

Epigenetics in plant breeding

Epigenetika u oplemenjivanju bilja

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Received: January 17, 2020; accepted: March 17, 2020

ABSTRACT

Current farming technology and advanced techniques of plant breeding are tending to enable high yields and cropping intensity, trying to alleviate the limitations of available arable land. However, rise in global population and climate changes could strain the ability to provide a stable food supply. Genetic diversity, which might be used for development of new, more resilient cultivars, could be a key for achieving better performance in agricultural production. New findings about how genes work and express, including the principles of epigenetics, could allow the advancements in breeding methods, and provide a new source of variability originating from epialleles. This paper provides a synopsis of the most significant epigenetic modifications, and particularities of plant species that impact epigenetic mechanisms, although it is mainly focused on application of epigenetics on plant breeding. Epigenetic aspects of breeding are described for increased yield in *Brassica napus* due to recursive selection for an epigenetic compound. Future application might be based on epigenetic recombinant inbred lines, similar to those in *Arabidopsis thaliana*, inhibition of DNA methylation in *Oryza sativa*, discovery of MSH1 system in *Glycine max* and *Solanum lycopersicon*. It also outlines the current issues and limitations of epigenetic breeding such as a lack of understanding of epigenetic mechanisms, interaction of epigenetic and stress responsive mechanisms, and the development of statistical models able to predict the impact and outcome of epigenetic modifications.

Keywords: epigenetic mechanism, genomic imprinting, epiallele

SAŽETAK

Postojeće tehnologije u poljoprivredi, kao i napredne tehnike u oplemenjivanju bilja nastoje omogućiti visoke prinose i intenzitet proizvodnje, uz ublažavanje ograničenja dostupnog poljoprivrednog zemljišta. Međutim, rast populacije u svijetu, ka i klimatske promjene, mogu ugroziti mogućnost stabilne opskrbe hranom. Genetska raznolikost koju bismo mogli upotrijebiti za razvoj novih, otpornijih kultivara, mogla bi biti ključna za ostvarivanje efikasnije poljoprivredne proizvodnje. Saznanja o načinu djelovanja gene i njihovoj ekspresiji, uključujući i principe epigenetike, mogla bi omogućiti napredak oplemenjivačkih metoda i pružiti novi izvor varijabilnosti koji proizlazi od epialela. U ovom radu pružen je pregled najznačajnijih epigenetskih modifikacija, kao i specifičnosti biljnih vrsta kod kojih postoje epigenetski mehanizmi, s naglaskom na primjenu epigenetike u oplemenjivanju bilja. Epigenetsko oplemenjivanje je opisano na primjeru porasta prinosa kod uljane repice, zahvaljujući ponavljajućoj selekciji epigenetske komponente. Buduća primjena epigenetike mogla bi se zasnivati na epigenetskim rekombinantama inbred linija, kao u primjeru arabidopsisa, inhibiciji DNA metilacije kod riže, ili otkriću sistema MSH1 kod soje i paradajza. Ovaj rad ističe aktualna pitanja u vezi epigenetskog oplemenjivanja, kao i njegova ograničenja, poput nedovoljnog razumijevanja epigenetskih mehanizama, interakcije epigenetskih faktora i mehanizama odgovora na stres, kao i nedostatka razvoja statističkih metoda u predviđanju utjecaja i djelovanja epigenetskih modifikacija.

Ključne riječi: epigenetski mehanizam, genomski otisak, epialeli

INTRODUCTION

According to the United Nations (UN) global population has risen from 5.3 billion in 1990 to 7.3 billion in 2015 and it is estimated that it will reach 11.2 billion people in 2100. Food and Agricultural Organization (FAO) claims that even though arable land per person is declining, technological advances allowed for higher yields and cropping intensity, enabling to meet rising food demands. A special report from Intergovernmental Panel on Climate Change (IPCC) named "Climate Change and Land" published in 2019, warns of climate change and its threat to a stable food supply. In order to ensure a stable and high quality food supply the humankind relies on advancements in fields and disciplines such as plant breeding. Plant breeding encompasses different fields of biology, using modern and traditional methods in order to improve plant performance, yield and quality as its primary goals (Sleper and Poehlman, 2006). Continual advancements in the field of biology provide novel tools needed to improve crops. One of these fields is epigenetics, that is today defined as "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence" (Wu and Morris, 2001 cited in Dupont et al., 2009, p.351).

