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Efficient mechanism of DNA repair stabilizes genome of *Arabidopsis thaliana* from the Chernobyl zone

Presented by Corresponding Member of the NAS of Ukraine E.L. Kordyum

Tolerance to radiomimetics and heavy metals has been investigated in Arabidopsis thaliana plants collected in the Chernobyl zone. Decrease of root growth and DNA damage level of a single cell have been evaluated. Tolerance of A. thaliana from the zone to the growth on genotoxic medium has been revealed. It is noted that certain Arabidopsis plants recover genomic DNA faster than control ones collected outside of the zone. Screening revealed plant lines expressing various levels of tolerance to genotoxins.

Keywords: *DNA damage and repair, Arabidopsis thaliana roots, single cell DNA gel electrophoresis, Chernobyl.*

30 years have passed since the explosion at the Chernobyl Nuclear Power Plant in 1986, and today, despite of heavy affection of radiation, flora is abundant even in the most contaminated sites of the Exclusion zone. This phenomenon proves plant adaptation to life in the chronic radiation environment and on soils polluted by heavy metals. Unfortunately, due to technological catastrophes and devastating economics, there is a continuous contamination by heavy metals and radiation throughout the world, which affects animals and plants and threatens human health. At the same time, despite its notoriety, the Chernobyl area along with other anthropogenically contaminated places could be considered as the unique area allowing the investigation of genetic changes in organisms after a long-time impact of genotoxins. Such anthropogenically polluted areas provide scientific challenges to researches, and the outcome of the investigations will contribute significantly to the biotechnology of plant stress tolerance, soil remediation, and human health protection.

Since plants cannot avoid environmental influences, their active vegetation in the Chernobyl zone evidences the application of extremely efficient mechanisms for minimizing harmful effects. As is known, radiation and heavy metals trigger a wide range of physiological and biochemical alterations causing DNA single- and double-strand breaks, and this results in the genome instability with potentially lethal consequences for the whole organism. During the first decade after the Chernobyl disaster (1986—1992), several studies have been performed to analyze plant genome

changes in the Chernobyl zone [1, 2]. Along with chromosome aberrations in rye and wheat [3, 4] they revealed the increased occurrence of DNA single-strand breaks [1] and pronounced dose-dependent genome destabilization [2].

Studies of the frequency of somatic intrachromosomal homologous recombination events in *Arabidopsis* plants [2, 5] have shown its tendency to return to the control level during the subsequent years after the explosion [5], evidencing in this way that changes in plant genomes have important roles in the ongoing process of plant adaptation after a short period of radiation impact. Nevertheless, a lot of questions still remain about molecular mechanisms of genome stability and how it is preserved over a longer period. Therefore, further studies are clearly needed to predict the long-term impact of radioactive contaminations on plants.

In connection with the above, we find it reasonable to check the adaptation ability of *Arabidopsis* plants from Chernobyl and to analyze to what extent the plant genome could be damaged and how fast it can be repaired. By this research, we would like to attract a wide attention of scientists and the social community to such an "open air laboratory" as the Chernobyl area and to promote investigations of the contamination impact on plants.

Up-regulation of DNA repair is one of the possible mechanisms of adaptation. By this research, we will investigate how efficiently this mechanism is functioning in the areas contaminated by radiation and heavy metals for thirty years. In particular, we investigated the ability of *Arabidopsis* to grow on a genotoxic medium (heavy metals and radiomimetics) and, in parallel, evaluated the kinetics of *Arabidopsis* genome recovery. For the latter task, we applied "comet assay", a versatile sensitive method for the evaluation of DNA damages and DNA repair capacity at a single-cell level [6]. The above method is used widely for checking the plant genome integrity after the influence of various toxins [7]. Our investigation has shown that *Arabidopsis* plants from Chernobyl tolerate the growth on a contaminated medium, and the genome of such plants is characterized by efficient repair capacity.

Materials and methods. Seed collection and growth conditions. Native *Arabidopsis* plants have been collected in 2009—2015 from areas with different levels of contamination (from 0.5 to 9 μSv/h) around the Chernobyl nuclear power plant (village of Kopachi, towns of Yanov and Prypyat). Control plants were collected in the village of Koncha Zaspa (KZ), where the level of radiation was 0.15—0.20 μSv/h. Columbia0 strain was used as a laboratory control.

Growth rate of *Arabidopsis* was assessed by germinating seeds for 10 days on square plastic plates with ½ MS medium including 0.5 % sucrose and cadmium chloride ($CdCl_2$) in the concentrations ranging from 100 to 300 μM (Fig. 1).

