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Research Article

The Effect of DNA Methylation Modification Polymorphism of Corn Seeds on Their Germination Rate, Seedling Resistance and Adaptive Capacity under UV-C Exposure

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Abstract:

Relations between epigenetic polymorphism of corn seeds, their germination rate, resistance and adaptation under UV-C exposure have been investigated. Association of various seed germination rate with the original differences in chromosome aberration yield and methylation patterns of transcribed and satellite DNA have been shown. In two series of experiments involving a acute single and repetitive UV-C exposure according to the scheme «adaptive - challenging», the investigation of possible biological importance of epigenetic polymorphism has been performed. Significant differences have been established in chromosome aberration yield and methylation pattern changes in satellite and transcribed DNA of seedlings from fast- and slowly-growing seeds under UV-C exposure. This data indicate existence of various resistances to UV-C exposure and adaptive capacity of seedlings from seeds with different epigenomes.

Keywords: epigenetic polymorphism; germination rate; DNA methylation pattern; transcribed DNA; satellite DNA; stress factor response

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Introduction

For any sample of seeds which are not in dormancy and belong to one species, variety and yield has particular germination rate variability after initiation process. Such phenomenon is interesting from both theoretical and practical perspectives, and affects the planting principles and harvest crop practices, complicating the fight against weeds, which results in significant financial costs. The efforts to increase the vigor led to the development of priming technologies [1, 2], which are based on empirically estimated dose and mode of stimulating exposure for each species and variety of cultivated plants. Significant progress of such technologies doesn't eliminate the need for theoretical investigation of the mechanisms that determine the variability of the seed germination rates, which seemingly belong to genetically homogeneous samples.

Nowadays this phenomenon is often explained by considering the causes and factors that determine the endogenous dormancy: differences in development of embryo, water- and air permeability of surrounding tissues, content of hormonal and non-hormonal growth inhibitors [2-6]. Variability of germination rates has important biological significance and may be due to accumulation of minor dose variations of these factors, however, it can also be a programmed effect.

This can be seen in increasing variability of germination rates and the phenomenon of heterokarpy under unfavorable ecological conditions [7-9], as well as in the capabilities of priming technologies [1].

Up-to-date population genetics along with the concept of genetic polymorphism uses the term of epigenetics which is understood as phenotype heterogeneity dependence on its epigenetic program variety maintaining the genotype identity. According to the present state of understanding molecular mechanisms determining expressed part of genome [2,10,11], it is possible to suggest that phenomena of epigenetic polymorphism is defined by a cascade of mechanisms, where the variety of genetically identical organism DNA methylation patterns takes a central role [5].

According to contemporary views about regulation of metabolic processes and their adaptation to various exogenous affects, the system of factors that determine the depth of endogenous dormancy and variety of germination rate might be the reflection of different epigenetic programs, i.e. epigenetic polymorphism of seeds that were formed in the population of homogeneous plants and even in one plant.

This paper is dedicated to investigation of the relations between corn seed epigenetic polymorphism and their germination rate and estimation of possible biological significance of these phenomena, which can be linked to different stability and adaptive capacity of plants with different epigenomes to the stress action. As a stress factor UV-C exposure was utilized. Currently molecular mechanisms of ultraviolet radiation- induced DNA damage and repair are studied comprehensively [12]. Ultraviolet radiation is one of the powerful agents that can alter the normal DNA state by inducing a variety of mutagenic and toxic DNA lesions as well as DNA strand breaks affecting genome integrity. There exist a number of highly conserved lesions and double-strand break repair mechanisms [12].

In our study we focus to estimate the following characteristics:

-relation between various seed germination rates and original differences in chromosome aberration yield and methylation pattern of

transcribed and satellite DNA;

-stress resistance of seeds with various germination rate and their adaptive capacity under repetitive stress exposure.

Material and Method

The subjects of the study were seeds and 3-7-day-old corn seedlings (the Polesska variety). Seeds were couched on plates, the bottoms of which were covered with the filter paper and incubated in a thermostat at +22-+230C. The experiment was performed 7 times.

