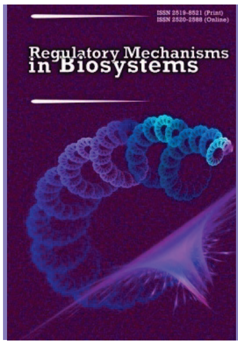

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Chromosomal disorders in *Triticum aestivum* subject to prolonged radionuclide pollution of soil from the Exclusion Zone of the Chornobyl Nuclear Power Plant

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Due to the large number of nuclear power plants around the globe and the growing threat of radioactive accidents, a complex assessment of the biological consequences of the Chornobyl disaster is a relevant issue. Study of mutagenic activity of prolonged and chronic action of radionuclide pollution of soil in the Chornobyl Exclusion Zone long after the accident will allow us to identify the current level of threat they pose to the human genome and can reveal specific cytogenic markers of radionuclide environmental pollution. For this purpose, we germinated seeds of wheat of soft winter varieties Smuhlianka and Bohdana in soil samples from the Chornobyl Exclusion Zone 35 years after the disaster. Specific activity of Cs-137 and Sr-90 accounted for 4.5–28.2 kBq/kg. To determine the frequency and range of chromosomal aberrations, we used the anaphase-telophase method. In aberrant cells, we studied extracellular distribution of chromosomal disorders. The frequency of aberrant cells was 3.53–7.55 times above the spontaneous level. High mutagenic activity remained in the conditions of the lowest density of radionuclide pollution of soil. The range of chromosomal disorders mostly included paired fragments and bridges and containd chromosomal acentric rings, binding of chromosomes, myocardium, and lagging and leading chromosomes. Radionuclide pollution in the Chornobyl Exclusion Zone induced multiple cellular aberrations in the rhizome meristem, the share of which was the highest in case of low specific radionuclide pollution of soil, and exhibited a tendency towards decrease against the background of the highest density of radionuclides. Pollution of the environment with radionuclides as a result of disasters at nuclear energy objects poses a threat to the human genome and is a global problem, requiring state support to solve through systematic genetic monitoring in readionuclide-polluted territories and areas adjacent to them.

Keywords: *Triticum aestivum* L.; radionuclide pollution; mutagenic activity; chromosomal aberrations.

Introduction

Emergence of life on Earth and evolution of living organisms was followed by adaptation of genomes to detrimental impact of ionizing radiation. Its existence is associated with cosmic rays and existence of initial and secondary natural radionuclides in the Earth's crust, whose half-life reaches $7 \cdot 10^8$–$2 \cdot 10^{19}$ years (Cinelli et al., 2019). Natural radioisotopes are involved in the formation of background level of ionizing radiation, which accounts for the average of 0.27 μSv/h. Some areas, for example, Ramsar in Iran, Morro do Ferro and Guarapari in Brazil, Mombasa in Kenia, have been observed to have over 100 times excess of the background level. Based on impacts on some species of plants, studies identified those levels of radiation as a gene-toxic factor (Saghirzadeh et al., 2008; Yakymchuk, 2017). However, the time of existence of natural radiation background, the duration of which is estimated on a timescale of geological periods, allowed populations of living organisms to adapt to it (Mousseau, 2021).

Nuclear disasters cause radionuclide pollution of the environment, threatening biological systems. The planet's population is subject to 50% dose of radiation as a result of radioactive breakdown of natural radionuclides. However, a special risk to the living organisms is posed by uncontrolled radioactive emissions that can lead to acute exposure to radiation, which can persist in polluted ecosystems for a long time (Duarte et. al., 2023). Starting from the second half of the twentieth century, anthropogenic activity resulted in significant increase in the areas of radioactively polluted territories, which raises concerns regarding protection of people and biota from radiation. An extremely dangerous source of growing radioactivity in the environment has been the disaster at the Chornobyl Power Plant (Ukraine) – the largest nuclear catastrophe of the twentieth century (Beresford et al., 2016). It emitted $1.85 \cdot 10^{18}$ Bq of radioactive isotopes into the atmosphere and polluted an area of over 200 thou km^2 in Europe and Eurasia, increasing the annual level of radiation for many people in the individual range of doses 100 times above the allowable threshold (Piguet et al., 2019). This radioactive material settled very unevenly, hence the differences in amounts of radiation doses even in small areas of the surrounding landscape (Goodman et al., 2022).

