National Academy of Science of Ukraine

Institute cell biology and genetic engineering

Epigenetic Polymorphism as the Way of Crop Biological Diversity Formation

Alexandra P. Kravets

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Key problem of food production at unstable climate

Optimization of interaction:

- Plant Biological diversity
- Cultivated plants breeding
- Agricultural industry

The Ecological Niche diversity is way to decline of plant competition and increase plant cooperation

The harvest of plant biomass from unit of area is maximal at the maximal diversity of species (Darwin, 1867*, 1876).**

This fact became the root of Ecological Niche concept development. Currently this conception mostly means the total set of abiotic and biotic factors provided existence of species.

Diversity of Ecological Niches inside multi species plant society is way:

1)to decline competition and results to effective use of resources;

2) To create cooperation in the Vegetable Kingdom.

These factors explain the phenomenon discovered by Darwin.

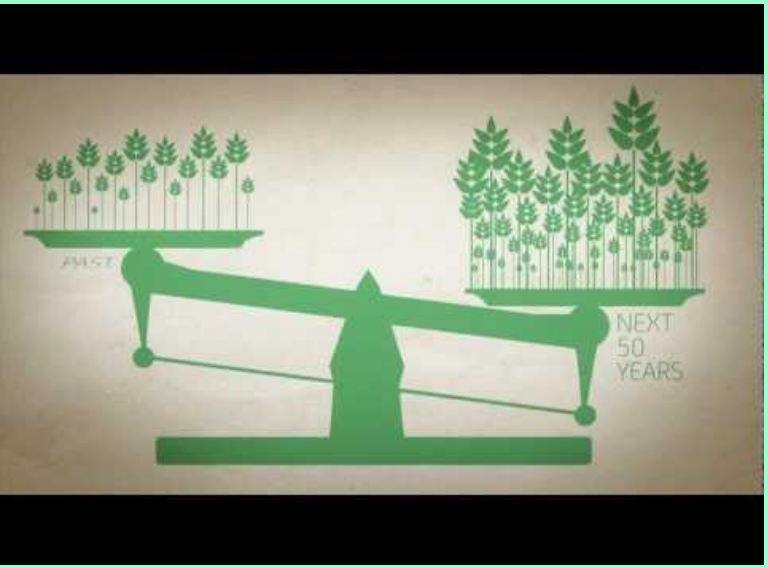
Ch.Darwin"The Variation of Animals and Plants under Domestication ,1868

Ch.Darwin The Effects of Cross- and Self-Fertilization in the Vegetable Kingdom, 1876)

Crops is plant society with one Ecological Niche and highest level of competition

- Crops are the basic source of food for human at last 8- 10 thousand years.
- Crops are one species, even one variety population, with one Ecological Niche and strong competition.
- An **INTRA** species competition is more powerful what **INTER** species, this is a competition between organisms, occupying one Ecological Niche with the same requirements in climate conditions and resources

Are there natural mechanisms to increase intra variety diversity?



Strong competition in crops partly decline by expensive agro technical tools (fertilizers, pouring, treatment from phytopathogens).

There is forecast you need to double the crops for the next 50 years, and, at list, to double these artificial efforts for this purpose.

Are there natural mechanisms to decline intra variety competition via increase intra variety diversity?

Many Faces of Biological Diversity

Any crop has phenotypic diversity. Any crop is variable on a lot of phenotypic characteristics and its cooperation. There are different seeds germination times, plant maturation, activity of primer and secondary metabolism, etc. All these distinctions connect with different expression of genes that control ontogenesis.

Main achievements of molecular genetics at last years are:

1) reveal of genes expression mechanisms control = mechanisms of epigenetic regulation, and

2) beginning of study interaction epigenetic mechanisms with environmental factors.

Today we can formulate the question: How does a phenotypic variability corresponds to the epigenetic pathways variability = epigenetic polymorphism or some kind of "epigenetic niche» variability?