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

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

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

c) «slowly-germinated» (SG-which didn't germinate on the 2nd day, or just have hatched, the prime root length 1 mm). 3-day-old seedlings were treated with UV-C irradiation (λ= 253 nm, the dose rate was 6, 2 W/m2). An OBN-150M bactericidal irradiator (Ukraine) equipped with Philips Special TUV-30W lamps was used.

The investigation of relations between changes in transcribed and satellite DNA methylation pattern with plant cell resistance to stress exposure was carried out in two series of experiments:

1. Acute single UV-C exposure (7,2 kJ/m2) of epigenetically different corn seedlings (EDS).

2. UV-C exposure of 3-day seedlings using «adaptive-challenging» exposure mode with 4-hour and 1-day intervals to investigate adaptive capacity of seedlings. The following variants of irradiation were used:

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.

Since all repeated experiments indicated significant dependence on season chromosome aberration yield of seedlings from seeds with middle germination rate, 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-growing seeds to analyze the connection between epigenetic polymorphism and specifics of adaptive reaction development. These groups showed stable results in both cytogenetic and molecular parameters.

Apical root meristem was used for 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: 1 1M HCl) for 16-18 hours. The stained material was washed with 45% CH3COOH, 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 was at least 300-500 cells per each preparation.

Isolation of DNA was performed from the 6-day-old corn seedlings with the set of reagents DiatomTM DNA Prep100 based on NucleoS-sorbent. The standard protocol for DNA extraction provided by the manufacturer was used.

The concentration of DNA in the obtained solution was measured spectrophotometrically by a standard methodology described in (Ausubel *et al*, 2004) using a BioPhotometer plus Eppendorf v.1.35 spectrophotometer [13, 14].

A 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') and to the 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 the Metabion Company (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 MgCl2, 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 [15].

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 MgCl2, 200 µM each dNTP, 0,1 µM each primer (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, 15s; primer annealing at 55°C, 15s and elongation at 72°C, 15s; fixing, consisted of denaturation at 94°C for 10s; primer annealing at 55°C for 10 s, and final elongation at 72°C for 5 min [14].

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 (5'C…C*CG, C…5'), HpaII (5'…C*CGG…3'; 3'G…G C*C…5'), and MboI (5'…C*CGC…3') (Fermentas, Germany). Experiments were performed in accordance with standard protocols for restriction analysis provided by the manufacturer.

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 a 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 a molecular weight standards. Statistical analysis of experimental findings – the mean value and variance value were calculated by traditional methods.

Results and Discussion

The first series of experiments

Cytogenetic data

The obtained cytogenetic data of seven experiments showed the dependence of behavior of cytogenetic characteristics on the season during which the experiments were carried out. The most essential differences in mitotic index parameter (MI, %) were observed in groups of fast-(FG) and slow-germinated (SG) seedlings. Major varieties in chromosome aberrations yield (Ab, %) were observed in groups FG and SG. The most unstable was the group of middle-germinated seedlings. The mitotic index parameter (MI, %) in this group was less than in slow-germinated and higher than the MI, % of FG group (Fig 1 a, b). This result indicated possible absence of significant connection between germination rate and cell division with mitotic index. It is known that germination is determined by both the stretching of embryo cells and their division. These two processes almost simultaneously start and run concurrently.

