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STUDY OF THE RADIOPROTECTIVE PROPERTIES OF RUTIN AND ITS COMPLEXES IN PLANT MODEL SYSTEMS

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Abstract: The radioprotective properties of complexes of rutin with iron, cobalt, ammonium and nickel have been studied. In model experiments with plants, the effect of rutin complexes on growth and development, on the number of photosynthetic pigments and malondialdehyde yield in seedlings of gamma-irradiated wheat seeds was investigated. The effect of the complexes on photosynthetic activity and the frequency of chromosomal aberrations was also studied.

Keywords: radioprotectors, complexes, photosynthetic pigments, malondialdehyde, frequency of chromosomal aberrations.

1. Introduction

In recent years, natural radioprotectors have been widely studied for their harmless and long-lasting effects. Studies have shown that Fe, Mn, Ni, Co complexes prevent the effects of ionizing radiation on the body. In the body, metals are involved in many biochemical processes in the form of complex compounds. Deficiency or excess of them leads to dysfunction of metal-containing enzymes. On the other hand, complexes containing metals are a source of micronutrient supply to the body. Studies in model systems have shown that complex compounds transform free radicals in inactive forms [1,2,3,4].

The biological effect of flavonoids is explained by the regulation of redox processes, stabilization of cell membranes, and modulation of the activity of enzymes and receptors. The anti-stress, anti-inflammatory, anti-fungal, anti-bacterial, antiviral, anti-ulcer, anti-toxic, anti-allergic, anti-arrhythmic, immunomodulatory, anti-carcinogenic, nephroprotective, the hepatoprotective spectrum of action of these compounds in the human body have been determined. These properties of flavonoids open up wide opportunities for their use as drugs that do not have serious side effects, unlike synthetic analogus [5, 6, 7, 8, 9].

2. Material and methods

The objects of the study were wheat seeds of the Barakatli 95 variety of the experimental base of the Research Institute of Agriculture. Wheat seeds were irradiated with a dose of 200 Gy on a Rhund 20000 device, the radiation source was 60Co. To study the prophylactic effect of complex compounds, wheat seeds were treated with 0.01% solutions of rutin complexes of cobalt, nickel and iron for 15 hours. After that, the seeds were dried and irradiated. The germination rate of seeds used in the experiments was 96%.

To determine the content of chlorophyll and carotenoids in the leaves of the studied plants, a sample of raw leaves was taken. Absorption was measured on a spectrophotometer Multiscan GO at wavelengths of 662, 644, and 440.5 nm. Served as control 80% acetone. Then, using Polevoy's formulas, the values of chlorophylls a and b, total carotenoids were calculated from the optical density of the solution.

Rutin was obtained from the plant Sophora japonica L. according to the known literature: the flowers of the plant were boiled in a Soxhlet apparatus with 70% ethanol solution for 2 hours. Then the solvent was evaporated in a vacuum in a water bath. The dry precipitate was treated with ether to remove resinous substances. After the ether had evaporated, the routine was separated with water. After cooling, the precipitated rutin crystals were separated by decantation. Complexes of rutin with iron, cobalt and nickel were obtained by a well-known method.

To study chromosomal aberrations, seeds were germinated on wet filter paper in Petri dishes at 24–28 ºC. When primary roots with a length of 1.0-1.5 cm appeared, they were fixed in a Carnoy mixture (ethyl alcohol: glacial acetic acid in a ratio of 3: 1) and stained with aceto-orcein. On cytological preparations, the number of aberrant anaphases was counted and the amount of CA per cell was determined. The frequency of aberrant anaphases was expressed as a percentage of the corresponding phases of mitosis examined (200 for each variant). For statistical processing of the data obtained, td and p were calculated according to the generally accepted method.

