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## DNA METHYLATION IN PLANT ADAPTATION TO CHANGING ENVIRONMENT

The article presents a mini review of the current and updated, significantly expanded in recent decades information on DNA methylation changes in plant responses to unfavorable environmental factors, which allows it to consider as ecological epigenetics (eco-epi). Epigenetic regulation of gene expression is considered as the main source of adaptive phenotypic plasticity. We emphasize a great potential of further studies of the epigenetic regulatory systems in phenotypic plasticity of a wide range of non-model species in natural populations and agroecosystems for our advanced understanding of the molecular mechanisms of plant existence in the changing environment and thus for forecasting the effects of global climate changes on biodiversity and crop yield. Specific taxa of the Ukrainian flora which, in authors' opinion, are promising and interesting for this type of research, are recommended.

*Keywords:* adaptation, biodiversity, DNA methylation, epigenetics, phenotypic plasticity

Understanding the mechanisms of the functional relationships of plants with the environment, which ensure their growth, development, reproduction and distribution in various regions of the world, not only remains a primary problem of theoretical and experimental biology, but also becomes more acute under the current conditions of global climate changes and anthropogenic pressure on the biosphere. Increasing air temperature, drought or flooding significantly change the conditions for plant existence in biotopes, to which sessile plants have to quickly adapt. Phenotypic plasticity, which is a fundamental property of all living organisms, is believed to be the basis for the survival and preservation of plant populations, as well as a key element of the evolution and ecological relationships of species in biotopes [1–3]. Phenotypic manifestations in gene expression are already defined at the transcription level and include a very broad spectrum of ecologically important traits — physiological, biochemical, anatomical and morphological, i. e. traits which influence fitness and specifically are studied by ecological genetics [4]. It is postulated that phenotypic

plasticity is carried out within the limits of the reaction norm on the basis of metabolic, hormonal, and epigenetic regulation of gene expression and provides two strategies of the adaptation process: (1) rapid adaptation (acclimation) in response to daily and seasonal fluctuations of environmental factors and (2) long-term adaptation (acclimatization) to moderate chronic effects of adverse changes in ecological factors often resulting in the appearance of a new ecotype.

The wide distribution of epigenetic regulatory mechanisms in plant development and a high plasticity of the epigenome compared to the genome and its sufficient stability to transmit adaptive changes in generations is assumed to be the main source of phenotypic plasticity [5, 6]. Epigenetic diversity in plant populations under unfavorable environment fluctuations sharply increases on an almost unchanged genetic background, that allows to say about ecological epigenetics (eco-epi) [7]. DNA cytosine methylation is regarded as a fundamental epigenetic mechanism of phenotypic variations [8–10]. Development of the efficient methods opened up new opportunities to examine this process in non-model wild species and crops with complex unsequenced genomes in the changing environment in an ecological aspect and for problem solving of plant breeding.

The authors briefly summarize the current information on the contribution of DNA methylation in response and adaptation of wild plants and crops to the action of adverse abiotic factors. Species of Ukrainian flora, which grow in diverse ecological niches, are proposed for such investigations.

### DNA methylation

In recent years, there has been tremendous progress in understanding the role of epigenetic regulation of plant responses to external stimuli, especially stress responses [11, 12]. It should be taken into account that the ways of perception and transduction of external signals in plants form the same basis for the simultaneous inclusion of these signals in the implementation of normal ways of their devel-

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opment and in adaptive responses to adverse environmental changes [13] that determines the complexity of epigenetic systems of plants and their unique components. Therefore, in our opinion, a key role of epigenetic changes in gene expression in plant adaptation to constant environmental fluctuations is determined by the peculiarities of plant biology, namely: widespread vegetative propagation by rhizomes, runners, bulbs, tubers and corms, as well as by vivipary and apomictic reproduction – adventitious embryony and aposporia, which seems to dominate in many perennials, but in fact, in combination with seed propagation, ensures in general the species performance; annual growth of perennial plants; modularity of organization and unlimited growth during a plant life cycle and finally by the variability of individuals in the population.

DNA methylation, histone modifications and small non-coding RNAs (interfering RNAs, microRNAs) are considered as components of epigenetic regulatory systems of gene expression. DNA methylation – a normal biochemical process of DNA modification after its replication. The most common site of DNA methylation is a cytosine (C) base immediately followed by a guanine (G), a base pair combination known as CG. An increase in the level of methylation in the 5'-region of the gene leads to a decrease or complete inhibition of gene expression, a decrease – to an increase in the level of expression. The primary structure of the gene does not change:

the gene with methylated bases encodes the same protein as the unmodified one. DNA methylation and gene silencing are largely dynamic during the ontogenesis of plants, with age and under the influence of external factors, changes in the level of DNA methylation occur. As we already mentioned, DNA methylation is considered the most stable mechanism of epigenetic control of transcription, i.e. the level of messenger RNA, recording and preserving epigenetic information [8, 9]. A textbook example of epimutation is the formation of actinomorphic flowers in *Linaria vulgaris* Mill. plants with zygomorphic flowers due to the silencing of the *Lcycloides* gene (a naturally hypermethylated *Lcyc* allele), which controls flower symmetry [14]. It was established that the silencing of the *Lcyc* gene is not caused by a mutation in the DNA nucleotide sequence, but by the stable transmission of the level of DNA methylation in this gene from generation to generation (Fig. 1).

