CHROMOSOMAL VIOLATIONS IN WHEATS FROM THE ZONE OF CHORNOBYL NPS

R. Yakimchuk[1], I. Chizhevskij [2]

[1] Pavlo Tuchyna Uman State Pedagogical University

[2]SSE «The Chernobyl Specialized Enterprise»

E-mail: peoplenature@rambler.ru

One of the main reasons for growing of global ecological crisis in modern conditions is man-caused contamination of the biosphere by radio nuclides of artificial origin, which leads to radiation destabilization of large territories.

Perceptible increase of radiation background on the large areas was caused by the accident at the Chernobyl Nuclear Power Station. All the Northern part of the Right-Bank Ukraine, 80 % of the area of Belarus, 17 regions and republics of the Russian Federation were affected because of it in various degrees. [4]. Radioactively contaminated territory of the exclusion zone has become a unique model system for the study of population-genetic transformations, caused by the changes in the direction and intensity of natural selection [3].The results of some studies show the preservation of the stable high level of mutation variability of organisms even after a long time since the accident at the Chernobyl NPS [1, 7]. Therefore, the aim of the work is to determine the level of mutagenic activity of radionuclide contamination, with the help of the frequency and spectrum of cytogenetic disturbances, in winter wheat of the exclusion zone in the long terms after the accident at the Chernobyl NPS.

To study the genetic consequences of radioactive contamination of the soil in the area near Chernobyl NPS in 18, 19, 20 and 25 years after the accident, was applied ana-telophaze method of cytogenetic analysis of chromosomal abnormalities in the meristem of primary roots of winter wheat (Triticum aestivum L.,) of such breeds as Odessa Albatross, Donetsk 48 and Zymoyarka. Seeds were grown in the samples of moist soil, which were selected from experimental sites in the exclusion zone: the disposal of radioactive waste (RWDF) of modal type «Pidlisnyi», RWDF of trench-type «Buryakivka », and in the vicinity of villages Kopachi, Chystohalivka and Yaniv of Chernobyl region. Total specific radioactivity of the soil exceeded the level of control in 17-713 times. For a control, were used soil, taken from the territory of experimental farm of the Institute of Plant Physiology and Genetics, Academy of Sciences of Ukraine (village Glevaha, Kyiv region), where the spontaneous mutation rate variability in winter wheat are studied for many years. Selection of soil samples were performed in accordance with standard methods.

Seeds were grown at t = 24-26 º C. Primary root with length 0.8-1.0 cm were fixed in the "vinegar alcohol" at t = 4 º C and subjected to maceration by action of 1 N hydrochloric acid. From the meristematic zone of roots, which were stained by atseto-orseyin, were produced temporary squash preparations [8]. The sample was at least 1000 cells for each option.

Maturing seeds of winter wheat of such varieties as Albatross Odessa and Donetsk 48 in the samples of wet radioactive soil in 18, 19 and 20 years after the accident has caused a significant increase in the frequency of chromosomal rearrangements in primary root meristem cells, exceeding the control, respectively in 2,5-4,3, 2,5-4,0, 2,8-3,5 times in Albatross Odessa and in 3,6-5,3, 3,1-3,3, 2,8-3,2 times in Donetsk 48. In the spectrum of chromosomal rearrangements dominated paired fragments and chromosomal and chrome bridges. Among the types of violations of mitosis cells of meristem because of action of radio nuclides from soil RWDF «Pidlisny" (plot № 2) on the seed variety Donetsk 48 in 19 years after the accident, a significant percentage (0,60 %) were microkernel.

The results of the analysis of the frequency and spectrum of chromosomal aberrations in conditions of prolonged effect of radionuclide pollution of moist soil on the seeds of winter wheat indicates that there is no direct dependency between the specific radioactivity of the soil and the frequency of chromosomal abnormalities. Typical evidence of this is the results of cytogenetic analysis, obtained after 18 years in the variant «Buryakivka»: at the slightest density of soil contamination with radionuclides observed the highest frequency of chromosomal abnormalities. The absence of a linear dependence between the level of radiation contamination of the soil and the frequency of chromosomal aberrations in cells of a meristem of primary roots of the plants may be the result of a reaction systems of reparation on the action of radiation in the range of low doses of [11].

