ISSN 1067-4136, Russian Journal of Ecology, 2016, Vol. 47, No. 1, pp. 1–10. © Pleiades Publishing, Ltd., 2016.
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Adaptation of Greater Plantain, *Plantago major* L., to Long-Term Radiation and Chemical Exposure

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Received February 2, 2015

Abstract—A comparative study of the greater plantain seed progeny was performed with samples from cenopopulations growing for a long time under conditions of radioactive contamination (in the Eastern Ural Radioactive Trace, EURT) or chemical pollution (in the impact zone of the Nizhny Tagil Iron and Steel Works, NTMK). The progeny of plants from the NTMK zone had low viability but proved to be resistant to the additional impact of a "new" factor (acute γ-irradiation) as well as of the "habitual" factor (heavy metal toxicity). Plantain seeds from the EURT area showed high viability and low heavy metal and radiation resistance; i.e., no preadaptation effect was revealed. In experiments on growing plants from different cenopopulations in plot culture, samples from the EURT zone were characterized mainly by morphoses of generative organs, while samples from the NTMK area, by morphoses of vegetative organs.

Keywords: heavy metals, ionizing radiation, low level doses, cenopopulations, *Plantago major* L., heavy metal resistance, radioresistance, preadaptation, adaptation

DOI: 10.1134/S1067413616010124

Ecological studies of natural populations in technogenic zones are of particular interest in light of increasing anthropogenic impact on the biosphere: on the one hand, the state of biological systems is indicative of the strength of this impact; on the other hand, long-term exposure to stress discloses the pathways of adaptation of organisms to various types of influence and the biological bases of their existence under variable environmental conditions. Each cenopopulation exposed to a certain kind of technogenic stress repeatedly passes through the stages of natural selection and, hence, acquires specific features. The effects of increased concentrations of heavy metals and doses of ionizing radiation on plants are described in a number of publications (Alekseeva-Popova, 1991; *Ekologicheskie posledstviya...,* 1993; Beresford et al., 2005; Bezel', 2006), but there are only a few studies where the effects of factors of different nature are compared over the same long period of time (Fuma et al., 2009; Pozolotina et al., 2012a).

The purposes of this study were as follows: (1) to comparatively characterize the viability of *Plantago major* seed progeny formed in a gradient of chemical or radioactive contamination; (2) to evaluate the adaptive potential of this seed progeny by exposing them to the additional effects of γ-radiation and heavy metals; and (3) to estimate the frequencies of morphological anomalies at different stages of ontogeny in plants from different contamination areas. A comprehensive analysis of such data would make it possible to estimate the degrees of universality and specificity in the adaptation of the test species to different technogenic factors.

MATERIAL AND METHODS

Study object. Greater plantain (*Plantago major* L.) is a herbaceous polycarpic perennial of the family Plantaginaceae Juss. (*Ontogeneticheskii atlas...,* 1997). Plants have stalked oval leaves growing in a rosette, generative shoots are leafless. The species is diploid ($2x = 12$, $n = 6$), self-compatible and wind-pollinated (Van Dijk et al., 1988), reproduces by seeds and vegetatively (in case of mechanical damage). Two subspecies are recognized, *P. major* ssp. *major* Pilger and *P. major* ssp. *pleiosperma* Pilger (syn. ssp. *intermedia*) (Morgan-Richards and Wolff, 1999). They can be reliably distinguished by the number of seeds per capsule: 4–13 seeds in *P. major* ssp. *major* and 14–30 seeds in *P. major* ssp. *pleiosperma* (Zhukova et al., 1996). While collecting the field material, we noted that this number did not exceed 12, with most plants containing 8 seeds per capsule. This and other morphological characters provided evidence that only *P. major* ssp. *major* was represented in the test populations.

Characteristics of test plots. *The zone of radioactive contamination* is in the Eastern Ural Radioactive Trace (EURT), which appeared in 1957 after the Kyshtym accident at the Mayak Radiochemical Plant, where a tank with radioactive waste exploded to release 7.4 × 10^{17} Bq of radionuclides, with 10% of this amount being discharged into the atmosphere and spread over a vast area in Chelyabinsk and Sverdlovsk regions. Short-lived radionuclides, which emitted strong radiation, decayed within the first 4 years after the accident, and today β-emitting ^{90}Sr and daughter ^{90}Y are the main contaminants in the EURT (their content in the isotope mixture at the moment of the accident was 5.4%). Additional contamination of this zone occurred in 1967 as a result of the wind transfer of radioactive silt and sand from the banks of shallowing Lake Karachay, which had been used as a reservoir for radioactive wastes (with ^{137}Cs being the main contaminant) (Aarkrog et al., 1997).

Current geobotanical and radioecological characteristics of test plots are described in our previous publications (Pozolotina et al., 2012b; Molchanova et al., 2014). Two radioactively contaminated plots, *EURT-1* and *EURT-2,* were established on the southern and northern shores of Lake Uruskul (55°49′ N, 60°55′ E and 55°51′ N, 60°55′ E, respectively). Soil samples were taken from the A1 horizon to a depth of 10 cm. Samples were analyzed for the contents of radionuclides at the Department of Continental Radioecology of the Institute of Plant and Animal Ecology (Ural Branch, Russian Academy of Sciences). The content of ^{137}Cs was determined using a Canberra 1510 γ-spectrometer (Canberra-Packard, United States), and that of ^{90}Sr, by a radiochemical method (Molchanova et al., 2014).

