Original Scientific Paper

EVALUATION OF TOXIC AND GENOTOXIC EFFECTS OF LOW-LEVEL ^{137}Cs IONISING RADIATION ON PLANTS*

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The aim of this study was to evaluate the impact of low internal exposure to ^{137}Cs on *L. sativum* meristem cells and *Tradescantia* stamen hair cells. It also compared the impact of ^{137}Cs internal and external irradiation of similar level on the plant seed germination and root growth. Compared to control, the tested internal (0.0007 mGy to 0.7 mGy) and external (0.04 mGy to 5.5 mGy) ^{137}Cs ionising radiation doses stimulated the elongation of *L. sativum* roots by 11 % to 12 % and 24 % to 33 %, respectively. Internal ^{137}Cs exposure (0.0003 mGy to 0.5 mGy) for 14 days caused 1.2 % to 1.6 % of somatic mutations and 19 % to 87 % of non-viable stamen hair in *Tradescantia*.

KEY WORDS: Lepidium sativum L., *roots, seeds, stamen hair cells, test plants,* Tradescantia

Nuclear Power Plants (NPPs) release various technogenic radionuclides (^{137}Cs, ^{90}Sr, ^{60}Co, ^{54}Mn, ^{14}C, and Pu isotopes) into the environment during operation. Because radionuclides accumulate in abiotic and biotic components of the environment, ionising radiation can cause toxic and genotoxic effects on organisms (1-3). It can directly disturb plant breathing, photosynthesis, growth, active transport as well as ionic balance and enzyme synthesis (4, 5). It has been determined that ionising radiation in plants can stop cell division (4). These changes point to the changes in biochemical processes which can decrease cell vitality.

After the Chernobyl accident, it was determined that in acute exposure to ionising radiation, the impact of radionuclides can be two to four times higher (6) in the cell, due to atom decay than in external irradiation. The biological impact of radionuclides depends on their accumulation level and localization in the organism and cells (7, 8). Radionuclides may enter the inner cell compartments, and sometimes bind to the DNA molecule. The genetic effects can be induced by ionising radiation due to the radionuclide decay and by transmutation. Transmutation is a change of the chemical nature of decaying atoms and ionising energy; it affects the site where radioactive decay takes place (7, 9-11).

Internal exposure in plants can increase with radionuclides accumulated in their tissues, especially in tissues with active cell division (12, 13). For example, radiocaesium, like its chemical analogue K, accumulates in relatively large amounts in both young and meristem tissues (8, 14). However, the plant response to the action of incorporated ^{137}Cs, particularly at low-level ionising radiation doses, has not yet been sufficiently investigated (15).

The purpose of this study was to determine - under experimental conditions - the toxic effects of low

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internal exposure doses of long-lived technogenic radionuclide ^{137}Cs on the plant's vegetative organ (root meristem cells) and the genotoxic effects on the plant generative organ (blossom stamen hair cells). We also compared the effects of ^{137}Cs ionising radiation on the plant seed germination and root growth when the plant was exposed to similar internal and external doses of radiation.

MATERIALS AND METHODS

We used two test plants for the experiments: *Lepidium sativum L.* and *Tradescantia* clone BNL 02. The experiments with *L. sativum* were conducted according to a modified Magone method (16) and lasted fortwo days. The toxic effects of radionuclides were evaluated through seed germination and root growth. The experiments with *Tradescantia* were performed applying the modified methods of I. Mericle and R. Mericle (17) and Osipova and Shevchenko (18). The experiment lasted for 14 days. The radionuclide genotoxic effects were evaluated by the number of somatic (colourless and morphological) mutations in the stamen hair (SH) cells and by the amount of non-viable stamen hairs (non-viable were those containing less than 12 cells).

Lepidium sativum L. is widely applied in toxicological investigations (19, 20). Due to intensive metabolic processes, the meristem cells of the plant roots are the most sensitive to the ionising radiation (12, 13).

Table 1 shows the ^{137}Cs activity concentrations used to assess its toxicity to *L. sativum* and genotoxicity to *Tradescantia*. It also shows the internal exposure doses for these plants. The activity of ^{137}Cs in aquatic solution and in plants was determined using gamma-spectrometry (21). Internal ^{137}Cs doses were calculated using the method presented in our earlier report (22).

In the experiment with external irradiation, the seeds of *L. sativum* were placed for two days in an irradiation chamber with a ^{137}Cs ionising radiation source. The chamber (758 mm x 618 mm x 1490 mm) was made of 16 mm steel sheets. The source was placed in a 100 mm thick lead block. The exposure ranged from 0.04 mGy to 5.5 mGy.

