European Journal of Biotechnology and Bioscience
ISSN: 2321-9122; Impact Factor: RJIF 5.44
Received: 27-11-2020; Accepted: 12-12-2020; Published: 28-12-2020
www.biosciencejournals.com
Volume 8; Issue 6; 2020; Page No. 40-42

Epr study of plants under the influence of radiation factors

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Abstract
In this paper, by using the method of Electron Paramagnetic Resonance (EPR) spectroscopy, the influence of some stress factors (ionizing gamma radiation, radioactive contamination, and UV radiation) on C-3 (wheat) and C-4 (corn) plants has been investigated. Under the influence of stress factors on C-3 plants, a stimulating effect is observed in their development, and in C-4 plants, stress factors have the opposite, inhibitory effect on the development of these plants. It is assumed that this process is related with photorespiration - with a protective system that prevents an increase in reactive oxygen species (ROS) during stress in type C-3 plants. In addition, the work shows that the stress factor has a stimulating effect on the generation of biogenic nanophase magnetic particles (Fe_3O_4 - magnetite and γ-Fe_2O_3 - maghemite) and leads to the appearance of anomalous magnetic properties in living systems. This effect can be used in the synthesis of functional magnetic iron oxide nanoparticles. The results show that stress factors play a stimulating role in the formation of paramagnetic centers in biological systems. This effect of exposure can be used as a bioindication parameter in environmental assessment and monitoring.

Keywords: radiation, radioactive contamination, stress factors, photorespiration, EPR signals

Introduction
At present the study of the effects of ionizing radiation is becoming an important task. Currently a large amount of data has been accumulated on the effects of ionizing radiation on plant growth and reproduction, and also about changes caused by ionizing radiation at the genetic level. Primary physicochemical reactions caused by ionizing radiation, including the formation of various reactive oxygen species (ROS), are the cause of the observed changes in the functional activity of plants. We have studied the influence of some stress factors (ionizing gamma radiation, radioactive contamination and UV radiation) on plants.

Materials and methods
The objects of our research were plants C-3 - wheat (Triticum L.) and C-4 - corn (Zea Mays L.). The studies were carried out by EPR spectroscopy (BRUKER - EMXPlus). When studying the effect of radioactive contamination and ionizing gamma radiation on the paramagnetic centers in Triticum L. and Zea Mays L. plants, morphological changes occurring in plants during these influences are of great interest. The morphological diversity and observed pattern in C-3 and C-4 plants during stress were confirmed in EPR studies. The role of photorespiration in the results obtained is not excluded. In the experiments, wheat and corn seeds were irradiated in different doses (50 Gy, 100 Gy, 200 Gy, 300 Gy) using a K-25 irradiation device. Then they were grown for 10 days at room temperature and under natural conditions (Fig. 1). When studying the effect of radioactive contamination, wheat and corn seeds were germinated in clean (control) and radioactively contaminated soil (Fig. 2).

Results and Discussion
Figures 1 and 2 clearly show that the influence of ionizing gamma radiation and radioactive contamination have a stimulating effect on the growth of wheat, which belongs to C-3 plants. In these plants, when exposed to stress, growth improves and the percentage of germination increases. But in corn plants we see the opposite. Stress factors have an inhibitory effect on corn.

