ISSN 2409-9066. Sci. innov. 2016, 12(2): 50—59 doi: http://dx.doi.org/10.15407/scine12.02.050

Kravets, O.P., Sokolova, D.O., Berestyana, A.M., Shnurenko, O.R., Bannikova, M.O., Morgun, B.V., Kuchuk, M.V., and Grodzinsky, D.M.

Institute of Cell Biology and Genetic Engineering, the NAS of Ukraine, Kyiv

CORRELATION BETWEEN ECOLOGICAL PLASTICITY OF ELITE WINTER WHEAT VARIETIES AND DNA METHYLATION PATTERN POLYMORPHISM WITHIN VARIETY

The correlation between the ecological plasticity of the eight premium winter wheat varieties and the DNA methylation pattern polymorphism within variety has been studied using the seedlings from seeds with different germination rate. Polymorphism degree or "epigenetic distance" in restriction fragment range of PCR products has been assessed using Nei index. The correlation between variety grade determined by its productivity and ecological plasticity and the degree of DNA methylation pattern polymorphism within variety (Rs=0.69) has been found. The prevalence of the greatest "epigenetic distance" (D≥0.1) in the most productive and ecologically plasticized varieties has been proved. The "epigenetic distance" assessment has been recommended to apply to the selection of new varieties for the areas with unstable climate conditions.

Keywords: *wheat, ecological plasticity, epigenetic polymorphism, Nei distance, and restriction analysis.*

The climate change and environment pollution are the challenges putting additional requirements for the design of new varieties. One of the most important objectives, is to speed up the breeding process and to create varieties that have not only a high productive capacity under optimal conditions, but also a good ecological plasticity and adaptability which enable getting rich harvests under the extreme conditions.

The advanced breeding techniques based on molecular genetic approaches widely use the genetic markers, which enable detecting the genetic polymorphism for a large amount of breeding material, speeding up and focusing on the design of new premium varieties possessing the required properties. At the same time, the recent studies [1–3] have showed that the adaptation depends on the modification of expression of large gene ensembles and substantial transformation of iso-enzyme spectra. These data have confirmed that the highly transformable varieties have a higher adaptability.

The advanced population breeding deals not only with the genetic polymorphism, but also with the epigenetic one that is dependence of heterogeneity of phenotypes on diversity of their epigenetic programs while preserving the genotype identity.

The recent research data testify to a significant role of climate factors in determining the population epigenetic polymorphism inherited by several generations and ensuring adaptability to unstable conditions of growth. By example of several industrial crops [4] it has been showed that the genetic polymorphism determines only several percent of the variety's adaptability, while the major contribution to the plasticity is attributed to epigenetic transformations and changes in epigenetic polymorphism which determine the plant organism reaction norm.

The fact of appearance and spread of epigenetic polymorphism between the populations of the same variety as a result of growing under different conditions raises a question whether in arbitrary population, a certain ***initial*** (existing at the stages of seed and seedling) ***epigenetic polymorphism*** enabling the population homeostasis, i.e. successful germination, growth, and reproduction of species and variety under unfavorable climate conditions typical for given region, exists.

Any sample of seeds that are not in the dormancy state and belong to the same species, variety, and harvest is characterized by a certain variability of germination terms after the initiation of this process. In fact, for the majority of species, no heterocarpy has been reported, with germination rate being a common phenotypic parameter enabling to switch from the phenotypic polymorphism to the epigenetic one. In research [5–8], the correlation between various germination rate of seeds from arbitrary sample with initial polymorphism of methylation profiles of different DNA sequences for the seedlings and their resistance to abiotic factors has been established by example of maize, which implies that DNA methylation can be deemed a factor of organism's individual resistance while methylation profile polymorphism can be considered a factor of the population resistance [5]. The adaptability of sub-populations has been showed to depend on the germination intensity and initial conditions of methylation of different DNA sequences of seedlings, whereas the adaptability of populations correlate with polymorphism of DNA methylation profiles [5–8].

Hence, one can conclude that the ecological plasticity ensured by epigenetic mechanisms is one of general protective reactions determining diversified responses to the action of stress factors and assuring viability and reproductive capacity of wild plants and crops. The data have showed that there are possibilities for applying the estimates of epigenetic polymorphism level as criterion of production successfulness.

This research is aimed at assessing the correlation between the ecological plasticity of premium winter wheat varieties and their epigenetic polymorphism i.e. initial distinction of DNA methylation profiles for the plants of the same harvest, within variety, and at developing a predicative criterion to be used in the breeding practice under instable climatic conditions, on the basis of estimates.