Growth tests with bleomycin were performed with the application of a microphenotyping system [8], which is the extremely useful tool for screening a bulk of material. In the experi-

Table 1. **Sites of *Arabidopsis* plant collection in the Chernobyl zone**

Location of sites	Doze of radiation	Geographical coordinates
10 km Chernobyl Zone, Kopachi village	~0.7 μSv/h	51°20'59.3"N; 30°07'33.4"E
5 km Chernobyl Zone, town of Pryp'yat'	~5 μSv/h	51°24'04.1"N; 30°03'54.8"E
5 km Chernobyl Zone, town of Yanov	~15 μSv/h	51°23'29.2"N; 30°03'23.6"E
Clean Control, Koncha Zaspa village, Kiev region	~0.20 μSv/h	50°29'N; 30°57'E

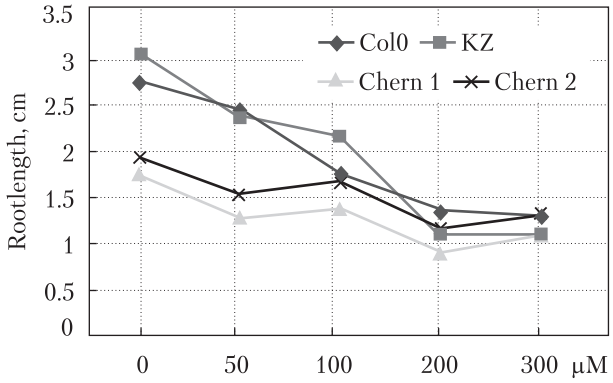


Fig. 1. Decrease of root growth in seedlings of *Arabidopsis* plants from the Chernobyl zone grown on the MS medium with addition of Cd^{2+} (Col0 — Columbia, KZ — *Arabidopsis* plants from Kiev region, Chern1 and Chern2 — *Arabidopsis* plants from radioactively contaminated sites)

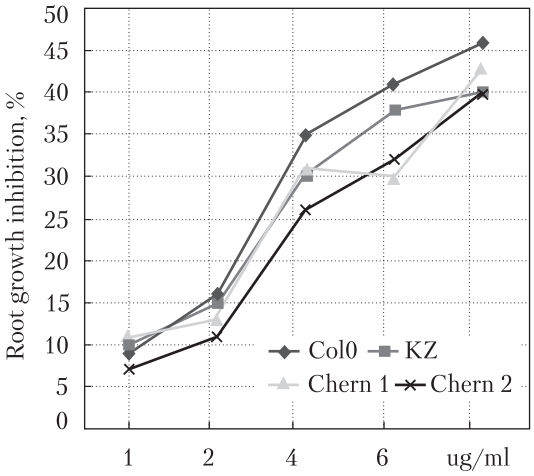


Fig. 2. Inhibition of root growth in *Arabidopsis* plants from the Chernobyl zone grown on a medium with addition of bleomycin

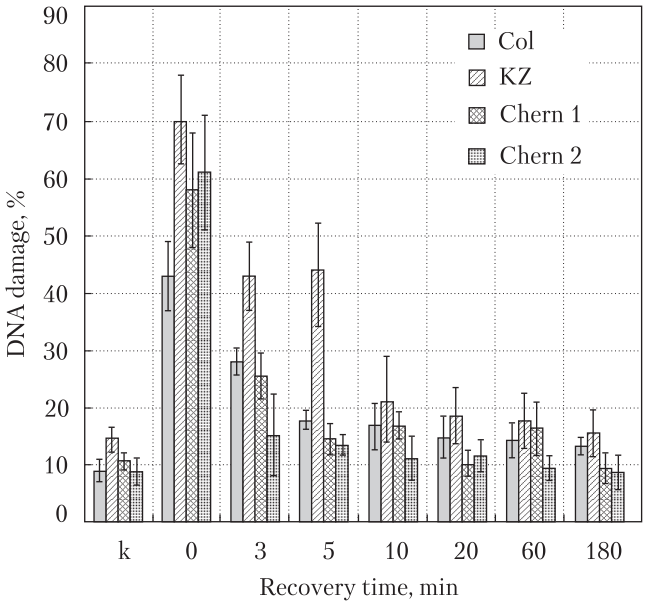
ments with growth inhibition, seeds were pre-germinated for 3 days on simple MS medium and then transferred to MS with bleomycin (1-7 μg/ml) for the next 3 days. Length of seedling roots has been measured by ImageJ programme (www.**imagej**.net), and the growth inhibition has been calculated using the formula $I\% = (\mu_c - \mu_t)/\mu_c \cdot 100$, where I% is the percentage of growth inhibition, μ_c — mean value for root length in the control, and μ_t is value for root growth rate under the treatment. The average (from 100 plants for each type of treatment) size of the roots in cm is shown in Fig. 2.

