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CYTOGENETIC EFFECTS IN THE NEEDLES INTERCALAR MERISTEM OF JAPANESE RED PINE IN THE REMOTE PERIOD AFTER THE FUKUSHIMA NPP ACCIDENT

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❀ **Background.** The study of the long-term effects of chronic radiation exposure on plants and animals, which are still the subject of scientific discussion, is necessary to understand the consequences of radiation accidents. After the Fukushima nuclear power plant accident, some of the young pines and spruces showed an increased frequency of apical dominance cancelling. The most probable cause of the observed morphoses is associated with damage to the apical meristem of coniferous plants by radiation in the first year of the accident, when they received the highest absorbed doses. If this hypothesis is true, then even 8 years after the accident it will be possible with high degree of probability to detect an increased level of cytogenetic abnormalities in the intercalary meristem of needles of plants from these populations.

The aim of this work was to verify this hypothesis.

Materials and methods. Five populations of Japanese red pine from territories contaminated with radionuclides as a result of the accident at the Fukushima nuclear power plant were investigated. The frequency and spectrum of cytogenetic abnormalities in the intercalary meristem of needles were determined by the ana-telophase analysis.

Results. The frequency of aberrant cells in the needles intercalary meristem of Japanese red pine from the contaminated with radionuclides territory statistically significantly exceeds the control level in all impact sites and increases along with the dose rate. Although there is no correlation between the frequency of cytogenetic abnormalities in needles and the presence of cancellation of apical dominance in plants, all pine populations from radioactively contaminated territories are characterized by an increased frequency of both cytogenetic abnormalities and morphoses associated with the cancellation of apical dominance.

Conclusion. Radiation damage to the apical meristems of conifers in the first year of the accident, when they received the highest absorbed doses, is the most likely cause of the increased frequency of cancellation of apical dominance in the studied populations of Japanese red pine from the zone affected by the accident at the Fukushima nuclear power plant.

❀ **Keywords:** accident at the Fukushima nuclear power plant; Japanese red pine; radioactive contamination; cytogenetic abnormalities; cancellation of apical dominance.

ЦИТОГЕНЕТИЧЕСКИЕ ЭФФЕКТЫ В ИНТЕРКАЛЯРНОЙ МЕРИСТЕМЕ ХВОИ КРАСНОЙ ЯПОНСКОЙ СОСНЫ В ОТДАЛЕННЫЙ ПЕРИОД ПОСЛЕ АВАРИИ НА АЭС «ФУКУСИМА»

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❀ **Введение.** Несмотря на значительный объем исследований, до настоящего времени отдаленные последствия хронического радиационного воздействия на растения и животных остаются предметом научных дискуссий.

После аварии на АЭС «Фукусима» у молодых сосен и елей была обнаружена повышенная частота снятия апикального доминирования. Наиболее вероятная причина наблюдаемых морфозов связана с повреждением излучением апикальных меристем хвойных растений в первые годы после аварии, когда они получили наиболее высокие поглощенные дозы. Если эта гипотеза верна, то даже спустя 8 лет в интеркалярной меристеме хвои растений из этих популяций с высокой долей вероятности можно будет обнаружить повышенный уровень цитогенетических нарушений.

Целью настоящей работы была проверка этой гипотезы.

Материалы и методы. Широкая распространенность на территориях, загрязненных радионуклидами в результате аварии на АЭС «Фукусима», и высокая радиочувствительность обусловили выбор красной японской сосны (*Pinus densiflora* Siebold et Zucc.) в качестве объекта наших исследований. Цитогенетические эффекты были оценены в пяти ее популяциях. Частоту и спектр цитогенетических нарушений в интеркалярной меристеме хвои определяли ана-телофазным методом.

Результаты. Частота аберрантных клеток в интеркалярной меристеме хвои статистически значимо превышает контрольный уровень на всех импактных участках и растет с увеличением дозы. Хотя корреляции между частотой цитогенетических нарушений в хвое и наличием нарушений апикального доминирования у растений не обнаружено, все популяции сосны с радиоактивно загрязненных территорий характеризуются повышенной частотой как цитогенетических нарушений, так и морфозов, связанных со снятием апикального доминирования.

Вывод. Повреждение излучением апикальных меристем хвойных растений в первые годы после аварии, когда они получили наиболее высокие поглощенные дозы, — наиболее вероятная причина повышенной частоты снятия апикального доминирования в исследованных популяциях красной японской сосны из зоны, подвергшейся радиоактивному загрязнению в результате аварии на АЭС «Фукусима».

