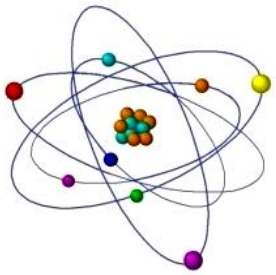
RADIOBIOLOGICAL ASPECTS OF PLANT EPIGENETIC POLYMORPHISM



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ABSTRACT: *The relationship of epigenetic variability with different individual radiosensitivity and adaptive capabilities was studied. Using a simple and convenient experimental model — maize seedlings with different germination terms and epigenetic patterns — the hypothesis was tested that genetically homogeneous but epigenetically different organisms have different radiosensitivity and radio adaptive capacity. Differences in the DNA methylation profiles of individual subpopulations of seedlings were used as a marker of epigenetic differences, and the yield of chromosomal aberration was used as an indicator of DNA vulnerability and its changes under different UV-C irradiation modes. In two series of experiments involving a UV-C acute single and exposure according to the scheme «adaptive - challenging», the investigation of the possible biological importance of epigenetic polymorphism has been performed. The study used a cytogenetic analysis of the yield of chromosomal aberrations and restriction analysis followed by ITS-ISSR- PCR. Significant differences have been established in chromosome aberration yield and DNA methylation profile in control and under UV-C exposure for seedlings of subpopulations differing in time of germination. The differences in the DNA methylation profiles and the yield of chromosomal aberrations in the control subpopulations of seedlings of different germination terms indicate the influence of the DNA methylation profile on DNA damage by regular metabolic factors, such as thermal vibrations or reactive oxygen species (ROS). This phenomenon can be explained by different chromatin conformation determining structural or "passive" resistance, which provides different DNA availability to damage. Methylation switching into de novo under different modes of radiation exposure could become a marker of gene expression changes due to induced repair and protection. The obtained data indicate the importance of epigenetic factors in determining the radioresistance and adaptive capacity of organisms. It points out that the epigenetic mechanisms that determine the choice of the metabolic pattern also contribute to the individual radiosensitivity and adaptive capacity of the organisms. This contribution is determined in two ways. First, the DNA methylation profile affects the initial damage processes, and second, the type of methylation switching into de novo is associated with the further development of protection and repair processes.*

Key words: radiation exposure, radiosensitivity, epigenetic polymorphism, DNA methylation profiles

INTRODUCTION

The issue of variability in species and population radiosensitivity and the identification of factors determining individual radiosensitivity is one of the key problems in radiobiology. Historically, the activity of metabolism, especially proliferative processes activity is the first of indicated and currently well-studied factors determining animal and plant radiosensitivity. The phenomenon is stated in the classic law of Bergonié J and Tribondeau L (1906). The connection between different radiation exposures with various DNA damage is intensively studied since the middle of the last century. Based on the study such representative markers of individual radiosensitivity as micronucleus test and chromosome aberration yield were suggested [1-3]. These markers are widely used from occupation medicine to investigation of plant radiation effects [1, 2, 4]. It is known that chromosome aberration yield is an integral index reflecting not

only DNA primary damage but also the effectiveness of protective and repair mechanisms associated with the functioning of the enzyme complexes. Individual radiosensitivity is now indicated to be connected with the polymorphism of more than 40 genes. They protect cells from mutagens and participate in their homeostasis. There are genes of the xenobiotic detoxification system, antioxidant protection, and DNA repair which polymorphism indicates different efficiency of the systems [5- 7].

Thus, genetic polymorphism is one of the reliable factors of population variability and individual radiosensitivity. The realization of genetic information (in other words gene expression) is related to a complex system of epigenetic regulation. Its mechanisms have been studied in recent decades. DNA methylation, covalent protein modification, and RNA interferences play reliable roles in epigenetic pattern choosing. Currently, the study of the mechanisms of gene expression is developed in different directions: from studying the organization of chromatin to identifying reactions to the effects of climatic factors and various stresses. DNA methylation is the most studied chromatin modification [8-12] and gene expression control factor [13-18].

This process is an integral component of a complex system of epigenetic regulation. Changes in DNA profiles associated with the switching of DNA methylation from maintenance to *de novo* mode are used as a marker of changes in gene expression upon environmental exposure [14-18]. In up-to-date biology along with the concept of genetic polymorphism, there is the concept of epigenetic polymorphism, which implies the existence of a variety of phenotypes while maintaining the unity of the genotype. The existence of epigenetic polymorphism in various biological communities from normal and cancerous human tissues to plant populations was shown in studies [15,17-21] However the biological role of this phenomenon, the connection between phenotypic and epigenetic heterogeneity with variability of sensitivity to environmental exposure and adaptation are still not studied.

A random sample of seeds of the same species, variety, and harvest is a simple and convenient experimental model to investigate the issue. It has some advantages. Firstly, there is data about germination time dependence on ecological factor effects [22]. This points to "gene – environmental" cooperation, i.e., the process is controlled not only genetically, but also epigenetically. Secondly, such a sample germinates asynchronously, individual seeds differ in germination term; this allows us to isolate different subpopulations of seeds and to study the relationship between the variability of germination time and epigenetic differences.