The zone of chemical pollution is near the city of Nizhny Tagil, at the site of the Urals' largest mining and metallurgical industrial complex, which has been operating for more than 300 years. The technogenic impact on natural communities has been especially strong since the mid-20th century, when the Nizhny Tagil Iron and Steel Works (the accepted acronym is NTMK) was put in operation. Priority pollutants in the region include heavy metals (Pb, Cu, Cd, Fe), formaldehyde, benzo[a]pyrene, nitrogen dioxide, ammonia, and phenol (*Gosudarstvennyi doklad…,* 2014). For detailed geobotanical characteristics of test plots, see the study by Zhuikova (2009).

Two test plots were established in this zone: *NTMK-1* (56°57′ N, 61°58′ E) and *NTMK-2* (57°57′ N, 60°54′ E). Soil samples were taken from the A1 horizon (see above), and heavy metals were extracted with 5% HNO_3 for 24 h. This method allows extraction of the mobile fraction, which is most easily accessible to plants. Concentrations of Zn, Cu, Pb, and Cd were measured in an AAS-6 atomic absorption spectrometer (Analytik Jena AG, Germany). Chemical analysis was performed at the Laboratory of Ecotoxicology of Populations and Communities, Institute of Plant and Animal Ecology.

Background plots were beyond the zones of chemical and radiation impact (*Background-1:* 56°47′ N, 61°18′ E; *Background-2:* 56°41′ N, 61°02′ E).

In all the plots, *P. major* occurred mainly along rarely traveled roads, on the roadsides and between wheel ruts, amid phytocenoses with signs of synanthropization that included ruderal pennycress–sow thistle–plume thistle or meadow–ruderal mixed herb–grass communities. Their species richness was relatively low (32–39 species), but total coverage reached 75%. The group of dominant and codominant species consisted of typical ruderal or meadow–ruderal plants such as *Thlapsi arvense, Urtica dioica*, *Artemisia vulgaris*, *Bromopsis inermis*, *Phleum pratense*, *Poa angustifolia,* and *Cirsium setosum*. It should be noted that *P. major* plants in the *EURT-2* plot showed gigantism of leaves and flower stalks. Pooled samples of seeds from 40–50 plants were collected from each of cenopopulations growing in zones with different levels of chemical and radioactive contamination.

Calculations of toxic loads and absorbed dose rates. The gradient of total chemical soil pollution was expressed through the toxic load index (Bezel', 2006), which allows reduction of information on the degree of soil pollution and shows by what factor the level of heavy metal pollution in the impact zone exceeds the background level:

$$K_i = \frac{1}{n}\sum_{j=1}^{n}\frac{C_{ji}}{C_{jf}},$$

where K_i is the pollution index of the *i*th point, C_{ji} is the concentration of the *j*th element at the *i*th point, C_{jf} is the concentration of the *j*th element in the background zone, and *n* is the number of elements included in analysis.

The absorbed dose rates for the maternal plants were calculated using the ERICA Assessment Tool 1.2 (Brown et al., 2008), in particular, the Tier 2 model that takes into account empirical coefficients of radionuclide accumulation, the fresh/dry mass ratio, and other parameters. Separate calculations were made for ^{90}Sr and daughter ^{90}Y and for ^{137}Cs based on their specific radioactivity in the upper soil layer (0–10 cm), because β-radiation from deeper horizons is completely shielded out (Karimullina et al., 2013).

Assessment of the quality of seed progeny. In the laboratory experiment, the seeds were germinated by a roll culture method in vessels with a suspension of soil from the background plots in distilled water (1 : 10), and the seedlings were cultivated for 3 weeks at constant temperature (24°C) and artificial illumination with a 12-h photoperiod. The vessels were rearranged in a random manner every day and supplemented with water to maintain the initial cultivation conditions. At the end of the experiment, the quality of the progeny was assessed by determining seed germination rate, seedling survival, and the proportion of seedlings with

Table 1. Specific ^{90}Sr and ^{137}Cs radioactivities in the soil ($G \pm GSD$, $n = 14$) and radiation loads on maternal *Plantago major* plants

Plot	Specific radioactivity, Bq/kg		Transfer coefficient		Absorbed dose rate, μGy/h
	^{90}Sr	^{137}Cs	^{90}Sr	^{137}Cs	
Background (average)	6.92 ± 2.60	9.89 ± 4.46	0.518	0.031	0.103
EURT-1	21496 ± 1.99	801 ± 2.67			5.85
EURT-2	14009 ± 2.65	375 ± 4.42			3.83

Designations: (*G*) geometric mean, (*GSD*) geometric standard deviation.

true leaves per vessel (i.e., per replicate of the experiment, which was performed in four replicates in each variant). In addition, root length in the seedlings was measured to an accuracy of 1 mm, and they were examined for the presence of lateral roots and morphological disturbances in different organs. In this case, each seedling was taken as a replicate.