3. Results and Discussions

At the beginning of the study, we studied the germination of seeds treated with rutin complexes and irradiated at a dose of 200 gray

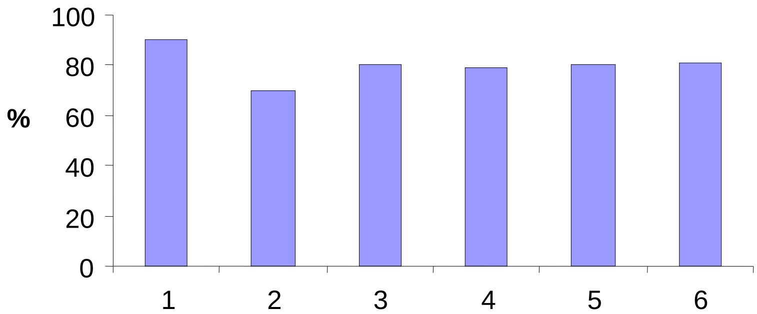

Fig. 1. Germination of irradiated seeds at a dose of 200 Gray: 1 - control, 2 - irradiated control, 3 - ammonium rutinate, 4 - cobalt rutinate, 5 - nickel rutinate, 6 - iron rutinate.

As can be seen from the figure, irradiation of seeds at a dose of 200 g reduces their germination. In the irradiated version, seed germination is reduced by up to 40% compared to the control. Treatment of seeds with solutions of complexes at a concentration of 0.001% before irradiation increased seed germination. From the experiments, it can be concluded that the treatment of seeds with the studied complexes with rutin before irradiation increases their resistance to ionizing radiation.

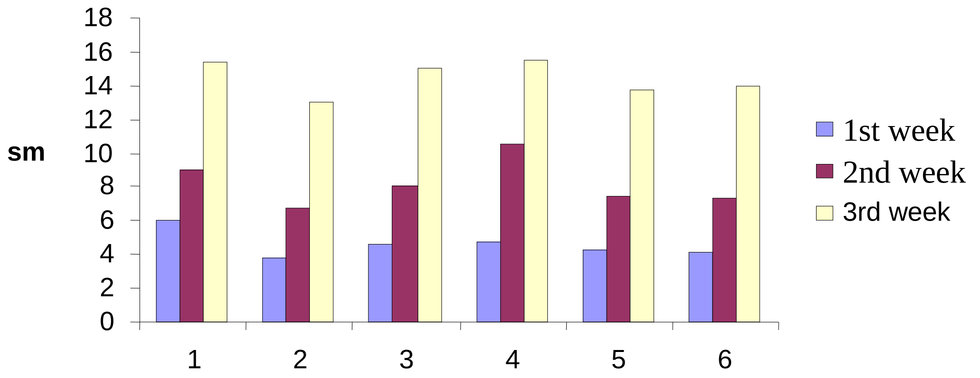

Fig. 2 Dynamics effect of complexes on the growth of seedlings obtained from irradiated seeds at a dose of 200 Gray (cm): 1 - control, 2 - irradiated control, 3 - ammonium rutinate, 4 - cobalt rutinate, 5 - nickel rutinate, 6 - iron rutinate.

As can be seen from Figure 2, in all variants there is a positive effect of rutin complexes on plant growth compared to the control irradiated variant. The variant with cobalt rutinate showed the best result in these studies.

In sprouts obtained from irradiated seeds, it is important to determine the number of photosynthetic pigments to determine the degree of damage to plants. We determined the number of photosynthetic pigments in sprouts obtained from seeds, irradiated in a dose of 200 grays. At the same time, an increase in the number of carotenoids occurs due to the activation of protective mechanisms in plants. In experiments, a relative increase in carotenoids in the affected variants may explain the activation of the repair system. To study the protective effect of rutin and its complexes of ammonium, cobalt, nickel and iron during irradiation, wheat seeds were treated in 0.001% solutions of these complexes and treated for 15 hours. Then the seeds were dried and irradiated with a dose of 200 Gray. The dynamics of chlorophyll a, chlorophyll b and carotenoid pigments were studied sequentially every week. The results are shown in the histogram below (Figure 3).

