



Effect of sodium and potassium humate in adaptive reaction of *Zea mays* under radiation stress

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Abstract

The effect of sodium and potassium humate on the activity of antioxidant system and photosynthetic pigment concentration of irradiated seeds was studied. The treatment of maize seed with 0.005% and 0.001% solutions of sodium and potassium humate caused significant reduction in processes initiated by radiation. A positive effect of sodium and potassium humate solutions on the amount of malon dialdehyde, chlorophyll pigments and carotenoids, fluorescent characteristics (the maximum quantum yield of PSII (photosystem II) in seedlings obtained from gamma-irradiated maize seeds was revealed.

Keywords: maize seedling, γ -irradiation, chlorophyll, lipid peroxidation, sodium humate, potassium humate malondialdehyde

Introduction

It is generally known that seeds during their germination are quite susceptible to the action of various environmental factors ^[1, 2]. This attribute facilitated the use of various physical or chemical agents prior to sowing, primarily focused on increase in productivity of agricultural plants ^[3].

Gamma irradiation damages initiated by free radicals are enhanced at the expense of reactive oxygen species (ROS) that cause oxidative modification of macromolecules, violation of the integrity of cellular structures. In lipids, mainly in polyunsaturated fatty acids, ROS cause chain reactions with accumulation of lipid, peroxyl, alkoxy and other radicals. Organisms are able to protect themselves from the damaging effects of free radicals due to highly active antioxidant system that includes low and high molecular substances capable inhibiting of free radical processes ^[5, 11] In recent years, in the radiotherapy treatment agents obtained from natural sources have been used. There were established stimulatory effect of humic compounds on the growth and development of plants, increasing their resistance to adverse environmental factors. Soluble forms of humates at low concentrations significantly stimulates protein and carbohydrate metabolism, increase crop yields. 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 radioactive 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. But their radioprotective function has not been studied enough. Under the protective effect of humic acids there are imply their ability to bind ions of radionuclides and heavy metals, and organic ecotoxicants to the stable complexes in contaminated water and soil environments. 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 ^[6-10]. Salts of humic acids with potassium, ammonium and iron protect plants under conditions of radiation and salt stress ^[11, 12]. Thus, obtaining and studying the biological activity and radioprotective properties of humic substances is quite actual direction.

Materials and methods

To obtain humate Na and K, the feedstock - peat - was treated with a 3% NaOH and KOH solution with constant stirring at a temperature of 35-40 degrees for 5 hours. After that, the dissolved sodium and potassium humates was separated in a centrifuge. The objects of research were maize seeds of the "Zagatala 68" variety. To study the radioprotective properties of humates, maize seeds were treated with solutions of sodium and potassium humate for 15 hours. Then the seeds were irradiated with a dose of 150 gray using the URĪ (K-25) device. In experiments to study the effect of sodium and potassium humate on the dynamics of growth and development of seedlings were used 0,005% and 0,001% solutions. Experiments to study the effect of sodium and 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, malonic dialdehyde, 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 95 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 maize 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 its discussion

At the beginning of the experiments were studied the effects of sodium and potassium humate solutions on the germination of seeds irradiated at a dose of 150 Gy. The germination rate of seeds that have not been treated with humate solutions has dropped to 62% due to radiation. As a result of irradiation, the germination rate of seeds was reduced by 40% compared to control. The germination rate of maize seeds irradiated at a dose of 150 Gray was - 76% in the variant treated with 0.005% humate solutions, 82% in the variant treated with 0.001% sodium and potassium humate solution.

The study of the dynamics of growth and development of seedlings obtained from gamma-irradiated maize seeds was carried out for 4 weeks. Seedling growth was measured every 4-5 days. The study of the effect of sodium and 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 of sodium and potassium humates solutions before irradiation.