Epigenetics and epigenetic modifications

Alleman and Doctor (2000) described genomic imprinting (GI) as "an epigenetic phenomenon in which the activity of a gene is reversibly modified depending on the sex of the parent that transmits it". GI is observed in both mammals and flowering plants. Key regulators of GI in plants are histone and DNA methylation. Biological role of imprinted genes is largely unknown, but it has been demonstrated that the majority of imprinted expression occurs in the endosperm (Alleman and Doctor, 2000; Rodrigues and Zilberman, 2015). DNA methylation is an epigenetic modification which involves a covalent transfer of a methyl group at the C-5 position of a cytosine base, creating a stable heritable epigenetic mark. This process is catalyzed by the enzyme class of methyltransferases (Law

and Jacobsen, 2010; Jin et al., 2011). In both plants and mammals, it is involved in: regulation of gene expression, genomic imprinting, suppression of repetitive element transcription, chromosome interaction, and silencing of transposable elements. Consequently, DNA methylation impacts genome stability and since it influences chromatin structure, it also controls the accessibility of genetic information (Jin et al., 2011; Bouyer et al., 2017; Zhang et al., 2018).

In plants it participates in gene imprinting and seed development, vegetative growth and pattern formation, fruit ripening, responding to environmental stimuli, responding to biotic and abiotic stress and in creation of heritable epialleles. Epialleles are heritable alleles that have been epigenetically modified. They usually occur in isogenic lines, but rarely happen spontaneously (Zhang et al., 2018). Unlike mammals, plants methylate both symmetrical CG, and asymmetrical CHG and CHH (H = A, T or C) sites (Jin et al., 2011; Bouyer et al., 2017). Another difference lies in the reprogramming of DNA methylation at germination and early embryogenesis. In plants, only the endosperm tissue shows significant reprogramming, while in sperm and egg cell, epigenetic marks are reinforced from neighboring nuclei via trans-silencing RNAs (Migicovsky and Kovalchuk, 2011; Pikaard and Mittelsten Scheid, 2014). Histones are small proteins that aid in packing genetic information into chromatin (Nelson and Cox, 2017). Histones are subject to different types of epigenetic modifications such as methylation, acetylation, phosphorylation, ubiquitination, sumoylation and ribosylation. Roles of these modifications are numerous, especially if they are combined (Peterson and Lanier, 2004).

Bourque et al. (2018) defined transposable elements (TE) as "DNA sequences that have the ability to change their position within a genome." They are separated in two classes, retrotransposons (class I) and transposons (class II). Main difference between the two classes is their intermediate, class I and class II use an RNA and DNA intermediate, respectively (Bourque et al., 2018). Transposable elements are the largest component of

the eukaryotic genome, especially in plants with large genomes, where only small fractions are active. Long terminal repeat (LTR) retrotransposons are the most represented component of plant genomes, and they can be activated under abiotic and biotic stress (Feschotte et al., 2002). TEs are under epigenetic control, and can beneficially affect gene expression if inserted near functional genes (Lisch and Benntzen, 2011). Non-coding RNAs (ncRNA) are RNAs that can contain information and have a function outside of encoding for proteins.

