CHAPTER 11

EVOLUTION PROCESSES IN POPULATIONS OF PLANTAIN, GROWING AROUND THE RADIATION SOURCES: CHANGES IN PLANT GENOTYPES RESULTING FROM BYSTANDER EFFECTS AND CHROMOSOMAL INSTABILITY

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Abstract: The viability of seeds growing around the nuclear power plant (NPP) can decrease up to 20–30%. We consider the appearance of both multiple secondary cells and chromosomes with abnormalities. We used the ideas of adaptation to explain these phenomena. **The aim** was the statistical analysis of the appearances of cells and chromosomes with abnormalities in dependence on radiation factor around the NPP and seeds' antioxidant status (AOS). **Methods**. The chromosome bridges and acentric fragments were registered as chromosomal abnormalities in root meristems of plantain seeds collected in tested populations. For sites within a 30 km radius of the NPP, the annual γ-radiation dose rates, ^{137}Cs soil concentrations and NPP fallouts were standard and did not exceed norms. Seeds were collected in years with normal and extreme high summertime temperatures. **Results**. The modelling showed that the appearances of a number n cells (or chromosomes) with abnormalities would be described by the formulas G_n, P_n, $G_n + P_n$, where P – Poisson and G – geometrical regularities. The parameters of distributions are the AOS- and NPP fallouts dose rate dependent, especially communicative G-component. Due to communication mechanisms, some of cells and seedlings accumulated abnormalities and many others died near the NPP. **Conclusions**. (i) statistical biomarker of stress effects is intensification of the communicative processes; (ii) dose-dependent microevolution process is observed (an appearance of new genotypes and their selection) in indigenous populations under the combined effect of NPP atmosphere fallouts and higher summer temperatures; (iii) the communicative processes in meristem are AOS-dependent; (iv) strategies of survival of populations are different for middle-lethal doses and those which do not exceed norms of their ecological niche (stresses).

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Keywords: low dose irradiation; seeds; cells with abnormalities; chromosomes with abnormalities; survival; adaptation, statistical analyses

Introduction

In the last decade, many investigations were devoted to radiation stress effects reviewed in (Mothersill and Seymour, 2005; Little, 2006). As a basic feature, researchers mark out the genetic instability including spatial intracellular (Mothersill and Seymour, 2005) and intercellular (Arutyunyan et al., 2001) spreading of the DNA changes. These phenomena could be described as the non-randomly processes of the appearance of multiple secondary cells with abnormalities (Korogodina et al., 2005) and chromosomal abnormalities (Chebotarev, 2000). At present, some biologists suggest an idea of an adaptation to explain the genetic instabilities (Mothersill and Seymour, 2005).

Earlier, the effects of radiation stress were studied on the plantain seeds collected in populations growing around the radiation sources in years with normal and extreme high summertime temperatures (Korogodina et al., 2000; 2004). The biological values prevented from the simple conclusion of the radiation effects on seeds and the mechanisms of damaging (Korogodina et al., 2004). We used an idea of adaptation to analyze structure of distributions of both seeds on the numbers of cells with chromosomal abnormalities (CCAs) in root meristem of seeds (Korogodina et al., 2005; 2006) and meristem cells on the numbers of the chromosomal abnormalities (CAs).

Our aims were to analyse statistically the mechanisms of the CCAs and CAs appearances in seedlings root meristems and to study changes in plant genotypes resulting from the intra- and intercellular communication processes in dependence on radiation factor around the nuclear power plant (NPP) and seeds' antioxidant status (AOS).

Materials and Methods

SEED

The plantain seeds (*Plantago major*) were used in the natural experiment (1998, 1999). For this kind of seeds, the reported quasi-threshold radiation dose, which corresponds to the inflection of the survival curve from a shoulder to mid-lethal doses, is 10–20 Gy (Preobrazhenskaya, 1971). The plantain populations were located in sites within 80 km of the Balakovo NPP and in Chernobyl trace area (Saratov region), and near the accelerator facilities in Moscow region. In 1999 the temperatures during daylight hours reached 30–32 °C in the Moscow region and 38–40 °C in the Saratov region (they are extreme for both provinces), and the seeds experienced elevated temperatures during the maturation period in nature (SCEPSR, 2000). The plantain populations were chosen in similar biotopes. Seeds were collected at the end of August in 1998 and 1999 from 20–30 plants. The seeds were refrigerated until the following April at T = 3–4 °C and relative humidity = 13–14%.