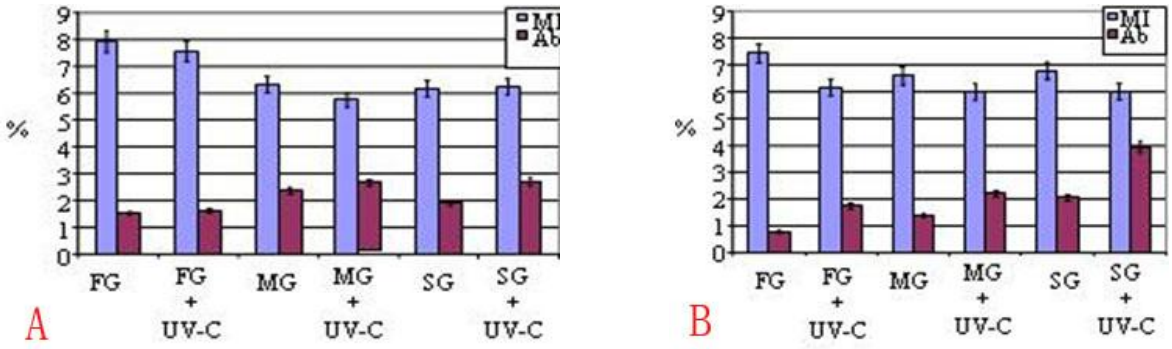

Figure 1 The cytogenetic parameters of meristematic roots under UV-C exposure of corn seedlings with various germination rates; (A-summer; B-autumn). MI-mitotic index; Ab-chromosome aberration yield; SG- slow-germinated; MG- middle-germinated; FG-fast-germinated.

DNA methylation data

The electrophoregram of isolated DNA nativity is shown in Fig 2. These data demonstrate the absence of considerable DNA fragmentation that occurs during apoptosis, which is potentially possible under UV-C

exposure.

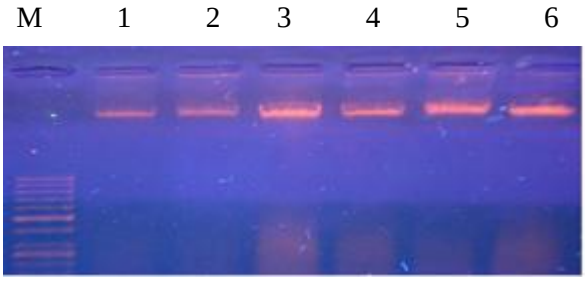

Figure 2 The electrophoregram of isolated DNA quality. (The first series of experiment). M – high-molecular-weight marker; 1-«FG» sample; 2-«FG+UV-C» sample; 3-«MG» sample; 4-«MG+UV-C» sample; 5-«SG» sample; 6-«SG+UV-C» sample.

The electrophoretic analysis of the amplification products of native DNA with both ISSR (a) and ITS (b) – primers showed the absence of differences within all experimental variants (Fig 3 a, b).

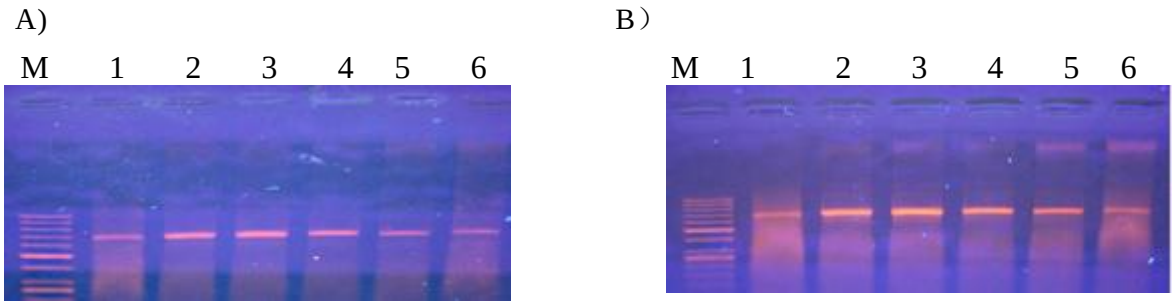

Figure 3 The electrophoregram of native DNA amplification with ISSR (a) and ITS (b) primers. M-high-molecular-weight marker; 1-«FG» sample; 2-«FG+UV-C» sample; 3-«MG» sample; 4-«MG+UV-C» sample; 5-«SG» sample; 6- «SG+UV-C» sample.

Not only this confirms the high level of extracted DNA quality and its primary structure integrity, but also indicates that following participation of molecules in PCR didn't lead to detection of latent injuries i.e. single-strand breaks, the number and position of which may change according to functional statement of seedlings and UV-C exposure.