Radionuclide pollution caused by the accident at the Chornobyl Power Plant has caused irreversible genetic disorders in living organisms. Due to the large number of nuclear power plants around the world and high probability of new accidents, complex assessment of biological implications of the Chornobyl catastrophe is becoming a relevant issue (Piguet et al., 2019). Results of numerous scientific studies laid groundwork for mathematical models for predicting environmental impacts of the largest nuclear disaster of this century – at Fukushima Daiichi (Japan) (Cannon & Kiang, 2022; Ludovici et al., 2022), where the radiation emissions accounted for 10% of those associated with the Chornobyl disaster (Steinhauser et al., 2014; Skalozubov et al., 2022). The impact of radionuclide pollution is found at all levels of organization of biological objects

– from viruses to ecosystems. Numerous studies assessed the genetic implications of radiation for people and wildlife. Increased level of mutations was found in liquidators, their descendants, and also children born in polluted areas. Similarly, studies demonstrated a heightened level of mutations in wild populations of animals and plants living in radionuclide-polluted areas (Baker et al., 2017; Volkova et al., 2018; Fuller et al., 2019; Morgun & Yakymchuk, 2021). Negative genetic consequences of acute or chronic effects of radioactive emissions from the ruined reactor included point mutations and chromosomal rearrangements (Dillon et al., 2023), changes in expressions of DNA-reparation genes (Jernfors et al., 2018; Kesaniemi et al., 2019), damage to mitochondrial DNA (Kesäniemi et al., 2020), decline in cellular proliferation (Cannon & Kiang, 2022), inhibition of growth of trees, heightened frequency of tumors and development abnormalities (Skalozubov et al., 2022), intellect decline (Tronko et al., 2019), enhancement of aging (Reste et al., 2016), fertility disorders (Einor et al., 2016; Mousseau, 2021), decrease in genetic diversity and number of population (Car et al., 2023; Dillon et al., 2023), and vanishing of some species (Fuller et al., 2019; Mousseau, 2021), etc.

The broad range of radiation levels in the Chornobyl Exclusion Zone gives the opportunity to research genetic processes under the influence of acute and chronic radiation in high and low doses. Most studies conducted in the Exclusion Zone in the first years after disaster focused only on various biological objects of genetic consequences of large radiation doses (Fuller et al., 2019). Over time that has passed since the Chornobyl explosion, the radiation state of radionuclide-polluted areas has significantly improved. This has been being promoted by processes of physical breakdown of most radionuclides, their migration into the environment, protective measures carried out in agriculture, deactivation work, and prevention of radionuclide emissions outside the Exclusion Zone (Ivanuta, 2021). However, even relatively low levels of radiation can induce significant genetic disorders and heightened rate of mutations (Cannon & Kiang, 2022), as confirmed by numerous studies in laboratories or precisely controlled conditions, which lay the ground for the linear non-threshold model (Caplin & Willey, 2018; Shore et al., 2018). Quite likely, mechanisms of correlation between dose-effect in cases of acute and prolonged radiation in the range of low doses significantly vary, and modeling genetic consequences in the natural conditions of the Exclusion Zone is complicated by many other variable factors that are unrelated to radiation impacts of the disaster (Skalozubov et al., 2022). Comparisons and generalizations of results of studies conducted in the areas affected by nuclear disasters, nuclear bomb test sites, and naturally radioactive regions of the world give unique opportunities for analyzing mutagenic effects of individual radionuclides and types of ionizing radiation (Mousseau & Møller, 2020). It is generally recognized that the most informative and sensitive indicators of environmental pollution with mutagens, radionuclides in particular, caused by chronic ionizing low-dose radiation, are parameters of cytogenetic disorders, particularly chromosomal rearrangements in plant cells (Maluszynska & Juchimiuk, 2005). When studying genetic impacts of mutagenic factors, results of cytogenetic analysis correlated with the results of analysis of isoenzymes using the electrophoresis method (Ramzaev et al., 2008).

An important aspect of the complex of relevant studies that are being conducted by the global scientific community is study of mutagenic activity of prolonged and chronic action of radionuclide pollution of soil in the Chornobyl Exclusion Zone long after the accident. Such studies will reveal the current threat in this area for genomes of living organisms, dynamics of induction of mutations as specific radioactivity of soil decreases, and frequency and range of mutation types so as to detect specific markers of radionuclide pollution of the environment.

Materials and methods

The mutagenic activity of radionuclide-polluted soil in the Chornobyl Exclusion Zone was studied on sprouts of soft winter wheat (*Triticum aestivum* L.) of the Smuhlianka and Bohdana varieties using the anaphase-telophase method. The seeds were germinated at the temperature of 24–26 ºC in samples of moist soil, taken in 2021 (35 years after the Chornobyl disaster) in the villages Kopachi, Chystohalivka, and Yaniv (Fig. 1). The specific activity of Cs-137 and Sr-90 was 4.5, 9.3, and 28.2 kBq/kg, respectively. As control, we used soil from conditionally clean territory of the Hlevaha village of Fastiv District, Kyiv Oblast, where the density of Cs-137 and Sr-90 pollution equaled 0.29 kBq/kg. Specific activity of Cs-137 and Sr-90 in soil was measured at the basis of Specialized State Enterprise Chornobyl Spetskombinat using γ-spectrometric complex and low-background β-radiometer. To fixate the primary roots, we used the Clarke's fluid – mixture of ethyl alcohol and glacial acetic acid in 3 : 1 ratio. For their chemical maceration, we used 1 n solution of hydrochloric acid. After maceration, the roots were held for 24 h in aceto-orcein solution at the temperature of 23–25 ºC.

