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PROTECTIVE EFFECT OF ZINC COMPLEX WITH HYPOXANTHINE-9-RIBOSIDE ON WHEAT SEEDLINGS GROWN FROM GAMMA-IRRADIATED SEEDS

Aim. The aim of the research was to obtain the zinc complex hypoxanthine-9-riboside and to study its effect during γ-irradiation on the biosynthesis of chlorophylls, carotenoids and on the release of chromosome aberrations in anaphase root hair cells in wheat seedlings. ***Methods***. The zinc complex was obtained by direct interaction of zinc chloride – $ZnCl_2$ with hypoxanthine-9-riboside. X-ray phase analysis and thermogravimetric measurements of the obtained complex were carried out. Before irradiation, seeds of durum wheat *Triticum durum* L. from a ^{60}Co source were treated with a zinc complex with hypoxanthine-9-riboside at concentrations of 0.1; 0.01; 0.001%. Structural changes in chromosomes were determined in the initial and final stages of anaphase. Determination of chlorophylls and carotenoids was carried out according to Shlyk. ***Results***. γ-irradiation at doses of 50, 100 and 200 Gy has a significant effect on the content of green pigments and carotenoids in wheat seedlings. Under the action of γ-irradiation, the content of chlorophyll decreases more than carotenoids. With an increase in the dose of radiation, a slight increase in the content of carotenoids is noted. In all variants, chromosomal abnormalities were found: the formation of fragments in metaphase and anaphase, bridges in anaphase, chromosome delays, uneven division of chromosomes. ***Conclusions***. For the first time, it was found that the Zn (II) complex of hypoxanthine-9-riboside at the indicated concentrations significantly reduces the damaging effect of γ-irradiation, helps to eliminate abnormalities in mitotic division in wheat root hair cells.

Keywords: zinc complex, hypoxanthine-9-riboside, gamma irradiation, chromosomal aberrations, chlorophylls, carotenoids.

Among the physiological processes that determine the growth, development and productivity of plants, photosynthesis is the most important. An analysis of the performed work shows that the photosynthetic apparatus (PhSA) of plants and the process of photosynthesis itself are very sensitive to the action of radiation, which manifests itself in the violation of many parameters of the functioning of the PhSA [1, 2].

As is known, photosynthetic pigments are involved in the absorption of light energy at the photophysical stage, carry out energy conversion in the photochemical reactions of photosynthesis, and are the most important components of the electron transport chain. The decrease in the level of photosynthesis in plants in the presence of stress factors, including radiation, is primarily associated with their negative effect on photosynthetic pigments [3, 4].

The main non-specific sign of the effect of radiation on plants is leaf chlorosis, which indicates a decrease in the amount of green pigments. A decrease in the content of chlorophylls a and b in plant leaves was found at high doses of radiation. The main reason for the decrease in the content of green pigments in plants under the action of radiation is the suppression of chlorophyll biosynthesis, which is associated, first of all, with a direct effect on the activity of biosynthesis enzymes. The main points of inhibition in this case are the formation of a photoactive chlorophyllide reductase complex and the synthesis of δ-aminolevulinic acid. At high doses of radiation, the binding of chlorophyll molecules to proteins in the light-harvesting complexes of photosystems slows down. It should be noted that with an increase in the irradiation dose, the proportion of chlorophylls in the substrate decreases, which leads to a deterioration in the light-absorbing properties of the photosynthetic apparatus of PhSA [5, 6].

Compared to chlorophylls, carotenoids are less susceptible to the negative effects of external stressors, including radiation, compared to chlorophylls. Considering that carotenoids are considered as one of the factors providing plant resistance to various types of stress, it can be assumed that the maintenance of their content at a constant level is associated with their protective role and is characteristic of more resistant species [7, 8].

A small number of works are devoted to the study of the formation of the photosynthetic apparatus, with the participation of metal complexes. In works [9-13], devoted to the study of defense mechanisms in plants, synthetic and natural substances are widely used that increase the vital activity of the body, restore metabolism, and eliminate genetic damage caused by irradiation. When studying the effect of salts of some metals on living organisms, their preventive and therapeutic effects were revealed [14–15]. It has been found that metals in organic complex compounds are less toxic than in the form of inorganic salts. The presence of an organic ligand imparts lipophilicity to metal complexes, neutralizes the electrostatic charge of metals, as a result of which their transport through cell membranes is greatly facilitated.

Based on the foregoing, the preparation of new biologically active preparations based on transition metal coordination compounds containing various classes of organic ligands is extremely important. The main objective of our research was to obtain the zinc complex hypoxanthine-9-riboside and to study its effect upon γ-irradiation on the biosynthesis of chlorophylls and carotenoids and on the yield of chromosomal aberrations in anaphase root hair cells in wheat seedlings.