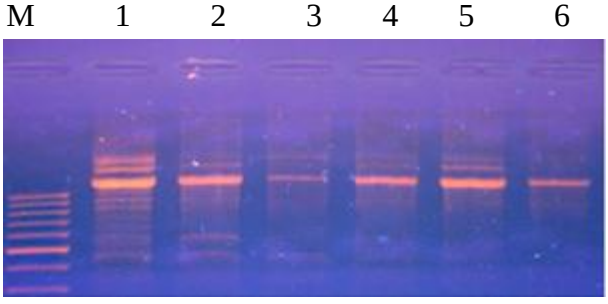

Figure 4 The electrophoregram of the ITS-amplification of the restriction products obtained with MspI endonuclease. M-high-molecular-weight marker; 1-«FG» sample; 2-«FG+UV-C» sample; 3-«MG» sample; 4-«MG+UV-C» sample; 5-«SG» sample; 6-«SG+UV-C» sample.

The electrophoregrams of the ITS-amplification of the restriction products

obtained with MspI endonuclease (Fig 4) demonstrated differences in the DNA methylation pattern among seedlings that had various germination rates (positions 1, 3, 5). The most obvious differences were observed between FG seedlings and other variants. The structure of amplicons in position 1 (FG) was the most complicated and showed various amplification products with both low and high molecular weight as well as substantial structural features of position of MspI restriction sites.

The electrophoregrams of the ITS-amplification of the restriction products obtained with MboI and HpaII endonucleases didn't show considerable differences in methylation patterns of their restriction sites.

A significant change in satellite DNA methylation pattern was showed by ISSR-amplification of MspI- and MboI-restricts.

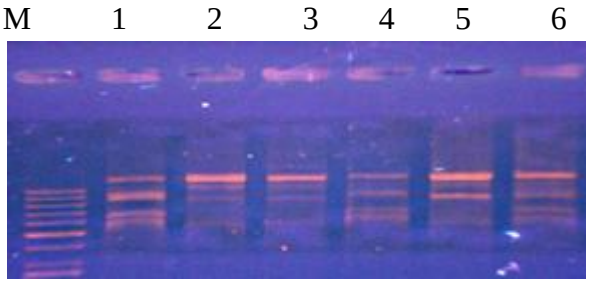

Figure 5 The electrophoregram of the amplification products obtained by ISSR-PCR of the MspI restriction products. M-high-molecular-weight marker; 1-«FG» sample; 2-«FG+UV-C» sample; 3-«MG» sample; 4-«MG+UV-C» sample; 5-«SG» sample; 6 -«SG+UV-C» sample

An electrophoregram of the amplification products obtained by ISSR-PCR of the MspI restriction products (Fig 5) illustrated the differences in DNA methylation pattern among seedlings with various germination rates (positions 1, 3, 5). The electrophoregram of FG seedlings (position 1) had for distinct groups of amplicons with almost the same number of DNA fragments. The groups of amplicons (positions 3 and 5) for variants MG and SG had the same molecular weight, but different number of DNA fragments.

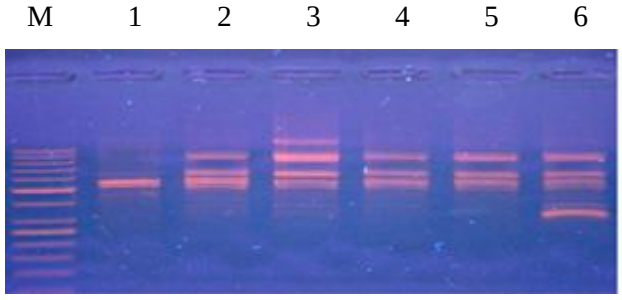

Figure 6 The electrophoregram of the amplification products obtained by ISSR-PCR of the MboI restriction products. M-high-molecular-weight marker; 1-«FG» sample; 2-«FG+UV-C» sample; 3-«MG» sample; 4-«MG+UV-C» sample; 5-«SG» sample; 6-«SG+UV-C» sample.

