SENSITIZING OF GENOME OF WELSH ONION (*Allium fistulosum* L.) BY ENVIRONMENTAL CONDITIONS

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Abstract

An effect of environment on the sensitivity of welsh onion genome to provocative mutagenesis with formaldehyde was studied. Seeds of welsh onion were collected from plants grown at localities with different values of ecological impact - “bad”, “middle” and “good”. Seeds (at the 19th month of storage) from three localities were germinated in formaldehyde solution (0,0004%) or in distilled water as a control. Formaldehyde increased the level of aberrant anaphases for seeds obtained from “bad” conditions: frequency of AA was 22.13% in comparison with 11.09% for control ($p < 0.001$). For seeds from “middle” and “good” localities mutagenic effect of formaldehyde was statistically insignificant. Another indicator of environmental impact, the age-related dynamics of chromosome instability, was different for these three localities, too. The increasing of frequency of aberrant anaphases (AA) with age of seeds from bad, middle and good localities was characterized by the regression coefficients 0.74, 0.38, and 0.16 respectively. Thus, both parameters, rate of increase of genome instability and genome sensitivity of seeds to mutagenic influences depend on ecological conditions of vegetation of plants. The relation between these two parameters is discussed.

Introduction

The pollution of environment is danger to all living beings, including humans. Besides of toxic effects, pollutants modify the mutation process, as a rule, increasing the mutation rate. The most common manifestation of mutagenesis at the cell level is chromosome aberrations (CA); increased level of CA is considered as chromosome instability. The chromosome instability is important factor of malignant neoplasm development; it also increases during aging. The increased level of instability revealed in organisms from radioactive (and other pollutants) contaminated places.

Genome stability may be defined as an ability to maintain stable its structure and function under changing conditions. Genome instability is any decline from the normal structure and function of the genome. The most usual indicators of instability are deviations from normal phenotype (instability of individual development) and abnormalities of genome structure (DNA damage, chromosome aberrations, aneuploidy, poliploidy, etc). The genome stability is a species-specific feature of genome and is under genetic control. The individual level of genome instability may vary depending on genotype of individual, age and environmental influences. On other side, it can be also modified by environmental conditions. Numerous studies were performed with plant species, animals and humans to study mechanisms of chemical, physical and other factors influences on genome instability. Special attention was devoted to radionuclid pollution due to A-bomb and nuclear power stations casualties.

Plants are perspective bioindicators of environmental impact (1, 2). Little is known, however, about mechanisms of influence of environment on plant genomes and their stability. Study of scabiose

centaury (Centaurea scabiosa L.) from the East Ural radioactive track showed increased level of chromosome aberrations, pigment mutations, rare and null alleles of enzyme locus *Lap* (3). Impact of Chernobyl radioactive contaminants on three plant species had manifested also in decreasing of fitness and respective growth of the level of fluctuating asymmetry and frequency of phenodeviant (4) - these characters are parameters of stability of individual development and respectively, they depend on genome stability.

It was reported that mutation events in barn swallows (Hirundo rustica) from Chernobyl were 2-10 times higher than in birds from control areas in Ukraine and Italy (5). High frequency of chromosomal aberrations was found in the common voles (*Microtus arvalis*) from East Ural radioactive track (EURT) and in the laboratory-reared F_1-offspring of common voles captured near the EURT. However, in the following generation under laboratory rearing this frequency decreased to control figures (6).

Data on hibakusha (survivors of the atomic bombing of Hiroshima and Nagasaki) and their children did not provide evidence of any statistically significant differences between exposed and control families (7, 8). Kodaira M. and coworkers (9) in a pilot study of effect of atomic bomb radiation on germ-line instability find no effect of parental irradiation on DNA minisatellite mutation rates in the children.

At the same time, studies of other human populations, suffered after radionuclid pollution, revealed high level of genome instability. Increased level of leukemia and lymphoma was found among young people near the Sellafield nuclear installation in West Cumbria (10, 11). The Chernobyl accident was followed by a significant increase in the incidence of thyroid cancer among children and adolescents in Belarus and Ukraine (12). Dubrova Yu. (13) reported that the rate of mutation involving a battery of DNA minisatellites was twice as high in children whose parents had been exposed in the Mogilev district of Belarus to fallout from the Chernobyl disaster than in controls. Also the controls were drawn from England, it seems more correctly to compare data from two European populations, than compare Europeans with Japanese or with mice (7). The genetic variability is a general phenomenon and one may suppose these controversies are result of ethnic differences.

