Pre-sowing irradiation of annual ryegrass seeds as a way to accelerate plant growth and adaptation to adverse conditions

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Abstract. As a result of pre-sowing irradiation of annual ryegrass seeds of Rapid variety, the effect of radiobiological stimulation was obtained, which manifested in root growth and an increase in the mass of seedlings and was most observed at a dose of γ-irradiation of 4 Gy. A similar effect when germinating seeds after storage for 90 days was obtained at higher doses of seed irradiation – 10 and 15 Gy; a dose of 4 Gy increased the sprout length. In case of imitation of arid conditions for ryegrass plants, seedlings from non-irradiated seeds of the control variant, under stress, experienced depression and lagged in growth from seedlings with optimal hydration. At the same time, pre-sowing γ-irradiation of seeds in doses of 15-20 Gy partially compensated for the negative effect of lack of moisture, ensuring the same plant development as with optimal hydration, confirming that radiation hormesis can be an example of an adaptive reaction to changing environmental conditions.

1 Introduction

Currently, the task of ensuring food security is acute in many countries of the world. The relevance of this issue is increasing due to the processes of global warming leading to climate change. Many studies have shown that an increase in temperature can reduce crop yields [1]. Therefore, it is necessary to develop technologies aimed at enhancing the adaptive potential of plants to changing environmental conditions. One of these techniques is pre-sowing irradiation of seeds, which allows not only to increase yields, but also to improve the quality of the products obtained [2]. Such a stimulating effect of ionizing radiation on the growth and development of plants has been called radiation hormesis. It was previously shown that the effect of radiation hormesis manifests not only at plant physiology level, but also affects deeper processes in organisms, such as gene expression, protein biosynthesis, and phytohormone formation [3, 4]; all this not only increases the yield of marketable products, but also increases the resistance of plants to adverse environmental conditions [5, 6].

Researchers draw a clear line between positive and negative effects after exposure to different doses of radiation. "Positive stress", or "eustress", occurs when plants are exposed to small doses of ionizing radiation (the effect of radiation hormesis); "distress", "negative

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stress", is formed when crops are irradiated with high doses of radiation, causes critical and sometimes irreversible damage to crops [7]. A range of low and high doses of ionizing radiation is allocated for each type of plant, variety, and development stage. It is believed that for most crops, exposure to radiation in the dose range of 5-20 Gy stimulates plant growth and development, the dose range of 20-100 Gy inhibits these processes[8]. A number of researchers have noted [9, 10] that radiation hormesis manifests itself in different ways in optimal and unfavorable conditions for plant growth, such as lack of moisture during the growing season or early frosts.

For almost every plant type, depending on the physiological state, certain dose ranges are allocated that cause stimulating effects. Nevertheless, this information is not available for all crops. In particular, there is insufficient data on the radiation effect on ryegrass seeds, thus, in our work we chose this crop as an object for study – annual ryegrass of the Rapid variety (*Lolium multiflorum* Lam., 1779). In addition, ryegrass (annual cockle) is a forage crop widely used in the Russian Federation, containing a sufficiently high amount of protein, is well eaten by all types of animals, and gives a good feed mass.

2 Materials and Methods

The purpose of our research was to study the pre-sowing gamma irradiation of annual ryegrass seeds (*Lolium multiflorum* Lam., 1779) of the Rapid variety in doses from 4 to 20 Gy for plant growth and development at the earliest stages. Subsequently, the value of morphometric parameters of seedlings was evaluated; the effect on these indicators of seed shelf life after irradiation; and the response to radiation of seedlings developing under conditions of optimal moisture and moisture deficiency was compared. Consideration of these issues has become the task of our research.

Seed irradiation was carried out at the Research Center Kurchatov Institute – All-Russian Research Institute of Radiology and Agroecology (ciy of Obninsk, Russia) at the UNU GUR-120 installation, where cobalt-60 served as a source of gamma radiation. The dose rate was constant and amounted to 60 Gy/h. The absorbed radiation dose was monitored using a universal dosimeter DKS-101 with an ionization chamber BMK-50, having a relative measurement error of 4%. Seeds in paper bags were placed in the field of γ-radiation for such a time that the radiation doses of the seeds according to the variants were 4, 8, 10, 15, and 20 Gy. The variant with non-irradiated seeds (dose 0 Gy) served as a control variant.