1. ROLE OF DNA METHYLATION IN ORGANIZATION OF POLYMORPHIC EPIGENOME

One of the functional mechanisms of epigenetic polymorphism as dependence of phenotype heterogeneity on diversity of epigenetic programs while keeping the genotype identity is chemical modification of DNA nitrogen-containing bases (cytosine methylation). This modification is inherited epigenetically due to the existence of system recognizing the hemimethylated sequences and transforming them into entirely methylated sections. The sequence epigenetic mark is removed together with methyl groups [9, 10].

Different activity of various methylation mechanisms in various cells causes the so called methylation mosaic. In addition to the supporting methylation that ensures post-mitotic modification of symmetric sites, methyltransferases perform the *de novo*, methylation, with arbitrary de-repression, as result of passive and active demethylation, added to preset selective removal of methylation [11].

DNA methylation occurs mainly on the target sequences (chiefly, CpG and CpNpG) and is an important form of polymorphism. Through these modification, new alleles, the *epi-alleles*, are produced. Their common feature is mandatory manifestation in phenotype and they are epigenetic basis for phenotype variability. However, not all epi-alleles and results of their transformation are visible as phenotype transformations, therefore, the study of epigenetic heredity in the entire genome has become relevant with development of molecular methods. Due to Methylation-Sensitive Amplified Polymorphism (MSAP), the natural populations have been showed to vary more significantly by DNA methylation level rather than by nucleotide sequence composition and to have a higher epigenetic diver-

sity as compared with genetic one [12, 13]. In addition, the genetic assignment of stress-induced epigenome transformations has been reported [6, 14–16, 17]. The use of high-productive sequence methods ensures a higher frequency of epi-alleles (as compared with genetic mutations) and reversibility of methylated DNA sections [8]. The metastability of epi-alleles shows that many of spontaneously created unique epigenetic features cannot exist for quite a long while and ensure the evolutionary changes of required duration. However, the epi-allele reversibility is the factor that makes them comply with general rules of phenotype breeding and assignment of features. Hence, the metastable epi-alleles induced by the environment ensure the phenotype diversity of population [5].

As mentioned above, different rates of seed germination have an epigenetic mechanism of appearance and maintenance; the seed epigenetic polymorphism correlates with germination rate and resistance of seedlings to abiotic factors [7, 5]. There is a substantial difference between the adaptability of seedlings and epigenetically different groups of the same species and variety [6–8]. This testifies to a certain biological sense of correlation between seed epigenetic polymorphism and germination rate: variability of germination terms and differentiation of seedling resistance are one of the mechanisms of keeping the population homeostasis under variable environment conditions.

2. ASSESSMENT OF EPIGENETIC POLYMORPHISM OF PREMIUM WINTER SOFT WHEAT VARIETIES

The research was made for 11-day seedlings of eight premium soft wheat varieties (*Smuhlianka, Podolianka, Sotnytsia, Natalka, Darunok Podillia Favorytka, Lymarivna, and Novokyivska*); the variety originators are the Institute of Plant Physiology and Genetics, the NAS of Ukraine, and the Remesl Myronivka Institute of Wheat the NAAS of Ukraine.

The correlation between epigenetic polymorphism and ecological plasticity was studied in several stages:

1) Analysis of certificate data of premium wheat varieties and their ranking by productivity and ecologic plasticity;

2) Variety description by ratio of subpopulation of seeds with different rate of germination;

3) DNA extraction, assessment of its nativity, restriction with further PCR;

4) Analysis of amplicon ranges, estimation of the Nei epigenetic distance with variety;

5) assessment of the Spearman rank correlation between the ecologic plasticity and epigenetic distance within the variety.

The ISSR-PCR, ITS-PCR reactions, and restriction analysis were carried out according to standard protocols. The reaction mix for ISSR-PCR (20 μl) contained: 1 unit (0.8 μl) Taq-polymerase inhibited for «the hot start», 10 μl PCR-diluent, 2.5 mM $MgCl_2$, 200 μM each dNTP, 0.1 μM primer (1.6 μl), 200 ng total genome DNA (2 μl), and 6.4 μl deionized water. The mix was coated with a paraffinic oil layer of 20 μl. The amplification with ISSR-primers included the following stages: initial denaturation during 5 min, at 94 °C (40 cycles); denaturation, at 94 °C during 45 s; annealing, at 52 °C during 45 s; elongation, at 72 °C during 90 s; final elongation, at 72 °C during 7 min [18].

