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RESEARCH PAPERS

γ-Irradiation of Barley Seeds and Its Effect on the Phytohormonal Status of Seedlings

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Abstract—We investigated the effect of γ-irradiation (4–50 Gy) of barley seeds (*Hordeum vulgare* L., cv. Nur) on the content of endogenous phytohormones–stimulators of plant growth and development: indol-3-acetic acid (IAA), indolyl-3-butyric acid (IBA), zeatin and abscisic acid (ABA). The ratio (IAA + IBA + zeatin)/ABA from the third to the seventh day of germination has been measured. It was shown that the changes in the content of phytohormones as a function of the radiation dose were nonlinear. In the dose range of 4–20 Gy, phytohormones balance was changed due to increased content of growth stimulators and decreased ABA content. Using a dose of 50 Gy led primarily to a decrease in the content of growth stimulators and an increase in ABA content, and the ratio (IAA + IBA + zeatin)/ABA shifted toward ABA content.

Keywords: Hordeum vulgare, γ-irradiation, hormesis, phytohormones, indoleacetic acid, indolyl butyric acid, abscisic acid, zeatin

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INTRODUCTION

The lifestyle attached to the substrate and the lack of possibility to evade the stressor caused the formation of a complex plant regulatory system that controls plant growth and development in changing environmental conditions. The main unit of regulatory mechanisms of plants is their hormonal system. Each of the phytohormones is possessed of unique functions that control a particular biological process [1]. The balance of endogenous hormones in the plant varies constantly, and its dynamic equilibrium is maintained by means of transport systems [2] and phytohormone transition from the bound forms to a free state [3]. In addition to participating in morphogenetic processes, phytohormones play an important role in protecting plants from stressful environmental factors, regulating many adaptive responses [4].

Almost any external action leads to a change in the ratio of endogenous phytohormones in the plant [5]. The ratio of phytohormones (stimulators to inhibitors) indicates a functional state of the plant, affecting its growth and development. Changing the intensity of biosynthesis of individual phytohormones, plant adapts to external influences, and hormonal changes may occur immediately after the start of the action of an irritating factor [6]. In the role of such a factor, ionizing radiation may act as the affecter of morphological, physiological, biochemical, and cytological indices of plants by inducing the formation of reactive oxygen species (ROS) in the cells [7]. Also, the direct effect of ionizing radiation on high-molecular compounds and cell membranes that may be realized in cascades of biochemical reactions affecting growth and development of plants cannot be excluded. In our previous work [8], the influence of γ-irradiation of barley seeds cv. Nur in doses of 8–50 Gy on the activity of the antioxidant enzyme system in seedlings on the third, fifth, and seventh days of germination were studied. It was shown that the activity of enzymes increased in the dose range that stimulated the development of seedlings (16–20 Gy), which indicates a possible rise in the level of ROS. It is known that ROS in a cell may act not only as a damaging agent but also as a trigger in seed germination. It was assumed that ROS, together with phytohormones, in particular ABA, play a key role in the process of seed germination [9]. During seed swelling, the ROS formed may lead to a decrease in the level of ABA and block the transmission of its signals, while enhancing the transduction of the signals of growth stimulated hormones. The data obtained in experiments with barley seeds indicate that ROS-dependent regulation of seed germination occurs due to the control of ABA catabolism [10]. It has also been shown that oxidative stress causes an increase in the expression of genes that positively regulates tryptophan-dependent auxin biosynthesis [11].

Large doses of radiation inhibit the growth and development of plants [7], while small doses may have

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Abbreviations: IBA—indolyl-3-butyric acid.

a stimulating effect [12]. One of the main effects of small doses of radiation is radiation hormesis. The concept of radiation hormesis suggests the emergence of positive biological effects as a result of exposure to small doses of radiation [12]. With radiation hormesis in plants, seed germination and germination energy, cell proliferative activity, stress resistance, yield, enzymatic activity, and intensity of oxidation processes increase, and the synthesis of RNA and DNA, proteins, and lipids is enhanced [12].