We tried to study these questions carry out the several series of experiments.

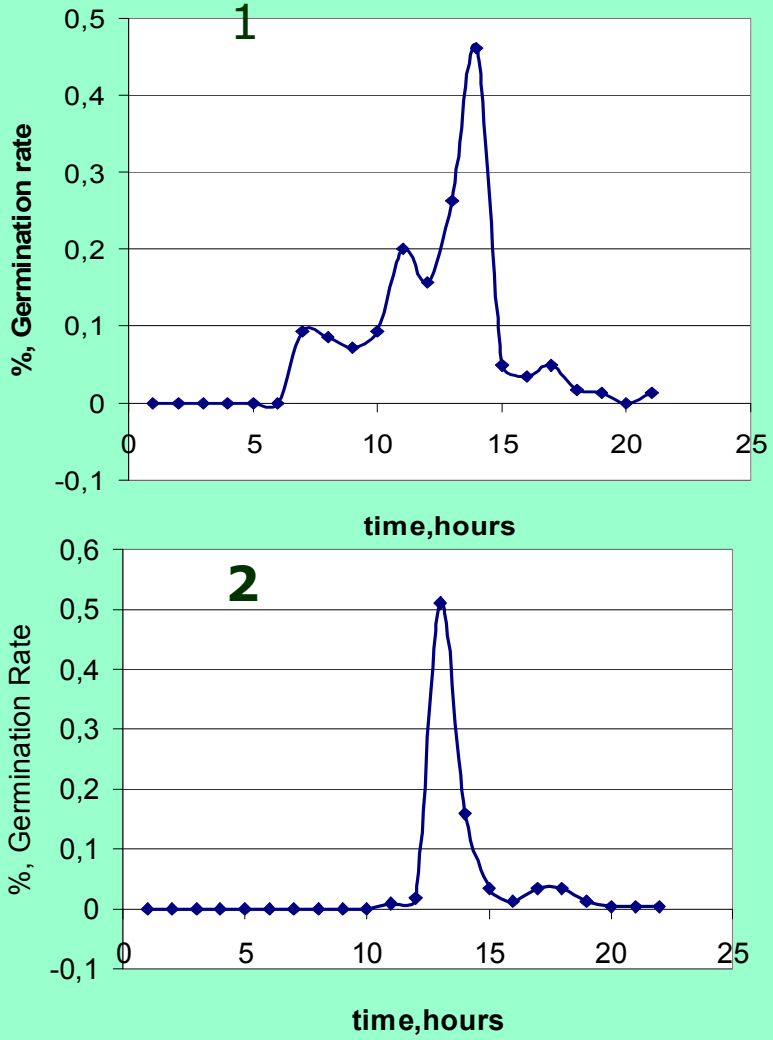
Object and Tasks

Object: assessment of epigenetic diversity= epigenetic polymorphism as the tool providing level of crop adaptive and productivity capacity.

The first task: Reveal possible connection physiological variability and epigenetic diversity (polymorphism)

The second task: Assessment of connection between level of epigenetic polymorphism and variety' ecological plasticity and productivity capacity

1. Reveal possible connection physiological variability and epigenetic polymorphism



Most visible crop variable characteristic is germination time of seeds. Asynchrony of germination for any seeds random sampling is wide-spread phenomenon both wild and cultivated plants.

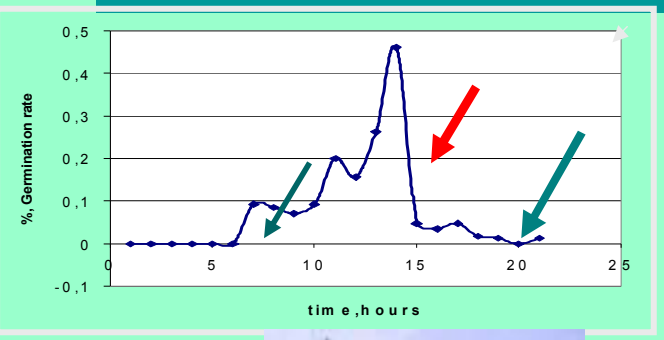
Example: Germination Rate Distribution winter wheat seeds "Podolyanka (1) and "Favouritka" (2) varieties.