The reaction mix for ITS-PCR (20 μl) contained: 1 unit Taq-polymerase inhibited for the «hot start»; 10 μl PCR-diluent, 2.5 mM $MgCl_2$, 200 μM each dNTP, 0.1 μM each primer (0.8 μl), 200 ng total genome DNA (2 μl), and 6.4 μl deionized water. The mix was coated with a paraffinic oil layer of 20 μl. The amplification with ITS-primers included the following stages: initial denaturation during 1.5 min, at 94 °C (5 cycles); additional 40 cycles consisting denaturation, at 94 °C during 15 s; annealing, at 55 °C during 15 s; elongation, at 72 °C during 15 s; fixation: denaturation, at 94 °C during 10 s; annealing, at 55 °C during 10 s; final elongation, at 72 °C during 5 min [19].

The restriction analysis (similar to amplification) was made in 4-channel Tercyc DNA amplifier (DNA technology, RF). Three types of restrictase were used: MspI, MboI, and HpaII (Fermentas, Lithuania).

The reaction of restriction with enzyme MspI runs in a volume of 25 μl, includes 0.6 unit enzyme (0.9 μl), 2 μl 10xBuffer Tango, 500 ng total genomic DNA (5 μl), and 17.1 μl deionized water. The reaction mixes for restriction analysis MboI and HpaII have a volume of 25 μl containing 0.2 unites enzyme (0.3 μl), 2 μl 10xBuffer Tango, 500 ng total genomic DNA (5 μl), and 17.7 μl deionized water. All reaction mixes were coated with a 20 μl layer of paraffinic oil. The restriction reaction lasted 16 hours, at 37 °C; the inactivation took 20 min, at 65 °C (for MboI and HpaII) and 20 min, at 80 °C (for MspI).

The obtained products of PCR and restriction analysis were separated in 1.7% agarose gel with TBE buffer in the presence of ethidium bromide and visualized in UV-trans-illuminator. For electrophoresis, the same volume of PCR and restriction (5 μl) was put into gel pockets; SMO373 GeneRuler 50 b.p. was used as molecular weight marker.

The epigenetic distance was estimated using the modified Nei approach [20].

X and *Y* were assumed to be two independent sets of restriction product amplicons, with x_i and y_i being frequencies of individual bands in these sets. The probability of two randomly chosen bands being identical is $j_X = \sum x_i^2$ —for set *X*; $j_Y = \sum y_i^2$ — for set *Y*. The probability of the *X* set band being identical with the band from the *Y* set is $j_{XY} = \sum x_i y_i$. Hence, the normalized identity of bands between *X* and *Y* is expressed as

$$I = \frac{J_{XY}}{\sqrt{J_X J_Y}}, \quad (1)$$

where J_X, J_Y, and J_{XY} are mean values of j_X, j_Y, and j_{XY}, respectively.

Epigenetic distance between *X* and *Y* is:

$$D = -\ln I. \quad (2)$$

Here, D = 0 in the case of absolute coincidence of bands in amplicon sets and D = 1 in the case of total divergence (amplicon weight).

The final stage was to calculate the Spearman correlation between the variety rank and the divergence of amplicon spectra across variety (epigenetic distance) and to statistically process the data using MS Office Excel.

Table 1

Properties of Soft Winter Wheat Varieties and Their Ranking by Productivity and Ecoplasticity

Variety	Yield capacity		Ecoplasticity, score	Total score	Rank of variety
	hundredweight/ha	score			
Smuhlianka	60.0—115.1	3+	0	3+	1
Podolianka	60.0—96.0	1+	6+	7+	5
Sotnytsia	50.2—102.6	2+	2+	4+	2
Natalka	50.3—93.6	1+	4+	5+	3
Darunok Podillia	50.4—91.4	1+	3+	6+	4
Favorytka	50.6—124.0	3+	3+	6+	4
Lymarivna	61.2—100.0	1+	6+	7+	5
Novokyivska	50.7—104.8	2+	6+	8+	6

Note. Yield capacity: 1+ maximum yield up to 100 hwt/ha; 2+ maximum yield up to 110 hwt/ha; 3+ maximum yield up to > 110 hwt/ha. Easiness to grow = ecoplasticity: 1+ soft requirements for sowing terms; 2+ soft requirements for sowing terms + soft requirements for mineral fertilizers; 3+ soft requirements for sowing terms + soft requirements for previous crop + soft requirements for mineral fertilizers.