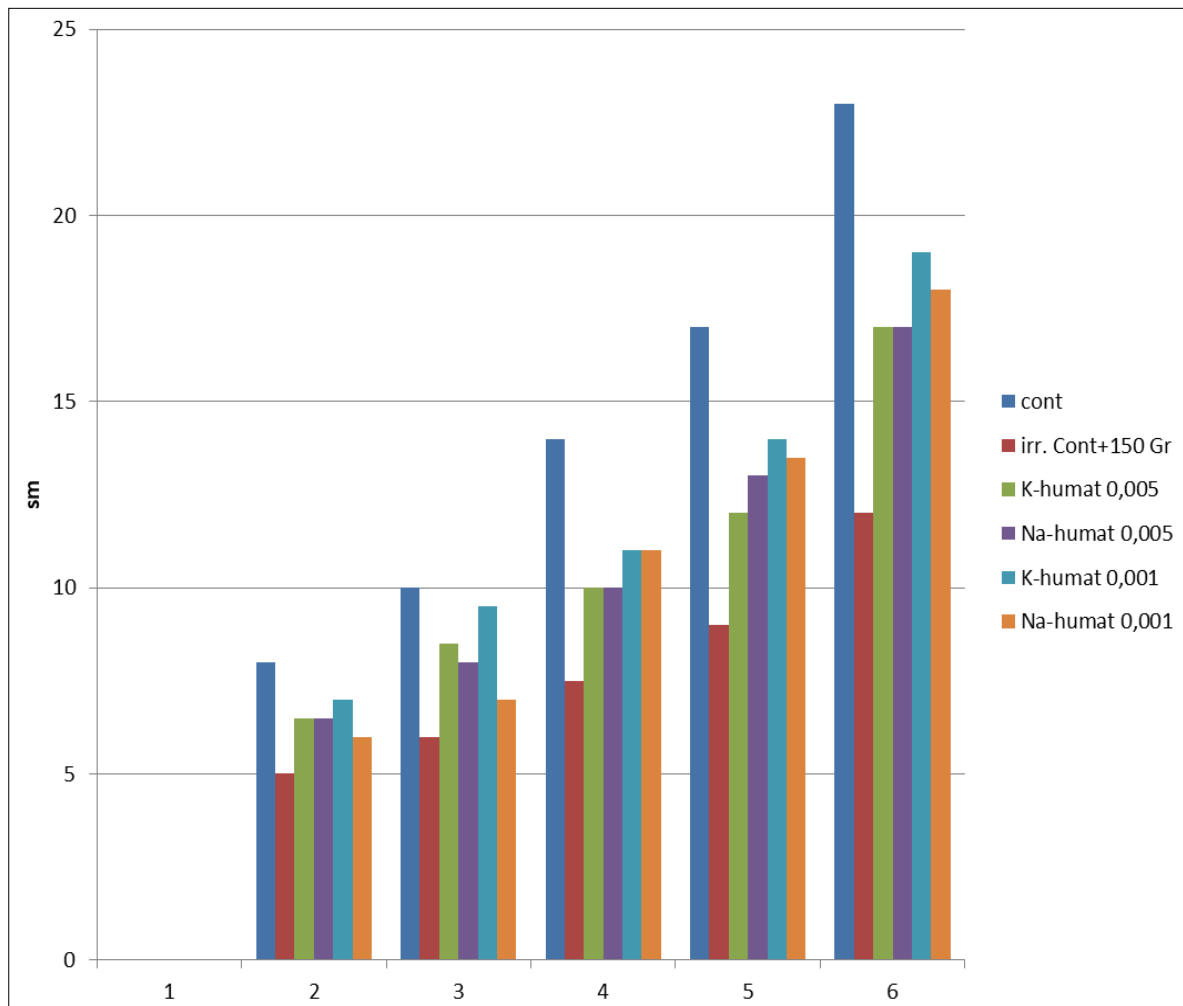


Fig 1: The effect of sodium and potassium humate solutions on the growth and development of seedlings obtained from gamma irradiated maize seeds (before irradiation).

The figure 1 clearly shows the effect of sodium and 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 0.001% of potassium and sodium

humate solutions before irradiation. But at further stages of development, the seedlings gradually equal each other.

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 CO₂ from the leaves. [13, 14]