Irradiated and control seeds of 100 pieces for each experiment variant were germinated under the same conditions according to the standard procedure in filter paper rolls at a temperature of 20°C according to the recommendations [3]. The experiment was repeated three times: one day, 21 and 90 days after irradiation of the seeds.

The germination energy was determined on the third day of sprouting, but germination and morphometric indicators of the development of seedlings (length of the sprout and the main root, the number of roots, the weight of the sprout) – on the seventh day. To study the adaptive reactions of seedlings to stressful conditions in an experiment conducted after 21 days, all experiment variants were duplicated by placing the germinated seeds in a 10.5% sucrose solution, where, due to high osmotic pressure, conditions of lack of moisture were created, simulating drought [11].

3 Results

The conducted studies have shown that during the germination of ryegrass seeds a day after irradiation, the stimulating effect of radiation manifested itself already at the earliest stages of

plant ontogenesis (Fig.1). The length of the main root, the number of roots and the mass of seedlings have significantly increased. Nevertheless, the stimulating doses were different. There was no increase in sprout length according to the variants. Annual ryegrass seeds had good germination (94-98%) and high germination energy, which in most variants repeated changes in seed germination. Both of these parameters were independent of the radiation dose.

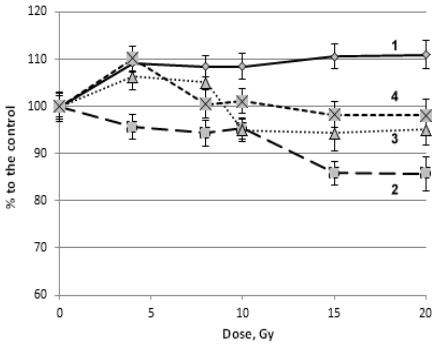

Fig. 1. The change in the length of the main root (1); the length of the sprout (2); the number of roots (3), and the weight of the seedling (4) during germination of *Lolium multiflorum* Lam. a day after γ-irradiation of seeds in various doses (in % of control) (Compiled by the authors).

It is obvious that the effect of radiation at the initial stages of the development of annual ryegrass plants was primarily manifested in accelerated root growth, increasing its length (by 8-11%) and the number of roots (by 5-7%), and, as a result, the weight of the plant increased (by 10-11% of the control). It is interesting to note that if radiobiological stimulation of the growth of the main root manifested itself when all the studied doses of γ-radiation were exposed to the seeds, showing the maximum effect at 15 and 20 Gy, then this effect with respect to the number of roots and the weight of the seedling manifested itself only at a dose of 4 Gy of seed irradiation. With regard to sprout length, small doses of radiation (4, 8, and 10 Gy) did not give significant changes, while the largest doses used showed, on the contrary, the effect of radiation inhibition (by 14-15% compared with the control). Perhaps the rapid growth of the root at an early stage inhibited the development of the sprout, and radiobiological stimulation would have manifested itself later than the first week of growth, during which measurements were carried out. Nevertheless, this reasoning requires experimental confirmation. Summarizing the above data, it can be noted that the effect of radiobiological stimulation was more pronounced at seed radiation dose of 4 Gy, affecting most morphometric indicators.

It is known that the growth and development of plants is influenced not only by the radiation dose, but also by the dose rate, seed moisture, the time between irradiation of seeds and the initiation of their germination, and other factors. In our study, the absorbed dose rate and seed moisture were constant. In addition to studying the effects of ionizing radiation on annual ryegrass seeds when germinating immediately after irradiation (the results are discussed above), we studied the features of the stimulating effect of radiation on seeds after storing them for 21 and 90 days (Fig. 2).