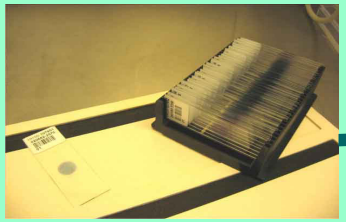
Research was focused on the study of connection of variability this characteristic with diversity of :

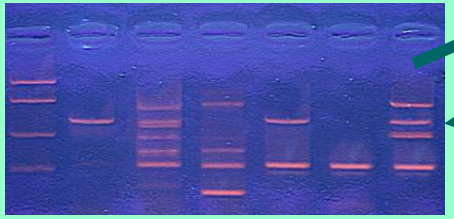
1) stress resistance and) epigenetic pathway;

DNA methylation pattern was used as marker of specific epigenetic pathway

General research methodology :







Every series of experiment include:

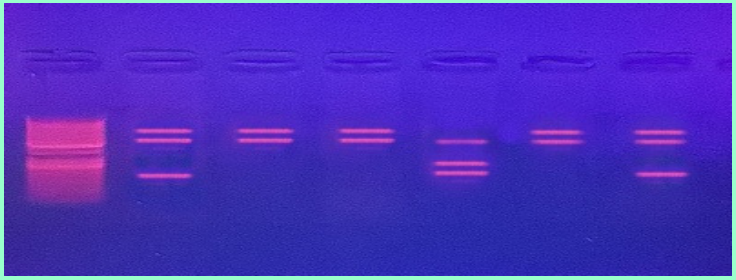
i) Selection seedlings with different time of germination: fast germinating subpopulation (FG), middling (MG) and slowly (SG) germinating subpopulation;

ii) Estimation of stability, adaptation capacity, state of immunity of different subpopulation in independent acute or chronic experiments;

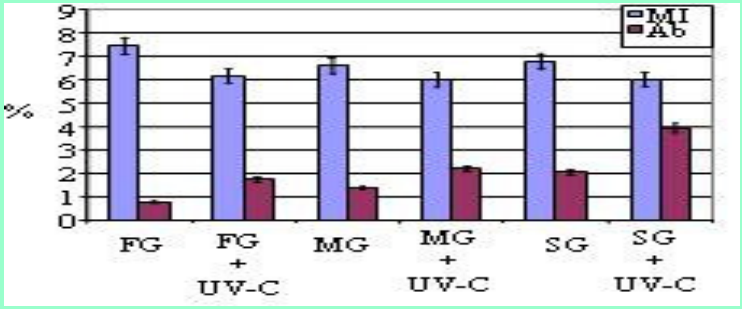
iii) DNA methylation pattern assessment with restriction analysis and PCR;

iiii) Estimation of "epigenetic distance', = DNA pattern methylation difference (D) with statistic methods.

Formation of Crop stability strategy



The electrophoregram of MspI- restricts' ITS - amplification. 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 cytogenetic parameters of root meristems under UV-C exposure of corn seedlings with various germination rates; MI – mitotic index; Ab – chromosome aberration yield; SG – slow-germinated; MG – middle-germinated; FG – fast-germinated.

Plant with different germination rate have <u>own epigenetic pathway</u> both control and UV-C exposed variants.

Plant with different germination rate have <u>own resistance capacity.</u> Plants fast germinated group (FG), in control and after UV-C exposure have lowest level of DNA damage compare with seedlings middle (MG) and slow (SG) germinated group.