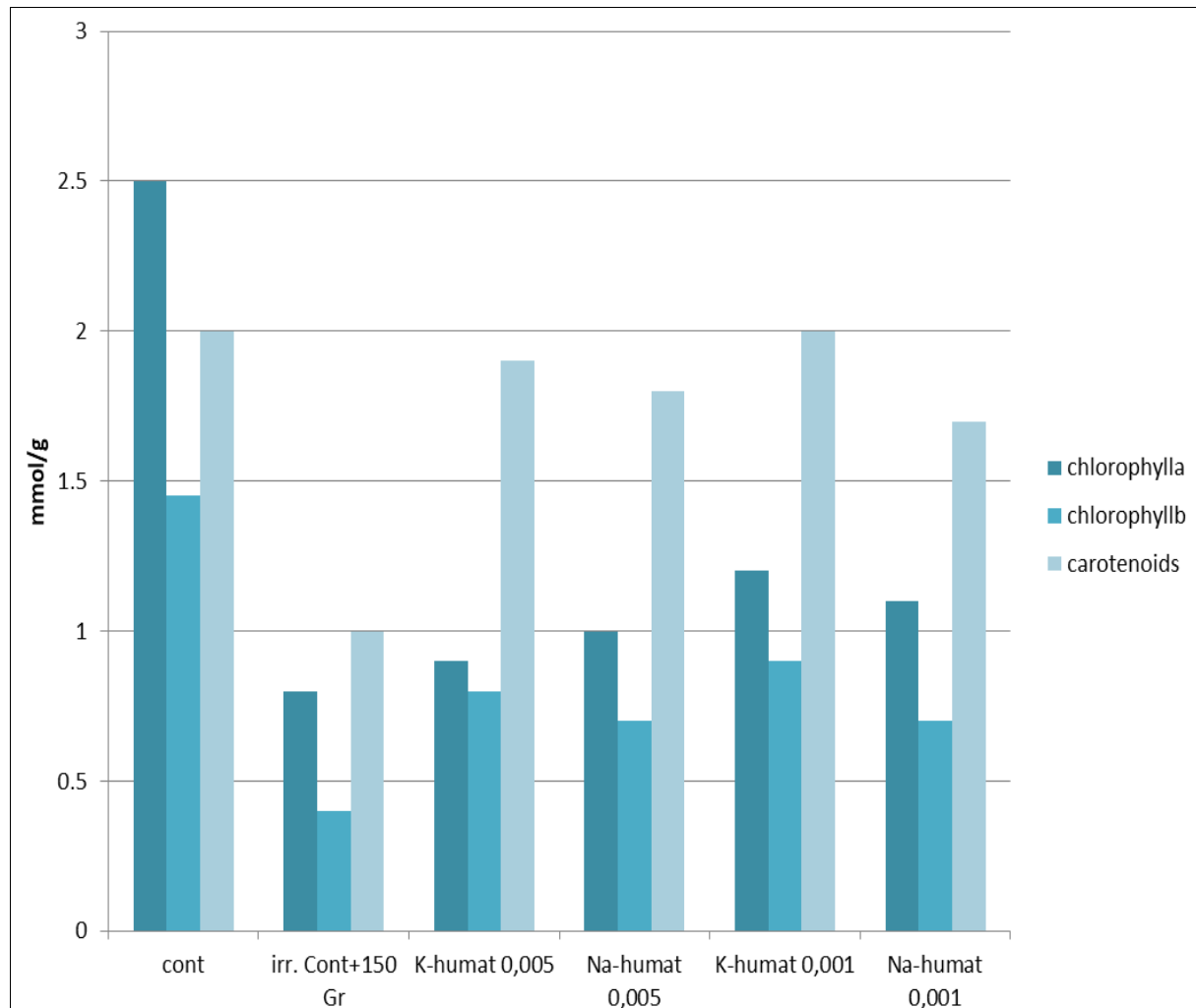


Fig 2: Effect of sodium and potassium humate solutions on the amount of chlorophyll pigments and carotenoids in seedlings obtained from gamma-irradiated maize seeds.

Experiments to determine the amount of photosynthetic pigments showed that the amount of chlorophyll pigments and carotenoids in seedlings obtained from seeds irradiated with 150 Gray decreased by an average of 55% compared with the control. In seedlings obtained from maize seeds treated with a sodium and 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 maize seedlings used as model plants. In the following experiments, the effect of sodium and 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 [23]. 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 maize 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.