Thus, the level of genome (and, respectively, chromosome) instability depends on both environmental and genetic factors. Age of an organism also make an important contribution to genome instability: the elder organism, the higher level of genome instability (age-related increasing of cancer frequency, Down syndrome and so on). The problem of special interest is a mutual effect of environmental conditions as external factor and age-related changes of genome as internal factor of genome instability. It is not easy to evaluate the partial contribution of age, genotype and environmental conditions to genome instability. One reason of this difficulty is that different authors in different localities use different indicators and test-systems. Main aim of our work was to study the age-related changes of chromosomal instability in seeds of welsh-onion (*Allium fistulosum* L.), grown in environments with different rate of pollutant load.

The purpose of our work is investigation of relation between conditions of plants (*Allium fistulosum* L.) cultivation, development of chromosome instability in seeds, obtained from these plants, and their sensitivity to the mutagenic influences.

Methods

Localities studied. Plants were cultivated at the three localities with different environmental conditions: A (good), B (common) and C (bad).

A – Village Vlasivka, Baryshivka area (rayon), Kyiv region - area with relatively good environmental conditions. A country place (summer cottage and dacha area), no chemical contamination, reasonable using of pesticides and other agricultural chemicals.

B – Town Oster, Chernigiv region – vacation resort, no significant chemical or industrial contamination, reasonable using of pesticides and fertilizers.

C – Town Vasylkiv, Kyiv region – suburb of Kyiv, close to military aerodrome, significant industrial and traffic (railway and highway) load. The place for plant cultivation was close to highway and located in lowland, thus had significant load of agricultural chemicals from surroundings.

Seeds for planting were harvested in 1990; they were from the same seed pool and were genetically homogenous. In 1993 these seeds were sawed out at the three places (localities A, B and C) with different environmental load. From the grown plants in 1995 were gathered seeds for testing. Seeds from different environments were stored together in nonhermetic vessels at the laboratory conditions, during more than three years they were periodically germinated and tested. Seeds germinating was performed at 24 °C in the darkness in Petri dishes (100 seed per dish) with filter paper wetted with distilled water. Germinating capacity was calculated as a proportion of germinated seed.

Slides preparation. Root tips (about 4-7 mm in length) of germinated seeds were cut off and fixed in ethanol:acetic acid (3:1). Fixed root tips were squashed and stained with acetoorcein; temporal slides were prepared according to standard methods, as described (14). Frequency of abnormal anaphase cells and a number of chromosomal aberrations in damaged cells were scored.

Results

The seeds' aging was followed with growth of chromosomal instability and changes of spectrum of chromosome aberrations. During the first six months of storage in a majority were chromatide-type bridges. "Old" seeds revealed chromosome-type bridges, other kinds of structural aberrations and increasing frequency of cells with multiple damages (fig. 1). The presence in cells of chromatid-type aberrations is an indicator of an ongoing production of primary structural changes, i.e. of chromosome instability. Chromosome-type aberrations indicate more considerable damages, since a major of them are derived of primary chromatide-type aberrations due to failures of reparative systems.