A significant excess of the level of violations of the integrity of the chromosomes and anomalies of mitosis in comparison with the indicators of control which were caused by radionuclide pollution of soil in near-Chernobyl zone (village Kopachi, Chystohalivka, Yaniv), was detected even after 25 years after the accident. The frequency of aberrant cells was higher in 2.0-7.6 times than the spontaneous level (table. 1). The maximum number of cytogenetic disorders – 2,89 % and 2,21 % in varieties of Albatross Odessa and Zymoyarka were detected under conditions of the exposure of seeds in moist soil with the highest summarized densities of radionuclide contamination (villages Chystohalivka, Yaniv). The effect on the seeds of soil radio nuclides with the lowest density of contamination (village Kopachi) has caused a significant increase in the frequency of aberrant cells and exceeding the indicators of control varieties of Albatross Odessa and Zymoyarka in 1,9 and 3,6 times, respectively. Radionuclide contamination of soil in village Chystohalivka, which is in 1,6 times higher than the total density of soil contamination of village Kopachi, induced cells with anomalies of mitosis and chromosomal aberrations, whose frequency is in 7,6 times higher than the reference level and in 3,9 times higher than their frequency under conditions of the influence of radionuclide's soil of village Kopachi in a variety Albatross Odessa. Despite the higher specific radioactivity of the soil in v. Chystohalivka, compared with soil in v. Kopachi, the frequency of aberrant cells in a variety Zymoyarka is still on the level 1,43 % (Table 1). The results of research conducted in other biological objects, such as Arabidopsis (Arabidopsis thaliana Henh. (L.)), skeredi roofing (Crepis tectorum L.) [14], Scots pine (Pinus sylvestris L.) indicates the absence of direct dependence of frequencies of aberrant cell from the density of radioactive contamination and exposure dose [13].

The range of chromosomal disorders which was detected during the cytogenetic analysis of the effects of radionuclide contamination of all investigated areas included most typical paired fragments and dicentric bridges for conditions of ionizing radiation [8, 11]. In comparison with the control, growth of frequency of chromatid aberrations (single fragments and chromatide bridges) explained by prolonged exposure on the root meristem of cells on replicative and postreplikative stages of cell cycle [6].Radionuclide contamination of soil in villages Kopachi and Yaniv accompanied by the induction of micronuclei in both varieties, numerous violations of mitosis and multyaberrant cells. Despite the fact that micronuclei because of radiation exposure formed mainly with acentric fragments [10], the quantitative relationship between the number of aberrant metaphases and cells with micronuclei were not found.

Table 1.

The frequency and spectrum of chromosome aberrations in winter wheat in conditions of prolonged impact of moist soil radionuclides

Place of samples selection	Mitosis with disabilities and chromosomal aberrations,%	The spectrum of violations of mitosis and chromosomal aberrations, %					
		Single samples	Double samples	chromatide bridges	chromosomal bridges	micronuclei	lagging chromosomes
Albatros Odessa							
v. Glevaha (control)	0,38±0,12	0,09±0,09	0,00	0,28±0,16	0,00	0,00	0,00
v. Kopachi	0,74±0,26	0,09±0,09	0,19±0,13	0,19±0,13	0,28±0,16	0,09±0,09	0,00
v.Chystohaliv-ka	2,89±0,52*	0,67±0,25	0,48±0,21*	0,87±0,29	0,87±0,29*	0,00	0,10±0,09
v. Yaniv	2,63±0,46*	0,33±0,16	0,49±0,20*	0,99±0,28*	1,07±0,29*	0,00	0,08±0,08
Zymoyarka							
v. Glevaha (control)	0,40±0,20	0,20±0,14	0,00	0,10±0,10	0,10±0,10	0,00	0,00
v. Kopachi	1,43±0,37*	0,67±0,25	0,10±0,10	0,10±0,10	0,48±0,21	0,10±0,10	0,00
v.Chystohaliv-ka	1,43±0,34*	0,24±0,14	0,48±0,19*	0,40±0,18	0,16±0,11	0,00	0,16±0,11
v. Yaniv	2,21±0,41*	0,55±0,21	0,47±0,19*	0,40±0,18	0,40±0,18	0,16±0,11	0,32±0,16*

* - the difference with the control is statistically authentic when $P < 0,05$

Among the cytogenetic violations were observed anomalies of cell division - triple mitosis and lagging chromosomes. Lagging chromosomes appeared only under conditions of influence of the high background values of radiation contamination of soil in v. Chystohalivka and v. Yaniv and show aneugene action of ionizing radiation which is related with damage of the intracellular mechanisms of control of chromosomes segregation and proper operation of the individual stages of cell cycle [5, 12].