Seeds of all populations were germinated on wet filter paper in petri dishes at 23 °C until seedling roots reached 3.5 ± 2 mm, a length corresponding to the first mitoses before which seedling growth is due only to swelling without cell division. After this point, a number of proliferated cells fluctuates at a constant level. Seedlings were fixed in ethyl alcohol and acetic acid (3:1) and stained with acetoorcein. Seedlings <1.5 mm after 13 days were scored as non-surviving (1–S) because too few cells reached the first mitosis (the mitotic index in the shoot zone is already known to increase in parallel with the size of the seedling (Gudkov, 1985). The first fixation was started when approximately 1/3 part of whole seeds' population was germinated. After 13 days we ended the fixation. Prolonging the germination period (up to 6 weeks) increases S by only 2–3%; some rootlets occurred too small and brown. The methods of seed sprouting and fixing have been described (Korogodina et al., 1998; 2004). Antioxidant activities were studied with a photochemiluminescence method (Korogodina et al., 2000). An amount of seed infusion, which inhibited chemiluminescence by 50% ($C_{1/2}$), was adopted as a measure of AOS.

Ana-telophases were scored for CCAs containing CAs. The chromosome bridges and acentric fragments were registered as CAs in ana-telophases. The mitotic activity (MA) of cells in meristem was scored as the number of cells in ana-telophases.

CHARACTERISTICS OF SITES AND WEATHER CONDITIONS

To investigate the possibility of radiation stress effects the sites near the NPP in Saratov region and accelerator facilities in Moscow region were selected. In Saratov region, a site in Chernobyl trace territory was also studied.

In 30-km zone of the NPP two sources of radioactivity are placed, which could be an influence on plantain populations: the atomic station (P2–P6 sites) (MAPRF, 1998) and the phosphogypsum dump (P8–P10 sites) (SCEPSR, 2000) (Fig. 1).The sites were chosen in the view of the wind rose. For the most part, the NPP atmospheric fallouts influence on populations P2–P6 resulting from the direction of the winds in summer. Perhaps the populations P7–P10 experienced the effects of phosphogypsum dump, which can influence a soil contamination in this area (Korogodina et al., 2000). Therefore, these populations (P7–P10) did not studied in 1999. The site P1 was at the left bank of the Volga (~80 km from NPP). The population P11 was chosen at the right bank of the Volga (100 km from the NPP) on a Chernobyl radioactivity-deposition track with well-characterized ^{137}Cs soil

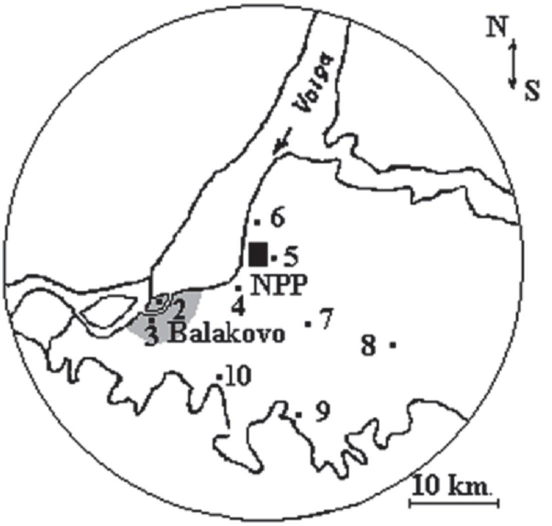

Fig. 1. Locations of the selected plantain populations in the vicinity of the Balakovo NPP: 2–10 (P2–P10). Populations P1, P11 are located 80 km and 100 km from NPP; P12 one is placed in the Moscow region.

contaminations (average concentration ~30 Bq/kg, EC, 1998). The Moscow region site P12 was selected in Moscow region to know in detail both radiation exposures from the accelerator facilities and soil pollution.

The annual rainfall near the Volga is 1.5 times higher than in steppe, therefore the microclimate of P1–P6 sites is damper than that of P7–P10 (SCEPSR, 2000). In 1999, the summertime high temperatures in the European part of Russia averaged 2–3 °C above normal (SCEPSR, 2000).

DETERMINATION OF RADIOACTIVITY

For sites within a 100 km radius of the NPP, the annual γ-radiation dose rates (DR) and ^{137}Cs soil concentrations (C_{Cs}) varied little from the ranges ~0.10–0.15 μSv/h and ~5–10 Bq/kg reported in independent radiological surveys (MAPRF, 1998; SCEPSR, 2000). In site P11 DR is ~0.10–0.15 μSv/h (SCEPSR, 2000) and C_{Cs} is 30 Bq/kg (EC, 1998). DR is ~0.10–0.12 μSv/h and C_{Cs} is ~5–10 Bq/kg in site P12 (Moscow region) (Zykova et al., 1995; Alenitskaja et al., 2004). These values (excluding the concentrations in site P11) do not exceed the average radiation values over the Saratov and Moscow regions (Zykova et al., 1995; SCEPSR, 2000; Alenitskaja et al., 2004).