In the course of this experiments, the seed progeny of plants from different zones were compared with respect to their adaptive potential, which was estimated from heavy metal and radiation resistance of seedlings. To estimate the effect of acute exposure to heavy metal toxicity, the seeds were germinated in vessels with the suspension of soil from plot *NTMK-2,* where the level of heavy metal pollution was the highest. Radioresistance of seeds from all cenopopulations was evaluated by exposing them (before germination) to γ-radiation from a ^{137}Cs source (in an IGUR device) at doses of 100–300 Gy (dose rate 41.1 Gy/s). The responses of samples from the EURT and NTMK zones were compared to that of background samples to estimate preadaptation of plants to the corresponding factors (i.e., the result of long-term exposure of maternal plants to technogenic stress). For each cenopopulation, parameters recorded in the variants with acute exposure to radiation or heavy metal toxicity were compared with the internal control (without such exposure) to estimate the potential of the seed progeny for adaptation to “habitual” and “new” factors. A total of 1800 seeds were used in the laboratory experiment.

In addition, we performed a greenhouse experiment in which 25–30 samples from each population were grown in isolated plots with a sufficient edaphic space and uniform agricultural conditions and evaluated for the following parameters: the numbers of leaves and flower stalks and their maximum length, survival rates at the end of the growing season and after overwintering, and the presence of morphological alterations (morphoses) in the vegetative and generative organs. The frequency of morphoses relative to the sum of all leaves and leaf stalks per plant and the frequency of plants with morphoses per sample were calculated.

Experimental data were processed statistically using Scheffe’s method for multiple comparisons, Mann–Whitney *U* test, and Kruskal–Wallis *H* test in Statistica 8.0 (StatSoft Inc., 2007), principal component analysis (PCA) in Past 2.11 (Hammer et al., 2001), and the method for determining the confidence interval (*CI*) of proportions (Newcombe, 1998) with macros created in MS Excel.

RESULTS

Toxic and Radiation Loads on P. major Plants

Data on specific ^{90}Sr and ^{137}Cs radioactivities in the soil and vegetative phytomass were used to calculate transfer coefficient for these main dose-forming radionuclides (Table 1). Taking into account the natural level of background radiation (BGR), which in the Ural region is about 0.1 μGy/h, we found that the absorbed dose rate for plants in the EURT zone was 37–57 times higher than in the background plots. The total absorbed dose over the growing season (April to October) for these plants amounted to 16.5–25.3 mGy. Since these values do not exceed 100 μGy/h, they are classified as low doses (Garnier-Laplace et al., 2004).

The toxic load in the NTMK plots was estimated from the contents of heavy metals in the soil (Table 2). As a result, positive correlations were revealed between the concentrations of Zn and Cu and also of Cd and Pb ($r = 0.69$–0.75, $p = 0.0195$–0.0376), indicating that the use of the toxic load index (K_i) was valid. Its values for the plots in the zone of chemical pollution exceeded the background level by a factor of 3–19.

Characteristics of P. major from Different Zones at Early Stages of Development

Viability of seed progenies of plants from different zones. The values of parameters “seed germination” and “seedling survival” were either identical (Fig. 1) or did not differ significantly ($\chi^2 = 11$, $df = 3$, $p = 0.611$). Hence, we then consider only the latter parameter, also taking into account the proportion of seedlings with true leaves and the root length. These parameters are important because they characterize the onset of the qualitatively new developmental stage related to the functioning of meristems. The background cenopopulations differed from each other in the rate of seedling survival and root length (Scheffe’ test, p =

Table 2. Heavy metal contents in the soil ($M \pm SE$, $n = 13$–20) and indices of toxic load in the NTMK zone and background plots

Plot	Metal, μg/g				K_i
	Cu	Zn	Pb	Cd	
Background (average)	12.3 ± 1.8	19.4 ± 3.4	7.9 ± 2.3	0.05 ± 0.04	1.0
NTMK-1	19.2 ± 4.31	42.9 ± 18.8	16.5 ± 4.9	0.4 ± 0.09	3.3
NTMK-2	78.6 ± 49.7	256.1 ± 92.3	115.3 ± 81.7	2.1 ± 1.64	18.9

Designations: (*M*) arithmetic mean, (*SE*) standard error.

0.0012–0.012). The two samples from the EURT zone also differed significantly in growth parameters, namely, the proportion of seedlings with true leaves and root length ($p = 0.000007$–0.00006), with their highest values being recorded in the *EURT-2* sample. Thus, the quality of the seed progeny formed in this plot differed significantly from that in all other samples, being superior in a set of parameters.

The viability of seeds from the NTMK zone was reduced, with both samples showing the lowest rates of leaf and root development ($p = 0.27$–0.99). Lateral roots were formed in only 3% of seedlings, compared to 19–53% in other samples.