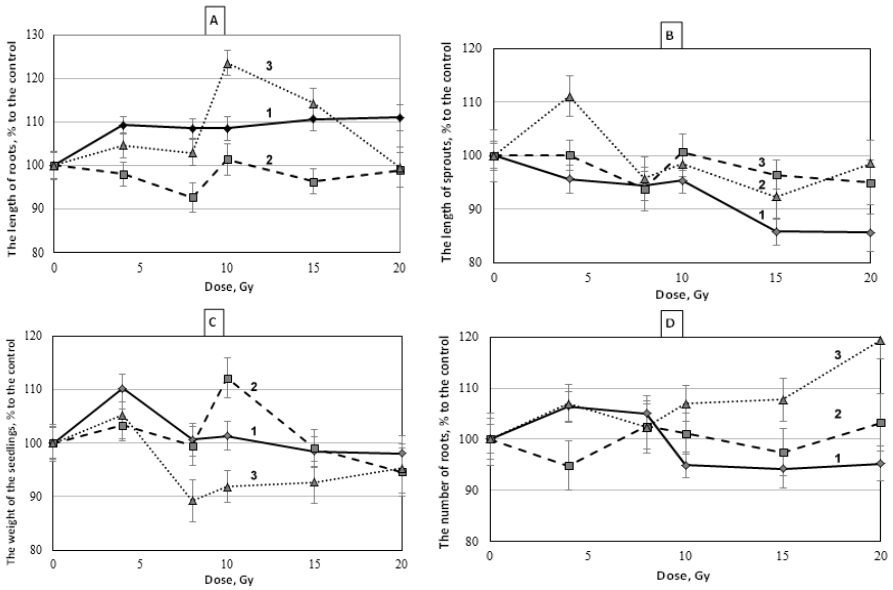

Fig. 2. The change in the length of the main root (A); the length of the sprout (B); the weight of the seedling (C), and the number of roots (D) during germination of *Lolium multiflorum* Lam. after day (1), 21 days (2), and 90 days (3) after γ-irradiation of seeds (Compiled by the authors).

As can be seen from the figure, storing irradiated seeds for three weeks did not enhance the manifestation of radiation hormesis. On the contrary, irradiation in the range of studied doses did not have a stimulating effect neither on the length of the main root and the number of roots, nor on the length of the sprout. The only indicator whose value changed was the weight of seedlings – at a dose of 10 Gy, it increased by 12.1% compared with the weight of seedlings from non-irradiated seeds.

The results of studying the effect of different radiation doses on common ryegrass seedlings after storing them for three months after irradiation turned out to be very unexpected. We observed radiation stimulation of plant growth in several parameters. At seed irradiation dose of 4 Gy, the length of the sprout increased by 11.1% compared to the control, which we did not observe in the two previous experiments described above. The length of the main root increased by 14-23% at doses of 15 and 10 Gy, respectively, which is significantly more than in the first experiment with ryegrass germination immediately after exposure to radiation. We observed a tendency to increase the number of roots at the same doses, while a significant increase in their number was recorded at a dose of 20 Gy. In relation to the mass of seedlings, the radiobiological effect, which we noted with shorter seed storage periods, on the contrary, was replaced by an inhibitory effect. Their weight significantly decreased in the dose range of 8-10 Gy, by 10.8% and 8.1%, respectively.

Another task facing us in conducting research was to study the effects of different doses of ionizing radiation on ryegrass seedlings in conditions of stress, manifested in a lack of moisture. We created these drought simulation conditions when germinating seeds after storing them for 21 days after irradiation. The choice was not accidental, because when germinating seeds under standard conditions, the effect of radiation hormesis was least pronounced during this period. Therefore, it was interesting to see if the radiation effect would change under stressful conditions of plant growth.

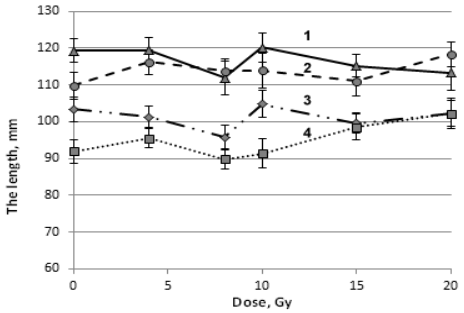

Fig. 3. The change in the length of the sprout (1,2) and the length of the main root (3,4) during germination of seeds of *Lolium multiflorum* Lam. under standard humidification conditions (1,3) and under simulated drought conditions (2,4) 21 days after γ-irradiation of seeds (Compiled by the authors).