Also considerable differences between methylation patterns of satellite DNA of seedlings that initially had various germination rates (positions 1, 3, 5) were observed in

separating amplification products of MboI restricts with ISSR primers (Fig 6). There was just one type of amplicons for FG seedlings and great differences between MG and SG variants. Electrophoregram for MG seedlings had four distinct groups of amplicons with comparatively more high-molecular fragments.

Indicated changes in methylation pattern of transcribed DNA confirm the assumption that difference in epigenetic programs which were reflected in transcribed DNA methylation patterns are connected to distinct germination rates.

Furthermore, these data do not contradict the results in [2] showing that exogenous control of germination changes metabolism activity, so the variability of germination rate could be determined by the same cause. It seemed possible that differences in activity of metabolic processes could be determined by both the accumulation of fluctuations in activities of seedlings' metabolism and the epigenetic polymorphism connected to induction of various isoenzyme activities.

More complicated for interpretation were the data showing the changes in satellite DNA methylation patterns. Since functional importance of satellite DNA was explained in part by conceptions, it was assumed that having a structural role in spatial organization of genome, and taking part in homologous chromosome conjugation during meiosis and replication of chromosome telomeric sites [16]. Perhaps, in this case different methylation patterns of satellite DNA implying various chromatin conformations cause different vulnerability, availability of this structure to the damages. Also, these results could have interactive character: specific methylation patterns of transcribed DNA may play role in repair and transcription processes only under definite conformation of all the chromatin.

Comparison of the results of cytogenetic analysis with changes in methylation patterns of transcribed and satellite DNA after irradiation indicated their connection with different resistance to UV-C exposure. As mentioned above the variant FG was characterized by the lowest initial level of chromosomal aberrations relative to other variants. It could result not only from efficient functioning of repair systems of spontaneous DNA injuries, but also from systems responsible for passing cell cycle checkpoints and complete repair of double-stranded DNA breaks. It was known, that effective repair of double-stranded DNA breaks with the mechanism of homologous recombination was possible only under conditions of certain level of chromatin relaxation [17], so it was also associated directly to the conformation of satellite DNA [16]. In the set of experiments it was demonstrated that general strategy of minor absolute parameters increases chromosomal aberration yield in variant FG under UV-C exposure (Fig 1, a, b). As it was noted above, the simplest explanation of these results is possible: different methylation patterns of DNA, which mean various chromatin conformations, cause different vulnerability, availability of this structure to the damages.

The second series of experiments

Consider the results obtained in the second series of experiments under repetitive UV-C irradiation with «adaptive-challenging» exposure mode.

Cytogenetic data

As in the first series of experiments, the significant difference is observed in the original chromosome aberration yield (Ab, %) in groups of seedlings from fast- and slowly-germinated seeds (Fig 7).

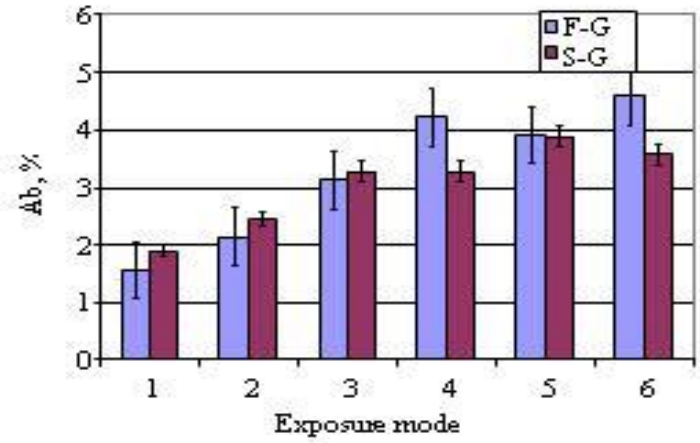

Figure 7 Chromosome aberration yield (%) for FG-and SG-seedlings. Key for position 1-6 as described in "Material and methods".