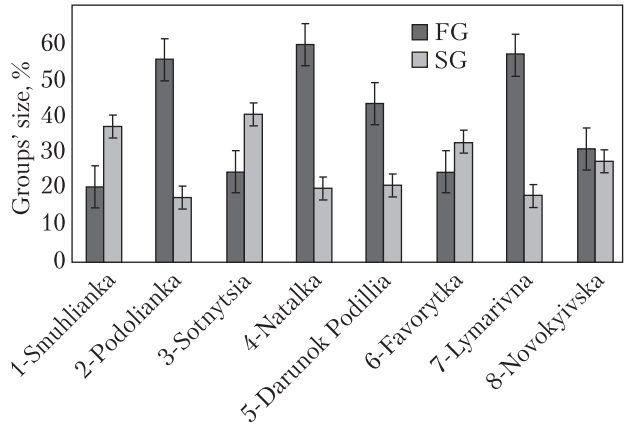

Fig. 1. The ratio between fast-growing (FG) and slow-growing (SG) seed groups of each variety.

The seeds germinate in thermostat, on substrates with wet filter paper, at 24–25 °C. On the second day, the germinated seeds were separated into two groups: the fast sprouting (sprout length >1 cm) and the slowly sprouting (the hardly germinated seeds). The equal samples of both groups were put into wet Petri dishes and kept in the thermostat (T = 24–25 °C) till the fourth day; then the seeds continued germinating in spacy transparent ware till the 11th day under 16/8 fluorescent lighting by *Electrum* 36W/54 (4 thousand lx).

The DNA was extracted from seedlings using reagent sets NeoPrep50 DNA (NeoGene, Ukraine) and GeneJET Plant Genomic DNA Purification Mini Kit (*Fermentas*, Lithuania) using standard procedures set by manufacturers. Concentration of DNA solution was measured using spectrophotometer BioPhotometer Plus Eppendorf v.1.35 by standard method [21].

The PCR was carried out in *Tertsik* 4-channel DNA amplifier (DNA-Technology, RF). Primers to mini-satellite ISSR sequences (inter simple sequence repeat 15-soro, 5′-AC-AC-AC-AC-AC-AC-AC-AC-<C>-3′), to ITS1 (internal transcribed spacer 1, 5′-TCC-GTA-GCT-GAA-CCT-GCC-G-3′), and to ITS4 (internal transcribed spacer 4, 5′-TCC-TCC-GCT-TAT-TGA-TAT-GC-3′) were used. Both primer types were synthesized by *Metabion* (Germany). Lyophilized dry mixes were used for DNA amplification (GenPak®PCR Core).

2.1. ANALYSIS OF CERTIFICATE DATA OF GIVEN VARIETIES AND THEIR PRODUCTIVITY AND ECOPLASTICITY RANKING

While analyzing the ecoplasticity of premium wheat varieties the certificates given by the originators were used. The certificate data show a significant heterogeneity of selected groups of premium wheat varieties by yield capacity and ecoplasticity. The certificates define some varieties as *for experienced farmer* i.e. sensitive to climatic conditions for sowing, previous crop, fertilizers, and treatment with pesticides; while other varieties are characterized as *easy to grow* (ecoplasticity). The existing data do not enable a direct economic analysis by *input/yield ratio*, therefore when classifying the varieties it is advisable to apply a simple ranking by yield capacity and easiness to grow or ecoplasticity (Table 1). Three parameters of ecoplasticity were used: requirements for sowing time, previous crop, and mineral fertilizers.

2.2. VARIETY PROPERTIES DEPENDING ON SHARE OF SEED SUBPOPULATION WITH DIFFERENT GERMINATION RATE

In the previous research [5–8], a procedure for sorting out the sample by germination rate was elaborated. The sample was divided into the three groups: with high, medium, and low germination rate. The size and cytogenetic parameters of seedlings using from the subpopulation of seeds germinating at a medium rate has been established to be irregular [6]. Therefore, the further study of epigenetic polymorphism was focused on the extreme groups (fast-germinating and slow-germinating seeds). This approach is recommended for studying epigenetic polymorphism of various wheat varieties, since it enables to expect and to identify the widest divergence and the largest distance in DNA methylation profiles.

For each variety, the seeds were sorted out according to germination rate (Fig. 1).