In order to evaluate the data statistically, the standard error (SE) of estimation was calculated and presented in the Figures 1 and 2.

RESULTS AND DISCUSSION

Regardless of the dose (from 0.0007 mGy to 0.7 mGy) of a two-day exposure to internal ^{137}Cs ionising radiation *L. sativum* seed germination did not significantly differ from the control, whereas root growth was statistically significantly higher (11 % to 12 %) (Figure 1).

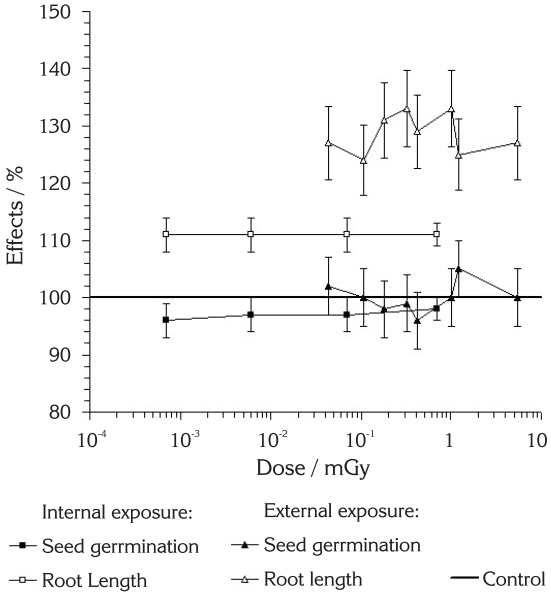

Figure 1 *Toxic effect of ^{137}Cs internal and external exposure on* L. sativum *(after two days)*

Table 1 *^{137}Cs initial activity concentrations and internal exposure doses for* L. sativum *and* Tradescantia *(clone BNL 02)*

***Lepidium sativum* L.**		***Tradescantia* (clone BNL 02)**	
Initial Activity Concentration / kBq L^{-1}	**Internal Exposure Dose (after 2 days) / mGy**	**Initial Activity Concentration / Bq L^{-1}**	**Internal Exposure Dose (after 14 days) / mGy**
0.4	0.0007	0.001	0.0003
4	0.006	0.01	0.004
40	0.07	0.125	0.05
440	0.7	1.25	0.5

The effect of external ^{137}Cs gamma-irradiation on seed germination of *L. sativum* was also insignificant when compared to control, irrespective of the dose (0.04 mGy to 5.5 mGy). Roots, however, were 24 % to 33 % longer than in control plants (Figure 1).

Our data show that the tested internal and external ^{137}Cs ionising radiation doses produce a negligible impact on seed germination in *L. sativum*. In contrast, both internal and external irradiation, irrespective of the exposure dose, stimulates the root growth.

Non-linear and non-monotonic dose-effect dependence was observed with in the studies of the effects of low external doses ($6x10^{-4}$ Gy to 1.2 Gy) on animal biophysical and biochemical properties (23) as well as in studies of genetic effects in the meristema cells of barley leaves induced by 4 cGy to 10 cGy irradiation doses (24). This diversity in dose-effect dependences because of low irradiation doses has been explained (23) as a change in the ratio between genetic damage and repair. According to *Burlakova et al*. (23), repair systems are not activated at low doses, as it takes longer for them to activate.

After 14 days of exposure, internal doses (0.0003 mGy to 0.5 mGy) of ^{137}Cs caused 1.2 % to 1.6 % of somatic mutations and yielded 19 % to 87 % of non-viable stamen hairs in *Tradescantia*. This indicates an inhibition of stamen hair cell reproducibility (Figure 2). *Shevchenko and Pomeranceva* (25) conclude that theone percent of somatic mutations induced in *Tradescantia* stamen hairshow genotoxic alterations that can cause the disappearance of a species sensitive to ionising radiation as well as changes in the whole ecosystem.

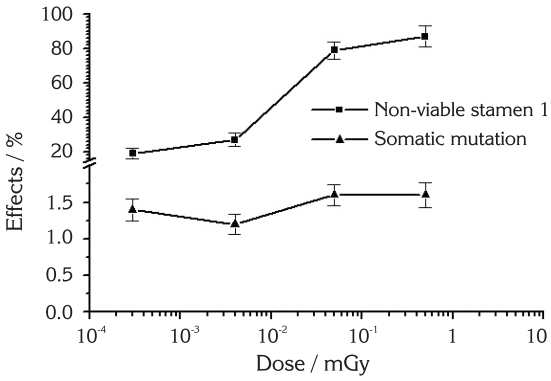

Figure 2 *Genotoxic effect of ^{137}Cs internal exposure on* Tradescantia *clone BNL 02 (after 14 days)*

Our data show the dependence between the amount of non-viable stamen hairs of *Tradescantia* and low-level exposure to ^{137}Cs, but this dependence was not established for somatic mutations.