A well-known example is also the silencing of the *SUPERMAN* gene in *Arabidopsis thaliana* L. (Heynh.), a result of which the number of functional stamens and carpels increases (Fig. 2).

A naturally occurring epigenetic change in the *Lycopersicon esculentum* L. SBP-box (SQUAMOSA promoter binding protein-like) gene resides at the Colorless nonripening (*Cnr*) locus resulting in formation of colorless fruits with a substantial loss of cell-to-cell adhesion was also found (Fig. 3).

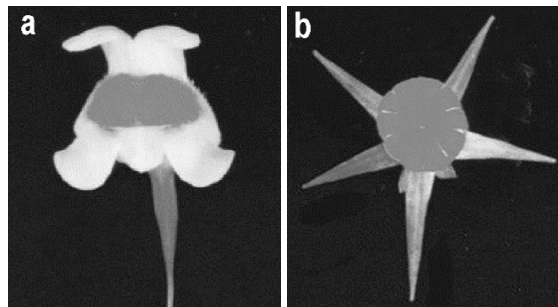


Fig. 1. *Linaria vulgaris*. Spontaneous epimutation: hereditary silencing of the *Lcyc* gene, which controls flower symmetry [14]. a – zygomorphic flower, b – pyloric flower.



Fig. 2. *Arabidopsis thaliana* flowers. a – wild-type with 6 stamens and 2 fused carpels forming the pistil, b – epimutant with 11 stamens and 3 incompletely fused carpels, c – epimutant with 9 stamens and 3 incompletely fused carpels [15].

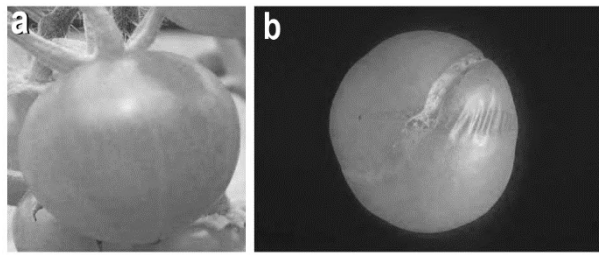


Fig. 3. *Lycopersicum esculentum* fruits. a – wild type, b – *Cnr* phenotype [16].

Until recently, the vast majority of research of a DNA methylation role in plant phenotypic plasticity has been performed with the model object *A. thaliana*. The development of the methylation-sensitive amplified polymorphism (MSAP) method, which is a modification of the amplified length polymorphism method, provided new opportunities for studying DNA methylation in plants with an unsequenced genome. The advanced MSAP-Seq method is based on conventional MSAP marker analysis and utilizes all major steps, such as methylation-sensitive restriction enzyme digestion, ligation of universal adapters, and polymerase chain reaction (PCR). However, the traditional gel-based separation of amplicons is being replaced by direct NGS next generation sequencing (NGS) of selected genome fractions. It was demonstrated its high efficiency for large-scale detection of DNA methylation in wild non-model and agricultural plants with large and complex genomes. It is emphasized that MSAP-Seq is as simple as the well-known and conventional MSAP, but using state-of-the-art NGS technology, enables high-throughput and direct analysis of DNA methylation modulation at hundreds of thousands of sites. Unlike traditional MSAP, it allows quantitative determination of DNA methylation changes, their direct localization and easy regulation of the number of obtained sequences [17].

#### Polymorphism of DNA methylation in species of natural flora and crops

In recent years, the database on the significant polymorphism of DNA methylation in plants of natural biotopes and agroecosystems in response to unfavorable environmental factors at the population and intra-population levels has been expanded, particularly in wild species: *Viola cazorlensis* Gand and *V. elatior* Fr. (Violaceae), *Laguncularia racemosa* (L.) C.F. Gaertn. (Combretaceae), *Hordeum brevisubulatum* (Trin.) Link., *H. murinum* L., *Arundo donax* L. (Poaceae), *Dactylorhiza traunsteineri* (Saut. ex Rchb.) Soó, *D. ebudensis* N.B. and *D. majalis*