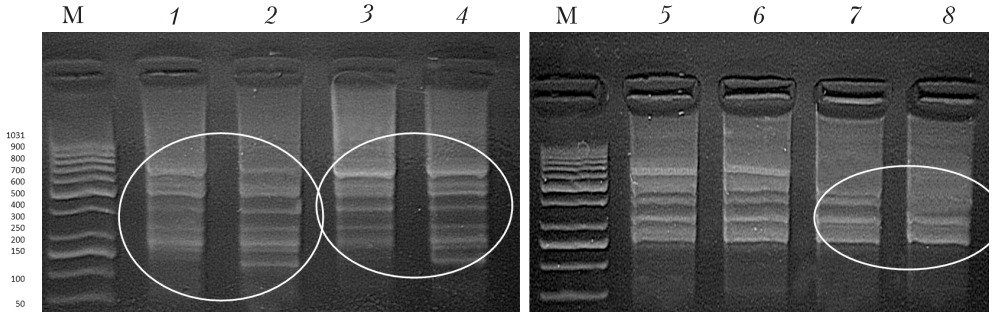

Fig. 2. The electrophoregrams of HpaII-restricts' amplification with ISSR-primers; M — molecular-weight marker Thermo Scientific GeneRuler 100bp Plus DNA Ladder. Tracks: *1* — SG, 1 — Smuhlianka; *2* — FG, 1 — Smuhlianka; *3* — SG, 2 — Podolianka; *4* — FG, 2 — Podolianka; *5* — SG, 3 — Sotnytsia; *6* — FG, 3 — Sotnytsia; *7* — SG, 4 — Natalka; *8* — FG, 4 — Natalka.

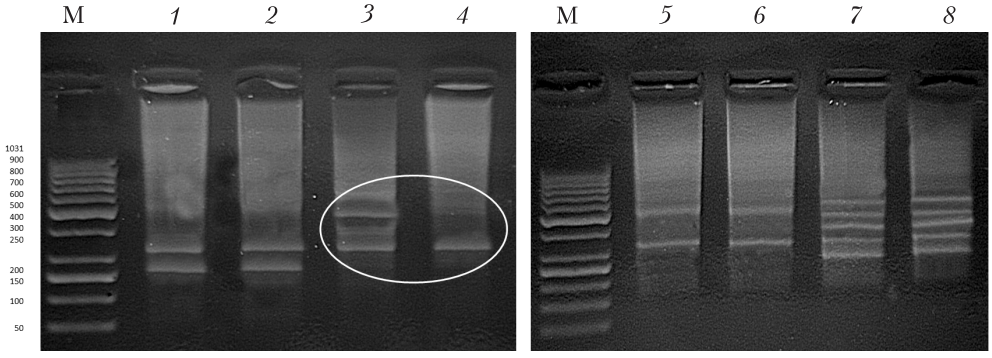

Fig. 3. The electrophoregrams of MboI-restricts' amplification with ISSR-primers; M — molecular-weight marker Thermo Scientific GeneRuler 100bp Plus DNA Ladder. Tracks: *1* — SG, 1 — Smuhlianka; *2* — FG, 1 — Smuhlianka; *3* — SG, 2 — Podolianka; *4* — FG, 2 — Podolianka; 5 — SG, 3 — Sotnytsia; *6* — FG, 3 — Sotnytsia; *7* — SG, 4 — Natalka; *8* — FG, 4 — Natalka.

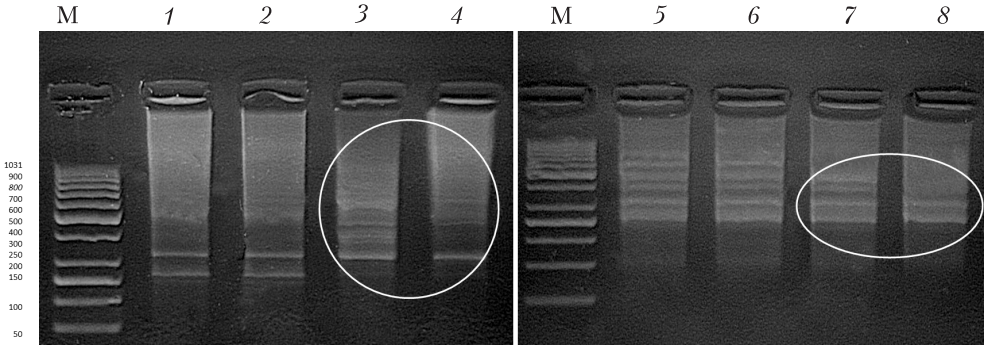

Fig. 4. The electrophoregrams of MspI-restricts' amplification with ISSR-primers; M — molecular-weight marker Thermo Scientific GeneRuler 100bp Plus DNA Ladder. Tracks: *1* — SG, 1 — Smuhlianka; *2* — FG, 1 – Smuhlianka; *3* — SG, 2 — Podolianka; *4* — FG, 2 — Podolianka; *5* — SG, 3 — Sotnytsia; *6* — FG, 3 — Sotnytsia; *7* — SG, 4 — Natalka; *8* — FG, 4 — Natalka.