We examined the upper 10–12 cm soil in tested sites. Measurements were carried out using the low-background γ-spectrometers with a NaI(Tl) crystal as well as a Ge one, which were described in (Alenitskaja et al., 2004). The

TABLE 1. Soil contamination of ^{137}Cs and ^{40}K (Bq/kg)

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
^{137}Cs	9	33	–	5	–	–	10	8	8	6	39	9
	5	4	3	5	5	5	–	5	–	–	15	10
^{40}K	36	33	–	55	–	–	47	45	46	70	45	58
	0	0	40	0	23	50	0	0	0	0	0	0
	40	34	0	41	0	0	–	41	–	–	32	60
	0	0		0				0			0	0

errors of detection efficiency of γ-quanta did not exceed 7%. Total errors of radioactivity determination for different isotopes were 20–40%. The artificial isotope ^{137}Cs soil contamination did not differ significantly in 1998 and 1999 (Table 1), although usually the fluctuations can be observed in the same site (Alenitskaja et al., 2004). The data on C_{Cs} agree with published values (Zykova et al., 1995; EC, 1998; MAPRF, 1998; SCEPSR, 2000; Alenitskaja et al., 2004) and do not correlate with NPP fallout. The accumulated doses were calculated using the Brian-Amiro model (Amiro, 1992) and were estimated to be ~1–3 cGy for seeds P1–P6. We accounted the secondary wind rising (SCEPSR, 2000) in P7–P10 populations by means of (Gusev and Beljaev, 1986). The results did not differ significantly from 1998 to 1999 (for accumulated radiation doses and soil concentrations) in tested sites.

CALCULATION OF THE NPP FALLOUT IRRADIATION OF SEEDS

Plantain seeds experienced the NPP fallout irradiation in nature (annual fallouts on isotopes: Kr ~2.5 TBq; Xe ~2.5 TBq, and I ~4.4 TBq (MAPRF, 1998), the dose rates are controlled by NPP administration). Distribution of the particulate emissions and gases were estimated according to the Smith-Hosker model (Hosker, 1974) based on NPP characteristics (MAPRF, 1998) and winds in summer near the ground in the NPP region (SCEPSR, 2000). The isotopes fallouts result in γ-irradiation mainly (mean energy ~1.1 MeV/γ-quanta (Ivanov et al., 1986)). The relative fallout dose rates (RFRD) values were calculated in the ratio to the dose in site P1. The RFRD value in P7 site is higher than in P2, P3, P8–P10 sites due to short half life of I isotopes, which do not reach the populations P2, P3, P8–P10. This fallout irradiation is not chronic and depends on location of populations. Intensity of irradiation is shown in Table 2. We used the γ-quanta' LEP-dependence on their energy (Ivanov et al., 1986) and the NPP characteristics (MAPRF, 1998) to calculate a mean γ-quanta energy deposition per plant cell nucleus, which is 1.4 keV. In our calculations, each seedling' meristem experiences an

TABLE 2. Relative daily Balakovo NPP fallout (Kr, Xe, I) dose rates (RFRD), experienced by populations, calculated in the ratio to that in the site P1

Populations	Relative dose rate RFRD	Intensity of γ-quanta per cell nucleus per $\times 10^{-7}$	Intensity of γ-quanta per cell nucleus per min for 3 months
P1	1	1.9×10^{-4}	2.5×10^{-6}
P2	80	1.7×10^{-2}	2.2×10^{-4}
P3	80	1.7×10^{-2}	2.2×10^{-4}
P4	560	0.11	1.4×10^{-3}
P5	5700	65	0.85
P6	1350	0.26	3.4×10^{-3}
P7	340	6.7×10^{-2}	8.6×10^{-4}

influence of even one g-quantum per 3 months. The expected irradiation dose was calculated after the accelerator facilities operation in the P12 site. In 1998 the calculated neutron dose level was 1 mSv (for two months), and the neutron dose rate level was 0.8 µSv/h (the neutron background dose rate ~ 9.3 nSv/h (Wiegel et al., 2002). In 1999, the neutron irradiation did not increase. The averaged neutron energy was 5.5 MeV (MCNP calculations).

STATISTICAL ANALYSIS

The data were processed using standard statistical methods (Van der Vaerden, 1957) and statistical criteria (Akaike, 1974; Schwarz, 1978; Glotov et al., 1982; Geras'kin and Sarapul'tzev, 1993). The maximum-likelihood method was used for approximations. For estimated regression the following criteria were used: R^2_{adj}- determination coefficient adjusted on the number of range of discretion; criterion AIC (Akaike criterion); criterion BIC (choice of the most probable model from an ensemble of ones on the assumption of their prior equal probability). The details of the approximation procedure are described in (Florko and Korogodina, 2007).