(Rchb.) P. F. Hunt & Summerh (Orchidaceae), *Polygonum persicaria* L., *P. aviculare* L. (Polygonaceae) *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae), *Glycine soja* Sieb. et Zucc. (Fabaceae), *Thlaspi arvense* L. (Brassicaceae), *Populus alba* L. (Salicaceae), *Ilex aquifolium* L. (Aquifoliaceae) and crops: *Vicia faba* L. (Fabaceae), *Citrus reticulata* Blanco cv. Clementine (Rutaceae), *Vicia faba* L., *G. max* (L.) Merr. (Fabaceae), *Solanum lycopersicum* L. (Solanaceae), *Morus alba* L. (Moraceae), *Triticum aestivum* L., *Oryza sativa* L., *Zea mays* L., *Hordeum vulgare* L. (Poaceae) (see reviews: [18–21]). Below certain examples of plant ecological diversity and adaptation determining by epigenetic regulation of gene expression in response to external stimuli are considered.

*Fragaria vesca* L. is a perennial species growing throughout Europe, northern Asia, North America, and northern Africa in disturbed forests, forest edges, and meadows. To test a role of DNA methylation in local adaptation in natural ecosystems, authors selected plants growing in natural habitats in Italy, Czechia and Norway along a climatic gradient ranging from warmest to coldest mean annual temperatures and their transplanted ramets across all localities as well as employing experimental demethylation [22]. In authors' opinion, epigenetic variation may contribute to plant adaptation to local conditions in natural ecosystems that occur faster than *via* random genetic processes. Significant correlations between environmentally induced DNA methylation changes and phenotypic variation were found in clones of three *Solanum kurtzianum* Bitter and Wittm genotypes growing in its natural habitats: the Villavicencio Natural Reserve in an arid region of Western Argentina in two Andean experimental gardens located at 1141 and 2113 m above sea level. DNA methylation changes in wild potatoes induced by specific environmental differences are believed to play a role in plant phenotypic plasticity [23]. A higher level of global methylated DNA was detected in *Festuca amethystina* L. tetraploids

in comparison with *F. tatrae* (Czakó) Degen. diploids that suggests that ploidy level can influence DNA methylation in responses to drought, high temperature and high insolation. These species are related mountain grasses – an endemic diploid *F. tatrae* and a more widely distributed diploid and tetraploid *F. amethystina* with different ecological niches [24].

The further development of ecological epigenetics requires parallel studies of both the phenotypic plasticity in various ecological niches or under man-made adverse factors and epigenetic modifications, primarily DNA methylation. In our opinion, the following species from Ukrainian flora can be adequate and interesting objects for this kind of research: *Pinus sylvestris* L., capable of growing in conditions of excessive swamps and limited moisture are of the greatest interest – arenas takes first place. *Quercus robur* L. populations of which are presented on a wide ecological gradient from sandy-loamy to chernozem non-saline and sandy-clay slightly saline soils and can also grow in the condi-

tions of a flood regime (flooded oak forests) and limited moisture (pine-oak and cypress forests). *Alisma plantago-aquatica* L and *Sium latifolium* L. – aerial-aquatic plants, capable to flower and fruit on the upland, and *Arnica montana* L., *Vaccinium vitis-idaea* L., *V. myrtillus* L. and *V. uliginosum* L. – species of mountain regions growing at varying heights 300, 1600 and 2060 m above the sea level.

### Conclusions

In general, a wide range of non-model plant species of natural populations and agrocenoses is a great potential to study a role of epigenetics in the phenotypic plasticity for obtaining the new knowledge on the molecular mechanisms of plant tolerance/resistance in the changeable environment as the basis to forecast the consequences of global climate change for state and productivity of phytocenoses. Future possible applications of induced and natural epigenetic features, as platforms for crop improvement via epibreeding, are discussed [20, 21]. For example, it is proposed a novel strategy for improving maize yield through epibreeding [25].

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**МЕТИЛЮВАННЯ ДНК В ПРОЦЕСІ АДАПТАЦІЇ РОСЛИН ДО МІНЛИВОГО ДОВКІЛЛЯ**

Наведено короткий огляд сучасних досліджень змін метилування ДНК, які значно поширилися в останні десятиліття, у реакціях рослин на несприятливі впливи довкілля, що дає підстави говорити про екологічну епігенетику (еко-епі). Епігенетична регуляція генної експресії розглядається як основне джерело фенотипічної пластичності. Підкреслюється, що подальше вивчення ролі епігенетичних регуляторних систем у фенотипічній пластичності широкого кола немодельних видів природних популяцій та агроценозів сприятиме поглибленню уявлень щодо молекулярних механізмів існування рослин у мінливому середовищі, безпосередньо пов'язаних із ключовими завданнями прогнозу наслідків глобальних змін клімату та підвищення врожайності сільськогосподарських культур. Авторами рекомендовано види флори України, перспективні, на їх погляд, для такого роду досліджень.

*Ключові слова:* адаптація, біорізноманіття, епігенетика, метилування ДНК, фенотипічна пластичність.