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EFFECT OF RADIOPROTECTORS ON POST-RADIATION REPARATION

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Abstract: Investigated the antioxidant activity of seedlings obtained from γ -irradiated seeds of maize. High doses of ionizing radiation reduced germination and stimulated lipid peroxidation in one-week seedlings. However, in the further development of seedlings differences in growth and activity of antioxidant system leveled. Obtained metal complexes of humic acids (sodium, potassium and ferric humates) reduced the damaging effects of ionizing radiation.

Key words: maize, γ -irradiation, lipid peroxidation, antioxidant activity, malon dialdehyde.

1. Introduction

It is known that radiation has a damaging effect on biological systems. Damage initiated by free radicals is 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, peroxy, 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 to inhibit free radical processes [Zenkov N.K., Menshikova E.B., 1993, Muslumova Z.H., 2013].

From literature many facts concerning influence of gamma radiation on seed germination and plant development are known. 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 is suppressed. With increasing radiation dose various repair systems of radiation damage are launched and probably different adaptive strategies are realized [Nikolaeva M.G., Razumova M.V., Gladkova V.N., 1985, Muslumova Z.H., Azizov I.V., 2013].

Stimulatory effects of humic compounds on growth and development of plants, increasing their resistance to adverse environmental factors were revealed. Soluble forms of humates (monovalent metal salts) at low concentrations significantly stimulate the growth and development of plants, increase flow of nutrients to plants and stimulate protein and carbohydrate metabolism, increase crop yields [Varshal G.M., Kashcheeva I.Y., Sirotkina I.S., 1979]. Influence of compounds of humic nature on the plants is particularly noticeable at deviation of values 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.

However their radioprotective functions have been poorly studied. By the end of the twentieth century chemical pollution of the environment and development of new radioprotective substances is one of the urgent problems. Humic acids, perhaps, realize their protective effect through binding ions of radionuclides and heavy metals, and organic ecotoxicants to form 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. Complex substance loses its toxicity. On this basis, the humic acids may be considered as natural detoxicants [UMKD "Environmental Physiology of Plants", 2008].

So, preparation and study of biological activity and radioprotective properties of humic metal complexes (sodium, potassium and ferric humates) is quite an actual problem.

2. Materials and methods

The object of the study was maize seeds. The process of obtaining humates is sequential processing of the selected substrate (raw material) with weak solutions of alkalis. To obtaining humates of potassium, sodium initial raw material – peat was treated accordingly with 3% solutions of KOH and NaOH under constant stirring. Synthesis of iron humate was 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 A1.].

While studying radioprotective properties of the humates, seeds were treated with 0.001% solutions of potassium, sodium and ferric humates for 15 hours. Thereafter, the seeds were exposed to even γ - irradiation from a ^{60}Co source at the “Rkhund” installation at a dose of 200 Gy. Lipid peroxidation (LPO) is an indicator of cell membranes damage. As a result of LPO, final metabolites (malondialdehyde, ethane, pentane, etc.) were formed which, in turn, reacted with thiobarbituric acid (TBA - reacting products) [UMKD "Environmental Physiology of Plants", 2008].

Changes in the intensity of lipid peroxidation were evaluated by measuring the amount of the secondary product of lipid peroxidation - MDA using the method by 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 MDA reacts with 2 - TBA forming pink trimethyl complex. The seeds were germinated in Petri dishes. To analyze the products of TBA and antioxidants, 1g 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 glass sand was added. The homogenate was transferred into a glass vial of with the small portions of reaction mixture. Samples were stirred and placed into a pre-heated 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 600 nm. The amount of TBA-reactive products were expressed in mM/g of wet weight. Statistical processing of materials was realized with application of statistical means of MS Office Excel.

3. Results and discussion

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

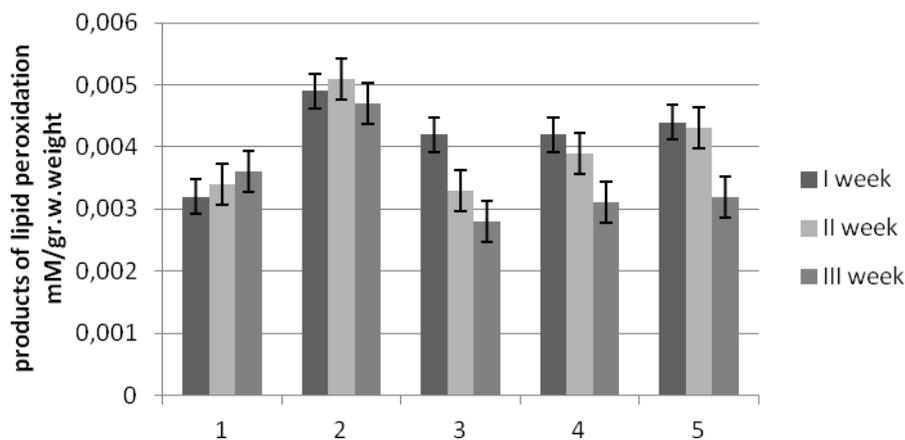


Fig. 1. Influence of gamma radiation on lipid peroxidation, mM/gr.w.weight
 1 – control; 2 – irradiated control; 3 – sodium humate; 4 – potassium humate; 5- ferric humate