It is known that plant growth is subject to daily and ontogenetic fluctuations [2], and the ratio of phytohormones is also subject to periodic fluctuations, since the maxima of the levels of individual phytohormones occur at different times of the day [13]. Therefore, to assess the effect of γ-irradiation on the levels of phytohormones in seedlings, the changes in the hormonal system over time were considered, which allowed us to take into account its high lability and features of ontogenetic oscillations of hormones ratio.

In our previous work, it was shown that γ-irradiation of barley seeds in small doses exerted a stimulating effect on the seedling development [14]. However, it remains unclear which biochemical processes are involved in the formation of the effect of radiation hormesis. In this regard, the aim of our study was to investigate possible changes in the phytohormone balance of barley plants after γ-irradiation of seeds in a wide range of doses (4–50 Gy).

MATERIALS AND METHODS

As the object of study, barley (*Hordeum vulgare* L., the elite cv. Nur) was chosen. Spring barley is an important food, fodder, and technical crop sown in Russia everywhere as well as a well-studied biological object. For the experiment, the seeds with humidity of 13–15% have been used. The seeds were irradiated with doses of 4, 8, 12, 16, 20, and 50 Gy at a dose rate of 60 Gy/h using a GUR-120 gamma irradiation device (All-Russia Research Institute of Radiology and Agroecology, Obninsk); the radiation source was ^{60}Co. Nonirradiated seeds were used as a control. Immediately after irradiation, the seeds were placed in rolls of filter paper (GOST 12038-84) [15]. Rolls were folded, put in small glasses with distilled water, and placed in an MIR-254 thermostat (Sanyo, Japan) with the temperature of 20°C.

The analysis of the qualitative and quantitative composition of phytohormones was carried out from the third to the seventh day of germination. On the third day, all intact seedlings were used for the study; starting from the fourth day, the seedling was divided into shoots and roots, which were analyzed separately.

Sample preparation. Extraction of phytohormones was carried out at the same time of day to exclude the influence of periodic fluctuations of phytohormone levels on the results of the experiment. We homogenized 1 g of plant material (tissues of 15–80 seedlings, depending on the day of germination) in a porcelain mortar with 1 mL of 80% methanol acidified to pH 3.5 (HPLC Gradient Grade, T.J. Baker, Netherlands) and cooled to 4°C. We added 2 mL of the same solution to the homogenate. To reduce the oxidative phytohormone degradation during the extraction, antioxidants were used: 100 μL of 0.1% ethylenediaminetetraacetic acid, 300 μL of 0.1% 2-mercaptoethanol per 3 mL of an extractant solution. The suspension was then sonicated for 1 min using an ultrasonic homogenizer Sonipuls mini20 (BANDELIN, Germany). In order to avoid heating with a sonication, a glass with a suspension was placed in an ice container. After processing, the suspension was transferred to three tubes (1.5 mL per each) with a pipette and placed in a refrigerator in a Sunflower minishaker of the 3D type (Biosan, Latvia) for 30 min. After 30 min, the suspension was centrifuged for 5 min at 14500 rpm in a MiniSpin minicentrifuge (Eppendorf, Germany); the supernatant was separated and placed in a refrigerator. The precipitate was resuspended in 1 mL of 80% cold methanol, which was acidified to pH 3.5, and reextraction was carried out under the same conditions. The resulting precipitate was discarded and the supernatants were combined.

