SOME EFFECTS OF PROTON IRRADIATION IN YOUNG SEEDLINGS OF WHEAT

L. OPRICA¹, S. SHVIDKIY², A. MOLOKANOV², G. VOCHITA³, D. CREANGA⁴*

¹“Alexandru Ioan Cuza” University, Iasi, Biology Faculty, Romania
E-mail: iasilacra@yahoo.com
²Dzhelepov Laboratory, Joint Institute for Nuclear Research, Dubna, Russia
E-mail: shvid@nusun.jinr.ru
³Institute of Biological Research, Iasi, Romania
E-mail: gabrielacapraru@yahoo.com
⁴“Alexandru Ioan Cuza” University, Iasi, Physics Faculty, 11 Blvd. Carol I, Iasi, Romania
*Corresponding author: dorina.emilia.creanga@gmail.com

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Abstract. Selected seeds were exposed to radiation dose array of tens of Grey the seedlings being investigated during their early ontogenetic stages. The changes in the activity of some antioxidant biomolecules, either enzyme – like peroxidase, catalase, superoxidedismutase or non-enzymes like the flavonoids and polyphenols were emphasized. The levels of photosynthesis pigments were also estimated to search for the influence of antioxidant enzyme impairing on the plant growth.

Key words: proton beam, antioxidant biomolecules, chlorophylls.

1. INTRODUCTION

The vegetation sensitivity to ionizing radiation seems to represent an important environmental issue as related to the atmosphere background fluctuations that result from cosmic and various terrestrial sources. People cultivate large surfaces with cereals in all parts of the world, being preoccupied by the plant quality and nourishing properties [1], so that all environmental factors influencing vegetation are important for the agrophysical research. From the viewpoint of environment sciences, the ionizing radiation, either from natural or artificial sources, is a pollutant factor with long term effects rather difficult to describe in detail [2].

The levels of radiation provided by the Earth itself are lower today than during ancient geological eras – about eight times diminished as estimated in [3] but nowadays, huge amounts of nuclear waste are spread all over the world, making the radiation safety a threatening issue. It is generally accepted that, when vegetation has covered the nursing soil [4], the ionizing radiation background was still higher than today so that plants adapted to those conditions thus there is an actual challenge to describe the radiation bioeffects in plants. Particular attention should be paid when studying vegetal organism response to radiation, because relatively little is known upon the radiosensitivity of different species. Opposite to
animals, in plants the permanent changes and mutations of DNA affect mainly the biomass yielding but not the basic aspects of growth [5]. Ecological statements have scaled the plant species radiosensitivity, beginning with trees and ending with grassy plants. It was found that the radiosensitivity correlates with the ratio of non-photosynthetic to photosynthetic material [6]. Generally, the research on the bioeffects in the irradiated vegetal organisms has been organized under laboratory-controlled conditions [2] being then extrapolated to atmosphere life. Mostly plant seeds or young seedlings were studied after being treated with gamma radiation, in comparison to non-irradiated control samples [7]. The results of laboratory investigations appeared significantly different since some of them revealed positive effects of low radiation doses while others evidenced negative effects at higher doses. Not only ionizing radiation but also ultraviolet and microwave radiation were found to induce some bioeffects in the ecosystem components, either young plantlets or environmental fungi [8–12].

Searching for evident modifications induced by ionizing radiation in plants, the irradiation protocols were based mainly on acute exposures to doses of tens or even hundreds of Gy [13], quite improbable in the earth natural conditions. There were few controlled experiments based on chronic irradiation with lower doses [2]. As the bioeffects of ionizing radiation depend on certain factors mainly the linear energy transfer (LET), dose range and the type of radiation [14], the experimental designs need to consider the phenomena underlying the radiation interaction with living matter and the way of radiation energy deposition in the tissues [15]. In contrast with electromagnetic radiation, the high LET ion beams are able of localized energy depositions, so that DNA damages [16] are, generally, consistent with genomic material fragmentation and can result in genetic mutations, as in the case of rice studied in [17].

Apart from proton beam radiobiological studies in animals, where phenotypic and genomic effects were described comprehensively, there is still much to do in the research of proton irradiation bioeffects in plants [14].

In [18] the authors have exposed seeds and seedlings of Brassica rapa to 1 to 3 MeV proton ions, the analyses result suggesting that low energy protons did not induce significant effects in seeds but in the irradiated seedlings. Since such genome breaks could affect antioxidant enzyme synthesis as well as other antioxidant biomolecule production, we present a laboratory study dedicated mainly to such biochemical parameters in the seedlings grown from proton irradiated seeds of wheat.

2. EXPERIMENTAL PROCEDURE

2.1. IRRADIATOR AND BIOLOGICAL MATERIALS

Seed irradiation was carried out by sample exposure to the proton beam of the phasotron of the Dubna JINR facility. The average energy of the proton beam
at the entrance into the sample exposure cabin was of 171 MeV. The beam energy in the cabin was determined by the parcourse of the beam in water, $R$ ($R = 200$ mm of water).