Material and methods

The zinc complex was obtained by direct interaction of zinc chloride – $ZnCl_2$ with hypoxanthine-9-riboside – $C_{10}H_{12}N_4O_5$. The ratio of the starting reagents was 1 : 2. To complete the reaction, the mixture was heated to 50–60°C with constant stirring on a magnetic stirrer for 2–3 hours, then the reaction mixture was evaporated on a water bath to a syrupy state. The precipitate that formed was filtered off, washed with water-alcohol, water-acetone mixtures and ether on a filter. The complex was dried at room temperature.

X-ray phase analysis of the obtained complex was carried out. The nature of the diffraction pattern of the resulting complex differed from the diffraction pattern of the starting compound, which confirmed the individuality of the isolated complex.

To study the thermal stability of the complex in the temperature range of 20–850°C, thermogravimetric measurements were performed. The following patterns were revealed: at the first stage, decomposition and removal of crystallization water occur – at 120-160°C; at the second stage, in the range of 160-320°°C, decomposition of the organic part begins; at the third stage, in the range of 320-850°C, the organic part of the complex burns out, the end product of thermolysis – above 850°C – are zinc.

In order to obtain information about the presence of a metal-ligand bond and establish the method of coordination, IR absorption spectra were taken in the region of 4000 – 400 cm^{-1}. The spectra of the complex contain stretching vibrations of the =C=O- group 1719–1706 (1673-ligand) cm^{-1}; stretching vibrations C=C and C=N of the purine ring 1582–1556 (1523 ligand) cm^{-1}; stretching vibrations of the substituted pyrimidine ring 1614 – 1523 cm^{-1}, 1470–1464 cm^{-1}, 1412–1380 cm^{-1} (1590, 1520, 1470, 1341-ligand) cm^{-1} and Me-N bond vibrations 431–403 cm^{-1} Based on the analysis of the spectrum, it can be assumed that hypoxanthine-9-riboside interacts with Zn^{2+} metal ions due to the N(7) heterocycle and, apparently, due to the C=O group, thus forming a five membered cycle.

Seeds of drought-resistant durum wheat *Triticum durum* L were taken as objects of research. Seeds were subjected to general uniform gamma irradiation from a ^{60}Co source. Before irradiation, the seeds were treated with a zinc complex with hypoxanthine-9-riboside at concentrations (0.1; 0.01; 0.001%). Structural changes in chromosomes were determined in the initial and final stages of anaphase using an ORTOPLAN light microscope. Determination of chlorophylls and carotenoids was carried out according to Shlyk [4].

Results and discussion

Data on the determination of the content of chlorophylls and carotenoids in wheat seedlings, pre-treated with a complex of zinc with hypoxanthine-9-riboside in concentrations (0.1; 0.01; 0.001%), are given in table 1.

As can be seen, γ-irradiation at doses of 50, 100, and 200 Gy has a significant effect on the content of green pigments and carotenoids in wheat seedlings. There is a slight stimulating effect of γ-rays at 100 Gy. In seedlings irradiated with a dose of 200 Gy, a decrease in the content of carotenoids and chlorophylls is noted. It is interesting to note that under the action of γ-irradiation at this dose, the content of chlorophyll decreases more than the content of carotenoids.

Table 1. Effect of organic ligand and Zn (II) hypoxanthine-9-riboside on pigment content milligram/gram (mg/g) in seedlings of γ-irradiated wheat seeds