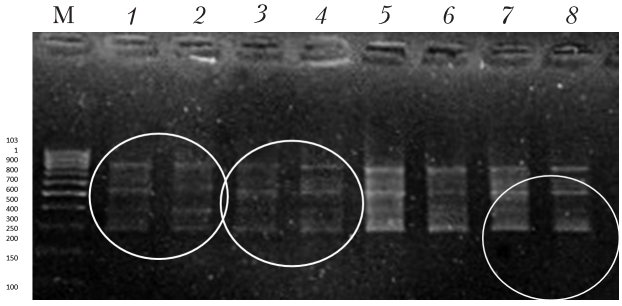

Fig. 5. The electrophoregram of MspI-restricts' amplification with ITS-primers; M — molecular-weight marker Thermo Scientific GeneRuler 100bp Plus DNA Ladder. Tracks: *1* — SG, 5 — Darunok Podillia; *2* — FG, 5 — Darunok Podillia; *3* — SG, 6 — Favorytka; *4* — FG, 6 — Favorytka; *5* — SG, 7 — Lymarivna; *6* — FG, 7 — Lymarivna; *7* — SG, 8 — Novokyivska; *8* — FG, 8 — Novokyivska.

On the basis of FG/SG seed ratio, the following facts have been established: SG seeds dominate in the *Smuhlianka*, *Sotnytsia*, and *Favorytka* varieties; FG seed prevail in *Podolianka*, *Natalka*, *Dar Podillia*, and *Lymarivna* varieties; approximately equal amount of SG and FG seeds are reported for *Novokyivska* variety. The results testify to possible heterogeneity of adaptation strategies in the bred cultivars. All 8 varieties being classified as the premium ones and having a high yield capacity, their divergence and similarity were studied while analyzing DNA methylation profile polymorphism.

2.3. STUDY OF DNA METHYLATION PROFILES OF PREMIUM WHEAT VARIETIES ORIGINATING FROM SUBPOPULATIONS OF SEEDS WITH DIFFERENT GERMINATION RATE

The DNA nativity control electrophoregrams have showed a high quality of DNA extraction, which enables the further analysis of methylation character.

A set of electrophoregrams is given as example of obtained initial data on DNA methylation specific features for the seedlings from seeds having different germination rate. A clear difference between FG and SG groups in the spectra of amplified restriction digests has been reported on the amplification electrophoregrams of HpaII restriction digests with ISSR-primers for the *Smuhlianka*, *Podolianka*, and *Sotnytsia* varieties. For the *Sotnytsia* variety, no difference between the range of amplified restriction products has been recorded, that pointed to the absence of difference in DNA methylation pattern of the seedlings from FG- and SG-groups (Fig. 2).

The amplification electrophoregrams of MboI restriction digests with ISSR show no difference in the number of bands for the *Smuhlianka*, *Sotnytsia*, and *Natalka*, while for the *Podolianka* variety, a difference has been recorded (Fig. 3).

Table 2

Properties of Soft Wheat Varieties, Their Ranking and Epigenetic Distance Across Each Variety

Variety	Yield capacity		Ecoplasticity	Total score	Rank of variety	D, epigenetic distance
	hwt/ha	score				
Smuhlianka	60.0—115.1	3+	0	3+	1	0.020
Podolianka	60.0—96.0	1+	6+	7+	5	0.126
Sotnytsia	50.2—102.6	2+	2+	4+	2	0.004
Natalka	50.3—93.6	1+	4+	5+	3	0.108
Darunok Podillia	50.4—91.4	1+	3+	6+	4	0.010
Favorytka	50.6—124.0	3+	3+	6+	4	0.0056
Lymarivna	61.2—100.0	1+	6+	7+	5	0.210
Novokyivska	50.7—104.8	2+	6+	8+	6	0.098—0.100

The amplification electrophoregrams of MspI restriction digests with ISSR indicate a difference in amplicon range for the *Podolianka* and *Natalka* varieties and no difference for the *Smuhlianka* and *Sotnytsia* (Fig. 4.).

The obtained data testify to the identity of amplicon range of products of restriction with HpaII-endonuclease that means that there is no difference in the methylation of respective sites not only across the variety, but also across the group of selected varieties. The *Darunok Podillia, Favorytka, Lymarivna,* and *Novokyivska* varieties have showed different spectra of amplicons for amplification of restriction digests of MboI-endonuclease with ISSR-primers. This means that there is a difference in the methylation profiles of satellite DNA of seedlings from the SG- and the FG-groups. For separating the amplification products of restriction digests of MspI-endonuclease with ISSR-primers, a difference in amplicon range has been reported for the *Lymarivna* variety only. The electrophoregram of separation of amplification products of restriction digests of MspI-endonuclease with ITS-primers testifies to polymorphism of amplicon range having different molecular weight for the following varieties: *Darunok Podillia* (5 and 4 amplicon types), *Favorytka* (3 and 5 types), and *Novokyivska* (5 and 4 types) (Fig. 5).