Hypotheses

PROCESSES IN MERISTEMS AND IN CELLS

At germination of seeds, the adaptations of rootlet meristems as well as meristem cells are required by the environment, which can result in process of some changes (reconstructions) in meristems and cells. Irradiation of meristems can be over by stage of first mitoses that can be considered as an "adapted stage of seedling" (Fig. 2A), or their death. Some of the irradiated cells come into mitosis, which can be considered also as "adapted stage of cells" (Fig. 2B), and the others die. The reconstructions, which are necessary for adaptation, can be provided by communication processes ($I^{com}/I^{rp2} > R_{ad} > I^{rad}/I^{rp1}$) or primary

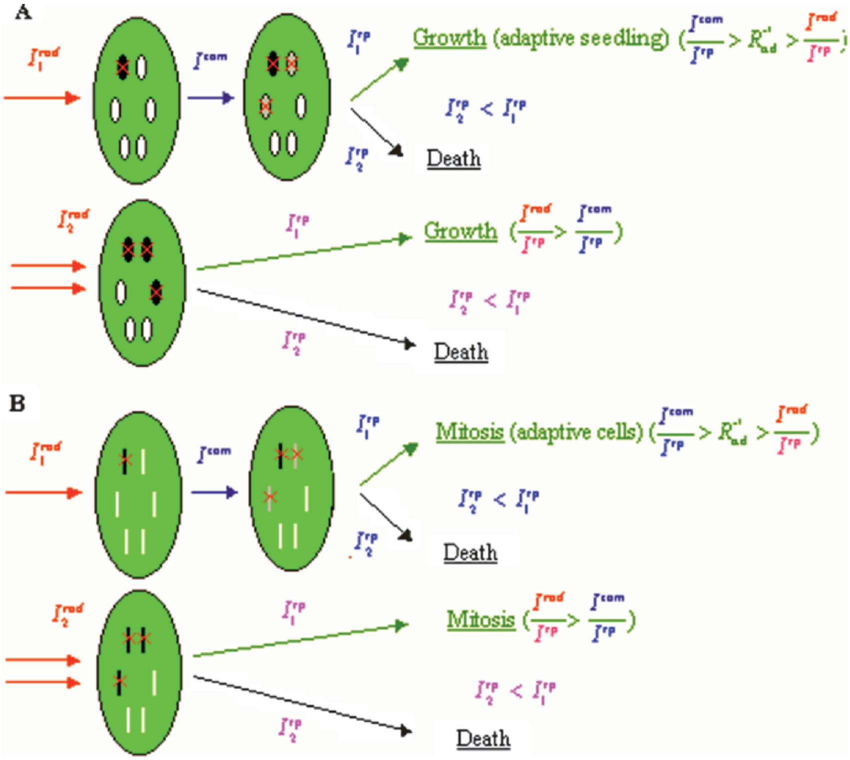

Fig. 2. The processes in meristems (A) and cells (B). I^{rad} – intensity of irradiation; I^{com} – intensity of communications; I^{rp} – intensity of repair. Primary damaged cells (A) or chromosomes (B) are crossed black, communicative ones – crossed gray, and undamaged ones – white colored.

damages ($I^{rad}/I^{rp1} > I^{com}/I^{rp2}$), where I^{rad} – intensity of irradiation; I^{com} – intensity of communications; I^{rp1}, I^{rp2} – intensities of repair. The abnormalities lead to death of seedling or cell if repair systems are not effective.

THE ADAPTATION HYPOTHESES IN MATHEMATICAL TERMS

- Primary damages of cells or chromosomes are rare and independent events and can be described by Poisson distribution;
- The communication processes could induce the appearance of the secondary abnormalities. This increases the Poisson parameter a;
- These reconstructions in meristems and cells provide selection of "adapted" seedlings and cells. A waiting period of adaptation is exponentially distributed that results in geometrical distribution on the reconstruction numbers.

Therefore, we conclude that an appearance of number n abnormalities could include Poisson and geometrical components (Florko and Korogodina, 2007).

A probability of a number *n* CCAs (CAs) appearance in adapted meristem (cell) could be described by the formulas

$$T_n = P_n + G_n$$

$$P_n = \frac{a^n}{n!}e^{-a}; G_n = G(1-\theta)\theta^n,$$

where P_n and G_n are probabilities of a number *n* CCAs (CAs) appearance in *P*, G-distributions; parameter *a* is the sample mean of the *P*-distributions; *q* is a part of seedlings (cells) without CCAs (CAs); *G* – value of this subpopulation.