Solid-phase extraction (nonretentive) was performed on a VacMaster-20 (Biotage, Norway) using ISOLUTE C18 SPE Biotage columns (1 mL). To solvate the column, 1 mL of methanol was passed through it, and 1 mL of 0.1% acetic acid was passed through it to equilibrate. The ultrapure water used in the experiment was purchased from Panreac. The extraction was carried out at a pressure of 0.35 bar. The flow rate of the liquid through the column was controlled by the shut-off valve of the facility. During the activation steps, the flow rate through the column was 0.5 mL/min. After this, the whole supernatant volume was introduced portionwise in the column, reducing the flow rate to 0.2 mL/min. The purified fraction was collected in small glasses and placed in a glass chamber of the facility. Glasses with purified extract were put on a water bath and evaporated for 7 min at temperature of 80°C.

Ultra-high-performance liquid chromatography. Qualitative and quantitative analysis of the extracts was carried out on a Nexera LC-30 high-performance liquid chromatograph (Shimadzu, Japan) with a SPD-M20A diode array detector (Shimadzu). The data were processed using the LabSolutions software (Shimadzu). We transferred 10 μL of the extract to a C18 analytical column with reverse phase (Shim-pack XR-ODSII, 2 μm, diameter of 3.0 mm, length of 100 mm, Shimadzu). The start conditions: solvent (A)—methanol; solvent (B)—0.1% acetic acid solution (10 : 90). Column oven temperature was 35°C and flow rate was 0.3 mL/min throughout the timesharing. To separate the four phytohormones, the optimum gradient elution conditions were selected (Table 1).

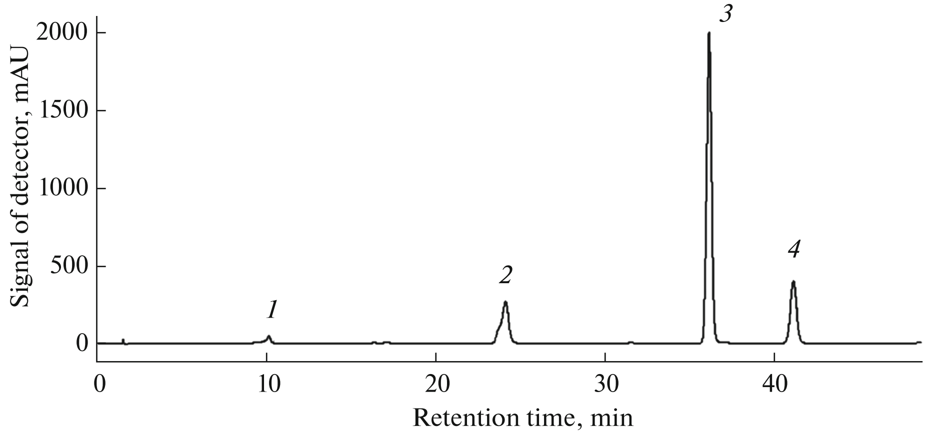

Fig. 1. Chromatogram of a mixture of standards of phytohormones with concentrations of 50 μM each (272 nm). (*1*) Zeatin, retention time (tR) corresponds to 10.196 min; (*2*) IAA, tR corresponds to 24.124 min; (*3*) ABA, tR corresponds to 36.118 min; (*4*) IBA, tR corresponds to 41.075 min.

The analysis was performed in triplicate (three samples of plant material per one experimental point), and each sample was analyzed twice to eliminate instrumental errors.

For identification and quantification, an external standard method was used based on the correlation of peaks of samples and standards. For the construction of calibration plots, phytohormone standards (Fig. 1) were obtained from Sigma-Aldrich (United States). Standard preparations were diluted in methanol in concentrations of 5–1000 μM.

Statistical data treatment. The analysis of the experimental data was carried out using nonparametric statistics using the Microsoft Office Excel 2003 and Statistica 6.0 programs. The experimental data were checked using the Dixon criterion for the presence of emissions, which were excluded from further analysis. The statistical significance of the differences was assessed using the Mann–Whitney *U*-test. The tables show average values.