The results of analyzing data on the viability of seed progeny by the PCA method showed that the first and second PC axes explained 87.4 and 11.7% of intergroup variance, respectively. As follows from Fig. 2, differences between the samples from the zone of chemical pollution were minimal, while the variance within the background and radioactively contaminated zones was considerable, and some populations from different zones proved to be more similar to each other than populations within the same zone.

Radioresistance of seed progeny of plants from different zones. To analyze the responses of plants from the two technogenic area to the acute impacts of "habitual" and "new" factors in comparison with the responses of background samples, the values of each parameter were expressed as a percentage relative to those obtained in the internal control (without acute treatment). This approach provided a clear idea of the adaptive response of seed progenies from each cenopopulation and allowed their comparison with each other.

Analysis of the dose–effect relationship showed that, with respect to seedling survival, the background cenopopulations and samples from the NTMK zone were resistant to acute irradiation (Scheffe's test, $p = 0.48$–0.99), but both *EURT* samples proved to be sensitive to the factor habitual for them ($p = 0.0003$–0.042). Differences in radiosensitivity between the samples became more apparent at the stage of true leaf formation: after irradiation at 100–300 Gy, the growth of true leaves was suppressed in all samples ($H_{(3;12)} = 8.2$–10.7, $p = 0.013$–0.042) except *NTMK-1* and *NTMK-2* ($H_{(3;12)} = 3.4$–4.1, $p = 0.25$–0.33). The root test confirmed this result: after irradiation at 100 Gy,

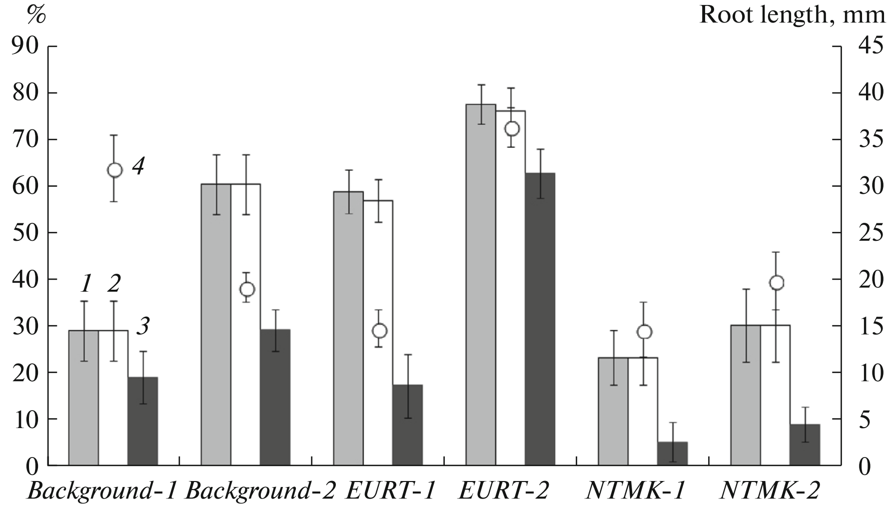

Fig. 1. Parameters characterizing viability of *Plantago major* seed progeny from background and impact populations ($M \pm SE$, %): (*1*) germination rate, (*2*) seedling survival, (*3*) proportion of seedlings with true leaves; (*4*) root length (right ordinate).

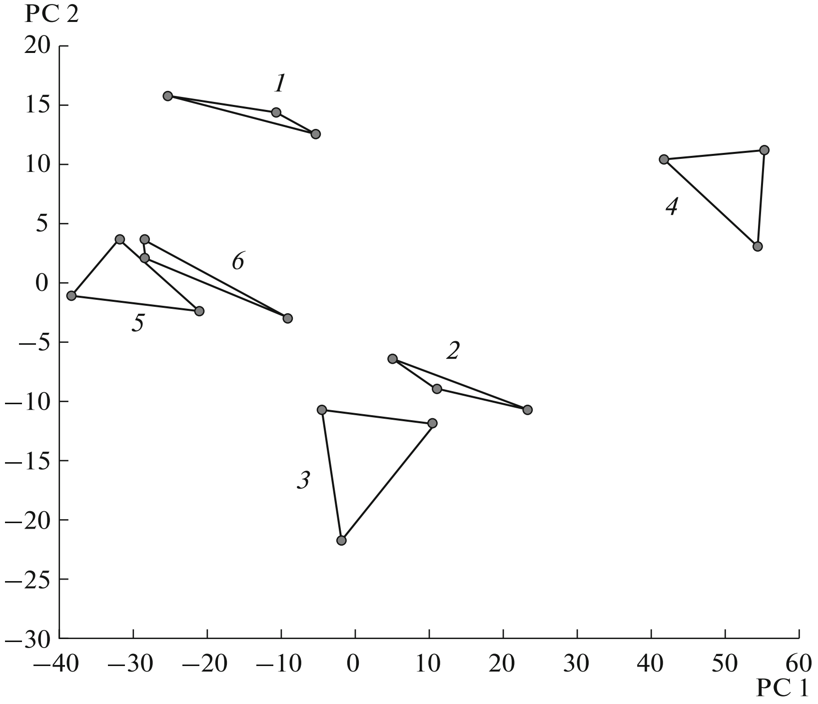

Fig. 2. Distribution of samples from *Plantago major* cenopopulations in the space of two principal components (PC): (*1*, *2*) background samples, (*3*, *4*) the EURT zone, (*5*, *6*) the NTMK zone.

the test parameter in the *NTMK* samples decreased by only 43–54%, compared to more than 85% in the *EURT* samples and background populations. Thus, samples from the chemical pollution area showed the highest resistance to the factor new for them. Radioresistance of the seed progenies of plants from the zone of radioactive contamination was not increased; therefore, no preadaptation effect was revealed.