Our verification of the assumption showed that seedling subpopulations from seeds with various germination terms have different methylation patterns. In other words, they are epigenetically different. The following studies showed a change in germination time and methylation pattern in subpopulations with different germination times under chronic gamma-radiation exposure [23]. Different germination terms could be connected with both various metabolic pathways of seed before germination or different ripening degree. According to Woddingtone' conception (1944) germination time difference could be connected with different "epigenetic trajectories" or various positions on the same "epigenetic trajectory". The great majority of researchers explain the phenomenon with different ripening degrees i.e., differences in "physiological age" In other words, these are different positions on the same "epigenetic trajectory" [24-26]. Testing the hypothesis showed that differences in DNA methylation patterns are observed both within the subpopulation of seeds with the same ripening and germination terms and between subpopulations with different germination terms. Thus, it was shown that different seed germination times reflect both different "epigenetic trajectories" (metabolic pathways) along which maturation occurred and different positions on them [27].

The paper is devoted to the first stage of studying the relationship between epigenetic variability and both different individual radiosensitivity and adaptive capacity. The question of the relationship between DNA methylation polymorphism with different radiosensitivity and the formation of adaptive reactions under radiation exposure will be analyzed. The experimental model described above was used.

The chromosomal aberrations yield was used as a marker of DNA sensitivity to genotoxic factors, radioresistance, and its changes under radiation exposure.

MATERIAL AND METHOD

The subjects of the study were 3 – 7-day-old corn seedlings (the Polesska variety). Seeds were couched on plates, the bottoms of which were covered with filter paper and incubated in a thermostat at +22-+23^{0}C. The experiment was performed 7 times and had two experimental series. The goal of the first series of experiments was to study the relationship between germination term variability, DNA methylation pattern, and seedlings' radiosensitivity by the index of chromosome aberrations yield.

On the 2nd day germinated seeds were separated into 3 groups:

a) «fast-germinated» subpopulation (FG – the prime root length more than >1 cm);

b) «middle-germinated» subpopulation (MG – the prime root length more than > 0,1cm);

c) «slowly-germinated» subpopulation (SG – which didn't germinate on the 2nd day or just have hatched, the prime root length 1 mm).

3-day-old seedlings were exposed to 7,2 kJ/m^{2}UV-C irradiation (λ= 253 nm, the dose rate was 6,2 W/m^2). An OBN-150M bactericidal irradiator (Ukraine) equipped with Philips Special TUV-30W lamps was used.

In the second series of experiments, the goal was to study the adaptive response of seedlings from different epigenetic groups.

Two time intervals between adaptive and challenging doses were selected - 4 and 24 hours. The choice was based on our own results and literature data. First, it was shown that DNA methylation switched to de novo mode with an interval of more than 1 hour between exposures [23]. Second, we tried to take into account the duration of single- and double-stranded break repair. In the first case it takes from several minutes to several tens of minutes; in the second one – taking time is comparable to the duration of the cell cycle (from several hours to a day) [28,29]. The different intensity of the adaptive response under different intervals between adaptive and challenger dose effects indirectly gives information about the role of reparative processes in the formation of the chromosomal aberrations yield.

All repeated experiments indicated significant dependence on the season chromosome aberration yield of seedlings from seeds with moderate germination terms; this fact could be attributed to the heterogeneity index for the group. With these observations, we decided to use only two groups of seedlings from fast- and slowly-germinated seeds to analyze the connection between epigenetic polymorphism and the specifics of adaptive reaction development.

The following variants of exposure were used:

1). Non-UV-C irradiated seedlings;

2). Adaptive exposure (1 kJ/m^2);

3). Adaptive exposure, in 4 hours – challenging one (6,2 kJ/m^2);

4). Whole dose exposure (7,2 kJ/m^2); exposure simultaneously with the challenging irradiation of variant 3;

5). Adaptive exposure, in 1 day – challenging one (6,2 kJ/m^2);

6). Whole dose exposure (7,2 kJ/m^2); exposure simultaneously with the challenging irradiation of variant 5.

These groups showed stable results in both cytogenetic and molecular parameters.

Apical root meristem was used for the cytogenetic assay. Samples for cytogenetic assay were collected on the 4th day after irradiation. After separation from the roots, the apexes were placed in a Brodsky fixation solution (0,3 acetic acid: 1 ethanol: 3 formaldehyde mixture) for 2 hours and then washed with ethanol 3 – 4 times. Maceration was performed by alkaline hydrolysis with 20% NaOH for 2 hours. After that, the preparations were washed with distilled water for 15 minutes. Staining was performed with a mixture of acetoorcein and hydrochloric acid (1 acetoorcein: acetoorcein: 1 1MHCl) for 16 – 18 hours. The stained material was washed with 45% CH_3COOH, and squash preparations were prepared. To perform an analysis, ten parallel samples were prepared and 5000 – 10000 cells were analyzed. An analysis of chromosome aberrations was performed by the anaphase–telophase method, taking into account the specificity of plant tissues. The anaphase cell samples were at least 300 – 500 cells per preparation.