Results

BIOLOGICAL VALUES OF SEEDS AND SEEDLINGS

Table 3 presents non-survival of seeds and values that characterize processes in seedlings meristems and their cells. For the populations located near the NPP (P2–P6), the non-survival of seeds grew high (up to 80%) in 1999 in

TABLE 3. Antioxidant status and non-survival of seeds, frequencies of both cells with abnormalities in meristem and chromosomes with abnormalities in cells

Seeds	Number of seeds	Number of ana-telofases	AOS ($C_{1/2}$)	Non-survival 1-S,%	CAs frequency	CAs frequency	Mitotic activity
				1998			
P1	167	726	0.22	10.8	2.5 ± 0.5	0,05	9.7 ± 0.9
P2	152	942	0.16	34.2	3.1 ± 0.8	0,03	6.0 ± 0.6
P4	156	518	0.20	19.9	2.5 ± 0.6	0,04	6.3 ± 0.7
P7	149	763	0.50	13.4	1.3 ± 0.3	0,02	10.9 ± 1.0
P8	148	1047	0.80	31.8	3.2 ± 0.8	0,03	7.8 ± 0.8
P9	167	528	0.76	65.3	4.6 ± 1.4	0,07	6.1 ± 1.0
P10	153	231	0.66	29.4	3.2 ± 0.7	0,05	9.7 ± 3.0
P11	153	342	0.83	55.6	4.4 ± 0.9	0,08	7.3 ± 1.0
P12	148	1805	0.28	12.3	1.2 ± 0.3	0,01	14.9 ± 0.9
				1999			
P2	500	2228	0.16	72.6***	3.2 ± 0.4**	0,04	17.8 ± 1.2***
P3	500	3827	0.33	32.6	5.4 ± 0.6	0,06	21.9 ± 1.3
P4	500	1035	0.22	83.6***	6.8 ± 0.9***	0,09	17.5 ± 1.4***
P5	500	2209	0.25	67.8	6.3 ± 0.6	0,07	15.0 ± 0.9
P6	500	2385	0.25	71.6	5.1 ± 0.4	0,07	17.9 ± 1.1
P11	500	2220	0.50	43.6*	5.5 ± 0.5*	0,07	9.8 ± 0.5**
P12	200	832	0.31	37.5***	5.6 ± 0.8***	0,01	8.0 ± 0.6***

Note: Standard error of the CAs frequency does not exceed 0.01

Comparing 1998 and 1999 data: *p>0.1; **p>0.5; ***p<0.001.

comparison with 1998 and with those in control populations. The mitotic activity is higher than the same value over all studied populations in 1998 ($p < 0.05$, ~ threefold), and higher than in P11, P12 plants (F, $p < 0.05$, ~ twofold) in 1999 (Korogodina et al., 2006).

The correlations of both frequencies of cells with abnormalities and chromosomes with abnormalities with non-survival of seeds were examined. The correlation between these values could mean the predominance of one mechanism influencing the non-survival of seeds. If such correlation is absent, we can suspect that some mechanisms have approximately equal rights. In 1998, a strong correlation of the $(1–S)$ value with both frequencies of cells with chromosomal abnormalities ($|r_{1-S,\,\mathrm{CCA}}| = 0.92$, df = 7, $p < 0.001$) (Korogodina et al., 2006) and the CAs ($|r_{1-S,\,\mathrm{CA}}| = 0.76$, df = 7, $p < 0.02$) was observed. In 1999, the correlation between the $(1–S)$ value and CCA frequency disappeared ($|r| = 0.02$), as well as with CA frequency was non-confidence statistically ($|r_{1-S,\,\mathrm{CA}}| = 0.51$, df = 5). These data indicated that some mechanisms acted in seeds collected near the NPP in 1999. We can think that observed effects were induced by radiation and heat factors because the radiation factors were the same both years and the temperatures were extreme high in all sites in 1999.

The hypothesis of the combined effects of radiation and heat stresses is verified by our laboratory experiments on pea seeds (the methods are the same as in natural experiments) (Korogodina et al., 2005). Figure 3 presents a non-viability of seeds, a frequency of CCAs in root meristem of seedlings and a frequency of CAs in meristem cells at γ-irradiation (^{60}Co) with 7 cGy and dose rates 0.3; 1.2; 19.1 cGy/h. The first group of seeds stored 8 months at 4°C and then was tested. The second one stored 8 months in the same conditions and was stressed during the outdoor storage of seeds (2 months) at extreme high summertime temperatures (32–34°C in Moscow region) (Korogodina et al., 2005). The non-viability of irradiated seeds of the second group increased up to 28–62%, whereas frequencies of CCAs at dose rates 1.2; 19.1 cGy/h as well as CAs at dose rate 19.1 cGy/h differed