Comet assay method. DNA damage and kinetics of DNA recovery after treatment with radiomimetic bleomycin are evaluated by the means of single cell DNA gel electrophoresis (SCGE) or so called "Comet assay" [7]. In particular, DNA-DSBs were detected by a neutral comet assay [7, 9]. For this, *Arabidopsis* seedlings were cut with a razor blade in phosphate-buffered saline (PBS, pH8), and nuclear suspension was dispersed in melted 0.7 % LMT (low melting point) agarose (15510-027, GibcoBRL, Gaithersburg, USA). Aliquots of LMT agarose with nuclear suspension were immediately pipetted onto each of two agarose coated microscope slides (two duplicates per slide). After solidification of agarose, the slides were put in a lysis solution (2.5 M NaCl, 10 mM Tris–HCl, 0.1 M EDTA, 1% N-lauroyl sarcosinate, pH 7.6) for at least 1 h to dissolve cellular membranes and to remove attached proteins. After lysis, electrophoresis was performed at 45V for 3 min. Then slides were dipped for 5 min in 70 % ethanol and 100% ethanol and air-dried. DNA 'comets' were visualized in epifluorescence with a Nikon Eclipse 800 microscope after staining with a SybrGold stain (Molecular-Probes Invitrogen, USA) and evaluated by the Comet module of the LUCIA cytogenetics software suite (LIM, Praha, Czech Republic).

Table 2. **Time for fast DNA recovery accounting less than 60 % of damaged DNA**

A.thaliana plants	Time of recovery, min
Col0	3–5
KZ	10
Chern1	3–5
Chern2	3

The fraction of DNA in comet tails (% of tail-DNA) was used as a measure of DNA damage. In



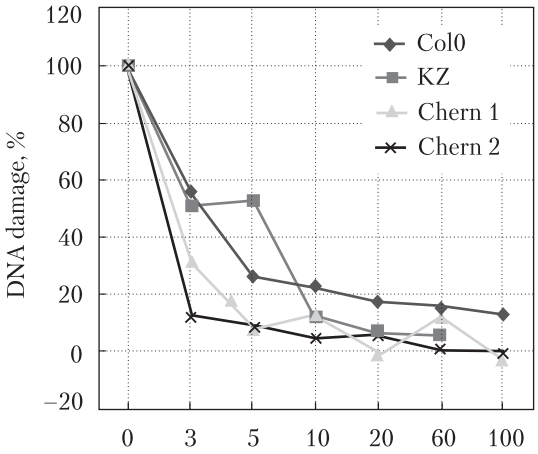


Fig. 4. Kinetics of genome repair in various strains of *Arabidopsis* plants after treatment with bleomycin

Fig. 3. DNA damage (%) in *A. thaliana* seedlings after treatment with bleomycin at the consecutive stages of genome recovery

each experiment, the % T DNA damage was measured at time points of 0, 3, 5, 10, 20, and 60 min after the treatment (1 h with 30 μg/ml bleomycin). Untreated plants were used as control. Measurements included four independent gel replicas of 25 evaluated comets (Fig. 3, 4). Repair time (*tx*) is defined as % damage remaining (*tx*) = mean % tail-DNA (*tx*) — mean % tail-DNA (control) mean % tail-DNA (*t*0) — mean % tail-DNA (control)* 100. Dose-response was calculated as the percentage of free DNA moved by electrophoresis into comet tail (% T DNA) (Fig. 3). Maximum damage is normalized as 100 % at $t = 0$ for all plants (Fig. 4).The rate of DSB repair was determined by measuring the proportion of fragmented DNA at intervals during a recovery period (Fig. 3). Data on the time of DNA recovery is presented in table 2.

Results and discussion. Root length measurements. Decrease of growth rate. In the set of experiments with tolerance to mutagens, we have tested approximately 10 *Arabidopsis* plants collected in the Chernobyl Exclusion zone. Control plants were collected from outside of the zone, where the level of radiation and soil contamination were negligible (Table 1). All plants expressed a dose-dependent decrease of growth (Fig. 1). Among Chernobyl samples, there were plants more and less tolerant to genotoxins (Chern1 and Chern2). In general, *Arabidopsis* from Chernobyl grew better on the medium with cadmium chloride (Fig. 1).

Calculation of relative growth inhibition under growth on bleomycin also has shown its lower percentage in plants from Chernobyl (Fig. 2).

Thus, the above results confirmed our previous investigations proving the tolerance of *Arabidopsis* from Chernobyl to the growth on a genotoxic medium [10].

