variant of seed treatment with 0.001% potassium humate solution. From Figure 4, we can conclude that under the influence of potassium humate solutions, the rate of lipid peroxidation, and, consequently, the rate of destructive processes occurring in the membrane, slows down compared to the irradiated control.

Catalase is an antioxidant enzyme that breaks down hydrogen peroxide into water and oxygen. Some organisms produce catalase to protect against hydrogen peroxide attacks (Loewen, *et al* 1985). Irradiation in small doses causes the activity of antioxidant enzymes. The activity of antioxidant enzymes increases depending on the dose and duration of exposure to gamma radiation (Min *et al*., 2014, Hanan *et al*., 2014). In our experiments, the activity of the catalase enzyme noticeably increases when seeds are irradiated at a dose of 200 Gy during the first two weeks (Fig. 5). As can be seen from the figure, seed treatment with potassium humate solutions at concentrations of 0.01% and 0.001% before irradiation significantly reduces catalase activity. The decrease in catalase activity is well observed in the third week.

The maximum yield of chlorophyll fluorescence - Fv/Fm reflects the maximum quantum efficiency of photosystem II (PSII). A decrease in this value indicates partial damage to PS2 (Schreiber, 2004). The advantage of using this indicator as a test function is due to its high sensitivity to pollution and stress.One of the most common methods for studying the activity of photosynthetic processes is PAM fluorometry based on pulse amplitude modulation (Pulse Amplitude Modulation). PAM fluorometers allow accurate measurements of the photochemical quantum yield of photosystems based on the kinetics of chlorophyll fluorescence quenching (Murchie & Lawson 2013, Kalaji *et al*., 2014].

The influence of potassium humate solutions on the measurement of the maximum quantum yield of PSII – Fv/Fm (Y) in seedlings obtained from gamma-irradiated wheat seeds was studied on the third week. Experiments were carried out in 3 repetitions. The purpose of these experiments was to study the effect of potassium humate solutions on the photochemical activity of photosystem II in the leaves of wheat seedlings, the seeds of which were irradiated at a dose of 200 Gy. Figure 5 shows the results of these studies. The highest activity of photosynthesis was observed in the variant where the seeds were treated before irradiation with 0.01% and 0.001% solutions of potassium humates.

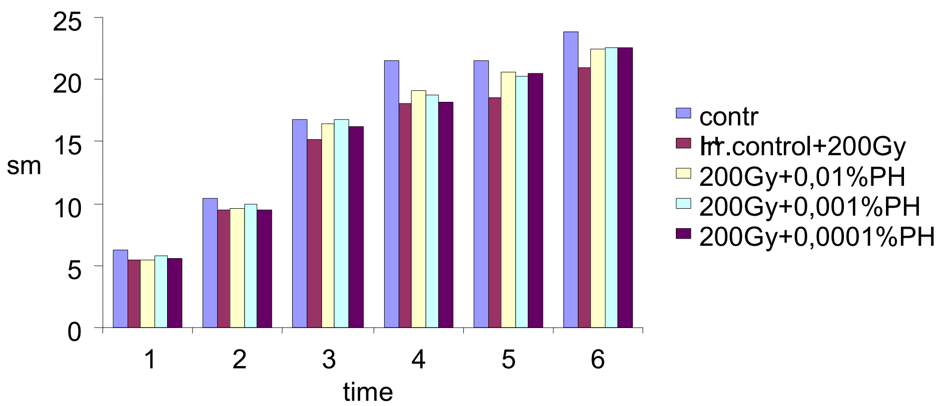

Figure 1: The effect of potassium humate solutions on the growth and development of seedlings obtained from gamma irradiated wheat seeds (before irradiation).