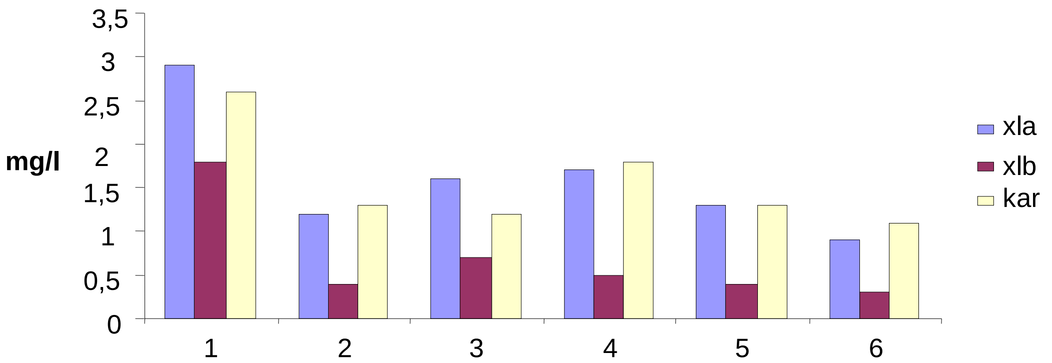

Fig. 3. The effect of routines on the number of photosynthetic pigments in wheat germ (mg / l): 1 - control, 2 - irradiated control, 3 - ammonium rutinate, 4 - cobalt rutinate, 5 - nickel rutinate, 6 - iron rutinate

Radiation at a dose of 200 Gray resulted in a 60% reduction in the biosynthesis of photosynthetic pigments compared to controls in seedlings derived from wheat seed .The mount of reduction in the biosynthesis of photosynthetic pigments in cobalt rutinate and ammonium rutinate was 42 - 45%. There was almost no difference in the biosynthesis of photosynthetic pigments compared to the radiation control in the nickel rutinate variant. The numder of pigments in this variant was 57%. In the variant treated with iron routine, there is a decrease in pigment biosynthesis compared to the control. As can be seen from the histogram, the amount of carotenoids in the irradiated variants was higher than the number of chlorophyll a and chlorophyll b. This can be explained by the fact that carotenoids perform certain protective functions in the plant organism, the biosynthesis of carotenoids is enhanced by radiation. At the same time, the amount of chlorophyll a and chlorophyll b increases in the irradiated variants and reaches peaks in the variant with iron rutinate.

From the experiments, it can be concluded that irradiation at a dose of 200 Gray negatively affects the biosynthesis of photosynthetic pigments in wheat germ. Solutions of rutin and its various complexes have a positive effect on the growth and development of seedlings, and also significantly reduce the harmful effects of radiation. From the complexes used in the experiments, the complexes of rutin, ammonium and cobalt have more effective radioprotective effects.

In the next studies, we studied the process of lipid peroxidation and the process of antioxidant protection, which is one of the components of the initial cell response to ionizing radiation. According to the literature, it can be assumed that gamma radiation acts as an inducer of the process of lipid peroxidation in wheat tissues. That is, the initial response of plant tissue to radiation is manifested in an increase in the secondary products of lipid peroxidation [10,11]. The results of these studies are presented below. The tests were carried out 1, 2 and 3 weeks after sowing. Seedlings of unirradiated seeds without radioprotectors were taken as controls.