Table 1. Gradient elution mode for analysis of phytohormones

Time, min	Methanol, %	0.1% acetic acid, %
0.01	10.0	90.0
5	10.0	90.0
7	20.0	80.0
10	30.0	70.0
20	30.0	70.0
25	35.0	65.0
35	45.0	55.0
45	45.0	55.0
50	95.0	5.0
55	95.0	5.0
60	5.0	95.0

RESULTS

Content of IAA

In seedlings grown from control seeds and seeds irradiated with doses of 4–20 Gy, the content of IAA was higher in roots than in shoots (Table 2); in control plant shoots, the IAA level increased statistically significantly with time and it decreased predominantly in roots. Due to this, the differences in IAA content in shoots and roots of control plants reduced with time. After irradiation of seeds with a dose of 50 Gy, no such regularity was observed in seedlings. In this case, the maximum content of IAA was fixed on the third day of germination. Irradiation of seeds with doses of 4–20 Gy increased the content of IAA mainly in 3-day-old seedlings and in rootlets from the fourth to the seventh day of germination. In shoots, the enhancement in IAA levels occurred only at certain doses and at certain stages of germination. The dose of 50 Gy caused a significant reduction in the content of IAA in both shoots and roots from the fourth day of germination. On the seventh day, a negative linear dependence of IAA content on the dose of irradiation in the roots ($r = -0.91$, $P < 0.05$) and in the shoots ($r = -0.81$, $P > 0.05$) was observed.

Content of IBA

Throughout the study, the content of IBA in the roots of seedlings grown from control and irradiated seeds in all the investigated doses exceeded its content in shoots significantly (Table 3). The only exception to this rule was registered on the fourth day at a dose of 8 Gy. However, IBA content, unlike IAA content, did not converge in roots and shoots over time. This is due to

Table 2. Effect of γ-irradiation on the content of IAA (μmol/g) in seedlings, shoots, and roots from the third to the seventh day of germination

Day	Localization	Dose, Gy						
		control	4	8	12	16	20	50
3	Seedling	1.31	4.96*	13.28*	7.96*	12.90*	10.13*	7.77*
4	Shoot	1.69	n. s.	2.30	2.67	4.61*	1.82	1.19
	Root	15.04	21.64	18.02	2.79*	34.61*	15.54	0.57*
5	Shoot	1.42	7.16*	1.74	1.69	2.17*	3.33*	1.00*
	Root	15.17	35.81*	30.80*	17.61*	18.09*	15.56	1.19*
6	Shoot	2.98	2.55	6.31*	6.31	4.18*	3.73	1.39*
	Root	12.54	15.84*	27.73*	24.24*	18.93*	19.71*	0.34*
7	Shoot	4.23	11.49*	5.38	6.19*	5.09	4.10	1.31*
	Root	12.05	34.36*	25.66*	22.12*	16.98*	12.57	3.14*

n. s.—not significant. * Differences are statistically significant compared to control values. Mann–Whitney *U*-test, $P < 0.05$.

Table 3. Effect of γ-irradiation on the content of IBA (μmol/g) in seedlings, shoots, and roots from the third to the seventh day of germination

Day	Localization	Dose, Gy						
		control	4	8	12	16	20	50
3	Seedling	1.86	2.28	2.56	3.42	1.33*	2.09	0.11*
4	Shoot	0.99	2.43*	2.42*	2.04*	3.71*	2.86*	0.05*
	Root	6.66	5.24*	1.81*	7.94	9.79*	9.82*	1.10*
5	Shoot	0.59	4.49*	4.04*	1.13	7.19*	5.75*	0.01*
	Root	11.78	8.98*	18.91*	11.02	11.20	16.35*	2.98*
6	Shoot	0.62	4.21*	6.68*	2.62*	4.71*	4.96*	0.04*
	Root	13.70	9.09*	17.50*	13.00	13.56	16.83	1.85*
7	Shoot	0.752	7.49*	7.49*	4.47*	3.78*	2.29*	0.03*
	Root	13.34	8.73*	13.10	10.43*	8.45*	13.20	3.65*

* Differences are statistically significant compared to control values. Mann–Whitney *U*-test, $P < 0.05$.

the fact that IBA levels in both roots and shoots in control and after irradiation at all doses other than 50 Gy showed a tendency to increase, sometimes statistically significantly.