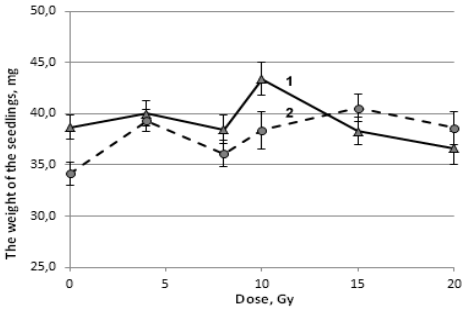

Fig. 4. The change in the weight of seedlings during germination of seeds of *Lolium multiflorum* Lam. under standard humidification conditions (1) and under simulated drought conditions (2) 21 days after γ-irradiation of seeds (Compiled by the authors).

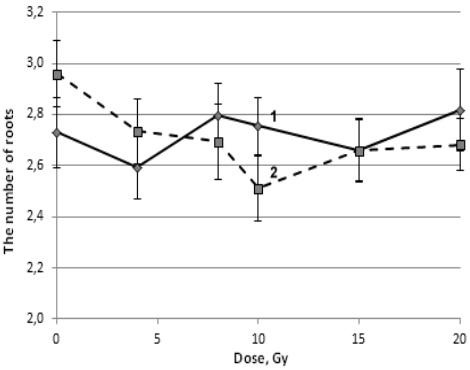

Fig. 5. The change in the number of roots during germination of *Lolium multiflorum* Lam. seeds under standard humidification conditions (1) and under simulated drought conditions (2) 21 days after γ-irradiation of seeds (Compiled by the authors).

As can be seen from Figures 3, 4, and 5, while in a sucrose solution with increased osmotic pressure, simulating conditions of physiological dryness, the seedlings grew in conditions of lack of moisture and experienced depression. In the control variant, all indicators characterizing the condition of seedlings significantly decreased compared to the indicators of plants of the control variant growing under normal humidification: the length of the main root – by 11.1%, the length of the sprout – by 8.0%; the average weight of the

sprout – by 11.5%. At the same time, it should be noted that with relatively high doses of radiation exposure to seeds, ryegrass seedlings felt almost the same as in conditions of optimal hydration. Thus, at doses of 15 and 20 Gy, the length of the main root, the length of the sprout (Fig. 3) and the average weight of seedlings (Fig. 4) practically did not differ from these indicators for seedlings developing in conditions of optimal physiological humidity, and in case of the last two indicators even exceeded them by 4-6%. The number of seedling roots was approximately the same and did not depend on the radiation dose and seed germination conditions (Fig. 5).

Thus, pre-sowing γ-irradiation of annual ryegrass seeds can partially compensate for the negative effect of lack of moisture at the earliest stages of plant ontogenesis.

4 Discussion

4.1 Germination of irradiated seeds

Seed germination is the initial stage of plant ontogenesis. It is characterized by growth resumption after a rest period. This stage takes place in the presence of water, oxygen, sufficient heat and light. The initial stage of seed germination is water consumption. Free water in the embryo stimulates the vital activity of cells, activates the enzyme system, increases hydrolysis and respiration intensity, while derepression of seed genome of the occurs [12]. During this period, all the physiological functions of the plant are initiated.

When seeds are irradiated in the early stages of ontogenesis, the embryos receive additional energy, which is converted into free radicals. In the presence of water and oxygen, in addition to free radicals, other strong oxidants and reducing agents are formed in the seed. All this affects the activation of the deeply repressive genome of embryo cells [13], increases the synthesis of nucleic acids, enzymes, increases the content of phytohormones, increases the intake of mineral nutrition elements [14, 15].

Small doses of ionizing radiation, which are used to obtain the effect of radiation hormesis, are not able to change the genetic program of plant ontogenesis, nevertheless, such an amount of radiation is enough to increase the rate of crop development in the early stages, and this further entails an increase in plant mass and an increase in yield.