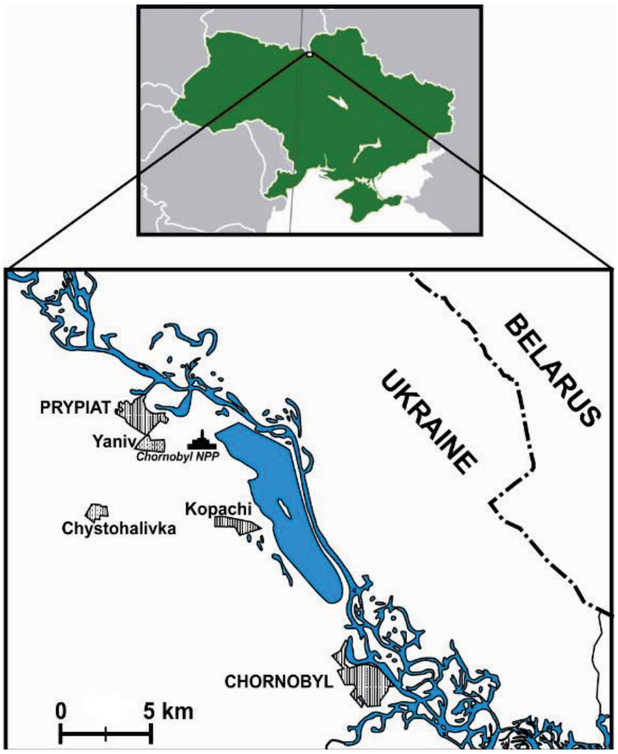

Fig. 1. Places of sampling radionuclide-polluted soil in the Chornobyl Exclusion Zone

Microscopic analysis was conducted on temporary squashed cytological preparations that were prepared using the generally approved methods (Singh, 2018). When making the compressed preparations, we used only the apical part of the 0.8–1.0 mm root. Microscopic study of cells of the root meristem was carried out using a JENAVAL microscope (Carl Zeiss Jena) under 600x increase. Chromosomal aberrations and mitosis disorders were seen in the cells that were in anaphase and early telophase. The analysis was conducted in 3 repetitions, the sampling was 900 cells, studied in 12–17 primary roots. Frequency of aberrant cells and abnormallities of mitosis were considered as percentage of cells in anaphase and early telophase, which contained the following disorders: singular and paired fragments, bridges of chromosomal and chromatide types, micronuclei, chromosomal acentric rings, binding of chromosomes, and lagging and leading chromosomes (Fig. 2). In the cells with chromosomal disorders, we studied distribution of aberrations in cells. When estimating average number of aberrations per aberrant cell, we analyzed cells with 1, 2, 3, and >3 aberrations and mitotic disorders. Statistical analysis of the research data was performed using the generally accepted methods (Atramentova & Utevska, 2007), and the significance of difference was identified according to the Student's Criterion taking into account Bonferroni's Correction. The data in tables are presented as x ± SE (mean ± standard error).

Results

The frequency of chromosomal aberrations and mitotic abnormalityes, induced by radionuclide pollution of soil in the territory of the Chornobyl disaster 35 years after the accident, was significantly higher than the control parameters. The level of aberrant cells was 3.53–7.55 times higher than spontaneous parameters and depended on wheat genotype and specific radioactivity of soil (Table 1). The highest levels of cytogenetic disorders – 6.04% and 4.17% in the varieties Smuhlianka and Bohdana, respectively, were found in the plants cultivated in soil with the highest general density of radionuclide pollution (the Yaniv village) (Fig. 3). Significant increase in the frequency of aberrant cells was also found in the plants grown in soil with the lowest density of radionuclide pollution (the villages Chystohalivka and Kopachi). The level of chromosomal rearrangements in the Smuhlianka and Bohdana varieties was 5.86–6.82 and 3.53–3.99 times higher than the control parameters, respectively.

Overall density of soil pollution with Cs-137 and Sr-90 in the Chystohalivka village was high, accounting for 9.3 kBq/kg, exceeding the specific radioactivity of soil in the Kopachi village by 2.07 times. Nonetheless, the frequency of aberrant cells against the background of radionuclide soil pollution of those areas did not significantly vary, and in the cells of the root meristem of the Smuhlianka variety we even observed a downward tendency. Therefore, subject to radionuclide pollution of soil from the Kopachi village, induction of chromosomal aberrations in the cells of root meristem of the Smuhlianka variety increased 6.82-fold compared with the control, and the effects of radionuclides of soil in the Chystohalivka village caused 5.86-fold increase in aberrant cells.