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

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

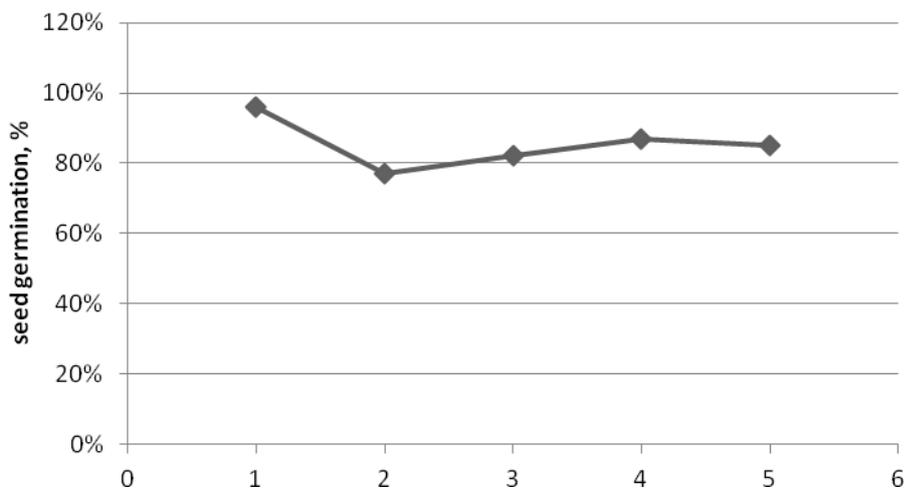


Fig. 2. Influence of gamma radiation on seed germination.
 1 – control; 2 – irradiated control; 3 – sodium humate; 4 – potassium humate; 5- ferric humate

As seen from Fig. 2, irradiation of seeds with high doses reduced germination of seeds, where as seed treatment with humic complexes compared with irradiated control improves seed germination. The best results were observed in seeds treated with potassium humate.

As seen from Fig. 3, the irradiation did not have strong pronounced effect on seedling growth, but inhibition effect were observed. Seed treatment with potassium humate gave the best result than other humates.

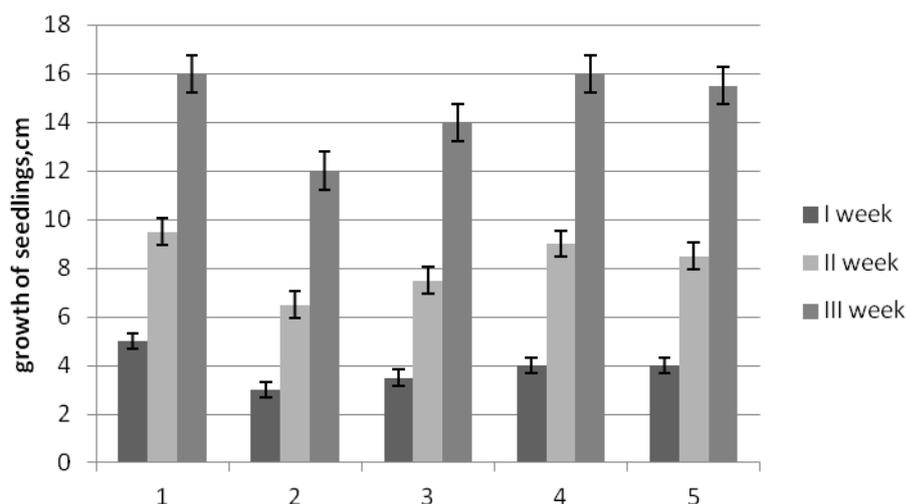


Fig. 3. Influence of gamma radiation on growth of seedlings, cm
 1 – control; 2 – irradiated control; 3 – sodium humate; 4 – potassium humate; 5- ferric humate

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 was reduced. Our data reveal that pretreatment of maize seeds with humic complexes of different metals reduces the effects of radiation on seed germination and plant growth. Treatment of seed with these complexes also inhibits the activation of lipid peroxidation and this way protects plants from the damaging effects of ionizing radiation. Among the used substances the most effective was the potassium salt of humic acid.

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ВЛИЯНИЕ РАДИОПРОТЕКТОРОВ НА ПОСТ-РАДИАЦИОННУЮ РЕПАРАЦИЮ

З.Г. Муслюмова

Резюме: Исследована антиоксидантная активность саженцев, полученных из γ -облученных семян кукурузы. Высокие дозы ионизирующего излучения снижают прорастание и стимулируют перекисное окисление липидов в однонедельных проростках. Однако, в дальнейшем развитии саженцев различия в росте и активности антиоксидантной системы нивелируется. Полученные комплексы металлов гуминовых кислот (натриевые, калиевые и железистые гуматы) снижают вредное воздействие ионизирующего излучения.

Ключевые Слова: Кукуруза, γ -облучение, перекисное окисление липидов, антиоксидантная активность, малоновый диальдегид

RADIOPROTEKTORLARIN RADIASIYADAN SONRAKI REPARASIYA PROSESLƏRİNƏ TƏSİRİ

Z.H. Müslümova

Hülasə: γ -şüalarla şüalandırılmış qarğıdalı toxumlarından alınmış cücərtilərin antioksidant aktivlikləri tədqiq olunmuşdur. İonlaşdırıcı radiasiyanın yüksək dozaları bir həftəlik cücərtilərdə boy artımı və inkişafı zəiflətməmiş, lipidlərin peroksid oksidləşməsi prosesini isə stimullaşdırmışdır. Sonrakı inkişaf mərhələsində boy artımı və antioksidant sistemdəki fərqlər azalmışdır. Humin turşularından alınmış metal kompleksləri (kalsium, kalium və dəmir) ionlaşdırıcı şüalanmanın zədələyici effektini aşağı salmışdır.

Açar sözlər: qarğıdalı, γ -şüalanma, lipidlərin peroksid oksidləşməsi, antioksidant aktivliyi, malon dialdeqidi