❀ **Ключевые слова:** авария на АЭС «Фукусима»; красная японская сосна; радиоактивное загрязнение; цитогенетические нарушения; снятие апикального доминирования.

INTRODUCTION

As a result of major radiation disasters, the human environment is deteriorating significantly, and some previously densely populated areas have become unlivable. One such disaster was the accident that occurred at the Fukushima Daiichi nuclear power plant in 2011. This accident was assigned to the maximum category 7 on the scale of the International Atomic Energy Agency (IAEA) [1]. The accident resulted in radioactive contamination of a large area, and the population along the northwestern trail was evacuated. The territories contaminated with radionuclides and abandoned by people provide a unique opportunity for assessing the consequences of long-term protracted exposure of wildlife to radiation. Despite the significant number of studies performed, the consequences of protracted irradiation for plants and animals inhabiting these territories have remained the subject of scientific discussion [2–5].

Analysis of the consequences of the Chernobyl disaster has shown that coniferous phytocenoses are the most sensitive to radiation effects [6, 7]. Studies conducted in the accident zone at the Fukushima nuclear power plant confirm this conclusion. In particular, it was revealed [8, 9] that young pine and spruce trees from territories contaminated with radionuclides are characterized by an increased frequency of morphological anomalies associated with the abolition of apical dominance. Questions arise about the reasons for the formation of such effects, and here genetic test systems can provide necessary assistance, since they are sufficiently sensitive and can integrate the action of all biologically significant agents in their response [10].

The most probable cause of the morphoses presented in previous works [8, 9] is associated with radiation damage to the apical meristems of conifers in the first years after the accident, when they received the highest absorbed doses. If this hypothesis is correct, then even 8 years after the accident, in the intercalary meristem of the needles of plants from these populations, an increased level of cytogenetic abnormalities can be detected with a high degree of probability. *This work aimed* to test this hypothesis.

MATERIALS AND METHODS

The studies were performed on the Japanese red pine (*Pinus densiflora* Siebold and Zucc), which is widespread in the area contaminated with radionuclides as a result of the accident at the Fukushima nuclear power plant. The Japanese red pine has high sensitivity to ionizing radiation, like the Scotch pine [11], as well as large, easily identifiable chromosomes, and is one of the reference species on which the modern system of radiation protection of biota is based [12].

We studied pine populations from areas contaminated with radionuclides as a result of the accident at the Fukushima nuclear power plant (Fig. 1). The average age of most trees in the populations studied was 5–8 years at the time of sampling. A plant population from the territory of the University of Fukushima (F) was selected as a control. In the contaminated areas, plant populations from the Akibadai (A), Tsushima Farm (T), Tsushima School (S), and Okuma (O) sites were studied. Three types of trees were identified in each population, namely those with impaired apical dominance (FA, AA, SA, TA, OA); those that had recovered following impairment of apical dominance (FR, AR, SR, TR, OR), and those without morphoses (FN, AN, SN, TN, ON). The share of abnormal trees in the population is presented in Table 1.

The absorbed dose rate in air was measured at a height of 1 m using a PDR-111 dosimeter (Hitachi-Aloka Medical, Japan). At each site, the average dose rate and standard deviation were determined from the results of 10–20 measurements. At the time of sampling, 8 years after the accident, ^{137}Cs made the main contribution to the dose absorbed by plants. Dose rates at the time of sampling and the coordinates of experimental sites are presented in Table 1.

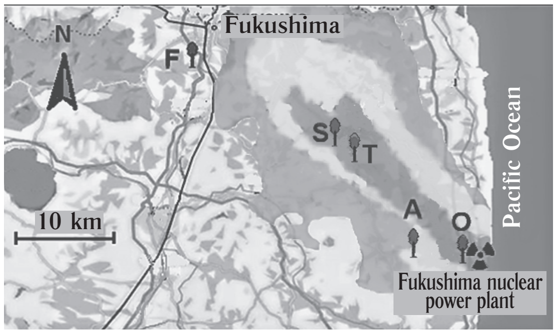

Fig. 1. Scheme of the study area. — sampling locations. Symbols in the text

Young shoots with needles were collected in May 2019. Shoots from 10–15 trees at a height of 1–2 m from the ground surface were taken from each site within the homogeneous forest stand. Ten young shoots were taken from each tree and fixed in vinegar alcohol (1 : 3).