The range of types of chromosomal disorders, which we found during cytogenetic analysis, contained paired fragments and bridges that are typical chromosomal rearrangements induced by ionizing radiation, while in the control we mostly found single fragments and dicentric chromosomes of chromatide type. Ratio of acentric fragments to bridges varied 0.98–7.19 with a tendency towards the largest share of single and paired fragments in the conditions of radionuclide soil pollution of the Chystohalivka village impacting the cells of root meristem of the Smuhlianka variety. In cases of highest density of radionuclide soil pollution (Yaniv village), the share of meristematic cells with acentric fragments and bridges was the same in the Bohdana variety and also we observed a tendency towards increase in the share of aberrant cells with acentric fragments in the Smuhlianka variety.

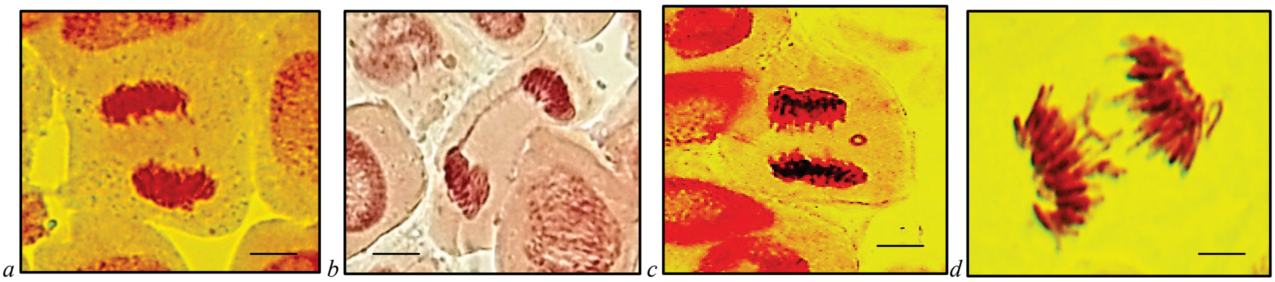

Fig. 2. Types of chromosomal aberrations and abnormalities of mitosis in anaphase-telophase cells of the root meristem of winter wheat: *a* – acentric fragment, *b* – chromosomal bridge, *c* – chromosomal ring, *d* – lagging chromosome; bar = 5 µm

Table 1

Frequency and range of chromosomal aberrations in anaphase-telophase cells of the root meristem of winter wheat induced by radionuclide soil pollution in the Chornobyl Exclusion Zone ($n_1 = 3$, $n_2 = 900$, $x \pm SE$)

Sampling location	Mitosis disorders and chromosomal aberrations, %	Range of mitosis disorders and chromosomal aberrations, %					
		fragments	bridges	bridges + fragments	micronuclei	chromosomal rings	lagging chromosomes
Smuhlianka							
Hlevaha village (control)	0.80 ± 0.02	0.74 ± 0.02	0.06 ± 0.04	0	0	0	0
Kopachi village	5.46 ± 0.89**	3.43 ± 1.00	1.46 ± 0.22**	0.04 ± 0.04	0.18 ± 0.12	0	0.35 ± 0.12*
Chystohalivka village	4.69 ± 0.50**	3.74 ± 0.70*	0.52 ± 0.18	0.10 ± 0.06	0	0.07 ± 0.03	0.26 ± 0.21
Yaniv village	6.04 ± 0.60***	4.12 ± 1.16*	1.73 ± 0.22**	0	0	0	0.19 ± 0.05*
Bohdana							
Hlevaha (control)	0.98 ± 0.08	0.34 ± 0.06	0.34 ± 0.11	0	0	0	0.30 ± 0.10
Kopachi village	3.46 ± 0.37**	1.93 ± 0.43*	0.97 ± 0.33	0.11 ± 0.05	0.15 ± 0.15	0.10 ± 0.05	0.20 ± 0.19
Chystohalivka village	3.91 ± 0.39**	2.21 ± 0.32**	1.39 ± 0.22*	0.06 ± 0.04	0	0.09 ± 0.08	0.16 ± 0.13
Yaniv village	4.17 ± 0.20***	1.72 ± 0.23**	1.75 ± 0.17**	0.16 ± 0.16	0.03 ± 0.02	0.11 ± 0.06	0.40 ± 0.15

Note: * – P < 0.05; ** – P < 0.01; *** – P < 0.001 difference compared to control is statistically significant, taking into account the Bonferroni's Correction.