SG-seedlings show higher chromosome aberration yield both in control and adaptive-exposed variants. Comparison of chromosome aberration yield under «adaptive-challenging» exposure mode with the one under simultaneously whole-dose exposure (7,2 kJ/m2) indicates distinct adaptive response under challenging exposure in both 4-hour and 1-day intervals after adaptive dose in FG seedlings. SG- seedlings do not show adaptive response under all intervals between adaptive and challenging exposure.

DNA methylation data

The electrophoregram of isolated DNA nativity is shown in Fig 8. These data indicate the disappearance of significant DNA fragmentation, which is potentially possible under UV-C exposure.

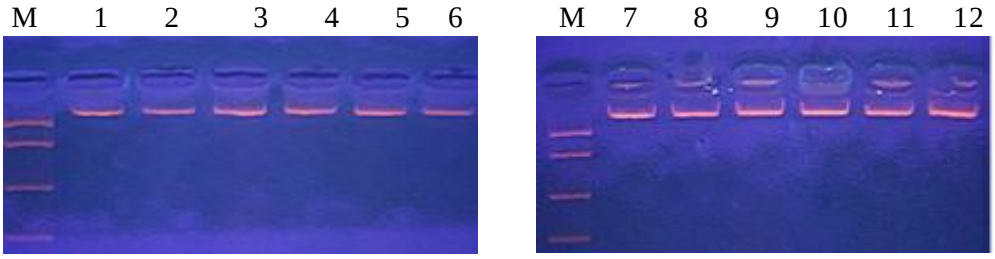

Figure 8 The electrophoregram of isolated DNA quality. (The second series of experiment).

For Figures 8-12: 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 variant 3 and 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.

Electrophoregram in Fig 9 shows great variety of amplicon ranges in both ITS-amplification products of HpaII restricts in control variants of FG and SG (positions 1, 7) and all variants exposed to UV-C.

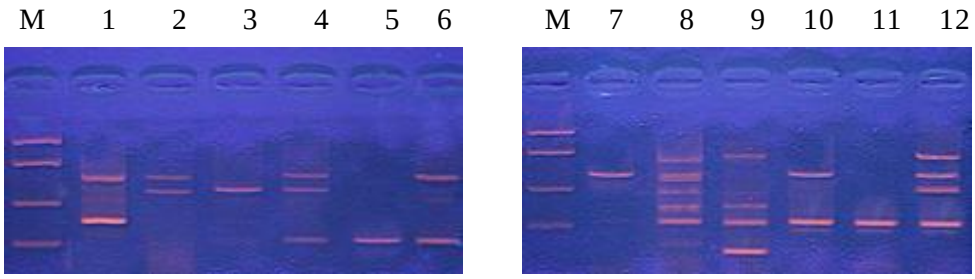

Figure 9 The electrophoregrams of HpaII-restricts' ITS -amplification.

Comparison of cytogenetic reaction to adaptive exposure (Fig 1) with amplicon rate changes for the variants (positions 2, 8) indicates that higher chromosome aberration yield of SG is connected to significant increase in amplicon number of larger and smaller molecular mass compared to the amplicons of control variant. Also major differences are observed in amplicon range at positions 3 and 9 - corresponding to «adaptive - challenging» exposure, positions 4 and 10, 5 and 11, 6 and 12.

Therefore major differences are observed in original methylation patterns of transcribed DNA on HpaII restriction sites and their changes in FG and SG seedlings under distinct UV-C exposure modes.

Electrophoregrams of MspI-restricts' ITS-amplification indicate varieties in amplicon range in both control variants (positions 1 and 7) and under all UV-C exposure modes.