Variants	Content of pigments mg/g		
	Chl a	Chl b	Carotenoids
Intact	2,8±0,06	0,8±0,04	1,7±0,05
treatment (0.1%) with hypoxanthine-9-riboside+ irradiation			
50Gy	2,4±0,07	0,7±0,02	1,4±0,06
100Gy	1,7±0,06	0,6±0,06	1,2±0,05
200Gy	1,5±0,02	0,5±0,02	0,8±0,07
Intact	2,8±0,06	0,8±0,04	1,7±0,05
treatment (0.01%) with hypoxanthine-9-riboside+ irradiation			
50Gy	2,5±0,03	0,6±0,04	1,8±0,04
100Gy	1,9±0,07	0,4±0,02	1,4±0,03
200Gy	1,6±0,05	0,5±0,05	1,2±0,05
Intact	2,8±0,06	0,8±0,04	1,7±0,05
treatment (0.001%) with hypoxanthine-9-riboside+ irradiation			
50Gy	2,1±0,02	0,5±0,01	1,6±0,08
100Gy	1,9±0,08	0,5±0,03	1,4±0,03
200Gy	1,4±0,05	0,6±0,04	1,1±0,07
Intact	2,8±0,06	0,8±0,04	1,7±0,05
treatment (0.1%) Zn (II) with hypoxanthine-9-riboside + irradiation			
50Gy	2,6±0,05	0,6±0,03	1,4±0,05
100Gy	1,8±0,06	0,4±0,02	1,2±0,04
200Gy	1,6±0,07	0,3±0,06	1,5±0,03
Intact	2,8±0,06	0,8±0,04	1,7±0,05
treatment (0.01%) Zn (II) with hypoxanthine-9-riboside + irradiation			
50Gy	1,9±0,02	0,7±0,05	1,5±0,04
100Gy	1,7±0,05	0,5±0,03	1,7±0,01
200Gy	1,3±0,04	0,3±0,02	1,1±0,07
Intact	2,8±0,06	0,8±0,04	1,7±0,05
treatment (0.001%) Zn (II) with hypoxanthine-9-riboside + irradiation			
50Gy	1,8±0,06	0,6±0,03	1,8±0,02
100Gy	1,4±0,05	0,3±0,04	1,5±0,03
200Gy	1,3±0,08	0,2±0,01	1,4±0,01
Intact	2,8±0,06	0,8±0,04	1,7±0,05
irradiated + treated (0.1%) with Zn (II) hypoxanthine-9-riboside			
50Gy	2,5±0,06	0,5±0,04	1,3±0,02
100Gy	1,7±0,04	0,3±0,05	1,1±0,08
200Gy	1,4±0,03	0,2±0,01	1,0±0,03
Intact	2,8±0,06	0,8±0,04	1,7±0,05
irradiated + treated (0.01%) with Zn (II) hypoxanthine-9-riboside			
50Gy	1,8±0,04	0,6±0,08	1,4±0,03
100Gy	1,6±0,06	0,4±0,07	1,7±0,05
200Gy	1,2±0,07	0,2±0,05	1,01±0,07
Intact	2,8±0,06	0,8±0,04	1,7±0,05
irradiated + treated (0.001%) with Zn (II) hypoxanthine-9-riboside			
50Gy	2,3±0,07	0,5±0,07	1,6±0,03
100Gy	1,5±0,08	0,2±0,01	1,2±0,04
200Gy	1,1±0,03	0,1±0,04	1.1±0,01

Note. *Statistically significant difference from control level ($p < 0.05$).

With an increase in the dose of radiation, a slight increase in the content of carotenoids is noted. It is known that, with an increase in the radiation dose, various compensatory systems, metabolic recovery processes and repair of radiation damage to cells come into action, that is, various adaptive processes are realized in plants [1, 2, 4].

One of the important mechanisms of plant adaptation to irradiation is the activation of antioxidant systems, which may result in the accumulation of carotenoids and flavonoid compounds [5, 7]. The applicable in various concentrations (0.1; 0.01; 0.001%) Zn (II) complex hypoxanthine-9-riboside has a protective effect, since the content of chlorophylls in this variant was almost at the control level, and the content of carotenoids at a dose of 200 Gy was higher than the control. The data obtained indicate that Zn (II) hypoxanthine-9-riboside contributes to the activation of chloroplast defense systems. The effect of different doses of γ-irradiation on the yield of chromosome aberrations in wheat seedlings in the presence and absence of the Zn (II) hypoxanthine-9-riboside complex is shown in table 2.

Table 2. The yield of chromosome aberrations in the presence of the Zn (II) hypoxanthine-9-riboside complex at 50, 100, and 200 Gy

Variants	Number of observed anaphase cells	Number of cells with aberrations	Number of aberrations	Delay of chromosomes	Fragments	Bridges	Unequal divisions	Other complex anomalies
Intact								
	1404	15	15	6	9	1	-	-
Irradiated (control)								
50 Gy	1415	123	134	51	54	18	7	6
100Gy	1476	139	154	66	68	22	8	8
200Gy	1498	172	211	87	76	28	10	6
treatment (0.1%) Zn (II) with hypoxanthine-9-riboside + irradiation								
50 Gy	1403	118	122	48	51	16	6	4
100Gy	1463	131	141	59	63	18	7	5
200Gy	1483	163	155	78	71	23	9	-
treatment (0.01%) Zn (II) with hypoxanthine-9-riboside + irradiation								
50 Gy	1398	116	120	45	48	15	5	3
100Gy	1455	127	138	56	60	17	7	4
200Gy	1471	157	151	73	69	21	7	1
treatment (0.001%) Zn (II) with hypoxanthine-9-riboside + irradiation								
50 Gy	1391	117	124	46	53	15	6	5
100Gy	1449	129	135	55	61	18	5	3
200Gy	1463	158	147	69	67	20	8	7
irradiated + treated (0.1%) with Zn (II) hypoxanthine-9-riboside								
50 Gy	1455	120	126	48	51	16	5	5
100Gy	1463	141	139	57	57	20	7	-
200Gy	1470	154	151	71	67	25	6	6
irradiated + treated (0.01%) with Zn (II) hypoxanthine-9-riboside								
50 Gy	1442	123	121	51	55	17	4	3
100Gy	1459	149	143	63	65	23	7	5
200Gy	1473	159	158	74	69	29	9	11
irradiated + treated (0.001%) with Zn (II) hypoxanthine-9-riboside								
50 Gy	1430	121	118	52	58	16	5	-
100Gy	1470	139	139	63	66	21	8	4
200Gy	1477	160	163	71	71	27	11	13