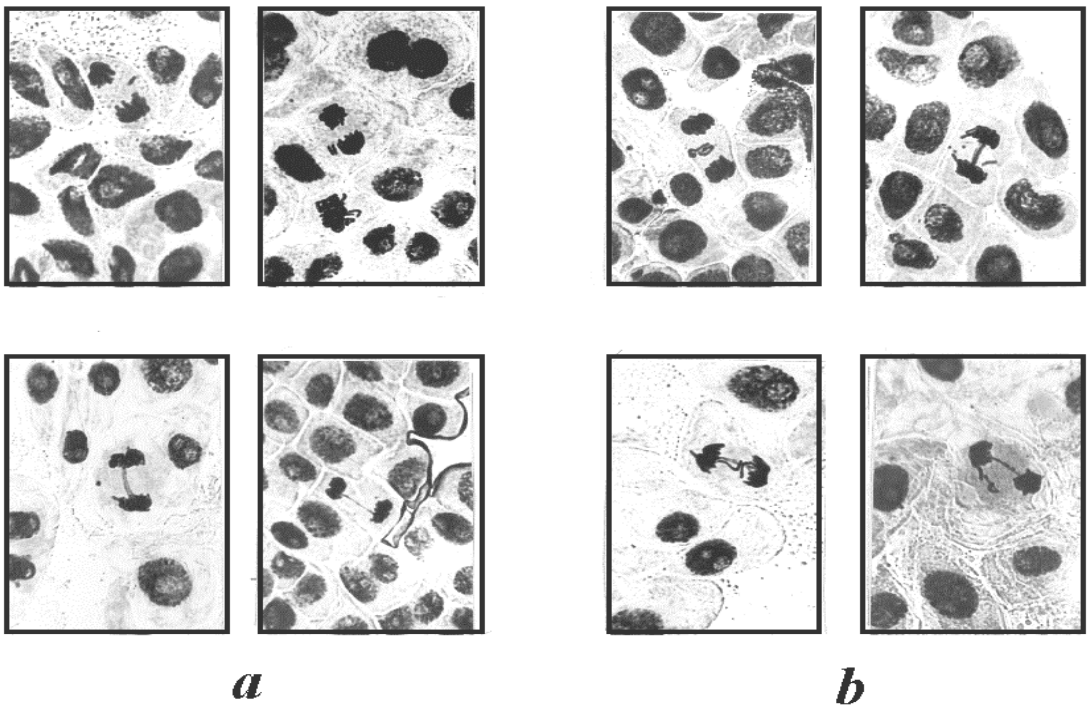

Fig.1 Some examples of observed chromosome instability in root tips of germinated "young" (***a***) and "old" (***b***) seeds of welsh onion (*Allium fistulosum* L.).

For further study of distinctions between young and old seeds from different environments we have chosen the following three indicators: the frequency of anaphase cells with chromosomal aberrations, "damage", as a number of chromosomal aberrations per one cell with aberrations and germinating capacity, as an indicator of "toxic" influence of age. To avoid influence of fluctuations, we pooled data on seeds of one and two months of storage, as well as data on seeds of 34 and 38 months and processed them as "young" and "old" respectively. Results of these comparisons are shown on Fig. 2. Data on statistical significance of these distinctions are presented in the Table 1.

The most impressive distinctions we have found in frequency of anaphase cells with chromosome aberrations (fig. 2). Young seeds from “good” environment revealed almost four fold higher level of chromosomal instability, than young seeds from “bad” environment (fig 2). After three-years of storage this relation changed to inverse: the level of chromosomal instability of old seeds from good environment was almost three times lower than that of old seeds from “bad” environment. Total level of chromosome instability grew up to 3.8 times for “good”, 15,3 – for “common” and 39,6 – for “bad” environments. These differences are statistically significant (table 1).