The frequency and spectrum of cytogenetic abnormalities in the intercalary meristem of the needles were assessed in ana-telophase. This method is recommended as a sensitive, informative, and highly reproducible tool for biological monitoring of the environment [10, 13], used effectively in our previous studies [11, 14].

For cytogenetic analysis, a section of needles with an intercalary meristem was pinched off with tweezers and stained with aceto-orcein, after which temporary squashed preparations were made according to a previously published method [15]. All preparations were coded. In each preparation, all cells were analyzed at the anaphase or telophase stages (3412–7958 ana-telophases per variant of the ex-

Table 1

Characteristics of the areas under study (mean ± SD)

Option	Description	Dose rate, mcSv/h^{-1}	Coordinates	Proportion of trees with radiomorphoses
F	University campus, hillside	0.33 ± 0.05	37.682921 N, 140.456635 E	0.14 ± 0.04
A	Abandoned rice field	3.4* ± 0.1	37.411508 N, 140.960887 E	0.52* ± 0.11
T	Abandoned agricultural plot	3.5* ± 0.3	37.553270 N, 140.786833 E	0.42* ± 0.07
S	Former athletic field	3.7* ± 0.4	37.562330 N, 140.768256 E	0.45* ± 0.06
O	Smooth slope along the road near an abandoned sports complex	6.4* ± 0.4	37.411911 N, 140.994318 E	0.42* ± 0.06

* Difference from the control is statistically significant: $p < 0,05$.

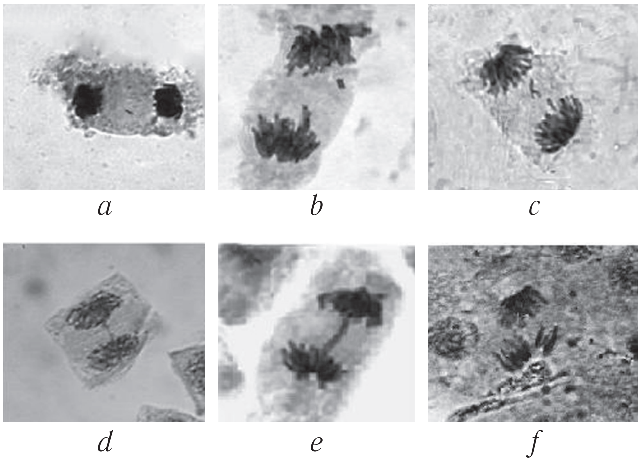

Fig. 2. Anomalies of mitosis in the intercalary meristem of red Japanese pine needles: *a* — single fragment; *b* — double fragment; *c* — lagging behind; *d* — single bridge; *e* — double bridge; *f* — multipolar mitosis

periment, 150–450 per plant), and the proportion of cells with cytogenetic abnormalities was calculated. When analyzing the range of disorders, chromatid (single), chromosomal (double) bridges and fragments, as well as multipolar mitoses and chromosome lagging were distinguished [15, 16] (Fig. 2). In difficult cases, changes in focus and lighting were used to identify accurately the type of abnormality. The preparations were examined using Nikon Eclipse 55i and Nikon Eclipse E200 microscopes (Nikon, Japan) at a total magnification of ×400–1000.

The experimental data were tested for the presence of outliers by the Dixon test. Four outliers exceeding the critical value at $p = 0.05$ were excluded from further consideration. Data analysis was performed in Microsoft Office Excel 2007 by methods of variation statistics (variance and correlation analysis). The hypothesis about the correspondence of the experimental data samples to the normal distribution was tested using IBM SPSS Statistics (Armonk, NY) using the Kolmogorov–Smirnov test. Since the experimental data were distributed in accordance with the normality assumptions, differences between mean values were determined by the Student's test and considered significant at $p < 0.05$. To optimize the sample size, the method of statistical analysis of empirical distributions was used [17].

RESULTS AND DISCUSSION

Empirical distributions of the data on cytogenetic effects in the populations studied were highly homogeneous both for the entire set of the trees under study (coefficient of variation 0.8–1.2) and between plants of each population (coefficient of variation 0.2–0.4). The frequency of cytogenetic disturbances in the intercalary meristem of needles in all areas contaminated with radionuclides was statistically significantly higher than the control level (Table 2). The highest fre-

Table 2

Frequency of aberrant cells in the intercalary meristem of needles and the relative contribution of different types of cytogenetic abnormalities