![Natural Proton Beam Depth Dose Distribution](image1.png)

**Fig. 1** — Proton beam depth dose distribution ($t$ — thickness of water).

![Horizontal distribution of proton beam](image2.png)

**Fig. 2** — Horizontal distribution of proton beam ($x$ — horizontal distance).

The measurements were carried out by a semiconductor silicon detector. On the way of the beam in the cabin there was permanently a suitable moderator piece while the irradiation of samples is usually carried out with an additional moderator, thus the beam energy at the point of irradiation of the samples was of 150 MeV, the average value of LET (Linear Energy Transfer) being like $\frac{dE}{dx} = 0.539$ keV $\mu$m. Proton beam dosimetry was based on the recommendations of the *International...*
Atomic Energy Agency (IAEA) [19, 20]. Dosimetric calibration of the beam at each point of the deep dose distribution was carried out by the PTW UNIDOS-E clinical dosimeter with TM30013 ionization chamber. The depth and horizontal dose distributions of the proton beam are shown in Figs. 1, 2.

The LET spectra were measured at different depths using the Liulin-4C semiconductor energy deposits spectrometer [21, 22]. Dose rate was of about 0.9 Gy/min while dose array was like 22.4–33.6–44.8–56.0–67.2 Gy.

The seeds (either irradiated or control, non-irradiated ones) have been cultivated in Petri dishes, on wet porous paper, being let to germinate in darkness, at a temperature of 22.0 ± 0.5°C (in Incucell room) in Iasi university laboratory. The administration of deionized water was carried out for 5 days, the growing of seedlings being achieved in controlled conditions of temperature (24.0 ± 0.5°C), humidity (90%) and lightness (light/dark cycle: 14 h/10 h).

2.2. BIOCHEMICAL ASSAYS

The activities of antioxidant enzymes, i.e., catalase (CAT), superoxidedismutase (SOD) and peroxidase (POD) were assayed spectrophotometrically based on specific biochemical protocols.

The CAT activity was assayed through the method described in [23]. SOD activity was assayed according to Winterbourn’s method with slight modifications [24, 25] POD activity (peroxidase) was assayed according to [26]. Activity of all enzymes was given as specific activity (U/mg – units of enzyme activity per gram of protein).

The assay of soluble protein content was done according to Bradford method [27]. The level of lipid peroxidation was measured by estimating the concentration of malondialdehyde (MDA) – the product of lipid peroxidation, in the green tissue from irradiated and non-irradiated plantlets, according to the method of Hodges [28]. The results were expressed as nmol of MDA per milligram of protein (nmol/mg protein).

The content of phenols in the green tissue was assessed based on Folin Ciocalteau procedure in accord with [29] while the total content of flavonoids was assessed by the method from [30]. The results are expressed as GAE/mg (gallic acid equivalent per tissue gram) and respectively as QE/g (quercetin equivalent per tissue gram).

The specific photosynthesis pigments, i.e. the chlorophyll a, and chlorophyll b were assayed spectrophotometrically by adjusting a method known from literature [31].

All reagents were purchased from Sigma-Aldrich. Shimadzu spectrophotometer 1800 PharmaSpec with quartz cells as well as with data acquisition and processing software was used. All experiments were carried out with four independent repetitions and the results were expressed as the mean values ± standard deviation. Anova test was applied for the statistical significance assessing in the samples corresponding to the irradiated seeds compared to control, non-irradiated ones.
3. RESULTS AND DISCUSSIONS

The results of antioxidant enzyme assays have revealed the decrease of CAT, SOD and POD activity in all seedling samples corresponding to the irradiated seeds in comparison to the control, non-irradiated ones. CAT activity (Fig. 3) was reduced with about 40% compared to the control sample (standard deviation of about 9%) with no evident correlation with the proton dose ($p < 0.05$).

In other author study [32] the barley seedlings were found to have increased catalase activity for gamma irradiation with similar dose array – but up to 50 Gy – probably due to the lower linear energy transfer in the case of gamma radiation compared to protons, as known, thus it is possible that the absorbed radiation caused less impairment in the genes controlling CAT enzyme synthesis. For higher doses of gamma radiations – of about 100 Gy some legume seedlings of *Vicia faba* L. were found with lowered catalase activity [33].

According to Fig. 4 the SOD activity was also significantly diminished in our wheat seedlings ($p < 0.05$) with 35 to 45% – the graph of the average values suggesting the highest reduction for the first proton dose (of around 22 Gy) and progressive moderate reductions for the next, larger doses – up to around 67 Gy (standard deviation of around 7%). This is different than for gamma radiation effect in *Vicia faba* L. where SOD activity was found enhanced probably through certain stimulatory effect of lower LET radiation [33].

In Fig. 5 the decrease of POD activity is presented, with similar graph as in the case of SOD, meaning the diminution with 35 to 45% in the samples corresponding
to the seedlings grown from the irradiated samples compared to those provided by control, non-irradiated seeds. Other authors [34] studied soybean exposed to tens up to several hundreds of Gy of proton beam and found that POD enzyme activity was non-significantly changed – proving that plant species actually influence the response to radiation impact.

Fig. 4 – Superoxidedismutase activity in the seedlings grown from proton irradiated seeds.

