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Radiation exposure of barley seeds can modify the early stages of plants' development

Stanislav Geras'kin*, Roman Churyukin, Polina Volkova

Russian Institute of Radiology and Agroecology, Kievskoe Shosse, 109 Km, 249020, Obninsk, Russia

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ABSTRACT

The reactions of barley seeds (Nur and Grace varieties) in terms of the root and sprout lengths, germination and root mass were studied after γ-irradiation with doses in the range of 2–50 Gy. The dose range in which plants' growth stimulation occurs (16–20 Gy) was identified. It was shown that increased size of seedlings after irradiation with stimulating doses was due to the enhancing pace of development rather than an earlier germination. The activity of the majority of the enzymes studied increased in the range of doses that cause stimulation of seedlings development. The influences of the dose rate, the quality of seeds, their moisture and time interval between irradiation and initiation of germination on the manifestation of the effects of radiation were investigated. The experimental data on the effect of γ-irradiation on seedlings development were significantly better explained by mathematical models that take into account the hormetic effect.

1. Introduction

It is well known that low doses of γ-rays stimulate cell division, growth, and development in plants (Cedergreen et al., 2007; Calabrese and Blain, 2009; Jan et al., 2012). This phenomenon, known as "hormesis", is commonly observed at subtoxic doses of different environmental stressors including ionizing radiation. The phenomenon is common and independent on a biological model, an endpoint measured, an inducing agent, and a level of biological organization (Calabrese and Baldwin, 2003; Tang and Loke, 2015). Although a conclusive explanation for the stimulation effects of γ-rays has not been available yet, several studies support hypothesis that mechanisms of hormesis induction by irradiation include changes in the enzyme activities and an increase in the antioxidant capacity of cells (Kim et al., 2004; Jan et al., 2012). Induced by ionizing radiation and other stressors reactive oxygen species (ROS) play an important role in intracellular signal transduction and activation of enzymatic antioxidant defense system (Wrzaczek et al., 2013). This would be particularly relevant for seeds since their genetic program runs into dramatic shifts, such as the transition from dormancy to germination. It is known that ROS can accelerate the transition of repressed seed genome to an active state (Poschenrieder et al., 2013). This manifests in radical changes in cell metabolism and activation of all physiological functions. A crucial role in this process is played by enzymes that control intensity of glucose metabolism and the oxidative pentose phosphate pathway (Kuehne et al., 2015).

Seeds are more resistant to radiation as compared with plants in vegetative phase (Sarapul'tzev and Geras'kin, 1993). Therefore, the region of stimulation doses for seeds is shifted to higher values of the absorbed dose, which leads to easier and more precise dosimetry. In addition it is easier to handle with seeds than with plants in vegetative phase. This makes seeds a convenient experimental object. In this regard, it was important to find out how irradiation of seeds affects the development of plants in the early stages of ontogeny.

In the present study, an attempt was made to elucidate the radiation induced changes in the early stages of plant development. Therefore, the aims of the present study were:

- to investigate the response of barley seeds to irradiation in the dose range of 2–50 Gy in terms of the root and sprout lengths, germination and root mass;

* Corresponding author.
E-mail address: stgeraskin@gmail.com (S. Geras'kin).

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- to estimate the dose range that causes stimulation of plant growth;
- to estimate the influence of γ-radiation on the activity of enzymes controlling the metabolic pathways and the antioxidant defense system in plant cells;
- to investigate the influence of a dose rate, quality of seeds, moisture content and storage time on the manifestation of radiation effects.

2. Materials and methods

2.1. Test organism

In the current study we used spring barley (*Hordeum vulgare* L.), an important crop and well-studied biological object. The varieties Nur (first reproduction, elite, super elite) and Grace (first reproduction) were used in our experiments. First reproduction is the first generation of plants grown from the elite seeds; elite seeds are the seeds obtained from the plants grown from the original seeds; and super elite seeds are the seeds of the first reproduction step that produces by the manufacturer of a variety.

2.2. Irradiation of seeds

The seeds were selected randomly from a given seed lot, then placed in the paper bags with a surface area of 25 cm^2 and irradiated prior to imbibition. Each bag contained 200 seeds, 2 bags per dose were used. This approach provides convenient geometry for irradiation. The seeds were irradiated with γ-rays of ^{60}Co (GUR-120, RIRAE, Obninsk) at room temperature. The radiation doses were assessed using DKS-101 dosimeter (Politehphorm-M, Russia) and were confirmed using a thermoluminescent dosimeter. The following doses were applied: 0 (the control), 2, 4, 6, 8, 10, 13, 16, 20, 25 and 50 Gy at dose rates of 20, 60 and 350 Gy/h. These dose rates were chosen to provide conclusive evidence of the differences in the response of plants on the different dose rates.