Option	VC	AC, % ± SD	Range					
			f' + m' ± SD		f'' + m'' ± SD		g + mp ± SD	
			f'	m'	f''	m''	g	mp
F	7958	1.08 ± 0.11	25.16 ± 0.05		32.08 ± 0.05		42.77 ± 0.05	
			13.21 ± 0.02	11.95 ± 0.02	3.77 ± 0.03	28.30 ± 0.03	38.36 ± 0.04	4.40 ± 0.01
A	4422	2.15* ± 0.21	17.95 ± 0.04		48.72 ± 0.06		33.33 ± 0.05	
			3.85* ± 0.01	14.10 ± 0.01	8.33 ± 0.02	40.38 ± 0.03	27.56 ± 0.03	5.77 ± 0.02
T	3467	2.83* ± 0.38	9.24* ± 0.04		63.59* ± 0.06		27.17 ± 0.06	
			4.35* ± 0.01	4.89 ± 0.01	6.52 ± 0.02	57.07* ± 0.02	23.91* ± 0.02	3.26 ± 0.01
S	4591	2.06* ± 0.21	11.30* ± 0.04		53.04* ± 0.06		35.65 ± 0.06	
			2.61* ± 0.01	8.70 ± 0.02	14.35* ± 0.02	38.70 ± 0.02	30.87 ± 0.02	4.78 ± 0.02
O	3412	3.88* ± 0.37	9.27* ± 0.03		47.10 ± 0.05		43.63 ± 0.05	
			4.63* ± 0.01	4.63* ± 0.01	11.20* ± 0.01	35.91 ± 0.02	35.52 ± 0.02	8.11 ± 0.01

Note. VC — the number of viewed cells in the anaphase and telophase stages; AC — aberrant cells; f'. m' — chromatid (single) fragments and bridges; f'', m'' — chromosomal (double) fragments and bridges; g — lagging chromosomes; mp — multipolar mitoses; the difference from the control is statistically significant: * $p < 0.05$.

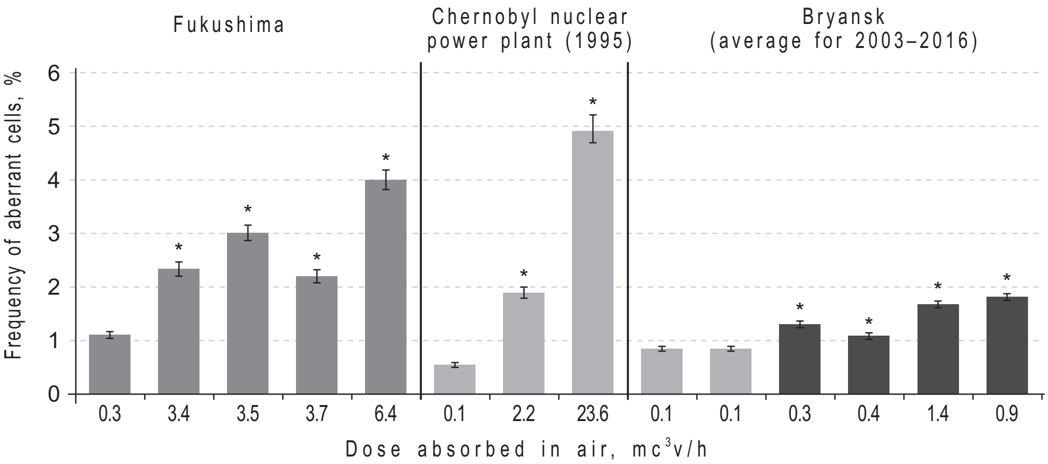

Fig. 3. Frequency of cytogenetic disturbances in populations of red Japanese pine (this study) and populations of Scots pine from the 30-km zone of the Chernobyl nuclear power plant [19] and the Bryansk region [14]. The difference from the control is statistically significant: * $p < 0.05$

quency of abnormalities was registered at site O located closest to the Fukushima nuclear power plant. The differences in the frequency of cytogenetic abnormalities between sites A, S, and T were statistically insignificant ($p < 0.05$), and the levels of doses absorbed into the air also differed insignificantly. The conclusion about the radiation nature of the changes registered confirmed the presence of a statistically significant correlation of the frequency of cytogenetic abnormalities with the dose ($r = 0.95$, $p < 0.05$). Thus, in populations of Japanese red pine, characterized by an increased frequency of apical dominance cancellation (Table 1), the frequency of cytogenetic disturbances in the intercalary meristem of needles was also statistically significantly increased (Table 2), which confirmed our hypothesis about the radiation nature of the formation of this type of morphoses.