Considering that the frequency of cytogenetic disorders in high degree correlates with the frequency of point mutations it is reasonable, in the future, to study the effects of chronic radiation exposure in the Exclusion Zone of Chernobyl NPP on the nature of the manifestation of visible mutations in successive generations of winter wheat and search among them specific types that may be used as biomarkers of radioactive pollution.

Literature

1. Артюхов В.Г. Цитогенетический мониторинг состояния окружающей среды на территориях, подвергшихся радиоактивному загрязнению в результате аварии на Чернобыльской АЭС (на примере пос. Уразово Белгородской области) / В.Г. Артюхов, В.Н. Калаев // Радиационная биология. Радиоэкология. – 2006. – Т. 46, № 2. – С. 208-215.
2. Беккер А.А. Охрана и контроль загрязнения природной среды / А.А. Беккер, Т.Б. Агаев – Ленинград: Гидрометеоиздат, 1989. – 286 с.
3. Глазко Т.Т. Хроническое низкодозовое облучение и полифакторность адаптации / Т.Т. Глазко, Д.М. Гродзинский, В.И. Глазко // Радиационная биология. Радиоэкология. – 2006. – Т. 46, № 4. – С. 488-493.
4. Захарченко М.П. Радиация, экология, здоровье / М.П. Захарченко, В.Х. Хавинсон, С.Б. Оникиенко, Г.Н. Новожилов – СПБ: Гуманистика, 2003. – 336 с.

5. Инге-Вечтомов С.Г. Фундаментальная и прикладная экологическая генетика // Фактори експериментальної еволюції організмів: зб. наук. пр. – Т. 10. – К.: Логос, 2011. – С. 33-37.

6. Мазник Н.А. Роль факторов нерадиационной природы в формировании цитогенетических эффектов у эвакуантов из 30-км зоны Чернобыльской АЭС / Н.А. Мазник // Цитология и генетика . – 2004. – Т. 38, № 6. – С. 33-44.

7. Моргун В.В. Генетичні наслідки аварії на Чорнобильській АЕС: [монографія] / В.В. Моргун, Р.А. Якимчук – К.: Логос, 2010. – 400 с.

8. Паушева З.П. Практикум по цитологии растений / З.П. Паушева – 4-е изд., перераб. и доп. – М.: Агропромиздат, 1988. — 271 с.

9. Шевченко В.А. Значимость цитогенетического обследования для оценки последствий чернобыльской катастрофы / В.А Шевченко, Г.П. Снигирева // Радиационная биология. Радиоэкология. – 2006. – Т. 46, № 2. – С. 133-139.

10. Шмакова Н.Л. Индукция хромосомных аберраций и микроядер в лимфоцитах периферической крови человека при действии малых доз облучения / Н.Л. Шмакова, Е.А. Насонова, Е.А. Красавин [и др.] // Радиационная биология. Радиоэкология. – 2006. – Т. 46, № 4. – 480-487.

11. Яримина А.А. Радиация и иммунитет / А.А. Яримина // Радиационная биология. Радиоэкология. – 1997. – Т. 37, № 4. – С. 597-603.

12. Bentley K.S., Kirkland D., Murphy M. et al. Evaluattion of thresholds for benomyland carbendazim-induced aneuploidy in cultured human lymphocytes using fluorescence in situ hybridization // Mutat. Res. – 2000. – V. 464. – P. 41-51.

13. Geras’kin S.A., Dikarev V.G., Zyablitskaya Ye.Ya. et al. Genetic consequences of radioactive contamination by the Chernobyl fallout to agricultural crops // J. Environ. Radioact. – 2003. – V. 66. – P. 155-169.

14. Kovalchuk O., Dubrova Y.E., Arkhipov A. et al. Wheat DNA mutation rate after Chernobyl // Nature. – 2000. – V. 407. – P. 583-584.