Global Journal of Science Frontier Research: C
Biological Science
Volume 20 Issue 1 Version 1.0 Year 2020
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Epigenetic Factors of Biological Variability and Individual Sensitivity to Biotic Stresses

By Kravets A. P. & Sokolova D. A.

Institute of Cell Biology and Genetic Engineering NAS of Ukraine

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GJSFR-C Classification: *FOR Code: 069999*

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Epigenetic Factors of Biological Variability and Individual Sensitivity to Biotic Stresses

Kravets A. P. [α] & Sokolova D. A. [σ]

Abstract- The variability of a wide variety of characteristics, including sensitivity to biotic and abiotic environmental factors, is one of the fundamental properties of living things. This study is a continuation of the investigation of the effect of epigenetic differences on the individual resistance of plants to abiotic and biotic stresses. The aim was to investigate the connection between epigenetic polymorphism of two wheat varieties and different sensitivity to fungal infection. Diverse DNA methylation patterns of seed subpopulations with various germination times used as a marker of epigenetic polymorphism. Showed that fast-grown seed subpopulation (Podolyanka variety) which characterized with a higher level of intravariety epigenetic polymorphism, had slow development and lowered final level of fungal infection compared to slow-grown seed subpopulation. The effect observed in plants of this variety for crops of different years. Favoritka variety was characterized with a lower level of intravariety epigenetic polymorphism and shown unstable results in fungal infection of both fast- and slow-grown seed subpopulations. The data obtained indicate the importance of epigenetic mechanisms in determining the individual sensitivity of plants to phytopatogens and the role of epigenetic polymorphism as a factor contributing to the biological diversity of crops. The revealed differences in resistance to pathogens confirm the previously obtained data on different radioresistance and adaptive capacity of plants with different epigenomes. Thus epigenetic polymorphism considered as an evolutionarily fixed phenomenon that increases biological variability and the nonspecific resistance of populations, varieties, and species.
The issue about connection intravariety differences in epigenomes with plant individual nonspecific resistance and the role of intravariety epigenetic polymorphism in the biodiversity of monocultures is discussed.

Keywords: DNA methylation, epigenetic polymorphism, phytopatogens, plant immunity, biodiversity, crop, mitigation competition factors in mono-crops.

I. Introduction

Variability of most diverse characteristics – time and dimensional parameters, sensitivity to both biotic and abiotic habitat factors is one of the fundamental properties of living things. A key focus of modern plant breeding is the study of the genetic nature of traits that are important for productivity, their polymorphism, and the use of these data in genetic engineering [2]. A significant limitation of this approach is due to the final result's unpredictability because of environmental factors influence gene expression realized with various epigenetic mechanisms.

Author α σ: Institute of cell biology and genetic engineering NAS of Ukraine, 32/17 Vasilkovska st, Kyiv 03022, Ukraine.
e-mail: kaplibra@gmail.com

Up-to-date biology, along with the concept of genetic polymorphism, has a concept of epigenetic polymorphism, which implies the existence of a variety of phenotypes while maintaining the unity of the genotype. Epigenetic diversity, like different genetic ensembles expression could be one of the scantily explored biological variability factors. One of the striking phenomena is a phenomenon of asynchronous germination of any seeds sample of the same species, variety, and harvest. The mechanisms defining this phenomenon are still not completely clear. It is significant from a practical point of view since affecting the seeding time and yield of cultivated plants and also complicating weed control, causing significant material costs. Noted for both wild and cultivated plants the level of asynchronous germination is dependent on climatic and environmental conditions. Thus it is controlled not only by genes (genetic factors) but also by environmental factors and points to epigenetic mechanisms participate in the determination of germination time.

A random sample of seeds of the same species, variety and harvest, is a simple and convenient experimental model to investigate the connection of both variabilities of phenotypic characteristic (germination time) and epigenetic polymorphism with sensitiveness to various environmental factors.

The vast majority of researchers explain asynchronous germination via various seeds ripening – differences in the "physiological age" from the same plant up to the time of harvesting due to different pollination terms.