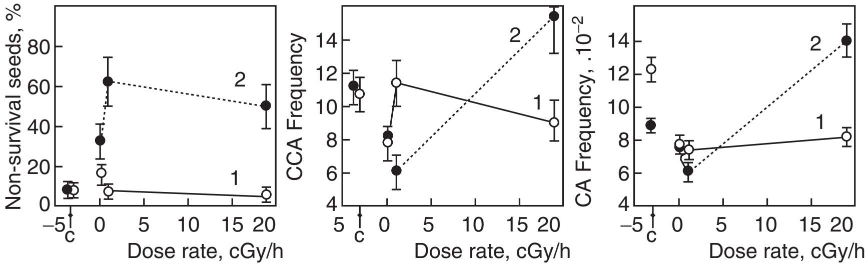

Fig. 3. The non-viability (A) of pea seeds, frequency of CCAs (B) and frequency of CAs (C) in seedlings meristem in dependences on dose rate lab irradiation. Explanation in text.

significantly in comparison with the first group (F-criterion) (Fig. 3). That is not surprising because high temperatures can induce DNA damages and apoptosis (Rainwater et al., 1996).

THE STATISTICAL MODELING

The statistical modeling was used to investigate mechanisms in plant seedlings and their dependence on irradiation. Table 4 presents the parameters of distributions of seeds on the number of cells with abnormalities and cells on the number of chromosomes with abnormalities in 1998 and 1999.

All distributions of seeds include Poisson and geometrical components, and the Poisson one predominated in 1998. In 1999, the *P*-value decreased and averaged sample means increased. It is expected because the heat stress (1999) increases an appearance of the reactive oxidative species (ROS)

TABLE 4. Parameters of distributions of plantain seeds on cells with abnormalities and distributions of meristem cells on chromosomes with abnormalities

	Distribution of seeds on cells with abnormalities				Distribution of cells on chromosomes with abnormalities					
Seeds	mG	G	mP*	P*	mG1*	G1*	mG2*	G2*	mP*	P*
					1998					
P1	1.10 ± 0.05	0.17 ± 0.03	0.22	0.41	0.01	0.42	0.30	0.07		
P2	0.05 ± 0.05	0.10 ± 0.03	0.17	0.45	0.03	0.26				
P4	0.07 ± 0.04	0.06 ± 0.02	0.17	0.64	0.04	0.38				
P7	0.14 ± 0.06	0.27 ± 0.04	0.19	0.38	0.02	0.52				
P8	0.10 ± 0.06	0.07 ± 0.02	0.26	0.39	0.03	0.34				
P9	2.40 ± 0.09	0.01 ± 0.01	0.36	0.19	0.07	0.39	0.28	0.04		
P10	2.30 ± 0.06	0.04 ± 0.02	0.32	0.46	0.03	0.35				
P11	0.70 ± 0.10	0.09 ± 0.02	0.45	0.21	non-confidence statistically data					
P12	0.06 ± 0.04	0.12 ± 0.03	0.21	0.69					0.01	0.9
av.	**0.77 ± 0.32**	**0.10 ± 0.03**	**0.26**	**0.42**						
					1999					
P2	1.05 ± 0.17	0.13 ± 0.01	0.36	0.11	0.02	0.43	0.14	0.12		
P3	1.85 ± 0.13	0.30 ± 0.02	1.20	0.37	0.02	0.40	0.11	0.55		
P4	2.01 ± 0.46	0.02 ± 0.01	1.26	0.12	0.03	0.25			0.53	0.03
P5	0.08 ± 0.31	0.04 ± 0.01	1.20	0.26	0.06	0.50	0.23	0.05		
P6	2.45 ± 0.42	0.04 ± 0.01	0.96	0.21	0.02	0.36			0.22	0.12
P11	0.32 ± 0.13	0.10 ± 0.01	0.84	0.33	0.02	0.36			0.20	0.17
P12	1.81 ± 0.34	0.12 ± 0.02	0.36	0.39	0.02	0.19			0.35	0.03
av.	**1.36 ± 0.34**	**0.11 ± 0.02**	**0.89**	**0.26**						

*Standard errors of the parameters do not exceed 20–30% (the sample means) and 10–15% (the relative values)

(Rainwater et al., 1996), which induce the DNA damages (Janssen et al., 1993*)* and apoptosis (Davies, 2000). The ROS increase the communicative processes (Burlakova et al., 2001) that result in the decreasing of the *P*-subpopulations also.

In 1998, some populations of cells are geometrical-distributed on the number of CAs. Two distributions of cells include the first and the second geometrical components (their plantain populations located in high chemical-polluted sites (Korogodina et al., 2000)). One cell population is *P*-distributed (these plants were growing near the accelerator). In 1999, all distributions are compound of two-geometrical or geometrical plus Poisson components. The Poisson component is observed in populations growing at the border of sanitary zone, in Chernobyl trace territory and near the accelerator.

It is interesting that cells' distributions on the number of CAs can be geometrical whereas seeds' distributions on the number of CCAs could have Poisson component. We explain this fact by more intensive irradiation of meristem as a whole than each separate cell. It suggests that irradiation effects are more pronounced in meristems than in cells.