Fig. 5 – Peroxidase activity in the seedlings grown from proton irradiated seeds.
As known, the mechanisms of action of the antioxidant enzymes consist in the annihilation of the oxidative free radicals generated, in this case, by the radiation interaction with the living tissues. At the time of the experiment the seeds were not germinated but in the latent state, the cell structure and functions being however still sensitive to radiation impact. The cell nucleus being the most radiosensitive cellular structure, the presented data, related to the antioxidant enzymes, could suggest the impairing of the enzyme synthesis in the seedlings grown from the radiation exposed seeds, probably because of the specific genes alteration in the cell nucleus.

The green tissues of the resulted plantlets contain less active antioxidant enzymes so that they seem to be less able to face the toxic effects of specific or non-specific enzyme substrates – the hydrogen peroxide (for CAT enzyme), the hydroxyl radical (for POD enzyme) and the superoxide radical (for SOD enzyme).

At the same time protein synthesis appears to be influenced by the interaction of the radiation with the wheat seeds. Indeed, the content of soluble proteins (including also other protein biomolecules beside the enzymes) appears to be significantly stimulated ($p < 0.05$) following the radiation absorption (Fig. 6); however the increase of the protein amount is lower that the decrease of the antioxidant enzyme activities – being of no more than 30% (standard deviation of about 8%).

The sensitivity to the oxidative processes triggered by various factors (of physical, chemical, or biological nature) could be described by the content of malondyaldehyde (MDA) that is an indirect measure of the lipid peroxidation in the cell membranes. In Fig. 7 one could see the slightly perceptible variation in this
parameter (statistically non-significant, \( p > 0.05 \)) which could also indicate the presence of other antioxidants in the cells, beside the antioxidant enzymes assayed above. It is the case of polyphenols and flavonoids that have been considered in this study.

In the studies of [35] carried out on cowpea legume seedlings the slight diminution at the level of MDA parameter was found – either for proton or gamma radiations of tens to hundreds of Gy however with less influence for gamma rays compared to protons.

![Fig. 7 – The results of MDA content assay.](image)

In Figs. 8–9 we present the contents of polyphenols and flavonoids – the most remarkable variation being consistent with the increase of flavonoids from the seedlings (Fig. 9). In the samples corresponding to the irradiated seeds the flavonoid content appeared increased with more than 100% \( (p < 0.01) \) – except for the sample corresponding to certain radiation dose of around 56 Gy where unexpected low average value was obtained (only about 10% increase compared to the control – which is comparable with standard deviation).

Thus, one can suppose that the green tissue of the seedlings grown from the proton beam exposed seeds could be rather protected against free radicals with oxidative properties by various antioxidant systems. Nevertheless, wheat is known as a radioreistant vegetal species (a grassy plant) so that our experimental work appears as a confirmation of this feature – for the radiation doses applied in the frame of the described study.
Fig. 8 – The results of polyphenol assay.

Fig. 9 – The results of flavonoid content assay.

The chlorophylls contents (Fig. 10), analyzed also from the viewpoint of proton exposure influence, indicate diminished levels ($p < 0.05$) of chlorophyll $a$, the main photosynthesis pigment (with around 25% – the standard deviation being of 9%), in all the samples corresponding to the irradiated seeds.

The chlorophyll $b$, as secondary important photosynthesis pigment, was less diminished (with about 8% comparable with the standard deviation) – consequently,
the ratio of the chlorophyll contents showed diminution trend ($p > 0.05$, data not shown here). Based on these findings one could assume that photosynthesis process, governing plant growth was not significantly affected by proton beam exposure of the wheat seeds. In [34] the soybean content of chlorophyll showed clear diminution for chlorophyll $a$ but insignificant variation for chlorophyll $b$, for proton beam doses of tens to hundreds of Gy.

![Fig. 10 – The levels of photosynthesis pigments.](image)

In [35] the cowpea legume green tissue showed decreased chlorophylls contents for gamma radiation exposure, but non-significant variations were noticed for proton beam exposure at the same dose array.

From all the mentioned studies the roles of radiation type and plant species are shaped related to the antioxidant enzyme and the chlorophyll levels from the green tissue of grassy plant seedlings. The importance of such studies continuation is basically related to the fact that plants are the only living organisms able to synthesize organic molecules nourishing all others Earth species, including people.

4. CONCLUSION

The experiment arranged to search for some bioeffects of wheat seed irradiation with proton beam doses of tens of Gy has allowed some insights in the response of antioxidant enzymes and some non-enzymatic antioxidant biomolecules. The catalase, the superoxidedismutase and the peroxidase have shown diminutions with 30 to 55% of their activities while flavonoid content appeared to be increased with more than
100%, so that the general result observed at the level of lipid peroxidation (by means of malonialdehyde content) did not indicate significant oxidative stress. The radiation response of the wheat seeds was also underlined through relatively slight diminution of the chlorophyll $a$ and $b$, the most important biomolecules in the photosynthesis complex process. Further studies could be developed based on the adjustment of the irradiation protocol and of the biological material with focus on the chromosomal changes induced by proton beam interaction with vegetal embryos.

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**REFERENCES**