In 3-day-old seedlings, a significant decrease in IBA content was recorded at doses of 16 and 50 Gy. The other doses led to an increase in the IBA content; however, it was not significant. When the seeds were irradiated in doses of 4–20 Gy, the content of IBA in the shoots increased greatly, and the dependence was observed: the higher the dose used, the earlier the maximum IBA content was observed. In the roots, there was a great variability in IBA content and a stable dependence of IBA content on the dose of irradiation was absent, but the difference between the content of IBA in the roots of irradiated and control seeds increased during germination. When seeds were irradiated by an inhibitory dose of 50 Gy, a decrease in the level of IBA in the roots and shoots of seedlings was observed. On the seventh day of germination, there was a negative linear dependence of the IBA level in shoots and the dose of irradiation ($r = -0.92$, $P < 0.01$).

Content of Zeatin

Zeatin in intact seedlings accumulated predominantly in the roots, while the levels of this phytohormone were so small on the fourth and fifth days of germination that they were outside the detection range (Table 4). The use of irradiation doses in the range of 4–50 Gy resulted in an increase in zeatin level of the 3-day-old seedlings and in the roots from the fifth to

Table 4. Effect of γ-irradiation on the content of zeatin (μmol/g) in seedlings, shoots, and roots from the third to the seventh day of germination

Day	Localization	Dose, Gy						
		control	4	8	12	16	20	50
3	Seedling	0.02	0.20*	0.30*	0.13*	0.16*	0.11*	0.04
4	Shoot	n. s.	0.08*	n. s.	0.10*	n. s.	n. s.	0.01*
	Root	0.05	0.12*	0.05	0.01*	0.21*	0.12*	0.20*
5	Shoot	n. s.	0.10*	1.26*	0.44*	n. s.	n. s.	0.02*
	Root	0.08	0.49*	0.14*	0.14*	0.14*	0.19*	0.21*
6	Shoot	0.01	0.12*	1.41*	0.21*	n. s.	1.04*	0.01
	Root	0.05	0.08*	0.22*	0.28*	0.26*	0.25*	0.18*
7	Shoot	0.01	0.39*	1.15*	0.49*	0.10*	0.48*	0.06
	Root	0.05	0.50*	0.10*	0.25*	0.23*	0.17*	0.15*

n. s.—not significant. * Differences are statistically significant compared to control values. Mann–Whitney *U*-test, $P < 0.05$.

Table 5. Effect of γ-irradiation on the content of ABA (μmol/g) in seedlings, shoots, and roots from the third to the seventh day of germination

Day	Localization	Dose, Gy						
		control	4	8	12	16	20	50
3	Seedling	1.59	0.44*	3.39*	1.03*	0.79*	1.11*	1.25
4	Shoot	2.67	0.89*	2.75	1.52*	1.17*	1.48*	1.59*
	Root	1.66	0.33*	2.60*	1.01*	0.54*	0.57*	1.95
5	Shoot	2.75	1.74*	3.69*	2.14*	2.04*	2.06*	2.06*
	Root	1.71	0.87*	2.64*	0.83*	0.68*	0.52*	2.41*
6	Shoot	3.13	1.78*	3.20	2.03*	1.86*	2.55*	2.79
	Root	1.33	0.68*	2.25*	0.96	1.10	0.66*	2.81*
7	Shoot	3.19	4.60*	3.17	1.98*	2.01*	1.95*	2.52
	Root	0.67	3.96*	1.69*	0.55	0.71	0.59*	3.65*

* Differences are statistically significant compared to control values. Mann–Whitney *U*-test, $P < 0.05$.