Isolation of DNA was performed from the 6-day-old corn seedlings with the set of reagents Diatom™DNAPrep100 based on NucleoS-sorbent. The standard protocol for DNA extraction provided by the manufacturer was used [30,31].

The concentration of DNA in the obtained solution was measured spectrophotometrically by a standard methodology described in the publications [30,31] using a Bio Photometer Plus Eppendorf v.1.35 spectrophotometer.

PCR analysis was performed in a four-channel Tertsik DNA amplifier (DNA-technology, Russia) with primers designed to minisatellite sequences ISSR (15-soro, 5'-AC-AC-AC-AC-AC-AC-AC-AC<C>-3'), transcribed sequences ITS1 (5'-TCC-GTA-GGT-GAA-CCT-GCG-G-3') and ITS4 (5'-TCC-TCC-GCT-TAT-TGA-TAT-GC-3'). Both types of primers were synthesized by “Metabion” (Germany). The set of reagents GenPak® PCR Core – the lyophilized dry mixes prepared for DNA amplification was used. The reaction mixture for ISSR-PCR (the total volume 20 µl) contained 1 unit of *Taq* polymerase inhibited for «quick start», 10 µl of PCR-diluent, 2,5 mM $MgCl_2$, 200 µM each dNTP, 0,1 µM primer (1,6 µl), 200 ng total genomic DNA (2 µl), 6,4 µl deionized water. The reaction mixture was covered with 20 µl of liquid petrolatum. The protocol for carrying out the reaction was provided by the manufacturer.

Amplification with ISSR primers included the following steps: 5 min initial denaturation at 94°C, 40 cycles; 45 s denaturation at 94°C, 45 s primer annealing at 52°C, 90 s elongation at 72°C; and 7 min final elongation at 72°C(Bartlett, 2003; Hernández, et.al.2013).

The reaction mixture for ITS-PCR (the total volume 20 µl) contained 1 unit of *Taq* polymerase inhibited for «quick start», 10 µl of PCR-diluent, 2,5 mM $MgCl_2$, 200 µM each dNTP, 0,1 µM each primer (per 0,8 µl), 200 ng total genomic DNA (2 µl), 6,4 µl deionized water. The reaction mixture was covered with 20 µl of liquid petrolatum. The protocol for carrying out the reaction was provided by the manufacturer.

Amplification with ITS primers included the following steps: 1,5 min initial denaturation at 94°C, 5 cycles; additional 40 cycles of denaturation at 94°C, 15 s; primer annealing at 55°C, 15 s and elongation at 72°C, 15 s; fixing, consisted of denaturation at 94°C for 10 s; primer annealing at 55°C for 10 s, and final elongation at 72°C for 5 min [32].

Experiments were performed in accordance with standard protocols for restriction analysis provided by the manufacturer.

Restriction analysis as well as amplification reactions were performed in a four-channel Tertsik

DNA amplifier (DNA-Technology, Russia). Three types of restrictases were used: MspI (C…C*GG; C…CGG), HpaII (C…CGG), and MboI (…GATC*) (Fermentas, Germany). The restriction endonucleases HpaII and MspI both cleave the nucleotide sequence CCGG, but the action of HpaII is inhibited if the internal cytosine is methylated. MspI is an isoschizomer of HpaII that cleaves both unmethylated and methylated HpaII sites.

The reaction mixture for restriction analysis (total volume 25 µl) contained 2 µl of 10xBuffer Tango, 500 ng of total genome DNA (5 µl), 17,1 µl (for reaction with MspI), or 17,7 µl (for reaction with MboI and HpaII) of deionized water, 0,6 units of the MspI enzyme (0,9 µl) or 0,2 units (0,3 µl) of MboI or HpaII. The mixture was covered with 20 µl of liquid petrolatum.

The conditions of the restriction reaction were as follows: incubation at 37°C for 16 hours, and reaction termination by incubation at 65°C (for HpaII and MboI) and 80°C (for MspI) for 20 min. The obtained PCR and restriction products were analyzed by electrophoresis in the 1,0% agarose gel supplemented with ethidium bromide in TBE buffer. The obtained gels were visualized by UV-transilluminator. To perform electrophoresis, the wells were filled with equal volumes of PCR and restriction products per 5 µl. A GeneRuler 50 bp DNA Ladder (Fermentas) containing fragments of 1000, 750, 500, 250 i 50 bp was used as molecular weight standards. Statistical analysis of experimental findings – the mean value and variance value were calculated by traditional methods.

RESULTS

The connection between germination terms variability, DNA methylation pattern, and radiosensitivity of seedlings. There are significant differences in chromosome aberration yield (Fig. 1) and DNA methylation pattern (Fig. 2) for control variants of FG- MG- and SG-subpopulations. The lowest chromosome aberration yield is indicated for FG-seedlings (1,6%) whilst MG- and SG 2,8%.