Heavy metal resistance of seed progeny of plants from different zones. The seed progeny from different samples showed a diversity of responses to additional heavy metal treatment (Fig. 4). Their comparison with the internal controls revealed no significant differences in seedling survival (Scheffe's test, $p = 0.93$–0.99), which was explained by high variability of the test parameter under conditions of acute exposure to heavy metal toxicity. A similar response to such exposure was also observed from growth parameters: the rate of development of true leaves (significant for the *Background-2* sample; $H_{(1;8)} = 4.1$, $p = 0.042$) and roots (significant for both samples, $H_{(1;8)} = 5.33$, $p = 0.021$) was lower than in the internal control.

A tendency toward accelerated true leaf formation after acute heavy metal exposure was observed in the *EURT-1* sample and both samples from the NTMK zone ($H_{(1;8)} = 0.09$–0.34, $p = 0.56$–0.77). The root length in all impact populations was decreased after such treatment, but no differences from the internal controls were revealed ($H_{(1;8)} = 0.08$–2.08, $p = 0.15$–0.77).

Thus, samples from the zone of chemical pollution showed the highest resistance to deleterious factors, both new (ionizing radiation) and habitual (heavy metal toxicity). This fact suggests that the mechanisms of resistance to different kinds of technogenic impact are not specific.

Frequency of morphological anomalies in seed progeniesy of plants from different zones. Experimental and field studies have repeatedly shown that radiation exposure leads to the development of morphological anomalies in plants and their progeny (Grodzinskii, 1989; Popova et al., 1992; *Ontogeneticheskii atlas...,*

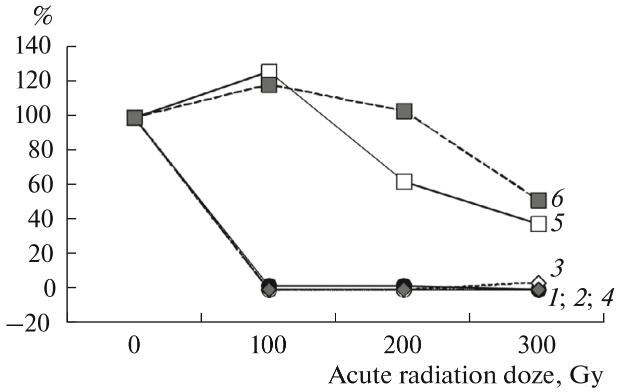

Fig. 3. Percentages of *Plantago major* seedlings with true leaves obtained after acute irradiation of seeds: (*1, 2*) background samples, (*3, 4*) the EURT zone, (*5, 6*) the NTMK zone.

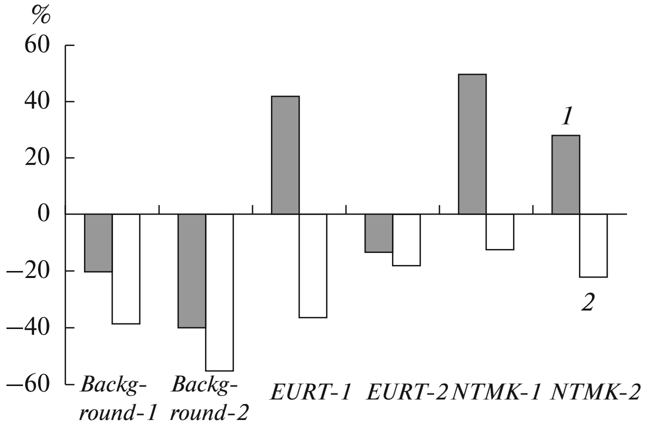

Fig. 4. Parameters characterizing heavy metal resistance of *Plantago major* seed progeny from background and impact cenopopulations: (*1*) percentage of seedlings with true leaves, (*2*) root length. Control values are taken as zero.

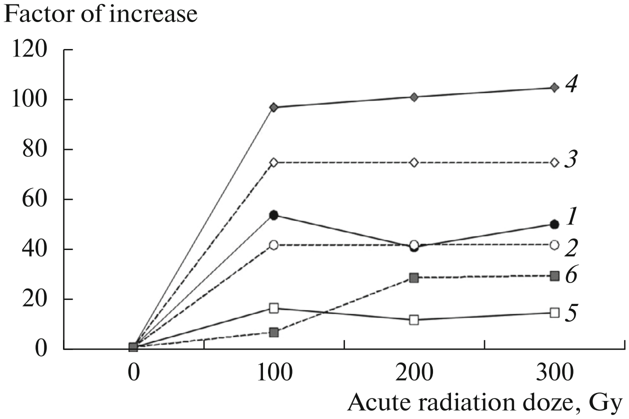

Fig. 5. Frequency of *Plantago major* seedlings with root necrosis obtained after acute irradiation of seeds: (*1*, *2*) background samples, (*3*, *4*) the EURT zone, (*5*, *6*) the NTMK zone.