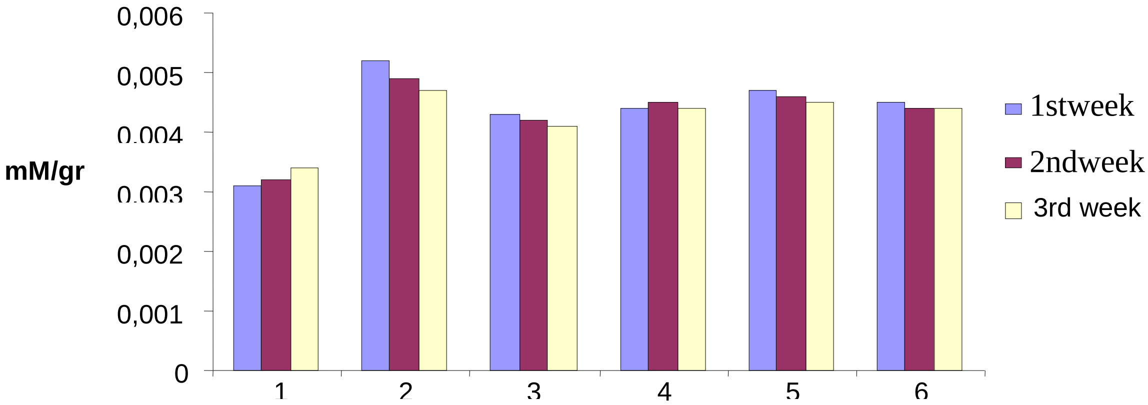

Fig. 4. Dynamics of lipid peroxidation in seedlings obtained from irradiated wheat seeds at a dose of 200 gray: 1 - control, 2 - irradiated control, 3 - ammonium rutinate, 4 - cobalt rutinate, 5 - nickel rutinate, 6 - iron rutinate.

As can be seen from Figure 4, the change in the dynamics of the accumulation of secondary products of the lipid peroxidation process after exposure to gamma radiation is clearly visible. Immediately after irradiation, the number of products that react with thiobarbituric acid in the tissues increased significantly. After two weeks, this difference is less noticeable, and in the third week it almost decreases. If we look at the variants treated with different rutin complexes, we will see that the amount of lipid peroxide oxidation products in the variant treated with ammonium rutinate is less than in other variants. Increasing the number of secondary products of lipid peroxidation is the initial reaction of plant tissue to radiation.

Many studies have shown disturbances in the electron transport chain in photosystem II under stress [12,13,14]. Table 1 shows the effect of gamma radiation on the activity of chloroplast photosystems in seedlings obtained from irradiated seeds..

Table 1

Effect of γ-radiation on the activity of photosystem II (mk mol O2 / mg xl · s-1

Variants	The activity of FS II
Control	96 ± 4,5
Irradiated control	61 ± 1,2
Ammonium rutinate	84 ± 2,4
Cobalt rutinate	76 ± 4,2
Nikel rutinate	74 ± 3,3
Iron rutinate	79 ± 3,5

As can be seen from the table, irradiation of wheat seeds with gamma rays at a dose of 200 Gray negatively affected the formation of the photosynthetic apparatus of seedlings. The above results show a decrease in the number of chlorophylls a and b, as well as carotenoids. In our experiments, the activity of photosystem II in seedlings obtained from irradiated seeds was determined by the ability of the isolated chloroplasts to release oxygen. As can be seen from Table 1, 200 Gray radiation dramatically weakens the activity of chloroplasts. Thus, in the irradiated variant, the activity of chloroplasts is reduced by 40% in comparison with the control. An increase in the activity of photosystem II was observed in the chloroplasts of seedlings obtained from seeds treated with ammonium rutinate in comparison with the irradiated control. The decrease in FSII activity was 13% compared to the non-irradiated sample. In the variants with cobalt rutinate and nickel rutinate, this decrease was 20% and 23%, respectively. In the variant with iron rutinate, similar results were observed with the variant treated with ammonium rutinate. Thus, in this variant, the decrease in the activity of the second photosystem was 17%. According to the results of the experiments, it can be assumed that the treatment of wheat seeds with conventional solutions before irradiation prevented the damaging effect of gamma radiation on the photosystem II.

Using the method of cytogenetic assessment, we investigated the effect of rutin and iron rutinate on the yield of chromosomal aberrations (CA) in the anaphase cells of Allium cepa L. Acute gamma irradiation of onion seeds in the dose range from 10 to 15 Gy had a significant cytogenetic effect, causing an increase in the yield of aberrant anaphases in the meristem onion sprouts (table 2). As can be seen from the table, the pre-irradiation treatment with rutin caused a significant decrease in the yield of aberrant anaphases and the amount of CA per cell. Even at relatively high radiation doses (10 and 15 Gy), the amount of CA decreased 1.9 and 2.4 times, respectively. A decrease in the yield of CA at the same irradiation doses in the case of pretreatment of seeds with an extract of rutin indicates that it has radioprotective properties.

Table 2

Influence of gamma irradiation and treatment with rutin on the yield of chromosomal aberrations in Allium cepa, c.u.