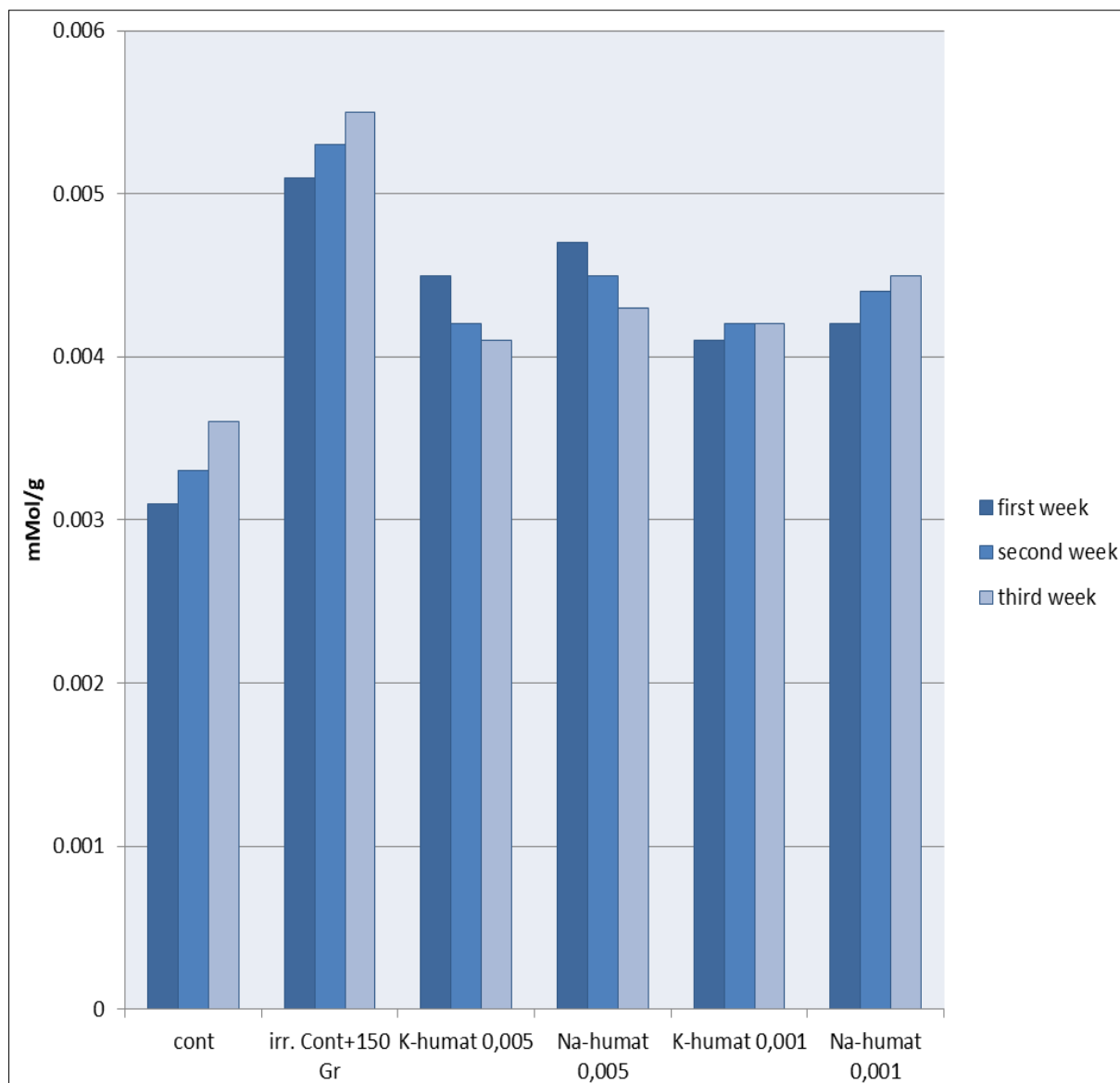


Fig 3: Effect of sodium and potassium humate solutions on the amount of malon dialdehyde in seedlings obtained from gamma-irradiated maize seeds.

On fig. 3 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 potassium humate solutions, 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 150 Gy 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 3, 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 [16]. 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 [17, 18].

In our experiments, the activity of the catalase enzyme noticeably increases when seeds are irradiated at a dose of 150 Gy during the first two weeks (Fig. 4). 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