Mutagenic action of radionuclide soil pollution expands the range of chromosomal aberrations and mitosis disorders by inducing chromosomal acentric rings, binding of chromosomes, micronuclei, and lagging and leading chromosomes. Cells with acentric chromosomal rings occurred singly and accounted for 0.07% of the total number of aberrations and mitosis disorders in the Smuhlianka variety after prolonged action of radionuclide pollution of soil from the Kopachi village and 0.09–0.11% in Bohdana cultivated in wet soil collected in the villages Kopachi, Chystohalivka, and Yaniv. Micronuclei were found in variants of impact of radionuclide pollutions where share of acentric fragments was the lowest – the Kopachi village for the Smuhlianka variety (0.18%) and the Kopachi and Yaniv villages for the Bohdana variety (0.15% and 0.03%, respectively).

Cytogenetic disorders included abnormalities of cellular division: lagging and leading chromosomes, and binding of chromosomes. Lagging chromosomes emerged in all variants of radionuclide soil pollution. Frequency of their induction in cells of the root meristem of the Bohdana variety was at the level of impact of background values of specific radioactivity of soil in the control, equaling 0.16–0.40%. As a result of radionuclide soil pollution in the Exclusion Zone towards the cells of the root meristem of the Smuhlianka variety, the share of lagging chromosomes significantly exceeded the spontaneous level, measuring 0.26–0.35% of the overall frequency of induced aberrations and mitosis abnormalities. Binding of chromosomes, which is a result of agglutination, was found in the variant with prolonged effect of radionuclide pollution of soil in the Yaniv village on the cultivated plants of the Smuhlianka wheat variety.

Genotoxic action of radionuclides in soil from the studied areas of the Chornobyl Exclusion Zone was accompanied by increase in the number of cells with multiple aberrations. If the share of anaphase cells with 1 detected aberration or mitosis abnormality had an upward tendency as the density of radionuclide soil pollution increased, the presence of 2 chromosomal disorders at the same time in meristematic cells was characterized by inverse correlation with specific radioactivity (Table 2). Therefore, in the root meristem of the Smuhlianka and Bohdana varieties, the share of aberrant cells with 1 chromosome disorder ranged 2.92–4.63% and 1.82–2.88%, respectively, and was the highest in the conditions of influence of radionuclide-polluted soil from the Yaniv village, and the highest level of cells bearing 2 aberrations was common in plants cultivated in soil from the Kopachi village, measuring 1.92% and 1.53%, respectively in the Smuhlianka and Bohdana varieties.

The share of aberrant cells with multiple aberrations, number of which was 3 and more, ranged 0.03–0.46%. In the root meristem of wheat subject to radionuclide soil pollution that had the largest specific radioactivity (the Yaniv village), we found no cells with multiple, more than 3, aberrations. The number of aberrations per aberrant cell significantly exceeded the control in plants subjected to prolonged influence of radionuclide-polluted soil from the villages Kopachi and Chystohalivka, measuring 1.37–1.59% and 1.36–1.46% in the varieties Smuhlianka and Bohdana, respectively (Fig. 4).

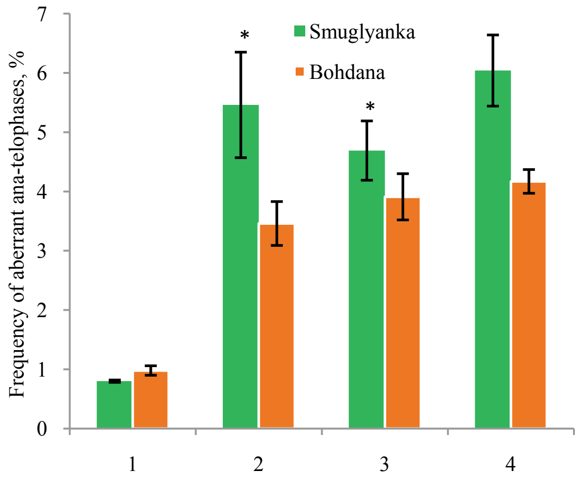

Fig. 3. Comparison of frequency of aberrant anaphase-telophase cells of the root meristem of winter wheat induced by various densities of radionuclide soil pollution in the Chornobyl Exclusion Zone: *1* – Hlevaha village (control), *2* – the Kopachi village, *3* – Chystohalivka village, *4* – Yaniv village; * – P < 0.05 difference compared with the Bohdana variety is statistically significant; $n_1 = 3$, $n_2 = 900$, x ± SE

In the conditions of various specific radioactivity of soil of the Chornobyl Exclusion Zone, change in the number of aberrations per aberrant-cell was not accompanied by a similar variation of frequency of chromosomal aberrations, which could be attributed to the fact that they reflect somewhat different parameters of interaction between mutagenic factor and chromosomes. Therefore, when assessing mutagenic activity of radionuclide pollutions of the environment, it is expedient to simultaneously take into account the level of cytogenic impairments and the degree of genetic damage to aberrant cell.