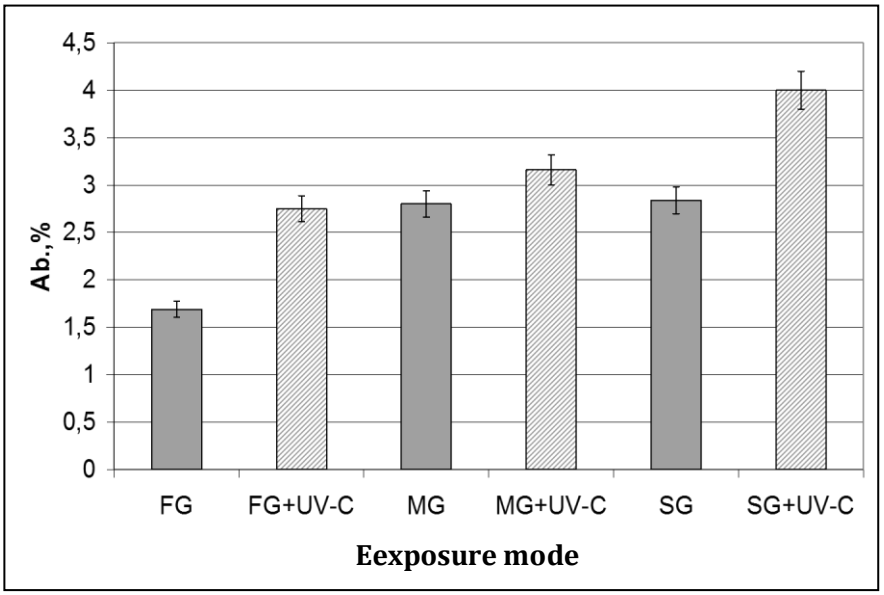

Fig. 1 Chromosome aberration yield (%) for FG – fast-germinated, MG – middle-germinated; SG – slow-germinated seedlings. Confidence interval, P = 0, 95.