THE AOS- AND DOSE-DEPENDENCE OF MODELS' PARAMETERS

The relative NPP fallouts dose is the same for populations P2 (AOS_{50} ~0.16) and P3 (AOS_{50} ~0.33), therefore it is possible to compare their characteristics in dependence on AOS of seeds. Figure 4 shows the AOS- and dose-dependence of distributions of seeds on the number of cells with abnormalities. The sample means and values of both P and G-distributions of seeds increase with seeds AOS ($p < 0.05$) (Fig. 4A, B). G-graphs split on two ones according to the sites distance from the NPP (AOS_{50} of seeds growing at the border and inside of sanitary zone are ~0.22–0.25). By means of interpolation, the distributions parameters for the "conventional" population in Balakovo (with AOS_{50} ~0.23) were determined. Near the NPP, the non-linear dose-dependence of the P- and G-parameters is observed (Figs. 4C, D). It is stronger for G-parameters: the sample mean is increased at the border of sanitary zone (not significantly) and decreased inside it ($p < 0.05$); the G-value is decreased dramatically for those populations ($p < 0.001$) in comparison with P2, P3 ones. P-parameters are dependent on NPP fallouts in this dose interval to a lesser degree, not significantly.

Figure 5 shows the parameters of distribution of cells on the number of chromosomes with abnormalities in dependence on seeds' AOS and NPP fallouts relative dose. The G1-sample mean is not dependent significantly on seeds AOS and dose irradiation (Fig. 5A, C). It can be suggested that G1-cells are more stable, in contrast with G2- and P-ones, which have fast changed genotype. Cells in meristem can be divided into two groups: the first - (G1) subpopulation and the second – (G2 + *P*) one.

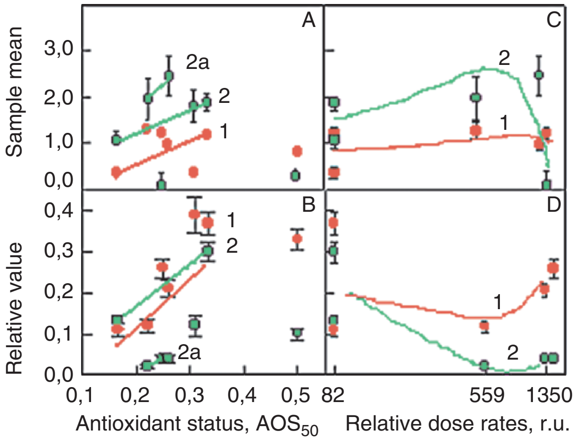

Fig. 4. The dependence of parameters of distributions of seeds on CCA numbers on antioxidant status (A, B) and the relative NPP fallout dose (C, D) in 1999. The parameters of subpopulations: P – red (1), G − green (2). The regressions, A: for P2, P3 $y = -0{,}48 + 5{,}04x$ ($p < 0.05$) (1), $y = 0.25 + 4.80x$ ($p < 0.05$) (2); for P4, P6 $y = -0.92 + 12.96x$ ($p < 0.05$) (2a); B: for P2, P3 $y = -0.15 + 1.43x$ ($p < 0.05$) (1), $y = -0.04 + 1.02x$ ($p < 0.05$) (2); for P4, P6 $y = -0.10 + 0.53x$ ($p < 0.05$) (2a); C: for P2–P6 polynomial fits (1, 2); D: for P2–P6 polynomial fit (1); $y = 100/x^{1.4} + 0.027$ (2).

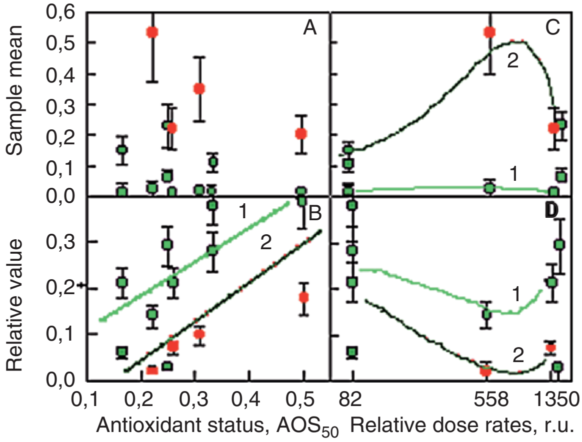

Fig. 5. The dependence of parameters of distributions of meristem cells on CCA numbers on antioxidant status (A, B) and the relative NPP fallout dose (C, D) in 1999. The parameters of subpopulations: P –red, G1 –light green; G2 –dark green. Subpopulation G1 – curve 1, G2 + P ones – 2. The regressions, B: for all populations $y = 0.73x + 0.02$ ($p < 0.05$) (1), $y = 0.77x - 0.12$ ($p < 0.05$) (2); C: for P2–P6 polynomial fits (1, 2); D: for P2–P6 polynomial fit (1); $y = 100/x^{1.4} + 0.027$ (2).