Previously, using DNA methylation patterns as a marker of epigenetic varieties it was shown that seedlings from seeds with other germination time had different DNA methylation pattern. Then was shown that variability of epigenetic patterns is associated not only with maturation and position on some "epigenetic trajectory" but also with diverse "epigenetic trajectories" – in other words, metabolic pathways along which this maturation has taken place [16]. Study of response to UV-C exposure shown that seedlings with different epigenomes and germination time had various sensitivity and formation of adaptive reaction [21].

The work is a continuation of studying plant epigenetic status effect on sensitivity to environmental factors and the formation of protective reactions with sensitivity to phytopatogens.

Crop contamination with phytopatogens is one of key problems both agriculture and food security [20]. Food contamination with spores of pathogenic and conditionally pathogenic fungi is a significant threat as a source of substances with genotoxic effects [8, 20,22,24]. Today grain contamination is studied from different sides because of a lot of various factors while the harvest is formed and stored lead to formation of both qualitative and quantitative characteristics of grain [8, 25]. Up to date, research are focused on several streams. It includes both assessment environmental factors effecting on plant immunity [4,8-11,19,22,25] and mechanisms determining constitutive or inducible way [12,13,17,18,22,] to protect and recognise plant pathogen [12,17,18]. Assessment of epigenetic factors effecting on contamination in one or another degree is a component of listed research areas.

II. Material and Method

The paper provides data obtained on two winter wheat varieties Podolyanka and Favoritka (the originators of both are Institute of plant genetics and physiology NAS of Ukraine and Myronivsky). According to our previous research, the wheat has different ecological plasticity – requirements for sowing, fertilizer application terms, and predecessor [15]. Analyzing different DNA methylation patterns of wheat seedlings with extreme - minimum and maximum germination using the parameter of "epigenetic distance" (D) by Nei. Indicated that plant varieties with increased ecological plasticity characterized with longer epigenetic distance thus wider epigenetic sifference. For example, Podolyanka variety in various experiments shown the parameter 0,1-0,3. The same index for Favoritka one is 0,01-0,056.

DNA methylation patterns from "fast-grown" sample compared with "slow-grown" one. DNA methylation polymorphism of two groups of seedlings was quantified with parameter D – "epigenetic distance" calculated by Nei [15,16].

Experiment repetition is four-time. There were used seeds harvested in 2013-2017 and stored in the refrigerator under +4 -5° C. Experiments were carried out during June-August. Seeds germinated on wet filter paper, ten seeds per Petri dish (to avoid their contact and cross contamination) in thermostat under +23 - +24 °C. As previously studying differences in DNA methylation patterns was carried out on extreme by germination time subpopulations of seedlings.

After 12-hour seed swelling and germination "fast-grown" group was selected, after 24 hours – "slow-grown" one. Then the groups were collected into different Petri dishes. thirty seeds taken per one variant. To determine the type of fungi contamination, we used both microscopy and taking photo of growing seeds with the subsequent zoom the image via a computer. Indicated that the most type of fungi contamination was *Mucor Fresen* – saprophyte belonging to *Zygomycetes*.

The DNA extraction carried out using reagent set NeoPrep DNA (Neogene, Ukraine).

PCR conducted in amplification "Tercik" ("DNA-technologies, Moskow"). Two types of primers used: RAPD-P6 (GAG-CAA-GTT-CAG-CCT-GG) and ISSR 5'-$(AC)_8$C-3'. Oligonucleotides were synthesized by "Metabion" (Germany). To carry out PCR was used reagent set GenPak® PCR Core – ready to use the dry mix for DNA amplification. Reaction mix for ISSR-PCR (20 μl) contained: 1 u Taq-polymerase, 10 μl buffer, 2,5 mM $MgCl_2$, 200 mM each dNTP, 0,1 mM primer, 200 ng total genome DNA, 6,4 μl deionized water. The mix covered with 20 μl vaseline oil. Amplification stages: prior denaturation 5 min under 94°C, then 40 cycles: denaturation under 94°C - 45 sec, annealing temperature 52°C - 45 sec, elongation under 72°C - 90 sec; final elongation 7 min under 72°C [17, 18].