Dry seeds with moisture content of 13–15% were used for experiments. A portion of the seeds was irradiated after increasing the moisture content to 40% by soaking in distilled water for 18 h. Another portion of the seeds after irradiation was stored in paper bags at room temperature and humidity for seven days prior to imbibition. The experimental set-up is presented in Table 1.

2.3. Seeds germination

Both irradiated and control seeds were placed in the rolls of filter paper. Each roll was put in a container with distilled water (200 mL) and grown in the thermostat (MIR-254, Sanyo, Japan) maintained at 20 °C. A seed was considered as germinated when the radicle elongated to 2–3 mm. Germination rate (percentage of normally developed seedlings on the 7th day), lengths of sprout and root, weight and number of roots were evaluated on the 7th day of germination. Additionally, we evaluated the beginning of active growth in the embryos of seeds during first 7 days estimating the radicle protrusion through the seed coat. All experiments were performed in four replicates of 400 seeds for each dose.

2.4. Enzymatic activity analyses

Activities of enzymes (superoxide dismutase (EC 1.15.1.1, SOD), catalase (EC 1.11.1.6, CAT), guaiacol peroxidase (EC 1.11.1.7, GPOX), pyruvate kinase (EC 2.7.1.40, PK), glucose-6-phosphate dehydrogenase (EC 1.1.1.49, G6PD), malate dehydrogenase (EC 1.1.1.37, MDH), and shikimate dehydrogenase (EC 1.1.1.25, SKDH)) in seedlings on 3rd, 5th, and 7th days of germination were evaluated. The choice of this time range is based on the fact that radiation-induced boost in cell division and seedling growth seems to be limited to a short timeframe (about 6–7 days) after irradiation (Gudkov, 1991).

After weighing, a sample of fresh plant material (2 seedlings per sample) was homogenized in 1 mL of potassium phosphate buffer solution using a mortar and a pestle. The pH of buffer solution depended on the enzyme that was analyzed in the moment. Only green parts of the seedlings (sprouts) were used for enzymes extraction. The homogenates were centrifuged in centrifuge MiniSpin Plus (Eppendorf, Germany) at 14 500 rpm for 10 min at 4°C. Supernatants were used for enzyme assays. Enzyme activities were evaluated using a "NanoDrop-2000" (Thermo Fisher Scientific, USA) spectrophotometer in accordance with the recommendations of Bisswanger (2004). 30 extracts were used for each enzyme for each experimental condition at 3rd, 5th, and 7th days of germination (90 extracts for each enzyme in total).

SOD activity was estimated using the rate of nitroblue tetrazolium (NBT) reduction by monitoring at 560 nm 100 μL of tissue extract was added to 0.2 mL of 1 mM EDTA and 0.4 mL of 0.01% of nitroblue tetrazolium. Absorbance was measured at 560 nm, then 0.4 mL of 2.4 mM hydroxylamine hydrochloride was added. The mix was incubated in the dark during 5 min, and then absorbance was measured again at 560 nm.

CAT activity was measured by the decomposition rate of hydrogen peroxide. A decrease of absorbance was measured in dynamics at 340 nm 100 μL of tissue extract was added to 1 mL of 30 mM H_2O_2 contained 100 μL of potassium phosphate buffer (pH 7.2). Absorbance was measured at 340 nm immediately, after 30 s of incubation, and after 60 s of incubation.

GPOX in presence of H_2O_2 catalyzes the transformation of

Table 1
The experimental design.

Dose rate, Gy/h		60	60	60 (40% moisture content)	20	350	60	60	Enzymatic activity analyses,
Days of storage, days		0	7	0	0	0; 7	0; 7	0; 7	60 Gy/h (7 enzymes; 3, 5 and 7 days)
Dose, Gy	0	✓	✓	✓	✓	✓	✓	✓	✓
	2	✓	✓						
	4	✓	✓						
	6	✓	✓						
	8	✓	✓	✓	✓	✓	✓	✓	✓
	10	✓	✓	✓	✓	✓	✓	✓	✓
	13	✓	✓	✓	✓	✓	✓	✓	✓
	16	✓	✓	✓	✓	✓	✓	✓	✓
	20	✓	✓	✓	✓	✓	✓	✓	✓
	50	✓	✓						✓
Type of reproduction		*the first reproduction*					*elite*	*super-elite*	*the first reproduction*
Variety		Nur	Nur						
		Grace							