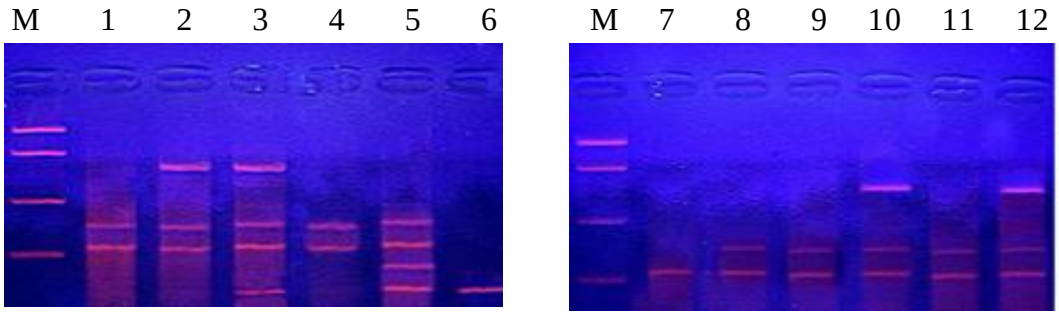

Figure 10 The electrophoregrams of MspI-restricts' ITS-amplification.

These data illustrate the differences in transcribed DNA methylation pattern of all variants. The most significant differences in DNA methylation patterns of FG seedlings are observed in all variants including the control one. Observed high-molecular-mass amplicons (positions 2 and 3) indicate the decrease of MspI-restriction sites, while observed low-molecular-mass amplicons indicate the increase of MspI-restriction sites. Based on changes in amplicon range, we conclude that resulting DNA methylation patterns change

MspI-restriction sites in FG seedlings rather than in SG group under different exposure modes.

Electrophoregrams of MboI-restricts' ITS-amplification show significant differences in methylation patterns of control variants from each group of seeds (positions 1 and 7) as well as changes of methylation patterns under various exposure modes.

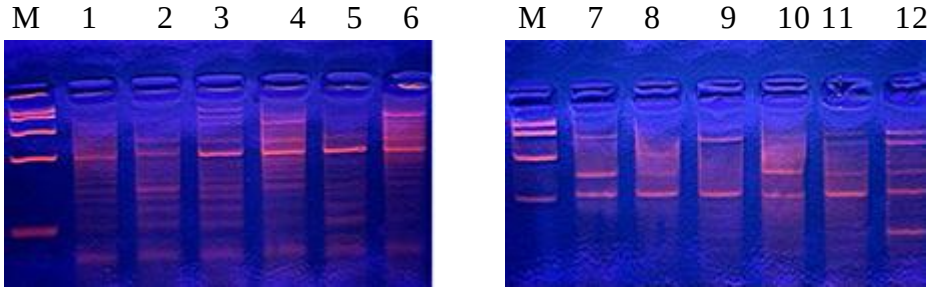

Figure 11 The electrophoregrams of MboI restricts' ITS-amplification.

Comparison of amplicon ranges under different UV-C exposure modes indicates that observed changes caused formation of amplicons with molecular mass in range of control variant. This is even more evident in FG seedlings. Observed amplicons of SG group are not in molecular-mass range of control variant (position 12).

Electrophoregrams of HpaII-restricts' ISSR-amplification are substantially different (Fig 12). Distinct DNA methylation patterns are observed in groups of seedlings with different germination rates (positions 1, 7).

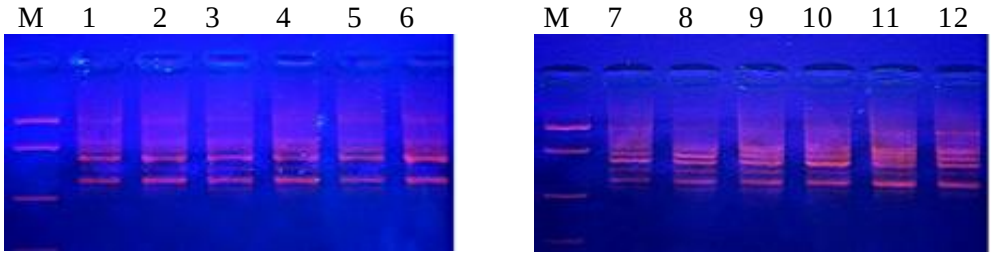

Figure 12 The electrophoregrams of HpaII restricts' ISSR-amplification.