DNA restriction following amplification was conducted in amplification "Tercik" (DNA-technology, Moskow) as well. Two restriction enzymes were used: MspI (5'-CC*CGC-3') and HpaII (5'-C*CGC-3') (Fermentas, Germany). Restriction reaction (25 μl) contained: 0,6 u MspI or 0,2 u HpaII, 2 μl 10xBuffer Tango, 500 ng total genome DNA, 17,1 μl deionized water. The mix covered with 20 μl vaseline oil. Reaction conditions: 16 hours under 37°C, stop reaction - 20 min under 65°C (for HpaII) and 20 min under 80°C (for MspI).

Obtained products of restriction and PCR were separated in 1,0% agarose gel in TBE-buffer with ethidium bromide and visualized in UV-transilluminator. The same volume of PCR products (5 μl) put into gel slots. As a molecular weight marker, GeneRuler 50 bp DNA Ladder (Fermentas, Germany) with fragments length 1000, 750, 500, 250, and 50 bp were used.

III. Results and Discussion

There is diverse initial level, dynamics of development and final level of infection in the subpopulation of seedlings with different germination time from Podolyanka and Favoritka varieties harvested in different years (Fig. 1a-c, 2a-c).

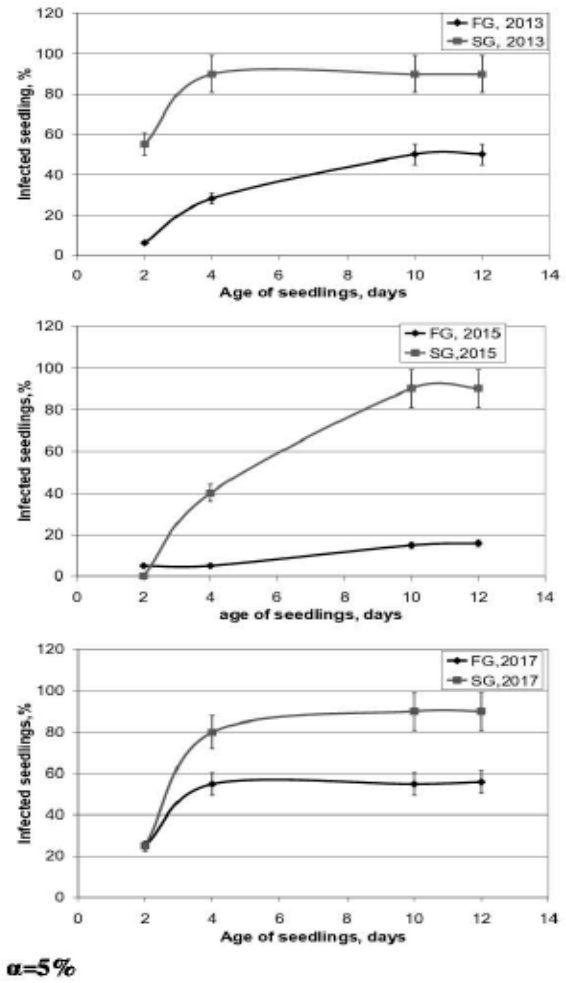

Fig. 1: The dynamics of infection of quickly (FG) and slowly (SG) germinating seedlings of wheat varieties Podolyanka crops of different years.