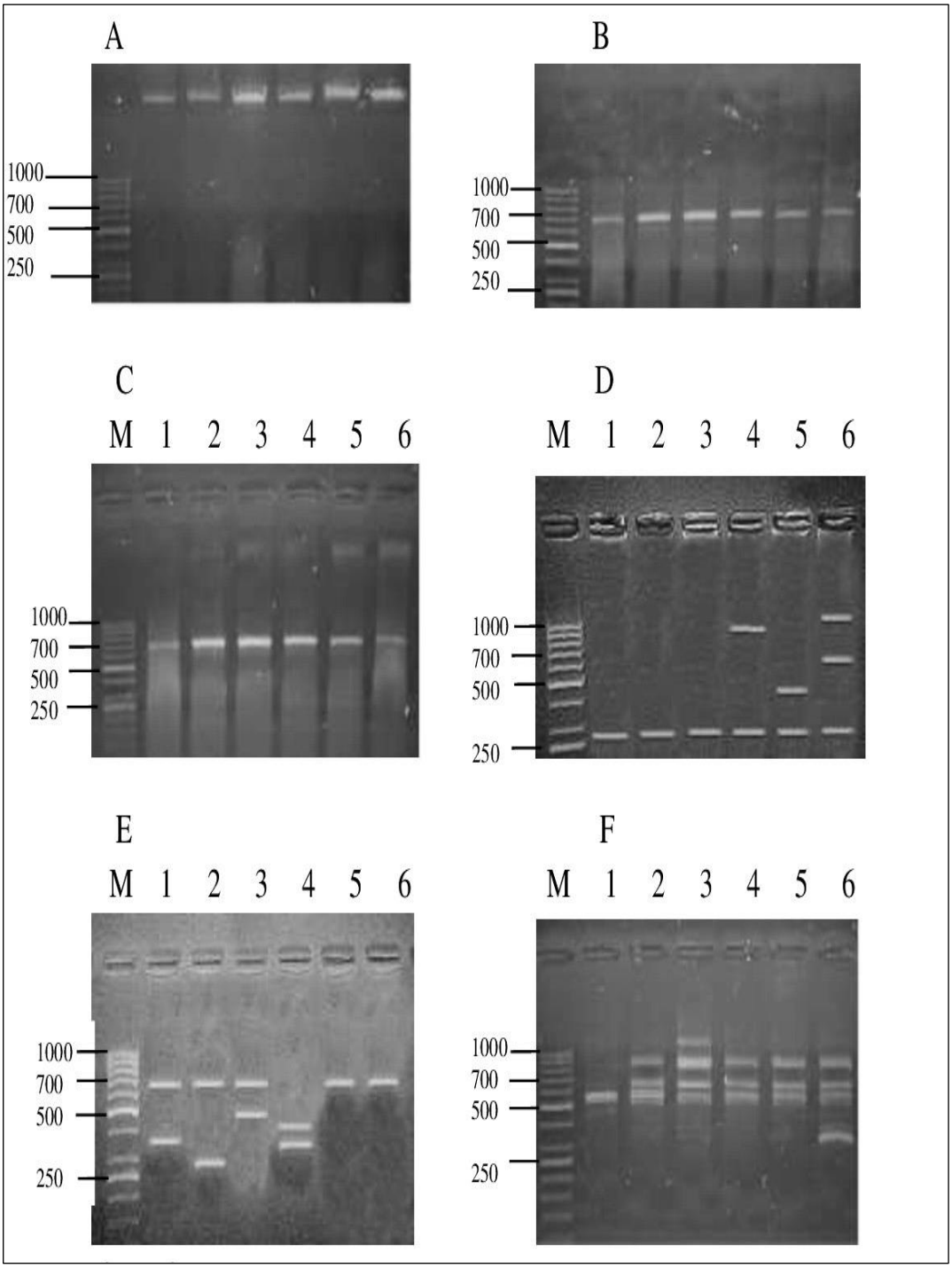

Fig.2. Connection between germination terms variability, DNA methylation pattern, and radiosensitivity of seedlings. DNA methylation data

A. The electrophoregram of isolated DNA quality control. **B**. The electrophoregram of native DNA ISSR- and. **C**. ITS – amplification; **D**. The electrophoregram of the MspI-restrict ITS-amplification. **E**. The electrophoregram of the MspI restricts ISSR –amplification; **F**. The electrophoregram of the MboI -restricts ISSR- amplification. **M** – high-molecular-weight marker; **1** – «FG» sample; **2** – «FG+UV-C» sample; **3** –«MG» sample; **4** – «MG+UV-C» sample; **5** – «SG» sample; **6** – «SG+UV-C» sample.

Differences in chromosome aberration yield are observed under radiation exposure to different subpopulations of seedlings. The lowest yield is up to 2,7% for FG- subpopulation, and the highest is for SG- ones (up to 4%).

For ITS-amplification of MspI-restricts within control variants from all subpopulations, there is one common amplicon 300 bp. It is the only band for FG- and MG- seedlings; for SG- there is another one 500 bp (Fig. 2D, positions 1, 3, 5). Radiation exposure causes the rearrangements of amplicon set for FG- and SG- seedlings by appearing longer amplicons 700 and 1000 bp.

ForISSR-amplification of MspI-restricts within control variants from all subpopulations there is only one common amplicon 700 bp. It is the only band for SG-seedlings; for FG- and MG- there are other bands 600 and 500 bp (Fig. 2E, positions 1, 3, 5). Changes for the SG- variant of amplicons are due to the appearance of a shorter amplicon of 300 bp; for MG- the disappearance of the 700 bp amplicon and appearing shorter bands of 600 and 450 bp.

The change in the set of amplicons for the variant FG - seedlings after radiation exposure is associated with the appearance of a "short" amplicon of 300 bp; for SG- seedlings, the amplicon 700 bp disappears, and shorter amplicons 600 and 450 bp. appear (Fig. 2E, positions2, 4, 6).

For all variants of the experiment, the amplicon 500 bp is common for ISSR-amplification of MboI - restricts. For control variants of SG - seedlings, amplicons 550, 600, and 1000 bp are still observed; for MG- seedlings -550 and 600 bp (Fig. 2 F, positions 1, 3, 5). The change in the set of amplicons for the variant of FG- seedlings after radiation exposure is associated with the appearance of longer amplicons of 550 and 750 bp; for SG seedlings the amplicon 1000 bp disappears; for the SG seedlings, a shorter amplicon 450 bp appears (Fig. 2 F, positions 2, 4, 6).

Thus, differences within chromosome aberration yield, in other words, the radiosensitivity of seedlings from the different subpopulations are related to various DNA methylation patterns through control and variety of their rearrangements under radiation exposure. Adaptive response of seedlings from different epigenetic groups. This experimental series also indicates differences in both chromosome aberration yield and DNA methylation pattern for control variants of seedlings from FG- and SG- subpopulations (Fig.3, 4)