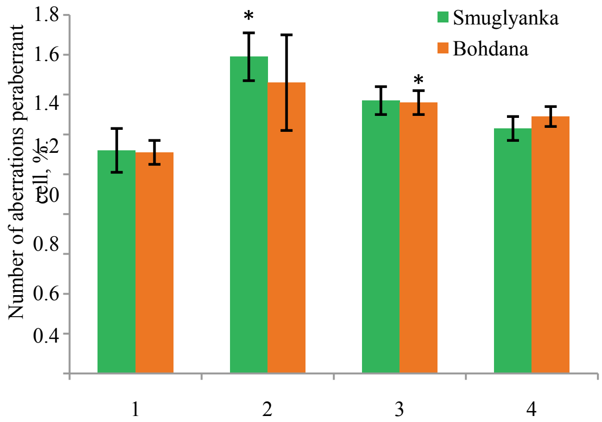

Fig. 4. Comparison of the numbers of aberrations per aberrant cell in anaphase-telophase cells of the root system of winter wheat, induced by various densities of radionuclide pollution of soil from the Chornobyl Exclusion Zone: *1* – Hlevaha village (control), *2* – Kopachi village, *3* – Chystohalivka village, *4* – Yaniv village; * – P < 0.05 difference, compared to control, was statistically significant; $n_1 = 3$, $n_2 = 900$, x ± SE

Table 2
Quantitative distribution of aberrations and abnormalities of mitosis in aberrant anaphase-telophase cells of winter wheat induced by various densities of radionuclide soil pollution in the Chornobyl Exclusion Zone

Sampling location	Frequency of anaphase-telophase aberrant cells, %			
	1 aberration or mitosis disorder	2 aberrations or mitosis disorders	3 aberrations or mitosis disorders	over 3 aberrations or mitosis disorders
Smuhlianka				
Hlevaha village (control)	0.64 ± 0.08	0.16 ± 0.09	0	0
Kopachi village	2.92 ± 0.12***	1.92 ± 0.32**	0.46 ± 0.13*	0.16 ± 0.09
Chystohalivka village	3.00 ± 0.25***	1.52 ± 0.47*	0.08 ± 0.07	0.09 ± 0.09
Yaniv village	4.63 ± 0.58**	1.33 ± 0.31*	0.08 ± 0.08	0
Bohdana				
Hlevaha village (control)	0.86 ± 0.01	0.12 ± 0.06	0	0
Kopachi village	1.82 ± 0.38	1.53 ± 0.52	0	0,11 ± 0,11
Chystohalivka village	2.73 ± 0.31**	1.00 ± 0.32	0.07 ± 0.07	0.11 ± 0.06
Yaniv village	2.88 ± 0.19***	1.26 ± 0.38*	0.03 ± 0.03	0

Note: * – P < 0.05; ** – P < 0.01; *** – P < 0.001 difference compared to the control is statistically significant taking into account the Bonferroni's Correction.

Discussion

Induction of chromosomal aberrations in the conditions of decrease in the radioactivity of soil in the Chornobyl Exclusion Zone. As a proliferating tissue, the apical root meristem is highly sensitive to genotoxic action of physical-chemical factors, and therefore can be broadly used in effective genetic biotests with chromosomal aberrations, and the analysis of their types is a reliable biomarker of radiation effect (Bolsunovsky et al., 2019). Cytogenetic studies in the Chornobyl Exclusion Zone were conducted since the first years after the disaster, under higher doses, and are carried out to this day in the conditions of low-dose chronic radiation (Hinton et al., 2007). In most of the territory of Ukraine, the radiation doses were no higher than the allowable values. However, in the Exclusion Zone, which has concentrated the largest amount of radioactive emissions from the disaster, ecological dosimetry monitoring will remain necessary for centuries, as well as assessment of mutagenic activity of radioactive component of complex of mutagenic factors (Dillon et al., 2023). Radionuclide-pollution-induced 3.53–7.55-fold increase in the frequency of chromosomal disorders in winter wheat indicates that even relatively low specific radioactivity of soil, which persists 35 years after the nuclear catastrophe, can cause significant genetic disorders. Some researchers substantiated the high mutagenic activity of radionuclide pollution in the Chornobyl Exclusion Zone long after the disaster, occurring through a combined ionizing radiation and mixtures of chemical components present in soil, including heavy metals, organic heterocyclic compounds, pesticides, and other xenobiotics (Dillon et al., 2023). However, the assumed possibility of significant mutagenic effects as a result of synergic interactions between radionuclides and chemical factors are doubtful considering the results of cytogenic studies that demonstrated significant increase in frequency of micronuclei – biomarkers of radiation impact in polychromatide erythrocytes of population of *Microtus arvalis* Pall., which lived in radionuclide-polluted areas of the Exclusion Zone (Garnier-Laplace et al., 2013).