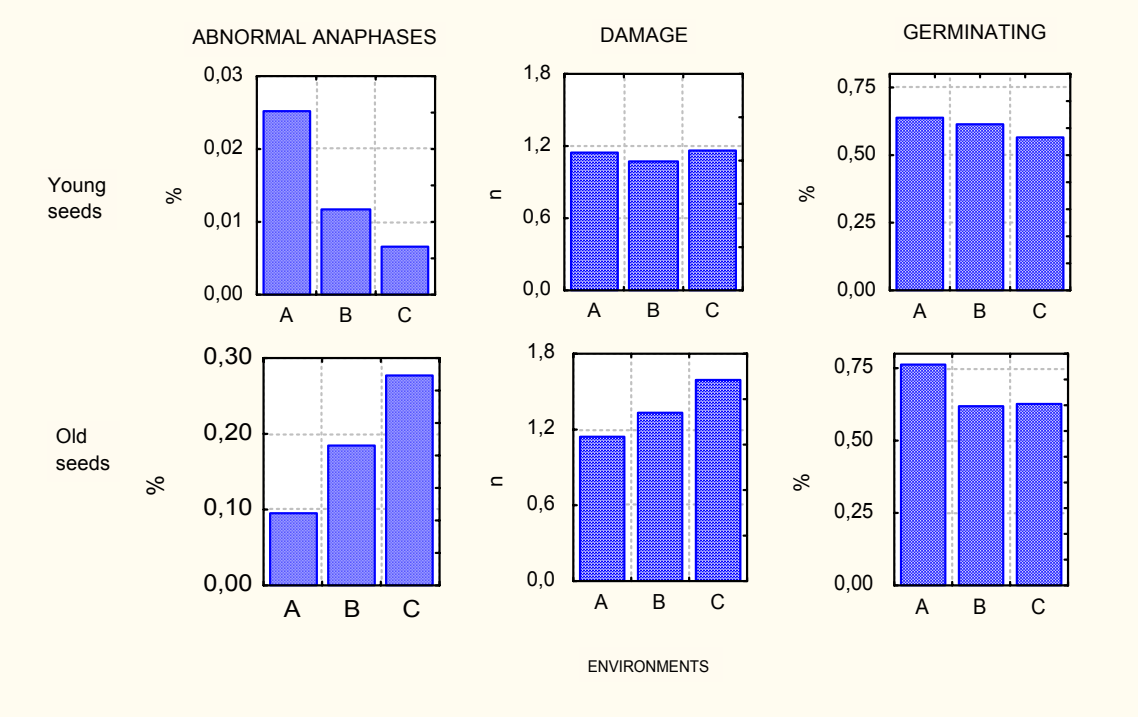

Fig. 2. Frequency of anaphase cells with chromosome aberrations (abnormal anaphases), cell damage (damage) and germinating capacity (germinating) in welsh onion seeds from “good” (A), “common” (B) and “bad” (C) environments; n - average number of chromosome aberrations per cell with abnormalities

Age-related change of another indicator of chromosome instability – cell damages – was less expressed. During storage the number of chromosome aberrations per one anaphase cell with aberrations did not changed for seeds from “good” environment, and slightly (1,23 fold) increased in seeds from “common” environment. The age-related increase of this indicator was more visible in seeds from “bad” environment (1,36 fold), but in all cases these changes did not reached statistically significant level (table 1). There were no significant differences among environments neither for young seeds, nor for old, also in the last case we observed clear and plain increasing of chromosome damage from good to bad environment (fig. 2). Due to statistical insignificance these results may be considered as a tendency only. In addition to real random outcome another possible reasons of this “insignificance” may be fluctuations of data, that increase with seed age, and “toxic” effect of storage that have an influence on seeds’ germinating capacity when seeds with more damaged genome do not germinate and are not accounted for studied indicators of genome stability.

In our study we did not find significant influence of seeds’ age on their germinating capacity (Fig. 2). On the contrary, this indicator even increased for seeds from “bad” and “good” localities; in last case it reached to statistically significant level (Table 1). Seeds from “common” environment did not changed significantly their germinating capacity during 38 months of storage. This fact let us suppose that observed differences of genome stability are not related to loosing of germinating capacity.

Table 1 Chromosomal instability (frequency of anaphase cells with chromosome aberrations and cell damage) and germinating capacity of welsh onion seeds (*Allium fistulosum* L) from "good" (A), "common" (B) and "bad" (C) environments

Indicator	Age	Locality			Statistical significance[1] of pairwise differences, *p*		
		A (Vlasivka)	B (Oster)	C (Vasylkiv)	A-B	A-C	B-C
Abnormal anaphases, %	1+2	0,025±0,005	0,012±0,003	0,007±0,003	0,036*	0,003**	0,330
(Mean±SE)	34+38	0,095±0,009	0,184±0,011	0,277±0,020	0,000***	0,000***	0,000***
Old:young ratio		3,80	15,33	39,57			
p		0,000***	0,000***	0,000***			
Cell damages	1+2	1,14±0,05	1,08±0,08	1,17±0,18	0,903	0,777	0,827
(Mean±SE)[2]	34+38	1,12±0,01	1,33±0,01	1,59±0,01	0,317	0,057	0,231
Old:young ratio		0,98	1,23	1.36			
p		0,93	0,73	0,80			
Germinating capacity	1+2	0,64±0,030	0,62±0,030	0,57±0,040	0,757	0,185	0,350
(Mean±SE)	34+38	0,76±0,030	0,62±0,034	0,63±0,034	0,004**	0,007**	0,916
Old:young ratio		1.19	1.00	1.11			
p		0,009**	1,000	0,221			

[1] - Yates corrected Chi-square criterion
[2] – according to Poisson distribution

The general level of chromosomal instability expressed as the proportion of anaphase cells with chromosomal aberrations positively correlate with age of seeds, also during storage the level chromosome instability in seeds increase by non-plane mode, it fluctuates. The age-related component of these fluctuations may be described by the first-order equation of regression: $y=bx+a$. Equations of regression for seeds from studied localities are shown at the fig. 3, close to respective trend-lines. The regression coefficient *b* corresponds to the general tendency (averaged increasing) of chromosome instability during seeds' senescence under their storage. For "good" (A), "common" (B) and "bad" (C) conditions they are equal: b_A=0.22, b_B=0.46 and b_C=0.84 (Fig. 3). Coefficients *b* are statistically significant for all cases (p<0.05 for C, and p<0.001 for A and B).