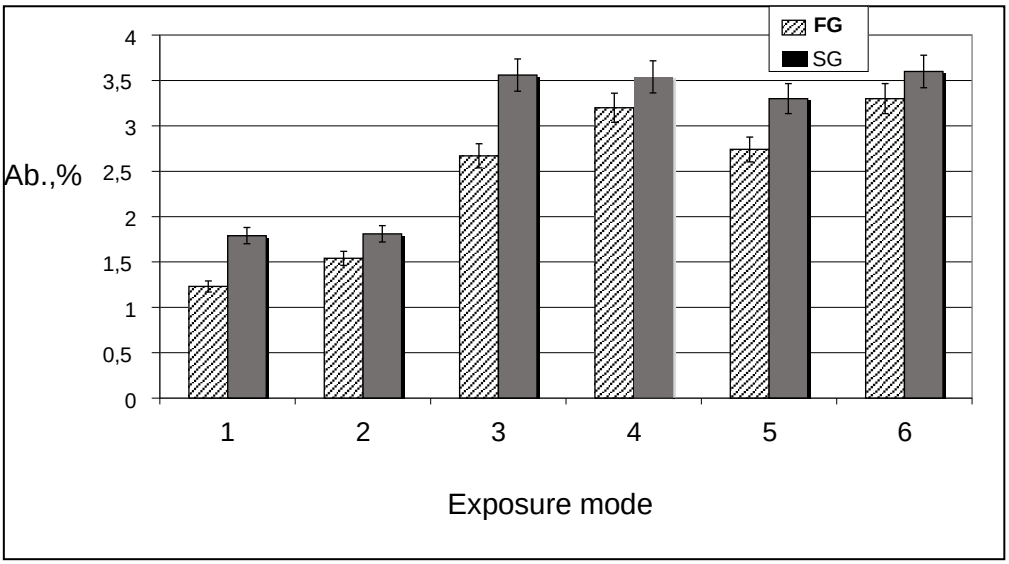

Fig.3. Chromosome aberration yield (Ab, %) for FG- and SG- seedlings.Confidence interval, P = 0,95.

1). Non UV-C irradiated seedlings;2). Adaptive exposure (1 kJ/m²);3). Adaptive exposure, in 4 hours – challenging one (6,2 kJ/m²);4). Whole dose exposure (7,2 kJ/m²); exposure simultaneously with the challenging irradiation of variant 3;5). Adaptive exposure, in 1 day – challenging one (6,2 kJ/m²);6). Whole dose exposure (7,2 kJ/m²); exposure simultaneously with the challenging irradiation of variant 5.

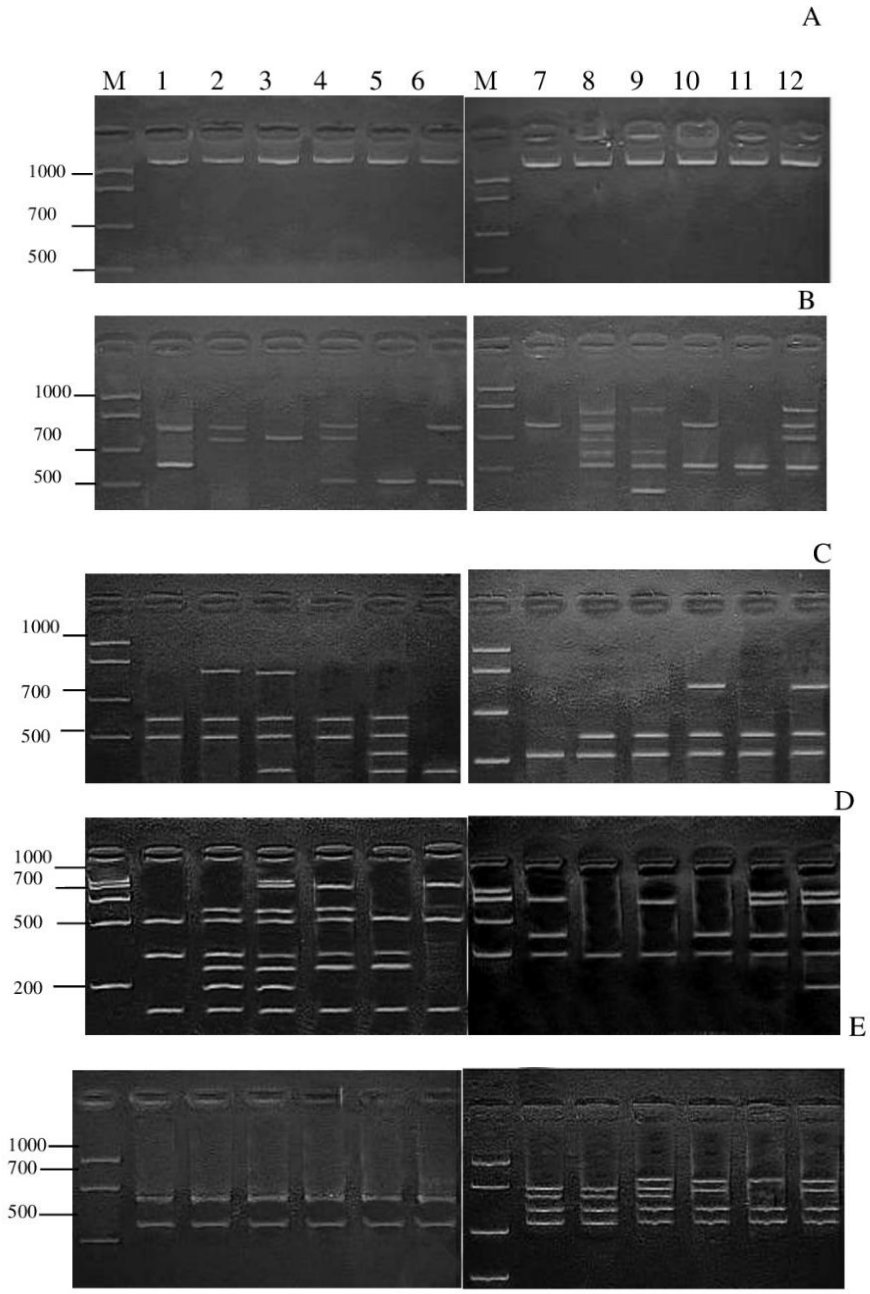

Fig. 4. Adaptive response of seedlings from different epigenetic groups.DNA methylation data

A) The electrophoregram of isolated DNA quality. B) The electrophoregrams of HpaII-restrict' ITS -amplification. C) The electrophoregrams of MspI- restricts' ITS -amplification. D) The electrophoregrams of MboI restrict ITS –amplification; E) The electrophoregrams of HpaII restrict ISSR-amplification.

