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Research Article

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Influence of Metal Humic Complexes on Activity of Antioxidant System of γ-Irradiated Wheat Germs

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Abstract: The activity of the antioxidant system of seedlings obtained from γ- irradiated seeds of wheat were investigated. The germination in high doses of ionizing radiation seed were reduced and the lipid peroxidation one week-old seedlings were stimulated. In the further development of seedlings, differences in the growth and activity of the antioxidant system was leveled. In case treatment with metal humic complexes (Na humate, K humate and Fe humate) the damaging effects of ionizing radiation on plants were reduced.

Keywords: *wheat, γ-irradiation, lipid peroxidation, antioxidant activity, malon dialdehyde.*

1. Introduction

γ- irradiated 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 [Rogozhin V.V., Kuryliuk T.T., Filippova N.P., 2000]. In lipids, mainly in polyunsaturated fatty acids, ROS cause chain reactions with accumulation of lipid, peroxyl, alkoxyl 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 [Zenkov N.K., Menshikova E.B., 1993, Muslumova Z.H., 2013].

From the literature there are known many facts about the influence of gamma radiation on seed germination and plant development. It is known that under the influence of low doses of gamma-irradiation generally the germination of seeds increased, while under the influence of high doses the germination of seed decreased and development of seedlings suppressed. With increasing radiation dose various radiation damage repair system comes into effect and probably there are realized different adaptive strategies [Nikolaeva M.G., Razumova M.V., Gladkova V.N., 1985, Muslumova Z.H., Azizov I.V., 2013].

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 (monovalent metal salts) at low concentrations significantly stimulate the growth and development of plants, increase the flow of nutrients to plants and stimulates protein and carbohydrate metabolism, increase crop yields [Varshal G.M., Kashcheeva I.Y., Sirotkina

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I.S., 1979]. Influence of compounds of humic nature on the plants particularly noticeable at deviation of external conditions from norm: at an increased temperature, oxygen deficiency, low humidity, mismatch of environment pH to biological characteristics of plants [Kononova M.M., 1963]. About secondary metabolic ability this substance, might on common.

But their radioprotective function has not been studied enough. By the end of the twentieth century, which one of the urgent problems are chemical pollution of the environment and the development of new radioprotective substances. 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. Adaptogenic properties of humic substances deserve particular attention, due to their ability to bind radionuclides, heavy metals, pesticides, herbicides and other ecotoxicants. By preventing chlorosis iron humate also exhibits stimulating effect on plants [Sorkina T.A., Kulikova N.A., Filippova O.I , Lebedeva G.F., Perminova I.V., 2007, Stevenson F.J.,1982]. Last fact is very important due to the fact that the free form of toxicant has a maximum activity. Combined substance loses its toxicity. On this basis, the humic acids may be considered as natural detoxicants [UMKD "Environmental Physiology of Plants", 2008].

Thus, the preparation and study of biological activity and radioprotective properties of humic metal complexes (humates Na, K and humate Fe) is quite actual direction.

2. Materials and Methods

The object of the study was wheat seeds. The process of obtaining humates is sequential processing of the selected substrate (raw material) with weak solutions of alkalis. For obtaining of humates of K, Na initial raw material – peat were treated accordingly with 3% solutions of KOH and NaOH at constant stirring. Synthesis of iron humate carried out by adding a solution of ferrous sulfate with ascorbic acid in to the solution of humate at constant pH with subsequent drying of the obtained preparation [Humic acid metallic compound, preparation thereof, composition, preparations containing same and use of said compound. Patent PCT WO 2005/ 042551 Al.].

To study the radioprotective properties of the humates the seeds were treated with 0,001% solutions of K, Na and Fe humate for 15 hours. Thereafter, the seeds were exposed to uniform γ- irradiation from a ^{60}Co source at the “Rkhund” installation at dose of 200 Gy. Lipid peroxidation (LPO) is an indicator reaction of damage of cell membranes. As a result of LPO, there are formed final metabolites (malondialdehyde, ethane, pentane, etc.) that react with thiobarbituric acid (TBA - reacting products) [UMKD "Environmental Physiology of Plants", 2008].