Note. *Statistically significant difference from control level ($p < 0.05$).

In the experiments, 31908 anaphase cells of 408 root hairs were analyzed. In all variants, chromosomal abnormalities were found: the formation of fragments in metaphase and anaphase, bridges in anaphase, chromosome delays, uneven, symmetrical and asymmetric division of chromosomes. When studying the yield of chromosome aberrations after γ-irradiation, it turned out that at a dose of 200 Gy, the yield of chromosome aberrations significantly increases.

As can be seen from Table 2, upon irradiation at a dose of 50 Gy, 134 chromosome aberrations were found in 1415 samples of anaphase cells, while almost no aberrations were observed in the unirradiated control sample. When irradiated at a dose of 100 Gy, 154 aberrations were found, and at a dose of 200 Gy, 211 aberrations. The use of the Zn (II) complex hypoxanthine-9-riboside leads to a significant decrease in the frequency of chromosomal aberrations.

Among the applied concentrations of the Zn (II) hypoxanthine-9-riboside complex, all three concentrations showed a noticeable antimutagenic effect on the wheat genome, which contributed to a significant reduction in chromosomal aberrations. It should be noted that the antiradiation properties of Zn (II) hypoxanthine-9-riboside were also found during seed treatment and after irradiation.

Conclusions

For the first time it was revealed that the Zn (II) complex of hypoxanthine-9-riboside at concentrations of 0.1; 0.01; 0.001% significantly reduce the damaging effect of γ-irradiation, cause the formation of the optimal photosynthetic apparatus of wheat in the post-radiation period. The same concentrations of the complex contribute to the elimination of anomalies in mitotic division in the cells of root hairs, stimulating the reparative mechanisms of the whole organism.

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ЗАХИСНА ДІЯ КОМПЛЕКСУ ЦИНКУ З ГІПОКСАНТИН-9-РИБОЗИДОМ НА ПРОРОСТКИ ПШЕНИЦІ, ВИРОЩЕНІ З ГАММА-ОПРОМІНЕНОГО НАСІННЯ

Мета. Метою дослідження було отримати цинковий комплекс гіпоксантину-9–рибозиду та вивчити його дію за γ-опромінення на біосинтез хлорофілів, каротиноїдів і на появу хромосомних аберрацій в анафазних клітинах корневих волосків проростків пшениці. ***Методи.*** Комплекс цинку отриманий прямою взаємодією хлориду цинку – $ZnCl_2$ з гіпоксантином-9-рибозидом. Проведено рентгенофазовий аналіз і термогравіметричні виміри отриманого комплексу. Перед опроміненням від джерела ^{60}Co насіння твердої пшениці *Triticum durum* L. було оброблене комплексом цинку з гіпоксантином-9-рибозидом в концентрації 0,1; 0,01; 0,001%. Структурні зміни хромосом визначали на початковій і кінцевій стадіях анафази. Визначення хлорофілів і каротиноїдів проводили по Шлику. ***Результати.*** γ-опромінення в дозах 50, 100 і 200 Гр істотно впливає на вміст зелених пігментів і каротиноїдів у проростках пшениці. Під впливом γ-опромінення вміст хлорофілу знижується більше, ніж каротиноїдів. З підвищенням дози опромінення відзначається незначне збільшення вмісту каротиноїдів. У всіх варіантах виявлено хромосомні аномалії: утворення фрагментів в метафазі та анафазі, мости в анафазі, відставання хромосом, нерівномірний поділ хромосом. ***Висновки.*** Вперше було виявлено, що комплекс Zn (II) гіпоксантину-9-рибозиду у вказаних концентраціях суттєво знижує пошкоджуючи дію γ-опромінення, сприяє усуненню порушень мітотичного поділу у клітинах кореневих волосків пшениці.

Ключові слова: комплекс цинку, гіпоксантин-9-рибозид, гамма-опромінення, хромосомні аберрації, хлорофіли, каротиноїди.