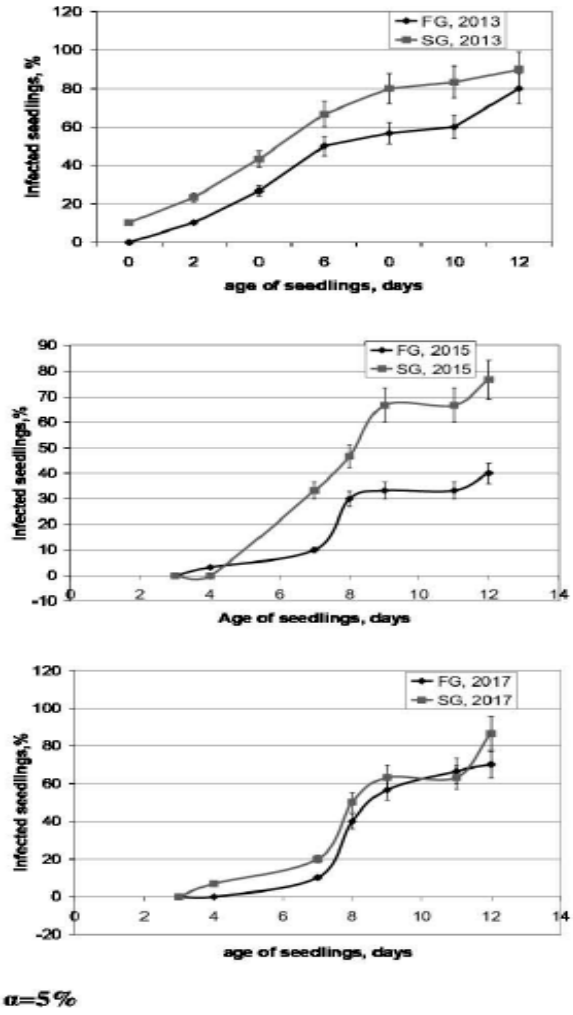

Fig. 2: The dynamics of infection of quickly and slowly germinating seedlings of wheat varieties Favoritka crops of different years.

All the parameters for Podolyanka variety are the same from year to year; in other words, they are stable and therefore less dependent on the weather conditions of the year. Less stable parameters are there for Favoritka variety, but plants from seeds harvested in 2017 have not shown any differences through infection indexes. The phenomena might be considered as the different immune status of plants from other varieties and subpopulations through the same one according to its germination time.

Proceed to the analysis of DNA methylation two wheat varieties belong to subpopulations fast- (FG) and slow-grown (SG) seedlings.

Electroforegram of extracted DNA (Podolyanka variety) shows its nativity that leads carrying out restriction analysis following PCR (Fig. 3 a, b, c). Electroforegrams of P6 and ISSR amplification indicate no polymorphism through these genome elements (Fig.3 b, c).

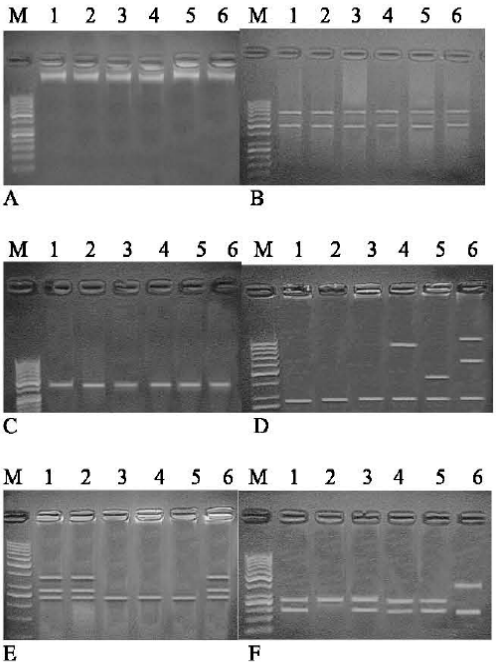

Fig. 3: Variety Podolyanka, harvests of 2013, 2015 and 2017. A. The electroforegram of isolated DNA quality control. B. ISSR-amplification of native DNA. B. P6 amplification of native DNA. G. P6 - amplification of HpaII restricts. D. ISSR - HpaII restriction amplifications. E. ISSR MspI restriction amplifications. **M**- molecular-weight marker GeneRuler 50 bp;1 – FG, 2013; 2 – FG, 2015, 3 – FG, 2017; 4 – SG, 2013; 5 – SG, 2015; 6 – SG, 2017.