The distribution parameters of these two subpopulations of cells show the similar regularities as parameters of seeds distributions. Their values increase with AOS ($p < 0.05$) (Fig. 5B). It is observed a partition of "$G2_{CA} + P_{CA}$" graphs into two ones. Figure 5C, D demonstrates a non-linear dependence of distribution parameters on NPP fallouts dose. The values of subpopulation ($G2_{CA} + P_{CA}$) are decreased in populations growing at the border and inside of sanitary zone ($p < 0.001$) (Fig. 5D). The dose-decrease of the subpopulations of cells with fast changed genotype is correlated with that of the subpopulation of seeds with cell-to-cell communication ($|r| = 0.97$; df = 3; $p < 0.01$).

Discussion

TWO EVOLUTION STRATEGIES OF SURVIVAL

In Poisson subpopulation, repair systems decrease the primary damages and mutations. So, survival is negative connected with a number of abnormalities and their frequency. In geometrical subpopulation, the communicative processes increase a number of abnormalities, which are accumulated. If values of geometrical and Poisson subpopulations are congruent quantities, survival become positive connected with a number of abnormalities and their frequency. Figure 6 shows correlations between survival of seeds and frequency of cells with abnormalities in root meristem. In 1998, a negative correlation is observed (Fig. 6A). In 1999, a correlation is positive for populations P2, P3, P11, P12 and negative for P4, P5, P6 ones (Fig. 6B). Table 3 shows that P-distribution is predominated in all populations in 1998 and at the border and inside of sanitary zone (P4, P5, P6) in 1999.

We can conclude that two strategies of survival are observed: at middle-lethal irradiation (Poisson distribution) and stressed low-intensity one (geometrical distribution). In the first case cells rid oneself of DNA damages

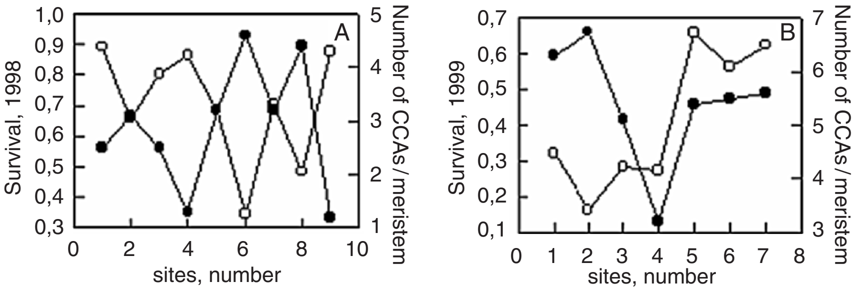

Fig. 6. Negative and positive connections of the numbers of CCAs in meristem with seeds survival. Survival marked in white and numbers of CCAs/meristem in black colors.

by protection systems. In the second one, stresses activate the repair systems, which maintain DNA changes. These repair systems are homologous to mismatch repair one of *E. coli* that influences multiple aspects of genetic stability, including genome rearrangement (reviewed in (Longerich et al., 1995)). In both cases the numbers of abnormalities increase whereas old genotype eliminate, especially at looking for new adaptive genotypes. In 1939 N.W. Timofeeff-Ressovsky pointed out for the first time (Timofeeff-Ressovsky, 1939) that microevolution process includes necessary both the increasing of the material for evolution and decreasing of a part of old population. Then Eigen and Schuster (1979) have shown that elimination of organisms with old genotypes is necessary for survival of new ones in the view of living resources of adapted population.

Conclusions

- Irradiation factor ~1.4 keV/nucleus influences the communicative processes;
- The irradiation effects are more pronounced in meristems than in cells;
- The communicative processes in meristem are AOS-dependent;
- Dose-dependent microevolution process is observed (an appearance of new genotypes and their selection) in indigenous populations under the combined effect of NPP atmosphere fallouts and higher summer temperatures;
- Statistical biomarker of stress effects is intensification of the communicative processes;
- Strategies of survival of populations are different for middle- lethal doses and those, which are not exceed norms of their ecological niche (stresses). The first maintains old genotypes, and the second look for new adapted ones.

Dedication

This article is dedicated to the memory of Prof. Vladimir I. Korogodin.

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The hypothesis of adaptation was evolved by V.I. Korogodin and discussed with V.B. Priezzhev. G.A. Ososkov suggested some statistical methods. We are grateful to these researchers, who contributed in these investigations. We would like to thank for discussions Yu.A. Kutlakhmedov and E.B. Burlakova. We are grateful to I.V. Kronstadtova for technical assistance.

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