For Figure 4 (A-E): M – high-molecular-weight marker containing fragments of 1000, 750, 500, 250 i 50 bp; 1. FG + non UV-C irradiation; 2. FG + adaptive exposure; 3. FG + adaptive exposure, in 4 hours - challenging one; 4. FG + whole dose exposure (7.2 kJ/m2); exposure simultaneously with the challenging irradiation of variant 3); 5. FG + adaptive exposure, in 1 day - challenging one; 6. FG + whole dose exposure; irradiation simultaneously with the challenging irradiation of variant 5; 7. SG + non UV-C irradiation; 8. SG + adaptive exposure; 9. SG + adaptive exposure, in 4 hours – challenging one; 10. SG + whole dose exposure; exposure simultaneously with the challenging irradiation of variants 9); 11. SG + adaptive exposure, in 1 day - challenging one; 12. SG + whole dose exposure; irradiation simultaneously with the challenging irradiation of variants 5 and 11.

Adaptive effect identification is by comparing results of exposure under "adaptive - challenging" and whole-dose mode. Comparison of variants 3 (adaptive dose 1 kJ/m2, challenging one 6,2 kJ/m2 after the 4-hour interval) and 4 (whole dose simultaneously with dose 6,2 kJ/m2 of the 3rd variant) shows different reactions of FG- and SG- seedlings. There is a lower yield of chromosomal aberrations for FG seedlings when exposed to an adaptive dose + stimulating dose (2.6%) compared with the full dose (3.2%), i.e., a clear adaptive response is observed. An adaptive response was not observed in SG seedlings. A comparison of variant 5 (adaptive dose of 1 kJ / m2, challenging exposure of 6.2 kJ / m2 after a 24-hour interval) and 6 (full dose at the same time as a dose of 6.2 kJ / m2 of variant 5) shows a significant adaptive response for FG- and negligible for SG seedlings.

For ITS amplification of HpaII-restricts, there is one common amplicon 800 bp within control variants of all subpopulations (Fig. 4B, positions 1,7). Adaptive radiation exposure causes rearrangements of DNA methylation of seedlings from different subpopulations: the disappearance of 600 bp band and appearance of 750 bp for FG-seedlings; appear 850, 775, 7,00, 600 and 500 bp for SG-group (Fig 4B, positions 2, 8).

Exposure under adaptive-challenging mode with a 4-hour interval also leads to rearrangements of methylation for FG- and SG-seedlings. 800 bp amplicon remains and appears new 500 bp band for FG-group; for SG-seedlings disappears 800 bp and in appears new 85,0, 600, 500 and 250 bp amplicons (Fig 4B, positions 3, 9). Exposure under adaptive-challenging mode with 24-hour intervals also leads to the same methylation changes for FG- and SG-groups. Common amplicon 500 bp appears there (Fig.4 B, positions 4, 10).

Two variants of the whole-dose exposure cause rearrangements of DNA methylation patterns of FG- and SG-seedlings. The 800 bp- amplicon maintains and 500 bp bands for both FG- and SG-groups (7,2 kJ/m2 simultaneously with challenging dose 4 hour-interval) appear (Fig, 4). Under whole-dose exposure (7,2 kJ/m2 simultaneously with challenging dose, 24-hour-interval) amplicons 800 and 500 bp were indicated for FG-group. These bands are common with the amplicons of SG-seedlings, where additional bands of 800 and 700 bp are observed (Fig 4B, positions 5 and 11, 6 and 12). For the ITS amplification of MspI - restricts there is one common amplicon 500 bp within controls of the subpopulations (Fig 4C, positions 1, 7). Under adaptive exposure the number of amplicons is different, only the amplicons 600 and 500 bp remain common (Fig 4C, position 2, 8). Underexposure mode 1+6,2 kJ/m2 with 4-hour intervals the same bands remain for FG- and SG-groups. Additional amplicons 850 and 250 bp appear for FG-seedlings. Underexposure mode 1+6,2 kJ/m2 with 24-hour intervals of 500 and 600 bp bands also remain for FG- and SG-groups. There are additional short amplicons of 350 and 250 bp for FG-seedlings (Fig 4C, positions 5).

Various rearrangements of DNA methylation patterns are observed for both whole-dose exposure modes. Methylation patterns of the FG-group have the same amplicons 500 and 600 bp with the control variant and a new one – 250 bp (Fig. 4C, positions 4, 6). Variant SG-seedlings have 500, 600 and 850 bp bands (Fig 4, positions 11 and 12).