Electroforegrams of HpaII restricts P6-amplification show the polymorphism of DNA methylation pattern through FG-SG seedlings (2013 - 2017 years) due to low-molecular-weight amplicons in SG-subpopulation. There is maintaining of DNA methylation pattern through FG seedlings over the years. All variants have low-molecular-weight amplicon 240 bp (Fig. 3 d) that may indicate existing some DNA sequences not conversing into *de novo* mode while forming immune reactions.

Electroforegrams of HpaII restricts ISSR-amplification show polymorphism (2013-2017 years) through both FG-SG seedlings and within each subpopulation with different germination time according to harvest time. As with the variation of methylation patterns in HpaII restricts P6-amplification (Fig. 3 e) there is low-molecular-weight amplicon 250 bp as well.

Electroforegrams of MspI restricts ISSR-amplification also indicate DNA methylation pattern polymorphism between both FG and SG groups of each year and within each subpopulation with different germination time due to the harvest time. There is a match in DNA methylation patterns between SG-subpopulation from 2013 and 2017 and between SG-seedlings from 2013 and 2015 year of harvest. There is observed no mutual amplicon for all variants(Fig. 3 f).

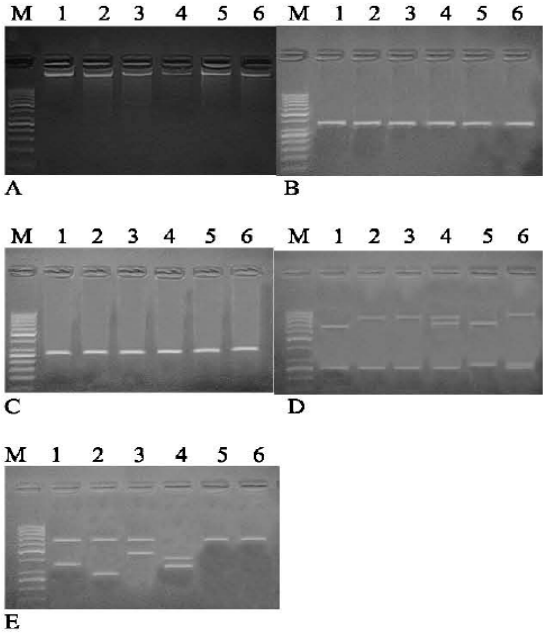

Fig. 4: Variety Favoritka, harvests of 2013, 2015 and 2017. A. Control of the origin of the isolated DNA. B. Electroforegram of native DNA ISSR-amplification. C. Electroforegram of native DNA P6 amplification. D. Electroforegram of HpaII restricts ISSR amplification. E. Electroforegram of MspI restricts ISSR amplification. **M**– molecular-weight marker GeneRuler 50 bp; 1 – FG, 2013; 2 – FG, 2015; 3 – FG, 2017; 4 – SG, 2013; 5 – SG, 2015; 6 – SG, 2017.

Electroforegram of extracted DNA (Favoritka variety) shows its nativity that leads carrying out methylation digest analysis (Fig. 4 a). Electroforegrams of P6 and ISSR amplification indicate no polymorphism through these genome elements (Fig.4 b, c).

Electroforegrams of HpaII restricts ISSR-amplification show polymorphism of DNA methylation pattern both between FG-SG seedlings and within each subpopulation with different germination time due to harvest year. Moreover there is low-molecular-weight amplicon 250 bp in all variants (Fig. 4 d).

Electroforegrams of MspI restricts ISSR-amplification show polymorphism of DNA methylation pattern (Favoritka variety) both between FG-SG seedlings and within with different germination time due to harvest year.

Visualizing infection development for FG- and SG-seedlings, we can conclude that higher steadiness to disease is for FG group. This effect is more stable for Podolyanka variety. At the same time, DNA methylation patterns changed in seedlings FG and SG for both Podolyanka and Favoritka in connection with the year of harvest. Thus it is no connection between phytopatogenes resistance and the specific structure of DNA methylation patterns. However epigenetic distance assessment indicates a general trend for the varieties: while varying DNA methylation pattern by years the

epigenetic distance for Podolyanka variety increasing the same parameter for Favoritka (Table 1).