The epigenetic distance, i.e. the difference in satellite DNA methylation patterns from seedlings of certain variety, which have different germination rate has been estimated on the basis of analysis of obtained electrophoregrams (Table 2).

Hence, the existence of significant polymorphism of DNA methylation patterns has been established for 8 premium soft winter wheat varieties. These data have confirmed the results of previous studies, which show the existence of DNA methylation polymorphism for the plants originating from the seeds with different germination rate, as well as the correlation of DNA methylation polymorphism, differences in resistance to abiotic factors, and adaptability of the plants.

The next stage of research was to estimate the Spearman statistical correlation between variety ranks and epigenetic distance. According to calculations, the rank correlation amounts to R_s = 0,69 at $\alpha > 0{,}05$ that testifies to a quite high density of correlation for small samples.

CONCLUSIONS

Hence, one can conclude that for the easy-to-grow varieties (those having a high ecoplasticity) have larger epigenetic distance between polymorphic spectra of lengths of DNA restriction digests originating from seeds of the extreme SG and FG groups. If $D \geq 0.1$ the variety belongs to the highest, 5th or 6th, rank by easiness-to-growth.

The quantitative parameter of polymorphism is epigenetic distance across the variety. It can be used as predicative index and marker of variety ecoplasticity. Its practical determination, i.e. algorithm for assessing the epigenetic marker of ecoplasticity that corresponds to its easiness-to-grow, can be divided into 4 stages:

1) Division of seeds into groups by germination rate;

2) DNA extraction;

3) Restriction followed by ISSR-PCR;

4) Analysis of diversity of amplicon spectra, estimate of Nei epigenetic distance across the variety.

The research was made within the framework of R&D project II-13-14 «Implementation of Molecular Systems for Identifying Genetic and Epigenetic Polymorphism of Winter Wheat for Obtaining High-Productive Special Varieties» (reg.no.0114U002736, Kyiv, 2014).

REFERENCES

1. Lekjavichus Je. *Elementy obshchei teorii adaptatsii*. Vilnjus: Mokclas, 1986 [in Russian].
2. Coleman M., Yin E., Peterson L. et al. Low dose irradiation alters the transcript profiles of human lymphoblastoid cells including genes associated with cytogenetic radioadaptive response. *Radiat. Res.* 2005. 164(41): 369—382.
3. Zhong L., Xu Y., Wang J. DNA-methylation changes induced by salt stress in wheat Triticum aestivum. *Afr J Biotech*. 2009. 8(22): 6201—6207.

4. Yi C., Zhang S., Liu X. Does epigenetic polymorphism contribute to phenotypic variances in *Jatropha curcas* L.? *BMC Plant Biol*. 2010. 10: 1—14.
5. Sokolova D., Vengzhen G., Kravets A. The Effect of DNA Modification Polymorphism of Corn Seeds on Their Germination Rate, Seedling Resistance and Adaptive Capacity under UV-C Exposure. *Amer J Plant Biol*. 2014. 1(1): 1—14.
6. Sokolova D., Vengzhen G., Kravets A. Vliianie epigeneticheskoho polimorfizma semian kukuruzy na skorost ih prorastaniia i ustoichivost prorostkov k UF-S obluchenniu. *Tsitologiia i henetika*. 2014. 48(4): 31—38 [in Russian].
7. Sokolova D., Vengzhen G., Kravets A. Rol epihenetycheskoho polimorfizma prorostkov kukuruzy v reaktsiiah na UF-S obluchenie. *Fiziologiia rastenii i henetika*. 2014. 46(3): 221—229 [in Russian].
8. Sokolova D., Vengzhen G., Kravets A. An analysis of the correlation between the changes in satellite DNA methylation patterns and plant cell responses to the stress. *CellBio*. 2013. 2: 163—71.
9. Tsaftaris et al. Epigenetic mechanisms in plants and their implications in plant breeding. *Proceedings of the International Congress «In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution»*. 2003: 157—171.
10. Salmon A., Clotault J. et al. Brassica oleracea displays a high level of DNA methylation polymorphism. *Plant Sci*. 2008. 174(1): 61—70.
11. Shan X., Wang X. et al. Analysis of the DNA methylation of maize (*Zea mays* L.) in response to cold stress based on methylation-sensitive amplified polymorphisms. *J. Plant Biol*. 2013. V.56: 32—8.
12. Scheid G., Scheid M. Epigenetic responses to stress: triple defense? *Curr Opin Plant Biol*. 2012. no 5: 568—573. doi: 10.1016/j.pbi.2012.08.007.
13. Luna E., Bruce T., Roberts M. et al. Next-generation systemic acquired resistance. *Plant Physiol*. 2012. V.158: 844—853.
14. Rasmann S., De Vos M. et al. Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant Physiol*. 2012. V.158: 854—863.
15. Becker C. et al. Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome. *Nature*. 2011. V.480: 245—249.
16. Herman J., Sultan S. Adaptive transgenerational plasticity in plants: Case studies, mechanisms, and implications for natural populations. *Front Plant Sci*. 2011. 2(102): 1—10.
17. Boyko A., Kovalchuk I. Genome instability and epigenetic modification - heritable responses to environmental stress? *Curr Opin Plant Biol*. 2011. 14(3): 260—266. doi: 10.1016/j.pbi.2011.03.003.
18. Tikunov Yu. M., Khrystaleva L. I. Application of ISSR Markers in the Genus Lycopersicon. *Euphitica*. 2003. V.131: 71—80.
19. Bartlett J., Stirling D. *PCR protocols*. Humana Press Inc., 2003.
20. Nei M., Crow F., Denniston C. A new measure of genetic distance. *Genetic distance*. 1974: 63—76.
21. Ausubel F. et al. Current Protocols in Molecular Biology. 2004.