Similar results were obtained by investigating the impact of low-level radiation doses on test organisms (26). *Shevchenko and Pomeranceva* (25) noted direct dose-effect dependence for *Tradescantia* when this plant was exposed to external irradiation of 2 Gy and higher.

The toxic effect of ^{137}Cs internal exposure on meristem cells and the genotoxic effect on the stamen hair cells can be explained by different radionuclide accumulation in plants and different distribution in plant tissues or cells. It is known that ^{137}Cs, as K analogue, chiefly accumulates in the areas of cell division and active metabolism in parenchyma and young tissues. Cellular protoplasm is the site where the largest amount of this radionuclide accumulates (27, 28).

The growth of plant cells involves three different processes: cell division, formation of protoplasm, and cell elongation. After the protoplasm has stopped growing, a cell can grow in length 10 to 50 times the initial size. Low ionising radiation doses can slow down cell division (4), which means that it can lead to a more intensive root cell elongation and therefore a longer root.

It is known that due to radionuclides can stimulate morphogenetic changes manifest in the early development stages (9, 17). Morphological changes in plants were observed after the Chernobyl accident in a 30 km radius from the NPP (6). Using the Scotch pine tree as bioindicators it was determined that the storage and reprocessing of low and intermediate activity waste were connected with an additional environmental contamination which induced cytogenetic disturbances of both the vegetative and reproductive organs of the pine tree (3). Reduced germination of matured seeds was due to the damage of a plant's reproductive organs. It was determined that toxicants stimulated plant metabolism and growth . However, the plant enzyme activity can be disturbed by metabolic products, and the more intensive is the metabolism the higher is the degree of such damage (29). Therefore, the stimulating effect of the investigated internal (0.0007 mGy to 0.7 mGy) and external (0.04 mGy to 5.5 mGy) ^{137}Cs exposure on plant root negatively influences further plant development.

CONCLUSIONS

Under laboratory conditions, low internal (0.0007 mGy to 0.7 mGy) and external (0.04 mGy to 5.5 mGy) ^{137}Cs exposure stimulated the root growth of *L. sativum* by 12 % and 33 %, respectively, regardless of the dose. A more prominent cell elongation caused by external and internal radiation exposure could affect the growth of a plant root.

After 14 days the studied ^{137}Cs internal exposure doses (0.0003 mGy to 0.5 mGy) reduced the viability of stamen hairs of *Tradescantia* and increased the amount of somatic mutations in them in a dose-dependent fashioin, but no such dependence was observed for somatic mutations.

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Sažetak

PROCJENA UTJECAJA NISKIH DOZA IONIZIRAJUĆEG ZRAČENJA IZVORA ^{137}Cs NA TOKSIČNOST I GENOTOKSIČNOST U BILJAKA

Radom je istražen učinak izlaganja čitavih biljaka ionizirajućem zračenju izvora ^{137}Cs na stanice meristema vegetativnog tkiva (korijen) i generativnog tkiva (stanice dlaka filamenata prašnika). Istraživanjem su se, također, pokušale utvrditi razlike u učinku zračenja na klijavost sjemena i rast korijena biljaka u ovisnosti o tome je li izvor zračenja u samoj biljci ili izvan nje.
Značajan toksičan učinak zračenja utvrđen je samo u biljaka vrste *Lepidium sativum L.*, i to na rast korijena. Neovisno o položaju izvora zračenja, doze od 0.0007 mGy do 0.7 mGy povećale su izduživanje korijena za 11 % do 12 %, a doze od 0.04 do 0.5 mGy za 24 % do 33 % u odnosu na kontrolu.
Interne doze zračenja izvora ^{137}Cs od 0.0003 mGy do 0.5 mGy tijekom 14-dnevnog izlaganja dovele su do pojave somatskih mutacija u 1.2 % do 1.6 % stanica dlaka prašničkih filamenata roda *Tradescantia*. Ujedno, 19 % do 87 % stanica izgubilo je vijabilnost, što upućuje na inhibiciju reproduktivne sposobnosti biljaka djelovanjem ionizirajućeg zračenja.

KLJUČNE RIJEČI: *korijen,* Lepidium sativum L., *pokusne biljke, sjeme, stanice dlaka filamenata prašnika*, Tradescantia

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