Problem of individual disease resistance was the subject of research for a long time. I. V. Michurin indicated different stability of cotton seed bolls from the top of the main stem to phytopatogens. Found that the position of cobs on the plant and seeds in the corn cob had immunological significance. These phenomena are associated with varying degrees of seed maturation at the time of harvest [19].

Table 1: Epigenetic distance changing for Podolyanka and Favoritka varieties by years

Variety	D, Epigenetic distance by years		
	2013	2015	2017
Podolyanka	0,23	0,28	0,12
Favoritka	0,02	0,051	0,024

The results suggest a connection between plant individual resistance and DNA methylation characteristics, i.e., some epigenetic features. Assessment of factors determining population epigenetic polymorphism in genetically homogeneous plants [20] indicates its determination not only by other grain maturation degree and factors affecting process but also acting earlier on different growth and development stages of parent organism including gametogenesis. Switching methylation into *de novo* mode is dependent on conditions of plant development. DNA methylation pattern formed to the ripening time of seed from new harvest brings information about the parental organism.

Known that rearrangement of methylated cytosine influence chromatin conformation. Thus the connection between DNA methylation pattern changes and individual plant radiosensitivity admits at least a partial explanation of the fact with different protection of both DNA and chromatin conformation [17]. Correlation between the level of epigenetic polymorphism variety and its unspecific ecological plasticity points into the importance of methylation as a factor of epigenetic regulation affecting various pattern of gene expression and metabolism [18].

Results in the paper also are confirming the role of DNA methylation as an epigenetic regulation factor leading to forming various patterns of gene expression within the genetically homogenous plant material.

The revealed relationship between resistance to phytopatogens and polymorphism of the DNA methylation pattern as a marker of epigenetic and metabolic differences does not indicate possible immune mechanisms that affect the process. Known that plants have both active inducible and passive constitutive (or structural) ways related to morphology and biochemical compound of biological structures [12,17,18] including shell grains. Development just saprophytic infection in grain surface and then in seedling root indirectly indicates epigenetic differences in structure of passive immunity type.

The obtained data indicate the importance of epigenetic mechanisms in determining the individual sensitivity of plants to phytopatogens and the role of epigenetic polymorphism as a factor contributing to the biological diversity of crops.

The issue about factors affecting crops biodiversity is significant. Charles Darwin (Darwin, 1876) was the first to note that the maximum biomass of the annual plant crop observed at their maximum species diversity [6, 23] per unit area. Then the observation formed the main concept of ecological niche. Up to date, it means all biotic and abiotic factors conditioning the existence of different species [1]. There is weakened competition for the resources with possible cooperative interactions among organisms with diverse ecological niches– that could explain the effect revealed by Darwin.

The concepts of life strategies are also widely used in the ecology of populations, one of the biological functions of which is to weak the interspecific competitive relations. At the same time, natural mechanisms reducing intraspecific competition level are not considered. The concept of a variety of life strategies does not apply to the population of cultivated plants that have undergone a lengthy breeding process and grown under artificial conditions of mono sowing. Competitive relationship as a one-species population is partially weakened with exogenous factors, first of all, agrotechnical effects (fertilizing, watering, pests' treatment). However, natural mechanisms decreasing intraspecific competition through organisms taking the same ecological niche are of significant interest.

One of the reasons could be genetic polymorphism appearing in various degrees in any population or planting crops. However, according to recent data, this parameter is relatively low and considerably below the epigenetic polymorphism index [26].

The data suppose that epigenetic polymorphism is efficient and evolutionarily fixed factor decreasing intraspecific competition, including relationship within mono sowing. It confirms with it widespread through various genetically homogeneous biological systems: from populations of wild [28] and cultivated plants [29] to differentiated animal tissues. The prevalence of this phenomenon indicates that the general mechanism increasing the functioning of biological communities any organization level aimed at reducing competitive relations and possibly strengthening cooperative ones.

Acknowledgements

The study was funded by the Academy of Sciences of Ukraine, Grant III-4-18 "Studying of mechanisms of resistance and adaptation of plants to abiotic and biotic stresses".

No Conflict of Interest

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