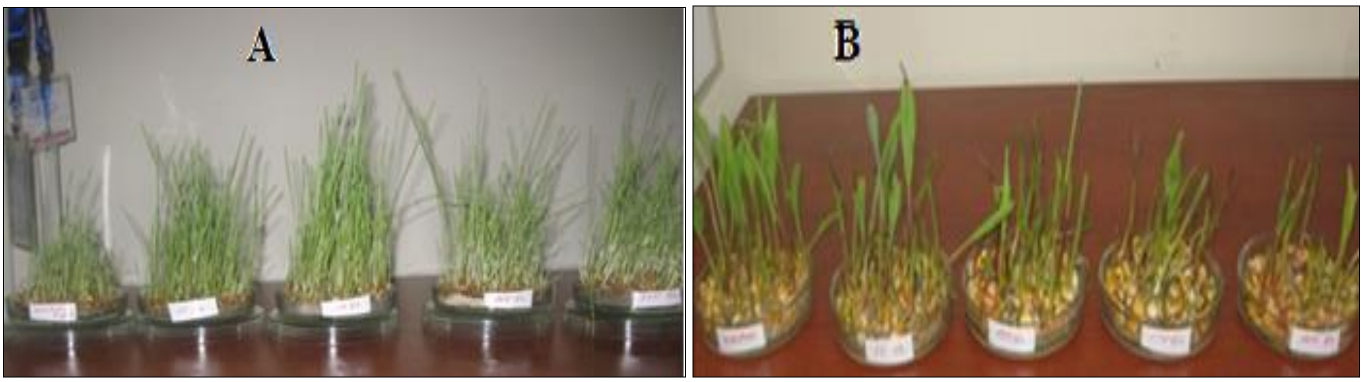

Fig 1: 10-days-old seedlings of control and irradiated with different doses of ionizing gamma radiation seeds of wheat (A) and corn (B)

We assume that photorespiration plays an important role in the resulting result. Analysis of our results and literature data indicates the high biological significance of the glycolate pathway of photorespiration. Photorespiration is a mechanism that removes molecular oxygen from the sensitizer site and reduces the formation of electrons [1, 2]. When exposed to stress in C-3 plants, photorespiration acts as a defense system, decreasing the concentration of ROS. According to the literature, it is known that photorespiration is enhanced under conditions when too many carbohydrate products of photosynthesis are formed. This condition leads to the oxidation of "excess" sugar and amino acids are formed. Thus, if, for some reason, with the use of the products of the light stage of photosynthesis in dark reactions, difficulties arise in the reduction of carbon dioxide or with the consumption of sugars, then the photooxidative processes are enhanced. As a result, no sucrose is formed, but amino acids are formed, which are used for growth processes in the leaf [3, 4, 5]. In our experiments, wheat seedlings under the influence of ionizing gamma radiation, as well as under radioactive contamination, significantly increase.

Fig 2: 10-days-old seedlings of wheat (A) and corn (B) seeds grown in control and radioactively contaminated soils

As for C-4 plants (in our case, corn), as it is known, they don't have a pronounced photorespiration [6, 7, 8]. Plants C-4 have a special structure and anatomy. The fact is that they have chloroplasts, which are present not only in the mesophyll cells, but also in the sheath cells. In the mesophyll cells, CO_2 assimilation takes place and a four-carbon compound is formed, which are then transferred to the sheath cells. In the cells of the mesophyll, CO_2 is absorbed, and in the cells of the sheath, it is restored to sugars. We believe that here the mesophyll cells play the role of a "transformer" regulating the absorption of CO_2 by the sheath cells. In the cells of the sheath, CO_2 is assimilated according to the usual mechanism of C-3 plants, but already at increased concentration of CO_2, formed during decarboxylation of malic or aspartic acid. This fact explains the absence of visible photorespiration in C-4 plants [1, 3, 4].

Then, by EPR spectroscopy, the spectra of the studied plant species were recorded. Under the influence of stress factors on plants, it was found that increase of the dose ionizing gamma radiation leads to the formation of broad EPR signals, which are characteristic of nanophase iron oxide magnetic particles ($g = 2.32$; $\Delta H = 320$Gs) and an increase in the intensity of the free radical signal ($g = 2.0023$) (Fig. 3A).