1997; Pozolotina et al., 2008; Igonina et al., 2012). Anomalies recorded in the course of our laboratory experiments were as follows: alterations in the color and shape of cotyledons, leaves, and roots; necrosis of roots and cotyledons; disturbances of heliotropism; multiple lesions in different parts of the plant; and some other aberrations (in single cases). However, the frequencies of anomalies at early stages of plant ontogeny were insignificant: e.g., the highest frequency of individuals with root necrosis in impact samples reached only 0.95–1.33% of the total number of surviving seedlings. Similar data are available on the closely related species *P. lanceolata,* in which the main proportion of anomalies was revealed at later stages of plant development in plot culture (Popova et al., 1992).

Previous experiments with other plant species have shown that acute irradiation helps to reveal latent mutability (Pozolotina et al., 2008; Antonova et al., 2014). In this study, the proportion of seedlings with root necrosis increased manifold after such treatment (Fig. 5; *CI* = –0.169 to –0.906), and the highest factor of increase was characteristic of samples from the EURT zone.

Characteristics of P. major from Different Zones at Later Stages of Development

In the first season of the greenhouse experiment (2013), plants of the middle-age generative state in all samples showed a high survival rate (71–100% of all plants in the sample). The highest values of all test parameters were recorded in plants from the EURT zone; the lowest values, in plants from the NTMK zone (Table 3), with plants from background cenopopulations occupying an intermediate position.

In the next season (2014) the survival of plants from the zone of chemical pollution and background samples was very low: only 9–24% of plants overwintered successfully. In contrast, this parameter in the *EURT-2* sample reached 100% (differences from all other samples are statistically significant: *CI* = 0.499–0.602 to 0.894–0.950). Significant differences in parameters of plant growth were revealed when this sample was compared with *EURT-1*, *NTMK-2*, and *Background-2* samples (Table 3). It is noteworthy that variation in the above parameters was at a medium level in 2013 (*CV* = 13–58%) but markedly increased in 2014 (*CV* = 25–85%).

On the whole, plants from the *EURT-2* sample proved most successful in terms of survival during the two growing season and growth characteristics of vegetative and generative organs. However, plants from the EURT zone showed a tendency toward increase in the frequency of anomalies in the generative organs, such as the presence of leaves on the flower stalk, forked spire, or multiple spire dichotomy (Fig. 6). Thus, the frequency of plants with an altered flower stalk shape in the *EURT-1* sample reached 9.5%, compared to a background value of 4.8% relative to the total number of flower stalks (the difference lacks statistical significance: *CI* = –0.025 to 0.14). No such anomalies were revealed in plants from the zone of chemical pollution.

Morphological anomalies of vegetative organs were more frequent than those of generative organs. In particular, the frequency of leaves with purple spots (relative to the total number of leaves) was increased significantly in samples from the NTMK zone, reaching 5.4–11.7% (*CI* = –0.065 to –0.077), while this proportion in samples from the EURT was the lowest (0.3–2.3%); background samples occupied an intermediate position (1.4–4.2%). The frequencies of leaves with an altered shape were distributed in a similar

Table 3. Variation of vegetative and generative organs of *Plantago major* plants from different zones during two seasons ($M \pm SE$)

Sample	Maximum leaf length, cm	Number of leaves	Maximum flower stalk length, cm	Number of flower stalks
2013 (n = 21–25)				
Background-1	15.48 ± 0.91	10.33 ± 0.37^{c}	27.48 ± 1.77	14.24 ± 0.68^{c}
Background-2	13.79 ± 0.90	13.67 ± 1.14^{c}	20.00 ± 3.50	11.38 ± 1.20^{c}
EURT-1	$19.48 \pm 1.06^{a,b,e}$	14.48 ± 0.89^{a}	$39.35 \pm 2.55^{a, b, e}$	$17.05 \pm 1.22^{b, e}$
EURT-2	$19.24 \pm 1.16^{a,b,e}$	$14.52 \pm 0.82^{a, f}$	$37.70 \pm 2.21^{a, b, e}$	$18.19 \pm 1.19^{a, b, e}$
NTMK-1	$12.82 \pm 0.56^{a,d,e}$	12.48 ± 0.50^{a}	$19.19 \pm 2.01^{a, e}$	$11.68 \pm 0.78^{a, e}$
NTMK-2	$9.71 \pm 1.12^{a,b,d,e}$	13.00 ± 0.79^{a}	22.50 ± 3.19^{e}	11.76 ± 0.90^{e}
2014 (n = 3–17)				
Background-1	7.25 ± 2.25	11.50 ± 3.50	No data	
Background-2	6.13 ± 1.64	9.50 ± 3.93		
EURT-1	10.17 ± 4.97	9.00 ± 3.06^{d}		
EURT-2	$18.35 \pm 2.06^{b,g}$	$31.82 \pm 6.39^{b, d, g}$		
NTMK-1	12.90 ± 3.57	17.40 ± 4.46		
NTMK-2	8.30 ± 1.54	11.20 ± 1.28		