Really every plant has own resistance strategy and capacity. <u>Crop resistance is cooperation of individual plant diverse resistance capacity.</u>

Formation of Crop adaptive capacity

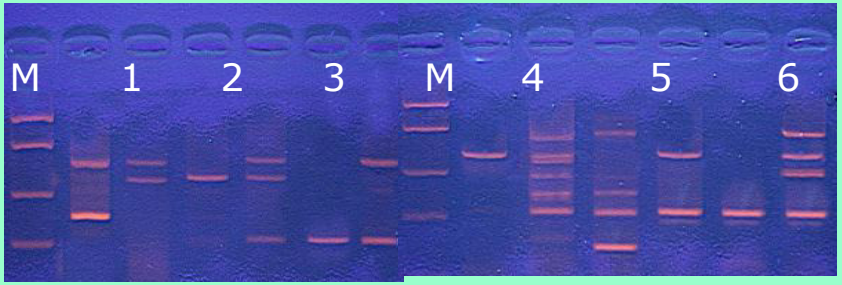
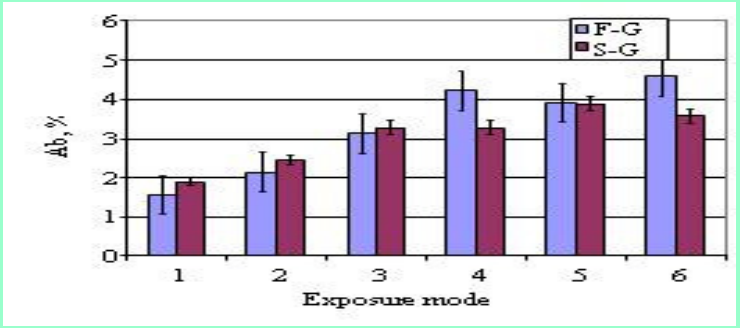


Figure 1. The electrophoregram of MspI- restricts' ITS - amplification.
1,2). K, (FG and SG) 3,4). Adaptive exposure (1 kJ/m2); 5,6). Adaptive exposure, in 4 hours – challenging one (6,2 kJ/m2); 7,8). Whole dose exposure (7,2 kJ/m2); exposure simultaneously with the challenging irradiation of variant 3; 9,10). Adaptive exposure, in 1 day – challenging one (6,2 kJ/m2); 11,12). Whole dose exposure (7,2 kJ/m2);

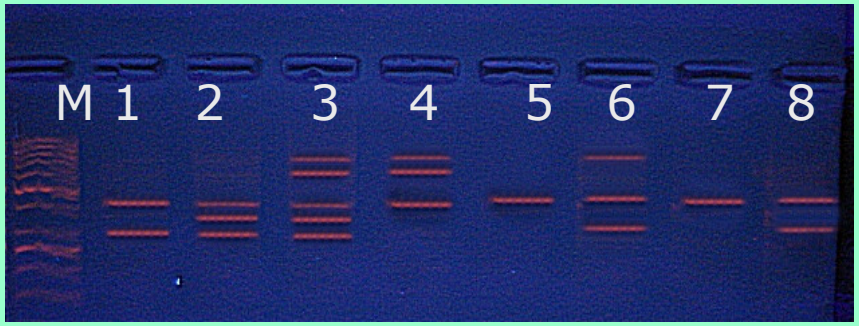


Chromosome aberration' yield (%) for FG- and SG- seedlings
1). K, (FG and SG) 2). Adaptive exposure (1 kJ/m2); 3). Adaptive exposure, in 4 hours – challenging one (6,2 kJ/m2); 4). Whole dose exposure (7,2 kJ/m2,4 hour); 5. Adaptive exposure, in 1 day – challenging one (6,2 kJ/m2); 6). Whole dose exposure (7,2 kJ/m2);

Seedlings with different rate of germination have different epigenetic pathway both control and at every mode of UV-C exposed variants. Fast germinating seedlings have less DNA damages in control and at adaptive exposure, visible reduction damages after challenger exposure compare with "full" dose.