Variants	The yield of chromosomal aberrations		
	0 Gy	10 Gy	15 Gy
Control	2.01±0.00 (1.96-2.05)	9.01±0.00 (8.95-9.09)	11.99±0.01 (11.83-12.14)
P (0 Gy)		<0.01	<0.01
Rutin	2.50±0.00 (2.46-2.55)	4.64±0.00 (4.60-4.68)	5.07±0.00 (5.00-5.13)
P (0 Gy)		<0.01	<0.01
P	<0.01	<0.01	<0.01

P - reliability of differences in relation to control

The data presented by us in Table 2 show that rutin has a pronounced gene-protective and antimutagenic effect.

Table 3

Influence of gamma irradiation and iron rutinate on the yield of chromosomal aberrations in Allium cepa, c.u

Dose, Gr	Control (without treatment)	P (0Gr)	Iron rutinate	P(0 Gr)	P
0	1.87±0.00 (1.86-1.89)		2.07±0.00 (2.00-2.11)		<0.01
1	2.99±0.00 (2.96-3.04)	<0.01	2.35±0.00 (2.27-2.41)	<0.01	<0.01
2.5	3.63±0.00 (3.59-3.68)	<0.01	2.91±0.00 (2.86-3.00)	<0.01	<0.01
5	3.05±0.00 (3.03-3.08)	<0.01	2.76±0.00 (2.70-2.80)	<0.01	<0.01
10	8.42±0.00 (8.37-8.46)	<0.01	5.41±0.00 (5.34-5.50)	<0.01	<0.01
15	8.60±0.00 (8.55-8.63)	<0.01	6.01±0.00 (5.91-6.06)	<0.01	<0.01

P – the significance of differences between control groups and with treatment with iron rutinate

Iron rutinate, like rutin, also shows a pronounced gene protective and antimutagenic effect. As can be seen from Table 2 and Table 3, iron rutinate, like rutin, significantly affects the decrease in the rate of chromosomal aberrations. This property can be used to create drugs with radioprotective properties, especially taking into account the availability of its production from plants.

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ИССЛЕДОВАНИЕ РАДИОЗАЩИТНЫХ СВОЙСТВ РУТИНА И ЕГО КОМПЛЕКСОВ В МОДЕЛЬНЫХ СИСТЕМАХ РАСТЕНИЙ.

С.А. Мамедли, З.Х. Муслимова, М.Ф. Фараджов

Резюме: Исследованы радиозащитные свойства комплексов рутина с железом, кобальтом, аммонием и никелем. В модельных экспериментах с растениями изучено влияние комплексов рутина на рост и развитие, количество фотосинтетических пигментов и выход малонового диальдегида в проростках гамма-облученных (200 грэй) семян пшеницы. Также было изучено влияние комплексов на фотосинтетическую активность и частоту хромосомных аберраций. Выявлены некоторые выраженные радиозшитные свойства комплексов в экспериментах.

Ключевые слова: радиопротекторы, комплексы, фотосинтетические пигменты, малоновый диальдегид, частота хромосомных аберраций.

BİTKİ MODEL SİSTEMLƏRİNDƏ RUTİN VƏ ONUN KOMPLEKSLƏRİNİN RADİOQORUYUCU XÜSUSİYYƏTLƏRİNİN TƏDQİQİ

S.A. Mamedli, Z.X. Müslümova, M.F. Fərəcov

Xülasə: Dəmir, kobalt, ammonium və nikel ilə rutin komplekslərinin radioprotektor xüsusiyyətləri tədqiq edilmışdır. Bitkilərlə aparılan model təcrübələrində qamma-şüalanmış (200 boz) buğda toxumlarından alınan cücərtilərdə rutin komplekslərinin bitkinin böyümə və inkişafına, fotosintetik piqmentlərin və malondialdehidinin miqdarına təsiri öyrənilmişdir. Komplekslərin bitkilərdə fotosintez aktivliyinə və xromosom aberasiyalarının tezliyinə təsiri də tədqiq olunmuşdur. Eksperimentlэк zamanı komplekslərin bəzi radio-qoruyucu xüsusiyyətləri aşkar edilmişdir.

Açar sözlər: radioprotektorlar, komplekslər, fotosintetik piqmentlər, malon dialdehidi, xromosom aberrasiyalarının tezliyi.