For the ITS amplification of MboI - restricts there are no common amplicons within controls of the subpopulations (Fig 4D, positions 1, 7). FG-seedlings contain 500, 350 and 100 bp bands; SG-group - 700, 300 and 200 bp. Different rearrangements of DNA methylation patterns are observed under adaptive exposure: FG-seedlings have amplicons 600, 500, 350, 300, 200 and 100 bp, SG-group - 500, 300, 200 bp (Fig 4D, positions 2, 8). Thus, the common band is 300 bp only. Underexposure mode 1+6,2 kJ/m2 with 4-hour interval FG-seedlings have 8 amplicons and SG -variant has only 2 bands 700 and 200 bp that are the same for FG-group (Fig. 4D, positions 3, 9).

Underexposure mode 1+6,2 kJ/m2 with 24-hour interval FG- and SG-seedlings have 4 amplicons both, only two of which (300 and 200 bp) are common for these groups (Fig. 4D, positions 4, 10). For both whole-dose exposure modes patterns of ITS amplification of MboI - restricts are different for FG- and SG-seedlings. There are 5 amplicons observed for FG-group under exposure with 7,2 kJ/m2, simultaneously with variant 3; for SG-seedlings, it is only 2 bands 300 and 200 bp, which are common with FG-variant (Fig 4D, positions 5 and 6, 11 and 12).

For ISSR-amplification of HpaII- restricts control variants of FG- and SG-seedlings are different with a number of amplicons (2 and 4 respectively) and have common bands (Fig. 4E, positions 1, 7). Under radiation exposure the difference maintains. Exposure under adaptive-challenging mode with a 4-hour interval indicates an extra amplicon of 600 bp for FG-group and 750 bp for SG one (Fig. 4E, positions 2, 9).

Exposure under adaptive-challenging mode with 24-hour interval shows the same amplicons as in control for FG-seedlings and decreasing number of bands, with only one amplicon of 750 bp for the SG-group. Both variants of the whole-dose exposure led to different changes in methylation for FG- and SG-seedlings. Under whole-dose exposure simultaneously with challenging exposure of variants 3 and 5 there is the same methylation pattern for FG-seedlings as in the control variant (4E, positions 5, 6). Itappearancering of a new band of 700 bp for SG-group under whole-dose exposure simultaneously with challenging exposure variant 3 and 700, 750 bp under whole-dose exposure simultaneously with challenging exposure of variant 5 (4E, positions 11, 12).

Thus, in the second series of experiments, with different intervals between adaptive and challenging exposure FG- and SG- seedlings demonstrate various rearrangements of the methylation profile and the difference in adaptive response by the criterion "yield of chromosomal aberrations". All variants of both series of experiments show a strong relationship between differences in DNA methylation profiles and the yield of chromosome aberrations as a well-established marker of radiosensitivity.

DISCUSSION

The results of the two experimental series indicate a strong connection between chromosome aberration yield and DNA methylation patterns of seedlings from subpopulations with different germination terms.

The seedlings differ not only with methylation patterns of transcribed and satellite DNA in control variants but also with changes under radiation exposure.

The yield of chromosomal aberrations as an indicator of radiosensitivity also significantly differs for seedlings of different subpopulations in the control and various modes of radiation exposure.

In other words, the methylation pattern is associated with epigenetic and phenotypic diversity, which manifests in different germination terms, different radiosensitivity of seedlings, and adaptive capacity.

These results correspond with the other new data [33]. When studying 263 maize genotypes, it was shown that with the same general methylation, greater phenotypic diversity is observed with a greater variety of methylation sites. Considering both groups of facts, we can conclude that the combination of methylated sites is the key factor associated with the pattern of gene expression, which determines the

phenotype, including resistance to environmental factors. However, these results are only a statement of facts and are far from understanding the relationship of biophysical and information processes associated with DNA vulnerability and its conformational transformations. Under the radiation exposure of DNA, it is also the main target and structure, informational supporting the processes of protection and recovery. Thus, obtained data allow different interpretations.

The difference in the DNA methylation profiles and the chromosome aberration yield in the control variants of different subpopulations points to the impact of methylation on DNA damage by regular intracellular factors. It may be, for example, thermal vibrations or reactive oxygen species effects. This may be explained by various chromatin packaging which indicates structural or "passive" stability related to different DNA availability to damaging factors.

The methylation switching into de novo mode under radiation exposure could indicate changes in gene expression related to induction of repair and protective reactions. Different formations of adaptive response under various time intervals between adaptive and challenging exposure of FG- and SG-seedlings may indicate different effects of both single- and double-stranded DNA breaks repair. Comparing the obtained radiobiological data and the known effects of other stress factors, we could expand the interpretation of the results. DNA methylation is sensitive to environmental factors and defines the "epigenetic memory", the inheritance of the epigenetic pattern [34-37]. Methylation profiles might contain information about the conditions in which the organism was developed; it determines the variability of epigenetic programs, individual radiosensitivity; organism protection, and restoration pathway.

Thus, according to the state of the art for regulating gene expression, simultaneous study of chromosome aberration yield and DNA methylation patterns under various radiation exposure modes points to the significant role of epigenetic factors for individual radiosensitivity and adaptive reactions.

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