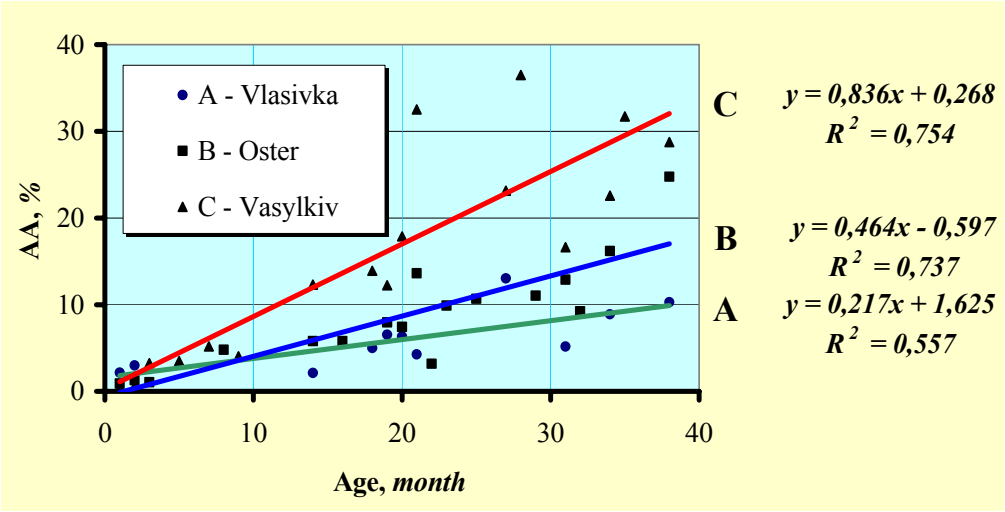

Figure 3 The age-dependent frequency of abnormal anaphases in root tips of welsh onion (*Allium fistulosum*) from different areas of maternal plants growing. A – "good", B – "common" and C – "bad" environments.

Seeds of *Allium fistulosum* L. from different localities had different responds to mutagenic action of 0,00004% formaldehyd: seeds, obtained from plants developed in more unfavourable conditions, were more sensitive to mutagene (Fig. 4).