the seventh day of germination. In shoots, only a few doses of irradiation caused a significant increase in zeatin content, which was especially noticeable against very low undetectable levels of phytohormone in intact seedlings. The maximum increase in zeatin content in shoots was recorded at dose of 8 Gy: on the sixth day zeatin content exceeded the control value by more than 100 times. It should be noted that the dynamics of zeatin content after irradiation with the lowest dose was significantly different from the dynamics of its accumulation at high doses of irradiation. At 4 Gy, abrupt changes in zeatin content during germination were observed with maxima on the fifth and seventh days of the experiment, whereas the change in phytohormone content at doses of 8, 16, 20, and 50 Gy was smooth with small variations in contents.

Content of ABA

Unlike hormones-stimulators, ABA accumulated predominantly in shoots and not in roots (Table 5). During germination, the content of phytohormone increased in the shoots of control plants, while it decreased in the roots, which led to an increase in differences in the levels of ABA in these organs. Only high-dose irradiation of 50 Gy changed this pattern in the opposite.

Irradiation of seeds in the experiment predominantly resulted in a decline in ABA content in all parts

Table 6. Effect of γ-irradiation on the ratio (IAA + IBA + zeatin)/ABA in seedlings, shoots, and roots from the third to the seventh day of germination

Day	Localization	Dose, Gy						
		control	4	8	12	16	20	50
3	Seedling	2.01	16.89	4.76	11.15	18.34	11.09	3.09
4	Shoot	1.00	2.83	1.72	3.16	7.12	3.16	3.79
	Root	13.12	82.69	7.65	10.60	83.31	44.92	2.26
5	Shoot	0.73	6.76	1.91	1.52	4.59	4.41	1.82
	Root	15.82	52.13	18.92	34.63	43.34	62.09	1.56
6	Shoot	1.15	3.87	4.49	4.50	4.78	3.82	3.61
	Root	19.81	36.76	20.22	38.96	29.76	55.58	1.60
7	Shoot	1.60	4.21	3.67	5.62	4.46	3.53	3.90
	Root	37.75	11.00	23.00	59.34	36.31	44.00	2.70

of the seedlings. However, when the dose of 8 Gy was used, the content of phytohormone in the seedlings increased on the third day of germination and on the fourth to the seventh day in the roots. The dose of 50 Gy increased the level of ABA in the roots. There was also a sharp increase in the content of ABA in the roots and shoots after irradiation with the dose of 4 Gy on the seventh day of germination.

Changes in the Ratio (IAA + IBA +zeatin)/ABA

In 3-day-old seedlings, the ratio (IAA + IBA + zeatin)/ABA was above the control level at all doses; the maxima were fixed at 4 and 16 Gy (Table 6). At the same doses, this ratio was maximal in the roots on the fourth day of germination and exceeded the control value by approximately six times; however, it decreased in the following days of the experiment. The reverse dynamics was observed at 12 Gy: on the fourth day of germination, the ratio (IAA + IBA + zeatin)/ABA was lower than the control value, but it was maximal in comparison with the other doses by the seventh day. On the fifth and sixth days of germination, the maxima of this ratio were observed at a dose of 20 Gy. In shoots, the ratio (IAA + IBA + zeatin)/ABA exceeded the control level at all radiation doses. When the seeds were irradiated with an inhibitory dose (50 Gy), the ratio of phytohormones in the roots was significantly lower compared to the control level.

DISCUSSION

Germination of the seed is a complex process, during which external factors may activate development of the embryo. Under favorable conditions (temperature, O_2), the water penetrating into the seed transfers seed from the state of forced dormancy to active growth [16]. This process is controlled by a number of factors, and phytohormones play an important role among them.