О.П. Кравець, Д.О. Соколова, А.М. Берестяна, О.Р. Шнуренко, М.О. Банникова, Б.В. Моргун, М.В. Кучук, Д.М. Гродзинський

Інститут клітинної біології та генетичної інженерії НАН України, Київ

ВЗАЄМОЗВ'ЯЗОК ЕКОЛОГІЧНОЇ ПЛАСТИЧНОСТІ ЕЛІТНИХ СОРТІВ ОЗИМОЇ ПШЕНИЦІ І ПОЛІМОРФІЗМУ ПРОФІЛІВ МЕТИЛУВАННЯ ДНК У МЕЖАХ СОРТУ

Досліджено зв'язок екологічної пластичності восьми елітних сортів озимої пшениці з поліморфізмом профілів метилування ДНК в межах сорту у проростків з насіння, що проростає з різною швидкістю. Рівень поліморфізму або «епігенетичну відстань» у спектрах рестрикційних фрагментів електрофореграм розподілу продуктів полімеразної ланцюгової реакції (ПЛР) кількісно оцінювали за показником Неї. Встановлено існування кореляції (R_s = 0,69) між рангом сорту, що визначається по продуктивності та екологічній пластичності, і рівнем поліморфізму профілів метилування ДНК у межах сорту. Показано, що найбільша «епігенетична відстань» (D ≥ 0,1) спостерігається у сортів з найвищим рангом по продуктивності та екологічній пластичності. Запропоновано використовувати оцінку показника епігенетичної відстані у селекції нових сортів для зон з нестійкими кліматичними умовами.

Keywords: пшениця, екологічна пластичність, епігенетичний поліморфізм, відстань за Неї, рестрикційний аналіз.

А.П. Кравец, Д.А. Соколова, А.Н. Берестяная, О.Р. Шнуренко, М.А. Банникова, Б.В. Моргун, Н.В. Кучук, Д.М. Гродзинский

Институт клеточной биологии и генетической инженерии НАН Украины, Киев

ВЗАИМОСВЯЗЬ ЭКОЛОГИЧЕСКОЙ ПЛАСТИЧНОСТИ ЭЛИТНЫХ СОРТОВ ОЗИМОЙ ПШЕНИЦЫ И ПОЛИМОРФИЗМА ПРОФИЛЕЙ МЕТИЛИРОВАНИЯ ДНК В ПРЕДЕЛАХ СОРТА

Исследована взаимосвязь экологической пластичности восьми элитных сортов озимой пшеницы и полиморфизма профилей метилирования ДНК в пределах сорта у проростков семян, прорастающих с разной скоростью. Уровень полиморфизма, или «эпигенетическое расстояние», в спектрах рестрикционных фрагментов электрофореграм разделения продуктов ПЦР количественно оценивали по показателю Неи. Установлено существование корреляции (R_s = 0,69) между рангом сорта, определяемым по продуктивности и экологической пластичности, и уровнем полиморфизма профилей метилирования ДНК в пределах сорта. Показано, что наибольшее «эпигенетическое расстояние» ($D \geq 0,1$) наблюдается у сортов с самым высоким рангом по продуктивности и экологической пластичности. Предложено использовать оценку показателя эпигенетического расстояния в селекции новых сортов для зон с неустойчивыми климатическими условиями.

Ключевые слова: пшеница, экологическая пластичность, эпигенетический полиморфизм, расстояние по Неи, рестрикционный анализ.

Received 27.07.15