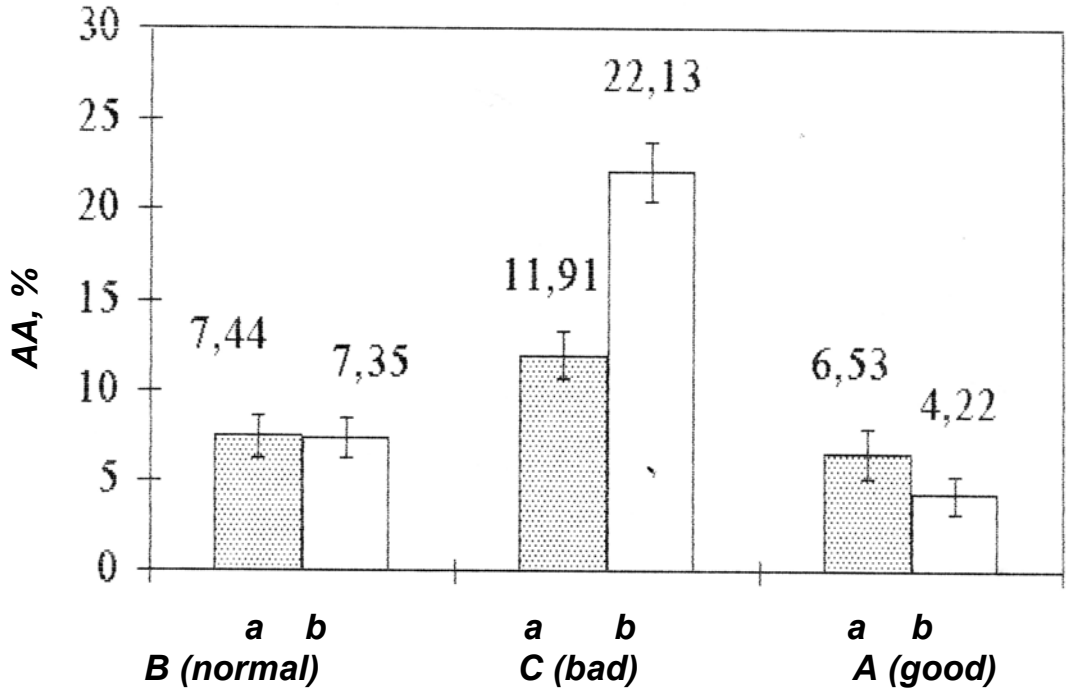

Fig.4. Ecological impact on seeds' genome sensitivity to formaldehyde.

AA - frequency of aberrant anaphases; ***a*** - H_2O; ***b*** - *formaldehyde*

Discussion

Summarizing obtained data, it may be noted that the frequency of abnormal anaphases in germinated seed and quality of environmental conditions where maternal plants were grown are inversely correlated: the worse conditions, the less stable are chromosomes in seeds. But at the first months of storage after harvest the frequency of anaphase cells with chromosome aberrations does not correspond to this rule. Actually, from fig. 2 it follows that “young” seed from ‘bad” environment are even more stable, than seed from “good” environment.

The superiority of young progeny of plants from “bad” environment in comparison with progeny of plants from ”good” environment (fig. 2) looks like manifestation of “stimulative effect” of “bad” environment. This superiority, however, is rather seeming, because after some time (3-5 months) the relation changed to inverse (fig. 3). Old-young ratios of studied indicators are good illustration for impact of environment of maternal plants vegetation on genome stability of these plants’ progeny (Table 1). We suppose that clear distinctions among young seeds (fig. 2) are one more manifestation of environmental impact on genome and that they may be related to chemical load.

After the harvest seeds need some time to ripe, unripe seeds reveal increased level of genome instability during germination. In natural conditions the period since harvesting till germination is about six months. The rate of ripening may be modified with substances, accumulated by seeds during maternal plant vegetation. Some of chemicals of the “bad” locality (Vasylkiv) could be useful or even essential for processes of ripening (this locality underwent a load of agricultural chemicals due to washing them down with water from surrounding hills. The direct influence of these chemicals on chromosome stability is not excluded, also.

The mechanisms of environmental influences of genome instability are little known. In our experiments we have used the seeds collected from the plants that were grown in different

environmental conditions from the same seeds pool and thus had the same gene pool. Processes of seed formation and ripening were gone through the certain environmental conditions and all changes in genome instability reflect the impact of these conditions, also seeds were stored and germinated under identical conditions.

The data obtained lets us suppose that environment of maternal plants vegetation have significant impact on chromosome instability of seeds, collected from these plants. However, mechanisms of this influence are not clear. The early observation on the transmissible genome instability (this term is used to refer to increasing of genome instability of intact progeny of the parents exposed to harmful factor) have received a strong support in literature (15). In mice parental exposure to ionizing radiation increased the frequency of detectable germline mutations, the rate of mutation in somatic cells and confer a predisposition to cancer in offspring (16). It is possible that effect on the stability of the progeny genome was transmitted through the germ line of the irradiated parents.

Another possible mechanism, that may be supposed, is mediated by cytoplasm of germinative cells - most likely by changing of chemical composition and, thus, disturbances of metabolic processes. This point of view may be supported by recent results of studies of alpha particles irradiated cells that testified the mutagenity of cytoplasm of irradiated cells (17, 18). In this case one should expect that transmission would be effective only if females were exposed to the harmful factor. However, in the very early study of this problem it was demonstrated increasing of genomic instability of progeny of males of mice, injected with ^{239}Pu (19). It may suggest the existence of several alternative mechanisms of transmissible genomic instability.

Conclusions

Environment of maternal plant vegetation has significant impact on chromosome instability of seeds, collected from these plants. The environmental impact on genome stability is manifested in age-related dynamics of chromosome instability: the worth environmental conditions, the more rapid increase of indicators of genome instability we have observed during three years of seeds' storing. The possible mechanism may be mediated by different chemicals, accumulated by seeds during vegetation of maternal plants.

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