Under the ionizing irradiation, radiolysis of water in plant cells occurs, which leads to the emergence of reactive forms of oxygen (ROS), the excess of which may cause oxidative stress. ROS change the structural and functional features of biological macromolecules, which are accompanied by cascading changes in the metabolic pathways. When exposed to ionizing radiation in low doses, ROS are formed in quantities insufficient for the development of oxidative stress [17]. Under certain conditions, these chemically active molecules are able to stimulate seed germination and seedling development, interacting with the signal pathways of phytohormones [18]. In turn, ROS are able to enhance gene expression by interacting with multiple transcription factors [9]. Some researchers attribute the interactions of ROS and ABA to a key role in the process of germination of barley seeds. The authors of [10] have shown that an increase in the level of ROS during the seed swelling leads to an increase in the expression of genes encoding the main catabolism enzyme ABA—8'-hydrolase ABA. In the seed, high content of ABA prevents the transition of seed from a state of dormancy to a state of germination. However, the appearance of additional amounts of ROS as a result of irradiation may lead to a decrease in the level of ABA or to block the transmission of its signal.

ABA performs a number of important functions in the plant, one of which is the induction of protective mechanisms in response to unfavorable factors. Since stresses cause the rapid accumulation of ABA [9], an increase in its quantity may be considered as an indicator of stress state of the plant and vice versa. Reduced ABA level indicates the favorable conditions for plant growth. The reduced ABA content in seedlings at irradiation doses of 4, 12, 16, and 20 Gy and high ABA content at dose of 8 Gy (Table 5) established in this study explain the two-vertebral nature of the dependence of shoot and root lengths on the dose of γ-irradiation of barley seeds obtained in our previous experiment [14]. Thus, the decline in the intensity of stimulating effect observed earlier [14] at intermediate doses of irradiation may result from the induction of increased ABA levels caused by these doses. High content of phytohormone in the roots of seedlings grown from the seeds irradiated at dose of 50 Gy indicates the inhibitory nature of this dose. However, no such effect was found in the shoots (Table 5). The decrease in the level of free ABA shown in our work may be due to its inactivation by hydroxylation [1]. Hydroxylated forms of ABA have a weak biological activity forming the conjugates with glucose.

Auxins play a key role in almost all fields of plant life. In most studies of natural auxins, IAA is the main endogenous member of this class of phytohormones, which accounts for 80–95% of all auxins in plant tissues [1]. IBA as a natural phytohormone has been known for more than 60 years [19], but the works devoted to its study are extremely few. IBA is widely used in plant growing as an inducer of root formation, since it has high ability to activate growth of adventitious roots in comparison with IAA due to its greater stability in solutions and tissues [20]. IBA is found in plants at a lower concentration than IAA [21]. This phytohormone may be synthesized by tryptophan-dependent pathway (using IAA as a substrate for side chain extension) and tryptophan-independent pathway, where indole acts as a distant precursor [22]. It was shown that the IAA is converted into IBA under natural conditions when seedlings are growing in the dark [23]. This is evidence of the large levels of IBA in our experiments with etiolated seedlings. A reverse process is also possible, where IBA is converted into IAA by peroxisomal β-oxidation [24].

γ-Irradiation may change the auxin content due to the change in the rate of their formation. Cojocaru and Revin [25] showed that γ-irradiation in small doses increases the content of tryptophan, which is a precursor of auxins during their biosynthesis, and enhances the activity of enzyme tryptophan synthase in maize, sunflower, and horse beans plants. They also demonstrated activation of IAA synthesis from β-indolylpyruvic acid and tryptamine at the stage of β-indolylpyruvic acid production from β-indolyl-3-propionic acid. There is evidence that ionizing radiation may change gene expression of the primary response to auxin [26].