Crop adaptive capacity is cooperation of individual plant diverse capacity.

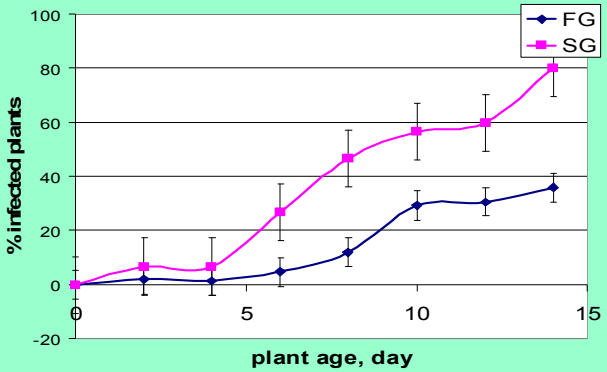
Formation of Crop immunity capacity



The electrophoregram of MspI restricts' ITS - amplification

1FG,2-SG -Podolianka; 3-FG,4-SG; Favoritka; 5-FG,6-SG; Lymarivna; 7FG,8-SG;-Novokyivska

Development of fungi infection the winter wheat plants «Podolianka" variety



Development of fungi infection the winter wheat plants «Favoritka" variety

Seedlings with different germination rate have different epigenetic pathway, different initial level of fungi infection and different rate of infection development.

Crop immunity is cooperation of individual plant diverse immunity

2.Assessment of connection between level of epigenetic polymorphism and ecological plasticity and productivity capacity of variety

Any variety have both phenotypic and epigenetic diversity and crop stress resistance is cooperation of individual plant resistance capacity. Plants belong to one variety have different stability to the environmental factors and pass ontogenesis different epigenetic pathway.

Question: How epigenetic diversity so-called epigenetic distance INSIDE variety corresponds to general, nonspecific stability to the environmental factors or the so-called ecological plasticity of variety. Meta analysis for different winter wheat varieties was carry out. Connection of ecological plasticity with level epigenetic polymorphism was explored. The grade of ecological plasticity was determined with use official «passport of variety»; one specified the variation of variety productivity, tolerance to terms of sowing, fertilizing, previous crop. Every variety have own rank and epigenetic distance (D). This permit carry out estimation of Spearmen correlation

Properties of Winter Wheat Varieties, Their Ranking and Epigenetic Distance across Each Variety

(Rs= 0,7)

Variety	Yield capacity		Ecoplasticity	Total Score	Rank of variety	D, Epigetetic distance
	centners/ha ners/ha	Score				
Smuhlianka	60- 115,1	3+	0	3+	1	0,02
Podolianka	60,0 - 96	1+	6+	7 +	5	0,126-0,21
Sotnytsia	50,2-102,6	2+	2+	4 +	2	0,004
Natalka	50,3- 93,6	1+	4+	5+	3	0,108
Darunok Podilia	50,4- 91,4	1+	3+	6+	4	0,01
Favorytka	50,6 -24,0	3+	3 +	6+	4	0,0056
Lymarivna	61,2 -100,0	1+	6+	7+	5	0,21
Novokyivska	50,7- 104,8	2+	6+	8+	6	0,1- 0,13

Note. Yield capacity: "1+"maximal yield > 90 centners/ha; "2+" yield > 100 centners/ha; "3+" maximal yield 110 centners/ha.Ecoplasticity: "1+" tolerance for sowing time; "2+" - I tolerance for sowing time + tolerance for mineral fertilizers; "3+"- tolerance for sowing time + tolerance for mineral fertilizers, + tolerance for previous crop

Conclusion

Future cultivated plant breeding needs to pay attention not only on the search of «good» genes but also on the search of «good» genomes, which successfully cooperate with an environment and create the higher diversity of epigenetic pathway. Crops with the higher diversity of epigenetic pathway (epigenetic distance) has a less competition and will give more high harvest at a less agritechnical care.

THANKS!