The superscripts indicate statistically significant differences: (a) from population *Background-1*: U = 57–165.5, z = 2.1–4.2, p = 0.001–0.031; (b) from population *Background-2*: U = 5–102, z = 2.2–3.6, p = 0.0001–0.025; (c) within the background zone: U = 94.5–132.5, z = 2.2–3.2, p = 0.001–0.026; (d) within the impact zone: U = 5–106, z = 2.2–2.3, p = 0.021–0.030; (e) between impact populations: U = 22.5–116.5, z = 3.1–4.5, $p \leqslant 0.002$; (*f*) from *NTMK-1*: U = 167, z = 2.1, p = 0.035; (g) from *NTMK-2*: U = 10–13.5, z = 2.3–2.6, p = 0.011–0.023.

pattern: 9.6–15% in the NTMK samples vs. 1–10.8% in the *EURT* and 4.2–8.3% in the background samples. It should be noted that relatively many plants in the NTMK samples had leaves with a twisted leaf stalk: their frequency reached 2.9–5.1%, compared to 0–0.9% in other samples. Significant differences were revealed between the samples from the two impact zones and between these and background samples (CI = –0.051 to –0.0004).

DISCUSSION

The data presented above show that the viability of *P. major* seed progeny from different zones have vary at the wide range. At early stages of ontogeny, the survival rate and growth parameters of plants from the zone of chemical pollution (NTMK) proved to be significantly lower than in the background cenopopulations, while the development of plants in the sample from the zone of radioactive contamination (EURT) was most successful by all test parameters. At later stages, samples from the latter zone were also superior to the background samples in all morphological characteristics, while their values in plants from the NTMK zone were the lowest. After overwintering, the survival rate of plants from the zone of chemical pollution and background plots decreased to 9–24%, whereas this rate in the *EURT-2* sample was 100%.

It could be expected that the adaptive potential of samples from the EURT would be also high, at least with respect to radiation as a habitual factor, since the phenomenon of radioadaptation has been revealed in different organisms exposed to long-term radiation stress (Shevchenko et al., 1992; Kovalchuk et al., 2004; Evseeva et al., 2007). However, the results obtained in this study are paradoxical in this respect: on the one hand, cenopopulations in the zone of radioactive contamination proved to produce radiosensitive progeny (i.e., the effect of radioadaptation was absent); on the other hand, seed progenies of plants from zone of chemical pollution showed increased resistance not only to heavy metals (the habitual factor) but also to radiation (a new stress factor).

A probable cause of these differences lies in the specificity of responses to the effects of different factors. It is known that ionizing radiation induces genomic instability, which is manifested in chromosomal rearrangements, gene amplification, micronuclei formation, aneuploidy, increased mutation rate, and eventual cell death; in addition, radiation exposure leads to changes in sensitivity to other stress factors (Marder and Morgan, 1993; Little, 1998; Mazurik and Mikhailov, 2001; Kovalchuk et al., 2004; Kim et al., 2006; Mukaida et al., 2007). A distinctive feature of genomic instability is that it persists for many cell generations after irradiation (Bychkovskaya et al., 2005), with the period of persistence after long-term irradiation at low doses being two to thee times longer than after high dose irradiation (Antoshchina et al., 2005).

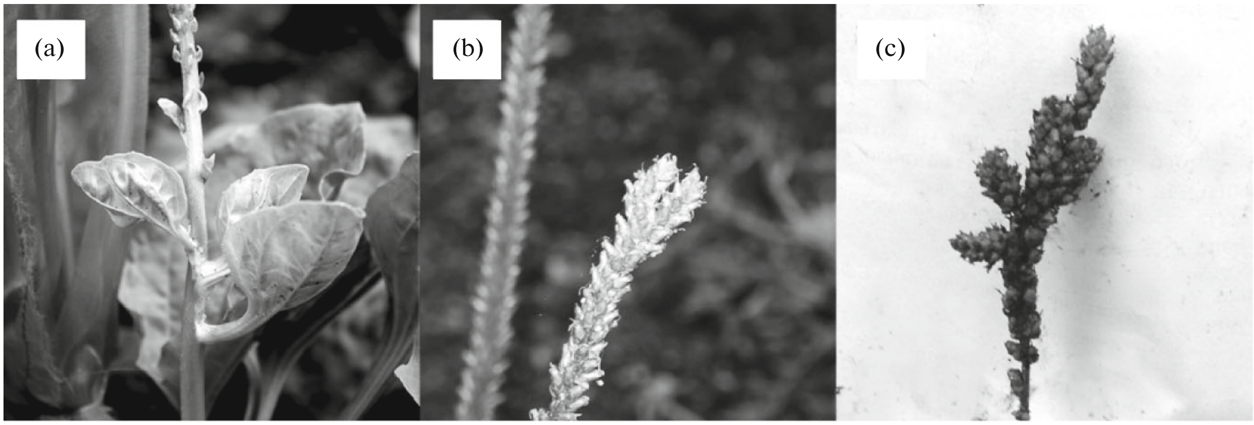

Fig. 6. Anomalies in the development of *Plantago major* generative organs: (a) leaves on the flower stalk, (b) forked spire, (c) multiple spire dichotomy.