The decrease in the auxin content observed in our experiments with an irradiation dose of 50 Gy may be due to the suppression of the systems of IAA biosynthesis and enhancement of metabolic degradation of IAA. When seeds were irradiated with stimulating doses, an increase in the auxin content in seedlings was observed, but the nature of IAA accumulation was different. The content of IAA increased mainly in 3-day-old seedlings and rootlets from the fourth to the seventh day of germination, while the IBA content was mainly accumulated in shoots. Such distribution of auxins may be caused by activation of the synthesis of these phytohormones in different parts of the plant, and, as a result, the conditions are formed that stimulate plant growth. Along with the increase in the content of IBA in the leaves, when the seeds were irradiated with stimulating doses, reduced levels of this phytohormone were observed in the roots indicating a weakening of basipetal transport. Irradiation in stimulating doses increased the content of IBA in shoots, and the maximum content of phytohormone was reached at earlier periods with increasing radiation dosage.

Cytokinins are known as one of the main classes of phytohormones regulating seed germination [27]. As zeatin was not detected in shoots of intact seedlings in the early period and its content was extremely small in the later period (Table 4), it may be suggested that cytokinins, which are synthesized in the roots and transported acropetally, in the leaves are the source for zeatin formation. There is evidence that cytokinins are formed during the ripening of seeds and then transported to shoots [28]. An increase in the content of zeatin in shoots as a result of low-dose irradiation indicates intensification of acropetal transport of zeatin to the site of its action, i.e., to the leaves. In 3-day-old barley seedlings and in 4–7-day-old roots, a high content of phytohormone indicates an increase in its synthesis as a result of the action of ionizing radiation in stimulating doses. However, when the seeds were irradiated with a dose of 50 Gy, an increase zeatin content was also observed in the seedling roots, which may be due to its protective action in the response to stress. It is known that cytokinins may capture free radicals or be included in the mechanism of purine protecting from damage [29].

The basis for the regulation of physiological processes in a plant is the balance of phytohormones, with a particular importance of the ratio of growth hormone stimulators to inhibitors [30]. The increase in the ratio (IAA + IBA + zeatin)/ABA relative to control in all parts of barley seedlings grown from irradiated seeds is probably responsible for the stimulation of growth and development of plants demonstrated in our previous work [14]. The radiation doses of 16 and 20 Gy, which stimulated the development of barley seedlings, caused the maximum increase of the ratio of phytohormones: growth stimulators to inhibitors (Table 6). A particularly most evident effect was observed in 3-day-old seedlings, shoots, and roots on the fourth day as well as on the sixth day in leaves for a dose of 16 Gy; for a dose of 20 Gy, the maximum ratio was found in the roots on the fifth and sixth days of germination. When exposed to an inhibitory dose of 50 Gy, the ratios were extremely low in shoots and roots, but this ratio exceeded the control level in 3-day-old seedlings, which is obviously associated with high IAA level recorded at this dose.

Analysis of the results of our study made it possible to conclude that γ-irradiation leads to disturbance in the phytohormonal balance of barley seedlings. When irradiating with stimulating doses, the content of growth activating hormones (IAA, IBA, and zeatin) increased and the content of inhibitor of germination ABA decreased. When irradiated in an inhibitory dose of 50 Gy, the balance was disturbed toward the ABA as a result of an increase in its content and a decrease in the content of growth stimulating hormones. The changes in the content of phytohormones during the germination were nonlinearly dependent on the dose of irradiation. The accumulation of phytohormones in leaves and roots of intact and irradiated seedlings was significantly different. For example, IAA accumulated in the roots in intact seedlings, and an increase in IAA content in the roots was also observed when seeds were irradiated with stimulating doses; however, IBA content, which normally accumulates in the roots also, mainly increased in the leaves after irradiation of seeds. The revealed differences in the content of phytohormones are probably associated with changes in their synthesis and transport as well as with the transition from bound state to free state.

The obtained results allow us to conclude that phytohormones actively participate in the formation of adaptive reactions of barley plants in the early stages of ontogeny, and apparently, play an important role in the formation of the effect of radiation hormesis after seed irradiation with stimulating doses. Significant changes in the phytohormonal balance create the necessary conditions for acceleration of plant growth and development, which are often observed in response to weak external influences of factors of a different nature.

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