Let us compare the results of this study with the data from our previous studies of Scotch pine populations from the territories contaminated with radionuclides as a result of the Chernobyl accident. These studies were performed in 1995 in the 30-km zone of the Chernobyl nuclear power plant (9 years after the accident, which is comparable in time to the start of this study, 8 years after the accident at the Fukushima nuclear power plant) and in the most radionuclide-contaminated areas of the Bryansk region (2003–2016). Despite the fact that in populations of Japanese red pine the frequency of cytogenetic abnormalities was estimated in the intercalary meristem of needles, and in populations of Scotch pine it was estimated in the roots of seedlings, the results obtained are qualitatively comparable (Fig. 3), although the frequency of cytogenetic abnormalities in the intercalary meristem is usually higher than in the apical meristem of seedling roots [14, 18]. In all three field studies, the frequency of cytogenetic abnormalities increased statistically significantly with increase in the dose rate of protracted irradiation (Fukushima: $r = 0.94$, $p < 0.05$; 30-km zone of the Chernobyl nuclear power plant: $r = 0.98$, $p < 0.05$; Bryansk region: $r = 0.85$, $p < 0.05$).

Additional information on the nature of the main factors contributing to the increase in the level of mutational variability can be obtained by analyzing the range of cytogenetic abnormalities. Since the frequency of induction of different types of structural mutations depends on the mechanism of action of the factor [20], it can be concluded from the change in the relative contributions of different types of mutations which factor is the main contributor to the increase noted in the frequency of mutations [10, 14]. In particular, ionizing radiation more often induces major genetic changes (deletions and rearrangements), which are expressed as an increased frequency of chromosomal aberrations [21], and most chemical mutagens cause mainly mitotic abnormalities and chromatid aberrations [10]. Indeed, in populations of Japanese red pine from regions T and S, a statistically significant increase in the frequen-

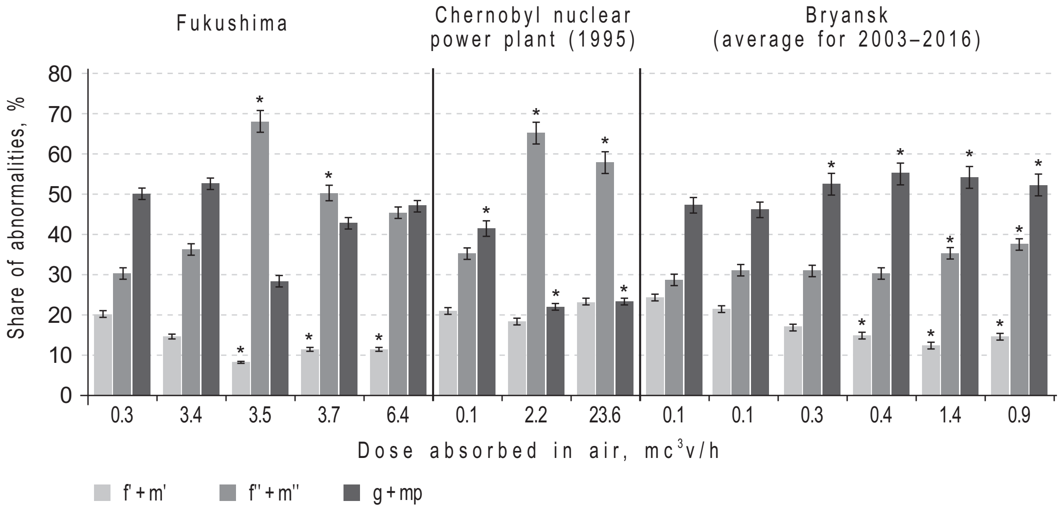

Fig. 4. The spectrum of cytogenetic disturbances in the populations of red Japanese pine (this study) and Scots pine from the 30-km zone of the Chernobyl nuclear power plant [19] and the Bryansk region [11]. f', m' — chromatid (single) fragments and bridges; f'', m'' — chromosomal (double) fragments and bridges; g — lagging chromosomes; mp — multipolar mitoses. The difference from the control is statistically significant: * $p < 0.05$

cy of chromosomal abnormalities was registered. At the same time, the differences in the range of disorders in trees within each population were insignificant (coefficient of variation 0.62–3.56). As in our previous studies on Scotch pine, there is a tendency for the contribution of chromosomal aberrations to increase with the increase in the dose rate (Fig. 4). Thus, the data on the ratio of different types of cytogenetic abnormalities in the studied populations of the Japanese red pine are another confirmation of the radiation nature of the changes noted.