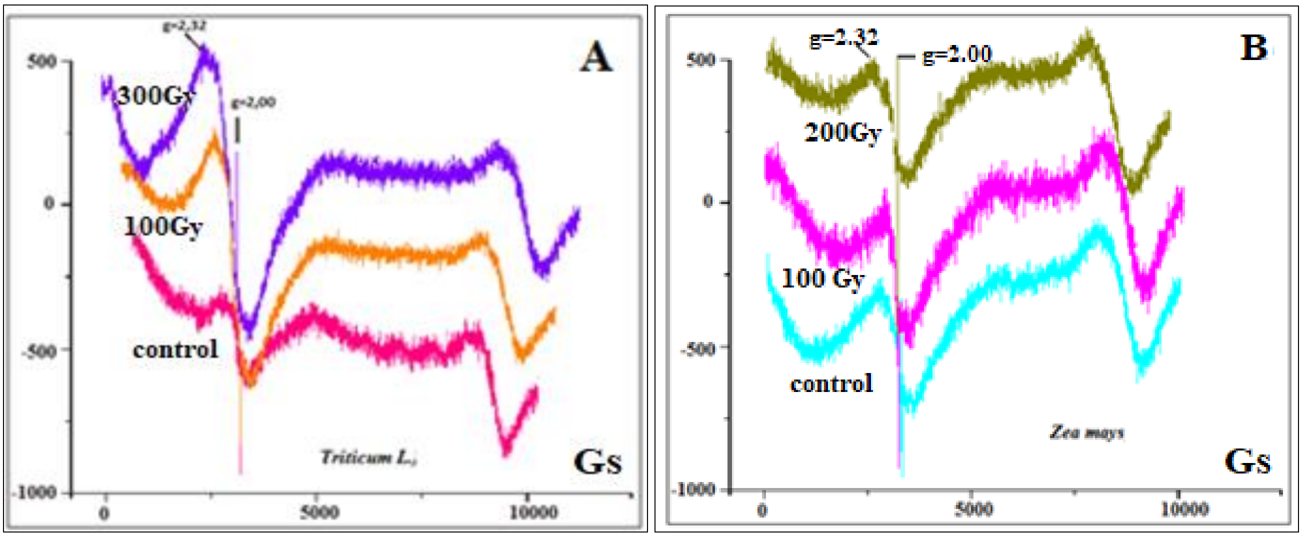

Fig 3: EPR spectra of seedlings of wheat (A) and corn (B) seeds irradiated with different doses of ionizing gamma radiation

The obtained results allow us to assume that a moderate dose of radiation stimulates the formation of magnetic iron oxide nanoparticles in plants [9, 10]. There is every reason to believe that the formation of magnetic nanoparticles giving broad lines in the EPR spectrum is associated with the operation of the photosynthetic apparatus in plant leaves. It can be assumed that the stimulation of the formation of magnetic nanoparticles in plants exposed to radiation may be associated with a partial disturbance of chloroplast intactness due to the action of gamma radiation. In this case, the accessibility of exogenous sources of nanoparticle formation (for example, iron ions) to the electron transport chains of chloroplasts will increase, thereby stimulating the formation of nanoparticles. Hence, as a result of biomineralization processes under the influence of stress factors, biogenic formation of magnetic nanoparticles occurs in plant systems, which leads to the formation and increase of their signals [10, 11, 12].

Such influence effects did not impact on the EPR spectra of Corn (Fig. 3B). This result is explained by the fact that the stress factor in C-4 plants suppresses the electron transport chain in thylakoid membranes. Despite the fact that the number of free iron ions increases, the number of electrons decreases. Therefore, the number of forming nanoparticles of iron ions does not change.

The results of our experimental work allow us to evaluate photorespiration as one of the key homeostatic defense mechanisms of plants. It is due to photorespiration that the rate of ROS formation decreases. In addition, in the glycolate pathways of photorespiration, energy costs and needs in reducing units of photosynthesis increase, so that they contribute to the protection of the electron transport chain.

Also, research results have shown that stress factors play a stimulating role in the creation of paramagnetic centers in plant systems. This effect can be used as a bioindication parameter in environmental assessment of the environment.

Experiments using EPR spectroscopy have shown that this method is a very promising method for detecting paramagnetic centers in plant systems and can provide new information in environmental assessment and biomonitoring [13-15]. The results will allow us to better understand the origin and role of biogenic paramagnetic centers in biological systems.

The author is grateful to prof. RI Khalilov for the helpful advice given in discussing the results of the experimental work.

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