Differences between methylation patterns were not detected under ISSR-amplification using other restrictases.

Results obtained in the second series of the experiments showed distinctive development of reactions to repetitive stress exposure in epigenetically different seedlings. Besides, major rearrangements in transcribed DNA methylation patterns are observed. These changes are different between seedlings from distinct epigenetic groups. More variable methylation patterns of transcribed DNA are detected in FG seedlings under all exposure modes. Comparison of changes in chromosome aberration yield (Fig 7) with transcribed DNA methylation data (Fig 8-11) confirms that changing methylations patterns are observed whether with or without adaptive response formation. One possible adaptation mechanism may be formation of less vulnerable DNA or whole chromatin structure after first, adaptive portion of exposure. Apparently, the original

conformation of transcribed and satellite DNA in FG-seedlings admits such chromatin rearrangement with formation of adaptation while DNA structure in MG-seedlings does not. The second adaptation path could be an improved accessibility of DNA to transcription and repair factors due to conformation changes. Most of research results on the role of DNA methylation in adaptation point out to precisely this mechanism [18].

The data from two experimental series suggest that connection between germination rate and DNA methylation pattern polymorphism is adaptive in nature. The polymorphism leads to decreased competition within one species, more efficient use of resources in one ecological niche and increased adaptive capacity of plants in whole population. The data in [19] illustrating epigenetic polymorphism in differentiated tissues also suggest adaptive nature of this phenomenon which leads to decreased competition for resources between related biological systems.

Conclusion

This study investigates two interrelated issues. The first one refers to the identification of the connection between the well-known biological phenomenon-the variability of germination rate of seeds from any sample that were not in dormancy and diversity of transcribed and satellite DNA methylation patterns.

The second question is dedicated to the study of possible biological role of the phenomenon and could be associated with different adaptive capacity and stress resistance of epigenetically different groups of seedlings.

Obtained results illustrate the existence of connection between germination rate and original differences of satellite and transcribed DNA methylation patterns. Furthermore, interrelation in prior variability of seedling DNA methylation pattern and diversity of their resistance and adaptive capacity was identified.

Contemporary views on the regulation types in living systems suggest several mechanisms that determine the influence of DNA methylation pattern on organism resistance and adaptation. Obtained data indicate the existence of correlation between changes in satellite DNA methylation pattern and chromosome aberration yield and give grounds to conclude that changing methylation patterns affect DNA conformation and determine its vulnerability to radiation exposure. Changes of transcribed DNA methylation pattern may reflect the differences in both DNA vulnerability and its availability to transcription and repair factors. In other words, it determines the epigenetic component of regulation.

A great interest has emerged in exploration of the mechanisms and factors that indicate the importance of different epigenetic programs in seeds formed in homogeneous population of plants, or even in one plant under the same conditions.

Nowadays, there are extensive data on the changes of epigenetic pattern under various biotic and abiotic stress exposures [10, 11, 20, 22, and 23]. The transgenerational pass of new-formed methylation patterns of transcribed DNA was confirmed by numerous research results [22]. It was shown that variety of climatic conditions led to current high level of epigenetic polymorphism, which is inherited and greatly exceeded the level of genetic one [23].

This important information allowed the assessment of mechanisms and factors potentially determining the variance of environmental effects and functional conditions of the plant on forming the epigenetic polymorphism of seeds germinated in one plant.

According to the specifics of fertilization and formation of plant embryo it was possible to identify at least a few factors determining activation of polymorphism in epigenetic programs of embryo, cotyledons, endosperm and their interaction during germination. First, the difference in time of pollination occurred under various climatic condition could be such a factor. The next important factor is ordering of seed in spike, ear or basket that determined the embryo provision with assimilations and its further program of interaction with spare tissues. It is possible that other mechanisms affecting plants were involved, which determined the set of seed epigenomes and their further germination rates and reaction to endogenous and exogenous factors.

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