Since our studies were performed on populations that were examined for the presence of radiomorphoses in 2014–2016 [9], the presence of a relationship between the cancellation of apical dominance and the frequency of cytogenetic abnormalities in the needles can be verified. For this purpose, the frequency of cytogenetic abnormalities at each experimental site was calculated separately for plants with apical dominance cancellation (FA, AA, SA, TA, OA), for plants that recovered after apical dominance cancellation (FR, AR, SR, TR, OR), and plants without radiomorphoses (FN, AN, SN, TN, ON). The analysis did not reveal (Fig. 5) statistically significant differences between plants from different groups in the frequency of cytogenetic abnormalities. Nevertheless, it should be noted that all areas contaminated with radionuclides were characterized not only by an increased frequency of cytogenetic effects (Table 2) but also by a high frequency of apical dominance cancellation (Table 1). The radiosensitivity of meristem cells is several orders of magnitude higher than that of differentiated and specialized cells; therefore, they are critical tissues under irradiation conditions [22] and are damaged at doses that do not have visible effects on the organs and tissues formed. Therefore, in the first years after the accident, when the plants received the highest absorbed doses, the apical meristems could be seriously damaged. Damage to the apical meristem stimulates the proliferative activity of more radio-resistant dormant buds which form lateral shoots, that is, it leads to the cancellation of apical dominance. In some cases, the restoration of apical dominance is registered when one of the shoots suppresses the growth of the others and takes a leading position.

Based on the results obtained, it can be concluded that the increased frequency of cytogenetic abnormalities in the needles of Japanese red pine growing in areas contaminated with radionuclides is due to radiation exposure. Even 8 years after the accident at the Fukushima nuclear power plant, the existing levels of radioactive contamination can have a negative impact on plants sensitive to ionizing radiation. The data obtained are comparable with the results of our studies

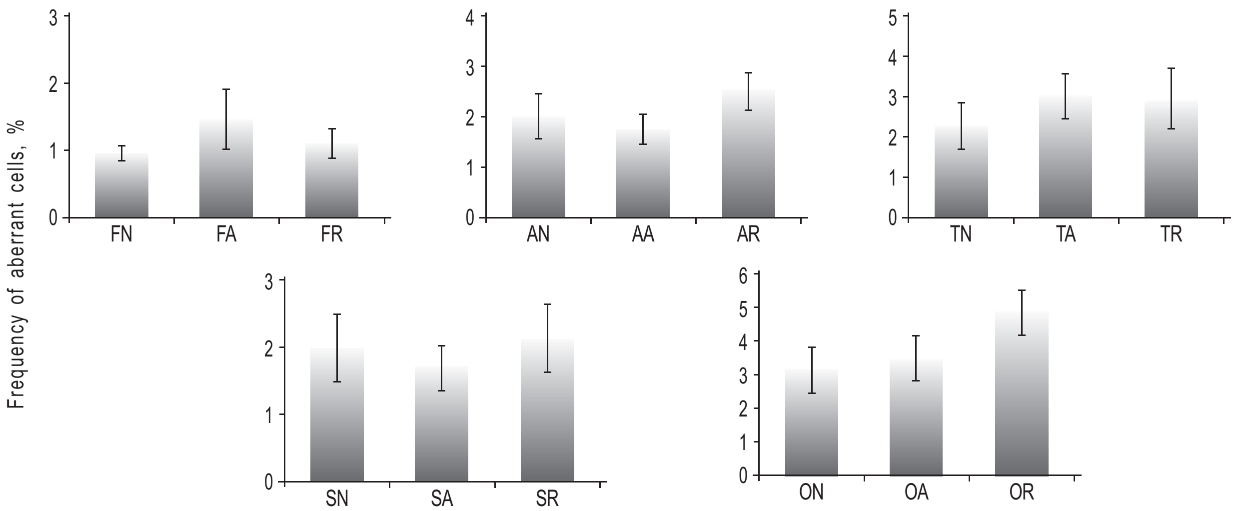

Fig. 5. The relationship between the frequency of cytogenetic effects and disorders of apical dominance. On the abscissa axis of the tree group: with impaired apical dominance (FA, AA, SA, TA, OA); recovered (FR, AR, SR, TR, OR); without morphoses (FN, AN, SN, TN, ON)

in areas exposed to radioactive contamination as a result of the Chernobyl accident and offer an explanation for the formation of morphoses associated with the cancellation of apical dominance in young conifers in the first period after the accident.

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