In our previous study on another plant species (*Taraxacum officinale* s.l.), the F1 progeny of plants from the EURT zone, grown in a clean environment, was found to be highly viable but sensitive to technogenic factors, while the F1 progeny of plants from the NTMK zone was characterized not only by high viability but also by improved adaptive potential (Pozolotina et al., 2012a). In the latter case, resistance acquired in the course of selection under the impact of a certain factor proved to be universal, making the plants resistant also to another kind of technogenic impact. In may be assumed that long-term exposure to heavy metals in plants does not lead to genomic instability.

The high adaptive potential of plants from the NTMK zone may also be due to the maternal effect, which has been observed in many plant species with respect to different factors (*Maternal Effects...,* 1998; Galloway, 2005; Youngson and Whitelaw, 2008). In particular, studies on three generations of *Plantago major* showed that the effects of environmental factors observed in the F1 and F2 generations were also manifested to different degrees in the phenotype of the F3 generation, depending on growing conditions for the latter (Miao et al., 1991). In the closely related species *P. lanceolata,* plant phenotypes were found to largely depend on the environmental conditions in which the maternal plants were growing (Latzel et al., 2014). The observed transgenerational effects were adaptive.

It has been shown that exposure to stress leads to the generation of mobile signal molecules (e.g., mRNA) that can enter the gametes and induce DNA methylation (Boyko and Kovalchuk, 2011). Hypermethylated DNA regions are prone to C-T mutations, while hypomethylated regions are characterized by higher frequency of homologous recombination. It is as yet unclear how many generations are required for epigenetic mutations to be converted into stable mutations, but organisms in which epimutations are useful for growing under given environmental conditions have more chance to survive and produce progeny. It appears that the transgenerational effect has both heritable and nonheritable components, with the latter including the accumulation of metabolites, proteins, and/or mRNA in seeds, thereby giving the progeny an advantage in growth.

It is important to take into account specific features of natural selection. Thus, *P. major* seedlings from the progeny of plants growing in the NTMK zone had a low survival rate (i.e., only the most resilient plants could survive), but this seed progeny proved to be resistant not only to heavy metal toxicity but also to radiation. Hence, it can be assumed that the corresponding adaptive mechanisms are not specific.

Analysis of mutability in *P. major* at early stages of ontogeny revealed an insignificant number of morphological anomalies, but acute irradiation helped to reveal latent disturbances. After this treatment, the lowest frequency of root necrosis was recorded in seed progenies from the NTMK zone, and the highest frequency, in samples from the EURT. In *Arabidopsis thaliana,* Tomilov et al. (2001) identified a gene mutation in which proved to result in root necrosis. It is probable that the causes of root necrosis in *P. major* also have a genetic component. The high proportion of latent disturbances in seed progenies from the EURT appears to be a manifestation of genomic instability, with such an effect being absent in samples from the zone of chemical pollution. However, the role of direct impact of high radiation doses on the meristems of seedlings cannot be excluded.

At later stages of ontogeny, plants with morphological anomalies occurred in all samples, with the frequency of anomalies in generative organs reaching a maximum in samples from the EURT. The highest frequency of plants with leaf discoloration due to the accumulation of anthocyanins was observed in samples from the NTMK zone. Anthocyanins play a major role in plant adaptation to both biotic stress (Kordali et al., 2005; Diaz-Vivancos et al., 2006; Franklin et al., 2009) and abiotic stress (Chalker-Scott, 1999; Treutter, 2005; Gordeeva et al., 2013; Khlestkina, 2013). Since these pigments are antioxidants that neutralize reactive oxygen species, their role is especially important under conditions of exposure to heavy metals and ionizing radiation (Ren et al., 1999; Treutter, 2005).

Anthocyanins in plant cells occur mainly in the form of chemical complexes with metal ions (Al, Fe, Mg, etc.) stabilized by copigments (flavones and flavonols), which are known as metalloanthocyanins (Yoshida et al., 2009). This is an additional argument that soil enrichment with metals can stimulate the synthesis of metalloanthocyanins, which accumulate in greater amounts and impart purple color to leaves. However, since the progenies of plants from all zones were grown in a clean soil, the matter at issue is not the direct effect of heavy metals but rather the ability to synthesize large amounts of anthocyanins that has been acquired by these plants and transmitted to their progeny. Hence, it may be assumed that the observed phenomenon is based on the maternal effect manifested as an adaptation in response to changeable environmental conditions.

ACKNOWLEDGMENTS

This study was supported by the Russian Foundation for Basic Research (project no. 15-04-01023), Scientific School Support Program (project no. NSh-2840.2014.4), and Basic Research Program of the Ural Branch, Russian Academy of Sciences (project no. 15-2-4-21).

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Translated by N. Gorgoluk