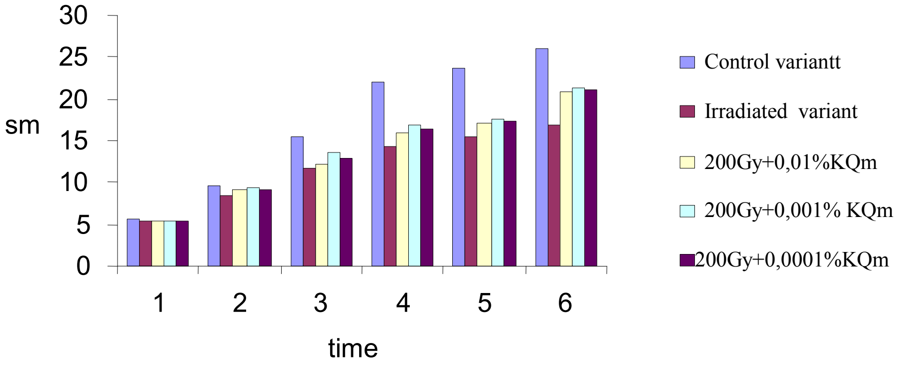

Figure 2: Effect of potassium humate solutions on the growth and development of seedlings obtained from gamma irradiated wheat seeds (after irradiation).

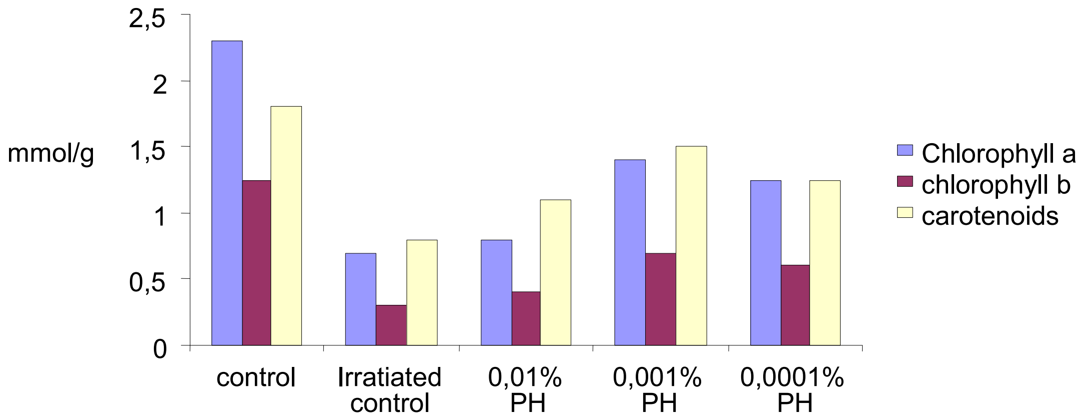

Figure 3: Effect of potassium humate solutions on the amount of chlorophyll pigments and carotenoids in seedlings obtained from gamma-irradiated wheat seeds.

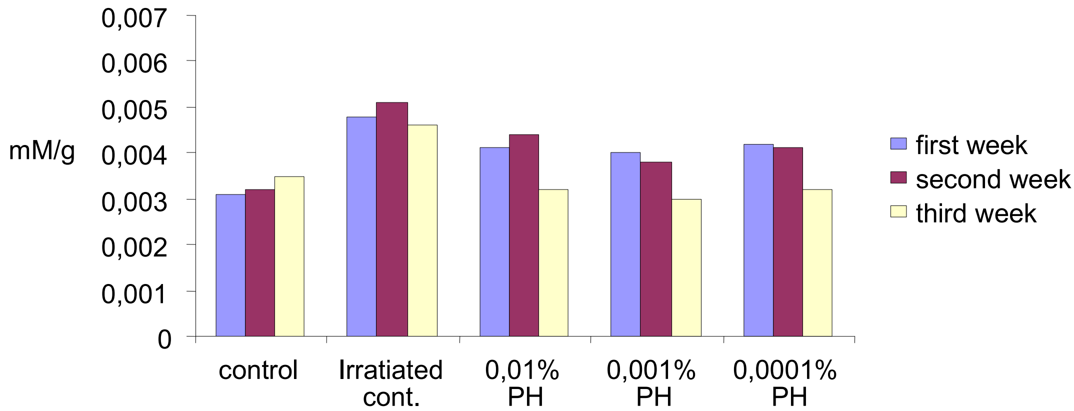

Figure 4: Effect of potassium humate solutions on the on the amount of malondialdehidein seedlings obtained from gamma-irradiated wheat seeds.