On the change in the intensity of lipid peroxidation was judged by the amount of the secondary product of lipid peroxidation - MDA using the method of Costa et al [Costa H., Gallego S.M., Tomaro M.L., 2002]. The method is based on the fact that at high temperature in an acidic medium the MDA reacts with 2 - TBA forming pink trimethyl complex. The seeds were germinated in Petri dishes. To analyze the products of TBA and antioxidants, 1gr fresh weight of seedlings were homogenized in a porcelain mortar with a small amount of the reaction mixture, consisting of 0,25% solution of thiobarbituric acid (TBA). For better grinding there were added glass sand. The homogenate is transferred into a glass vial of with the small portions of reaction mixture. Samples were stirred and placed into a preheated up to 95°C water bath for

30 min. Then, the content sample was transferred to centrifuge tubes and centrifuged for 10 min at 10,000 g. Optical density was measured in a spectrophotometer Multiscan Go, Thermo Scientific Finland, at 532 nm and 600nm. The amount of TBA- reactive products are expressed in mM/gr of wet weight. Statistical processing of materials made by use of statistical means of MS Office Excel.

3. Results and Discussion

As seen in Figure 1, changes in the dynamics of accumulation of secondary products of lipid peroxidation in tissue culture of wheat after the influence of gamma radiation detected clearly enough. Amount of TBA- reactive substances in the tissue immediately after irradiation significantly increased. After two weeks, this difference was of less pronounced, and three weeks later the difference disappeared altogether.

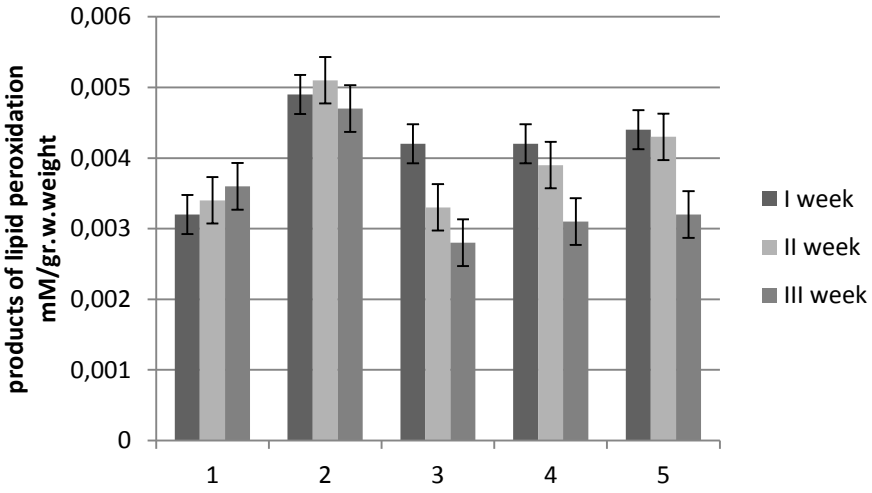

Figure 1. Influence of gamma radiation on lipid peroxidation , mM\gr.w.weight
1 – control; 2 – irradiated control; 3 – Na - humat 4 – K - humat; 5-Fe - humat

Literary data give the basis to assume that γ- irradiation may be an inducer of lipid peroxidation in wheat leaves. It was found that one of the primary responses of plant tissue to radiation expressed in elevated levels of secondary products of peroxidation.

In addition, to fully understand the effects of radiation on plant tissue, we also studied the dynamics of plant growth and seed germination.