When irradiating seeds, the energy of ionizing radiation is transformed mainly into chemically active forms of oxygen, which, if excessive, can cause oxidative stress, manifested in slowing plant growth. We observed such a decrease in the growth of ryegrass germ when exposed to radiation on seeds in doses of 15-20 Gy (Fig. 1, line 2). Nevertheless, it is worth noting that when irradiated seeds in small doses, such oxygen forms are formed in quantities that are not capable of causing significant harm to the crop; on the contrary, interacting with phytohormones, they can stimulate seed germination and further plant development [16], which was confirmed in our study. Starting with a dose of 4 Gy, we observed an acceleration in the growth of the main root, the number of roots and the mass of ryegrass seedlings (Fig. 1, lines 1,3,4). Other researchers have also noted such an acceleration in the growth of the root system of plants grown from irradiated seeds [17, 18].

4.2 Storage time of the irradiated material

The time between irradiation and the initiation of seed germination is one of the important factors influencing the manifestation of radiation hormesis. Previously, many researchers recommended sowing plant seeds immediately after irradiation. Nevertheless, it can be noted that when storing irradiated seed material, especially those that have received sufficiently high doses of radiation, the content of reactive oxygen species decreases over

time, the development of seedlings is improved compared to seeds that germinated immediately after exposure to radiation. This was confirmed in our study. Thus, when germinating seeds 3 months after irradiation, the largest increase in the length of the main root was noted at the highest doses used in the study (10 Gy and 15 Gy), and an increase in the length of the sprout (dose 4 Gy) was recorded, which was not observed when germinating immediately after irradiation (Fig. 2).

4.3 Moisturization conditions

The stimulating effect of small doses of ionizing radiation in the early stages of plant development depends on external environmental factors [10], the key of which are the sum of effective temperatures and precipitation during the growing season. Pre-sowing irradiation of seeds has a positive effect on the development of crops, namely, the root system is better formed, which in arid conditions improves plant access to soil moisture, nutrients and has a beneficial effect on subsequent stages of ontogenesis [6]. Thus, in our studies, an increase in the length of the main ryegrass root in drought conditions at a dose of 20 Gy (Fig. 5) and the weight of seedlings at doses of 15 and 20 Gy (Fig. 4) was confirmed. Thus, it can be noted that seed irradiation helps plants cope with adverse growth and development conditions, confirming that hormesis is an example of an adaptive response to changing environmental conditions [19].

Nevertheless, do not forget that radiobiological hormesis may not occur under extremely dry or very humid growing conditions.

5 Conclusion

An analysis of the results of the study showed that the dose range we selected (4-20 Gy) for pre-sowing irradiation of annual ryegrass seeds turned out to be successful and allowed to study the features of the stimulating effect of γ-radiation (^{60} Co) on the morphometric parameters of the seedling. The effect of radiobiological stimulation was most clearly observed at an irradiation dose of the initial seeds of 4 Gy. At doses of 15-20 Gy, even radiobiological inhibition was manifested in relation to the sprout length.

The manifestation of radiation hormesis was also influenced by the time between irradiation and the initiation of seed germination. With an increase in the shelf life of irradiated seeds, the stimulating doses shifted to a range of higher values. Thus, when germinating seeds after three months, an increase in root growth and the number of roots was observed already at 10-20 Gy, and a dose of 4 Gy caused an increase in the sprout length.

It was especially interesting to compare the manifestation of radiation hormesis under unfavorable growth conditions, with a lack of moisture. It turned out that under stressful conditions, the stimulating effect of γ-radiation was much more pronounced than under standard growth conditions. At radiation doses of 15-20 Gy, the development of the root system increased, which offset the negative effect of arid conditions.

The study of the peculiarities of the radiation hormesis manifestation in different plant growth conditions is of practical importance when choosing the dose of pre-sowing irradiation of seeds in production conditions. These are technologies that researchers and product manufacturers have been paying more and more attention to lately – technologies that allow increasing yields and product quality, especially in the zone of risky farming.

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