Comet assay results. Our results have shown that both *Arabidopsis* plants grown in the Chernobyl zone and outside of it expressed the dose-dependent DNA fragmentation, and there was no significant difference in DNA damage by bleomycin between plants (Fig. 3, time point 0).

All tested plants exhibited the sensitivity to genotoxin to a various extent. There were plants with significant tolerance to bleomycin, as well as more susceptible to it (Fig. 3).

Starting from the first minutes of the plant recovery, there was a difference in the percentage of DNA damage (Figs. 3, 4). Chernobyl plants expressed a more efficient repair capacity than the controls (Fig. 4). Moreover, the rates of DNA repair in all tested plants have a characteristic biphasic profile: an initial rapid phase (1—5 min) accounting for less than 60 % of the fragmented DNA followed by a slower phase (5—60 min) accounting for the remainder (Figs. 3, 4; Table 2). To our surprise, control plants (KZ) were even more susceptible to DNA damage than *A.thaliana* from Chernobyl and Col0 (Fig. 3).

Thus, it becomes evident that plants collected in the Chernobyl zone have efficient and fast repair capacity. As is known, genetic responses to irradiation mainly depend on the efficiency of DNA repair systems in restoring the integrity of genome and preserving the heritable information. Our previous investigations on the sensitivity of Chernobyl plants to genotoxic agents have shown the increased expression of marker genes connected with such DNA repair pathways as homologous recombination (HR) and non-homologous end joining (NHEJ) [10]. Both these DNA repair pathways are known to operate in plants [11—13], and molecular components of these pathways are highly conserved amongst eukaryotes [14]. Up-regulation of several key enzymes like CyclB1:1, Rad 54, and Ku80 suggests the involvement of both HR and NHEJ, as well as cell cycle regulation, in promoting plant genome stability in genotoxic environment of the Chernobyl zone [10].

Fast recovery has been noted also by Kozak et al. (2009) [13] in their research of *Arabidopsis* mutants providing evidence for a variation in the rate of DSB repair during the first 5—10 min.

Basing on our experience and emerging works in this field, we must stress out that the comet assay method can be efficiently used for the rapid monitoring of genotoxicity induced in *Arabidopsis s*eedlings.

To sum up, we would like to note that the stability of *Arabidopsis* genome in the Chernobyl zone depends upon the efficient work of DNA damage repair mechanisms. It is worth to mention that the research of long term impact of radiation and heavy metals on plants in the nature has been performed for the first time, and it is a great contribution into our understanding of the adaptation ability of plants. In addition, it also provides knowledge for the biotechnology of stress tolerant plants.

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ЕФЕКТИВНИЙ МЕХАНІЗМ ВІДНОВЛЕННЯ ДНК СТАБІЛІЗУЄ ГЕНОМ РОСЛИН *ARABIDOPSIS THALIANA* ІЗ ЧОРНОБИЛЬСКОЇ ЗОНИ

Досліджено стійкість проростків *Arabidopsis thaliana* із зони Чорнобильської АЕС до дії мутагенів (радіоміметиків і важких металів). Проаналізовано швидкість росту коренів і ступінь пошкоджень ДНК окремих клітин рослин. Виявлено стійкість росту *A. thaliana* із зони на генотоксичному середовищі і встановлено, що деякі рослини із зони ЧАЕС відновлюють свій геном швидше, ніж контрольні, зібрані поза зоною. За результатами скринінгу визначено рослини різного ступеня чутливості до дії генотоксинів.

Ключові слова: *пошкодження та відновлення ДНК, корені Arabidopsis thaliana, електрофорез ДНК окремої клітини, Чорнобиль.*

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ЭФФЕКТИВНЫЙ МЕХАНИЗМ ВОССТАНОВЛЕНИЯ ДНК СТАБИЛИЗИРУЕТ ГЕНОМ РАСТЕНИЙ *ARABIDOPSIS THALIANA* ИЗ ЧЕРНОБЫЛЬСКОЙ ЗОНЫ

Исследована устойчивость проростков *Arabidopsis thaliana* из зоны Чернобыльской АЭС к действию мутагенов (радиомиметиков и тяжелых металлов). Проанализированы скорость роста корней и степень повреждения ДНК единичной клетки растений. Обнаружена устойчивость роста *A. thaliana* из зоны на генотоксичных средах и установлено, что некоторые растения из зоны ЧАЭС восстанавливают свой геном быстрее, чем контрольные, собранные вне зоны. В результате скрининга определены растения различной степени толерантности к воздействию генотоксинов.

Ключевые слова: *повреждение и восстановление ДНК, корни Arabidopsis thaliana, электрофорез ДНК отдельной клетки, Чернобыль.*