Excess of the spontaneous level of aberrant cells and mitosis abnormalities were also found in previously conducted studies of prolonged action of radionuclide-polluted soils of the Chornobyl Exclusion Zone towards sprouts of winter wheat (Yakymchuk, 2013). Sometimes, results of analysis of mutations in natural populations of organisms that had been exposed to various anthropogenic effects vary. In particular, there was found increase in the frequency of chromosomal aberrations in *Microtus ar-*

valis Pall. subject to low doses of chronic exposure to radiation in radionuclide-polluted zone and absence of mutagenic effects after exposure to radiation in a 10-km area of the Chornobyl Exclusion Zone, the degree of which was ten times higher than the previous parameter (Hinton et al., 2007). High mutagenic activity persisted in the conditions of the lowest density of radionuclide pollution of soil in the Kopachi village, being comparable to the parameters of cytogenic disorders induced by radionuclides in soil from the Chystohalivka village with significantly higher specific radioactivity. This level of activity could be associated with non-linear pattern of genetic effects after low-dose radiation. Numerous experiments on plants and animals suggest a non-linear pattern of biological effects caused by the action of low doses of radiation, in particular in the Chornobyl Zone (Kalaev & Butorina, 2006; Hinton et al., 2007; Yakymchuk, 2018; Bolsunovsky et al., 2019; Yin et al., 2019; Ocolotobiche et al., 2021; Cherednichenko et al., 2024). Those studies confirmed that in natural conditions, when subject to technogenic radiation factors, a living organism has a significantly higher level of genetic disorders per unit of radiation dose than the classic laboratory models imply (Milacic, 2009; Mousseau, 2021). One may assume that persistence of high level of mutation in the conditions of decline in specific soil radioactivity is associated with specific mechanisms of repair of genetic disorders caused by low doses of radiation, which are regulated by both level and rates of formation of disorders of DNA and active oxygen species.

The literature includes numerous data on various levels of radiation-sensitivity of organisms and some cells that had been subjected to the same radiation dose (Applegate et al., 2020; Herate & Sabatier, 2020). Even when conducting cytogenic analysis right after radiation impact, which rules out a of chromosomal aberrations as a result of cell death, there was observed a quite significant variation of individual values of mutational disorders. The differences in parameters of frequency of chromosomal aberrations among genotypes of various varieties of winter wheat in the same conditions of mutagenic impact suggest that the Smuhlianka variety is more sensitive to radiation than Bohdana. During radioecological monitoring, using genotypes of soft winter wheat with various radioation-sensitivity produced highly reliable data regarding the level of mutagenic activity of radionuclide pollution of soil in the Chornobyl Exclusion Zone. The persisting high rate of chromosomal aberrations 35 years after the accident in the conditions of significant decline in specific radioactivity of soil suggests the existing danger to genomes of living organisms in the nearer area (approximately 10 km radius from the reactor) of Chornobyl Exclusion Zone. Further systematic genetic monitoring in radionuclide-polluted areas using various plant test objects will help in accurately assessing the level of mutagenic activity of radionuclide pollutions long-term after the nuclear disaster.

Radioactive component in the formation of the range of chromosomal aberrations. During radioecological studies, it is very important to analyze ratio of chromosomal aberrations of various types. The effect of relatively low stress factors first is accompanied by change in the spectrum of mitotic disorders, and only later causes increase in the number of pathologies (Shkarupa et al., 2011). An important class of disorders induced by ionizing radiation is double chain disruptions of DNA that occur in G1-period of cellular cycle and further lead to chromosomal exchanges of various complexity (Milacic, 2009; Anderson, 2019). Unstable chromosomal rearrangements such as dicentric and polycentric chromosomes, and acentric rings are usually used as biomarkers in assessing impact of ionizing radiation. However, in the conditions of chronic or prolonged radiation with low doses, the frequency of induced dicentric chromosomes significantly decreases and is unstable, when analysis of up to 3,000 anaphases of mitosis is needed to produce statistically significant results (Cherednichenko et al., 2024). In such conditions, the efficiency of mutagenic influence of ionizing radiation should be identified according to the level of induction of chromosomal-type aberrations (Nugis et al., 2019). Prolonged action of radionuclide soil pollutions in the territory of the villages Kopachi, Chystohalivka, and Yaniv increased in the frequency of cells with chromosomal aberrations through induction of mostly paired fragments and dicentric chromosomes, which form as a result of chromosomal disruptions and non-symmetric repeated joining events. In the control, cells with dicentric chromosomes in the root meristem of sprouts were recorded at a very low level (0.06–0.34%), and we also saw a tendency towards direct dependence on the density of radionuclide soil pollution in the Bohdana variety and its absence in the Smuhlianka variety. It is believed that induction of dicentric chromosomes increases linearly or in linear-quadratic manner as a result of radiation impact, respectively, with high and low linear energy transfers (Anderson, 2019). Considering the difference we found between the wheat varieties in the frequency of chromosomal bridges and paired fragments depending on intensity of radionuclide soil pollution, we may assume that increase in various types of chromosomal aberrations – biomarkers of radiation impact – has a genotype dependence. Expansion of the range of chromosomal rearrangements due to induction of single fragments does not rule out radiation component of their formation. There is an assumption that increase in the level of acentric fragments is a distinctive feature of prolonged impact of low-dose ionizing radiation. It was confirmed that radiation-induced disruptions of chromatides in G2-period form during condensation of chromatin and formation of chromatin fiber (Soni et al., 2019).