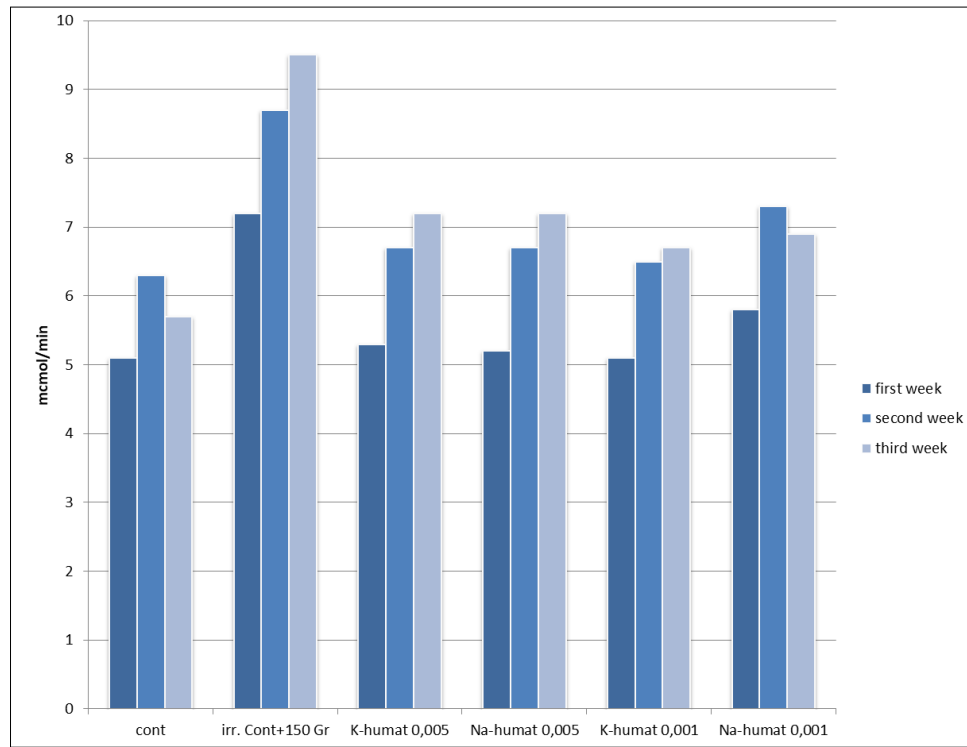


Fig 4: Effect of potassium humate solutions on the activity of catalase (CAT) in seedlings obtained from gamma-irradiated maize seeds.

The maximum yield of chlorophyll fluorescence - F_v/F_m reflects the maximum quantum efficiency of photosystem II (PSII). A decrease in this value indicates partial damage to PSII [19]. 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 fluorimeters allow accurate measurements of the photochemical quantum yield of photosystems based on the kinetics of chlorophyll fluorescence quenching [20, 21].

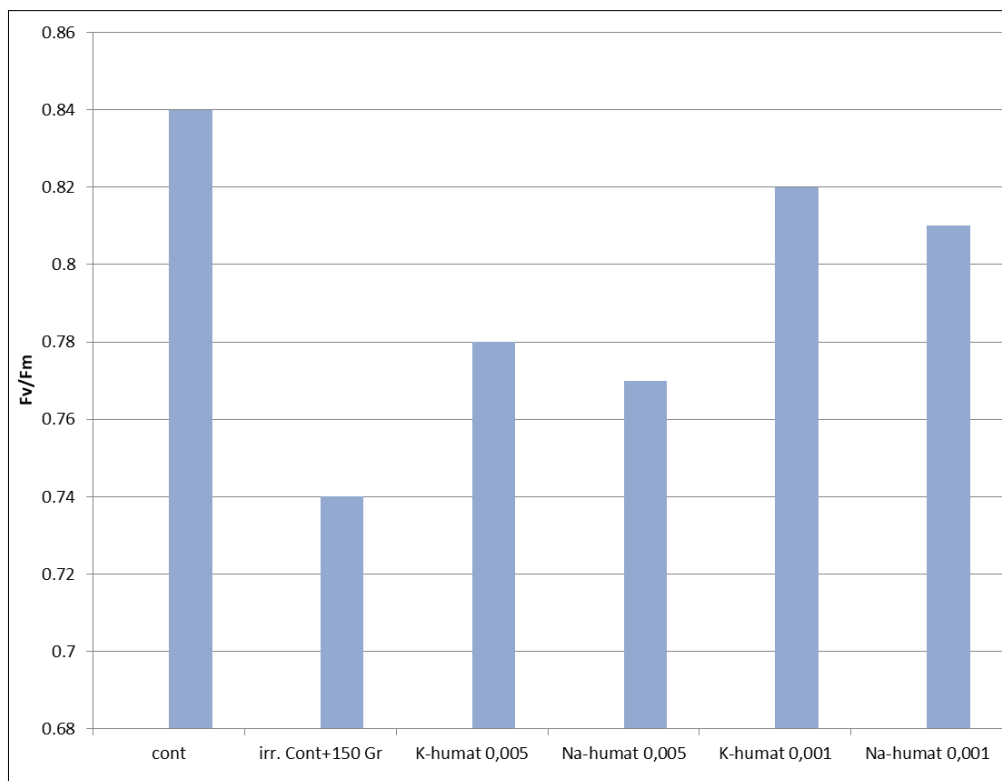


Fig 5: Effect of potassium humate solutions on the measurement of the maximum quantum yield of PS II - F_v / F_m (Y) in seedlings obtained from gamma-irradiated maize seeds.

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 maize 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 and sodium humate solutions on the photochemical activity of photosystem II in the leaves of maize seedlings, the seeds of which were irradiated at a dose of 150 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 0.001% solutions of potassium and sodium humates.

Conclusion

Thus in all experiments it was found that the use of potassium and sodium humate causes less damage to irradiated maize seedlings. The experiments solved the problems of determining the effect of sodium and potassium humate on seedling growth and development, productivity of chlorophyll pigments, catalase activity and studying the amount of malon dialdehyde. 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 and sodium humate showed the highest radioprotective activity. We suggest that the pre-sowing treatment of seeds with humate solutions can be used to increase tolerance to radiation stress.

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