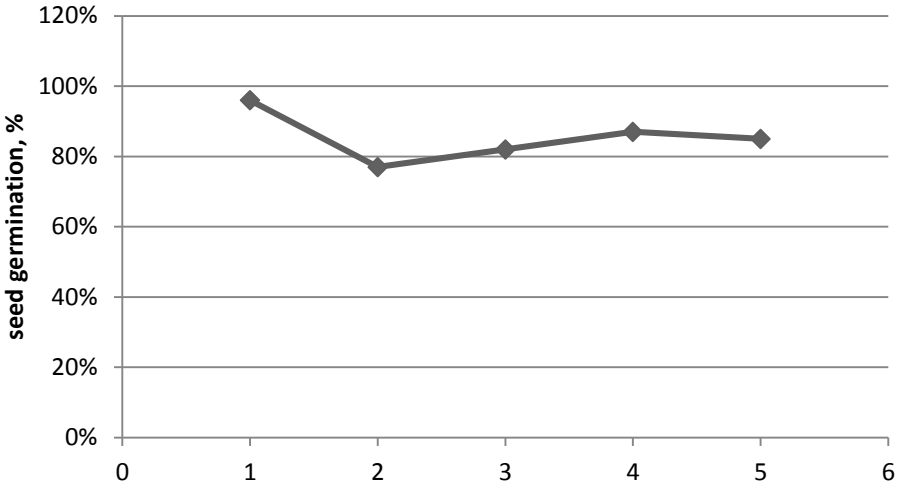

Figure 2. Influence of gamma radiation on seed germination.
1 – control; 2 – irradiated control; 3 – Na - humat 4 – K - humat; 5- Fe – humat

As seen in Figure 2 seeds irradiation of seeds with high doses reduces germination of seeds, seed treatment with humic complexes, compared with irradiated control improves seed germination. The best results were observed in seeds treated with K- humate.

As seen in Figure 3, the irradiation has not strongly pronounced effect on seedling growth, but inhibition action were observed. Seed treatment with K- humate gives the best result than other humates.

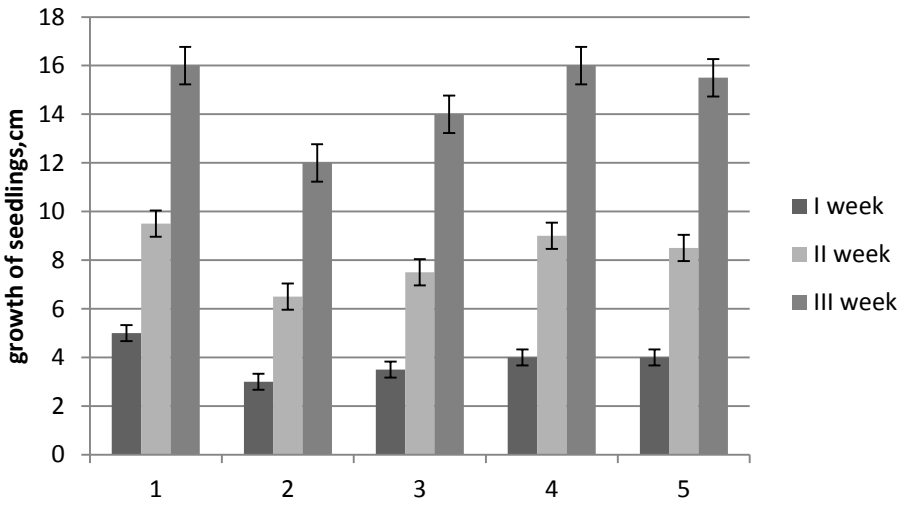

Figure 3. Influence of gamma radiation on growth of seedlings, cm
1 – control; 2 – irradiated control; 3 – Na - humat 4 – K - humat; 5- Fe - humat

We have shown that the irradiation of seeds in high doses increases the content of hydrogen peroxide, superoxide and hydroxyl radicals and thus activates lipid peroxidation and causes the activation of the antioxidant system of plants. The germination in high doses of ionizing radiation seed were reduced. Our date reveals that pretreatment of wheat seeds with humic complexes of different metals reduces the effect of radiation on seed germination and plant

growth. Treatment of seed with these complexes also inhibits the activation of lipid peroxidation and thus protect plants from the damaging effects of ionizing radiation. Among the used substances most effective was the potassium salt of humic acid.

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