Micronuclei were found in the variants of radionuclide pollutions with the lowest share of acentric fragments – the Kopachi village for the Smuhlianka variety (0.18%) and the villages Kopachi and Yaniv for the Bohdana variety (0.15 and 0.03%, respectively), which could be associated with their formation mostly from acentric fragments (Krupina et al., 2021). The frequency of detected micronuclei did not significantly exceed the control parameters and ranged within the statistical error, which is consistent with other studies, in particular those that dealt with effects of low radiation doses on the apical root meristem of *Allium cepa* L. (Bolsunovsky et al., 2019).

Subject to radionuclide-polluted soil of the Chornobyl Exclusion Zone, the range of chromosomal aberrations of cells of root meristem of wheat expanded and included chromosomal rings that are markers of radiation impact as much as paired fragments and dicentric chromosomes (Anderson, 2019; Jeong et al., 2023). There is a point of view that chromosomal rings result from exchange of areas of two arms of one chromosome, with further binding of its proximal ends (Burssed et al., 2022). Emergence of lagging and leading chromosomes as a result of prolonged radionuclide pollution of soil suggests aneugenic action of mutagenic factors of soil. It is caused by impaired mechanisms of chromosomal segregation and errors in control of the normal course of certain phases of cellular cycle (Siri et al., 2021).

Induction of multiple aberrations depending on density of radionuclide pollution. Quantitative cytogenic analysis of multiple chromosomal aberrations that results from subsequent bonds of the complex of independent disruptions is able to reveal the degree of genetic damage to aberrant cells from acute, prolonged, or chronical effect of radionuclide pollution (Anderson, 2019). Radionuclide pollution in the Chornobyl Exclusion Zone induced multiple aberrations in cells of the root meristem, the share of which was the highest in case of low specific radioactivity (Kopachi village) and exhibited downward tendency in case of the highest specific radioactivity of soil (Yaniv village). Against the background of lower values of specific radioactivity of soil (Kopachi and Chystohalivka), there was also found statistically significant increase in the number of aberrations per aberrant cell. This parameter characterizes the intensity of induction of multiple aberrations and does not directly correlate with the frequency of aberrant cells, and also is an individual quantitative characteristic of mutagenic activity of radionuclide pollution of the environment. According to the classical radiation target theory, distribution of aberrations in cells in the conditions of low-density radiation follows Poisson's law. However, there are data that probability laws do not always apply to the distribution of the general number of aberrations or disruptions of chromosomes in radiation-subjected cells (Kutsokon et al., 2003). Some mutagens or certain conditions of their influence are able to increase frequency of cells with aberrations, while other mutagens or conditions increase the number of new genetic disorders in aberrant cells. This indicates various mechanisms and ways of realization of disorders of inherited material at chromosomal level.

Conclusions

Despite gradual improvement in the radioecological situation in the nearest zone of the Chornobyl Power Plant, radionuclide soil pollution

35 years after the disaster retains mutagenic activity, as indicated by a 3.53–7.55 excess of the spontaneous level of chromosomal aberrations. No direct correlation was found between yield of aberrant cells and density of radionuclide pollution of the areas of the Chornobyl Exclusion Zone. Considering persisting high level of cytogenic disorders in the territories characterized by significant decrease in specific radioactivity of soil, identification of boundaries of radiation-safe areas of the Exclusion Zone should consider not only density of radionuclide pollution and power of exposure dose, but also mutagenic activity. The range of chromosomal disorders included paired fragments and bridges, typical for the conditions of impact of ionizing radiation, ratio of which ranged 0.98–7.19. Mutagenic action of radionuclide soil pollution expanded the range of chromosomal aberrations and mitosis abnormalities, including chromosomal acentric rings, micronuclei, and lagging and leading chromosomes. Induction of lagging and leading chromosomes by prolonged impact of radionuclide soil pollution suggests aneugenic action of the mutagenic factor. Mutagenic action of radionuclides in the soil of the Chornobyl Exclusion Zone caused emergence of cells with multiple aberrations, the share of which exhibited inverse correlation with density of radionuclide pollution, which should be considered when performing radioecological monitoring of the environment. Results of multiple studies of genetic implications of the Chornobyl disaster and accidents at nuclear objects in other countries indicate long-lasting radiation threat to stability of genomes of living organisms. Therefore, systemic genetic monitoring of territories that have been affected by radionuclide pollution and near radiation-dangerous objects should be included in the state ecological program.

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