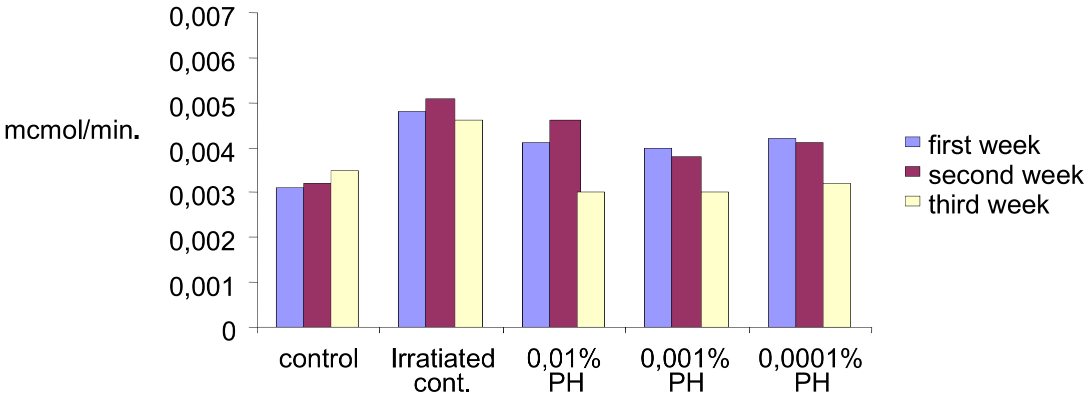

Figure 5: Effect of potassium humate solutions on the acnivity of catalase (CAT) in seedlings obtained from gamma-irradiated wheat seeds.

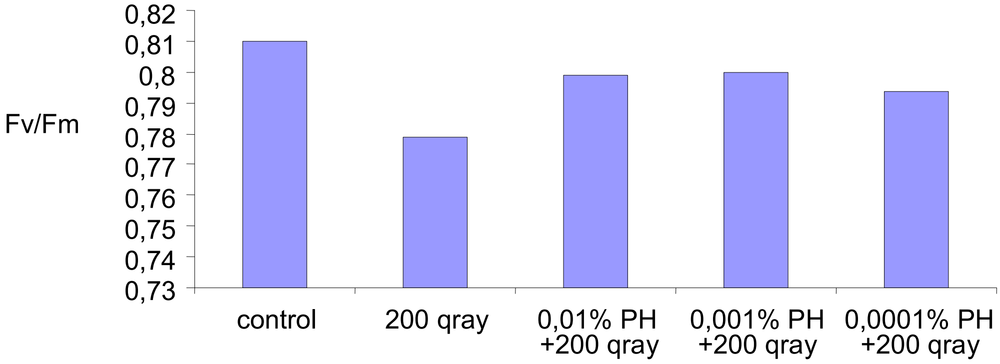

Figure 6: Effect of potassium humate solutions on the measurement of the maximum quantum yield of PS II - Fv / Fm (Y)in seedlings obtained from gamma-irradiated wheat seeds.

CONCLUSIONS

The results obtained in all experiments suggest that the use of potassium humate causes less damage to wheat seedlings grown from irradiated seeds, compared with seedlings obtained from irradiated samples not treated with humate solutions.On the basis of the obtained results, it was concluded that potassium humate solutions resulted in lower levels of lipid peroxidation products, and the maintenance of normal concentration of malondialdehyde, chlorophyll pigments. 0.001% solution of potassium humate showed the highest radioprotective activity, reducing the harmful effects gamma irradiation at a dose of 200 gray.

CONFLICTS OF INTEREST

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

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