There is increasing evidence that suggests ncRNAs fulfill regulatory roles during development and assist in responding to stress and different environmental stimuli. There is differentiation between infrastructural and regulatory ncRNAs. Infrastructural ncRNAs include ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs). Regulatory RNAs include microRNAs (miRNAs), Piwi-interacting RNAs (piRNAs), small interfering RNAs (siRNAs), and long non-coding RNAs (lncRNAs). Recent evidence shows that infrastructural ncRNAs have certain regulatory functions (Mattick and Makunin, 2006). Regulatory ncRNAs are strongly linked to epigenetic processes like directing of cytosine methylation and histone modifications, and regulation of gene expression by small RNAs. siRNAs participate in management of TE activity and silencing, X-chromosome inactivation, genomic imprinting, and paramutations (Costa, 2008). Paramutation is an epigenetic phenomenon that involves transfer of information from one allele of a gene to another, which establishes a state of gene expression that is heritable for generations (Chandler, 2007). Paramutation violates Mendelian rules of heredity, because it enables changes in expression of an allele under the influence of the other allele in a heterozygote, which in turn leads to a non-Mendelian inheritance pattern (Grant-Downton and Dickinson, 2005).

Plant development and its impact on epigenetic mechanisms

Plants have a haplodiplontic life cycle that consists of multicellular haploid and multicellular diploid stages (Gilbert, 2000). The male and female gametophytes

are made up of multiple cells produced by mitotic division. Since epigenetic information is reinforced from neighboring nuclei, it can be passed on through meiosis. Plants also lack a defined germline in early embryogenesis, which could allow transference of epigenetic marks that occurred in the meristem onto germ cells produced later in plant development (Pikaard and Mittelsten Scheid, 2014). Plasmodesmata (PD) enable transport of compounds between plant cells, among them also mRNAs, siRNAs and sRNAs. Discovery of their transport is significant since sRNAs have a role in gene expression via transcriptional and post-transcriptional RNA silencing (Hyun et al., 2011). PD also allow the exchange of diffusible epigenetic signals between distant plant organs, suggesting the possibility of epigenetic modifications occurring in meristem cells and gametes due to such signals. Somaclonal variation is a phenomenon that can occur in tissue culture when cloning is done via somatic embryogenesis, and it is ascribed to epigenetic modifications. Such modifications could prove useful for plant breeding and selection of adaptive traits (Pikaard and Mittelsten Scheid, 2014).

Applying epigenetics in plant breeding

Epigenetics could provide a push in plant breeding via selection for favorable epigenetic states, creation of new epialleles, and regulation of transgene expression (Springer, 2013). Development of new tools will allow researchers to access and quantify existing epigenetic variations that contribute to important traits like respiration and energy use efficiency, especially agronomical traits such as yield and seed quality (Gallusci et al., 2017). Deeper understanding of epigenetic mechanisms could provide a more efficient way of improving crops (Springer, 2013).

Use of epigenetics in plant breeding until today

Although epigenetic breeding is in its infancy, new discoveries and further understanding of epigenetic mechanisms and pathways, advancements in screening methods, and use of advanced genetic tools becoming more readily available, allowed certain strides. An example of accomplished epigenetic breeding was demonstrated by increasing the yield of *Brassica napus*

via selection through an epigenetic energy use efficiency (EUE) component Hauben et al. (2009). They have achieved higher yield by recursive selection from an isogenic population. Another important discovery was the creation of epigenetic recombinant inbred lines (epiRILs) in *Arabidopsis thaliana*. That was accomplished by the use of DNA methylation mutants (Zhang and Hsieh, 2013). Crosses of epiRILs and wild type resulted in hybrids that showed a stable significant phenotypic variation in traits like flowering time, plant height and fruit size (Schmitz and Ecker, 2012). The same is currently not possible for crop plants due to the lack of DNA methylation mutants, but they might be created by inhibiting DNA methylation or causing histone modifications. This was demonstrated for *Oryza sativa ssp. japonica* by Akimoto et al. (2007). Via the use of a methylation inhibitor, they created a stable phenotypically diverse population, in which resistance to *Xanthomonas oryzae* pv. *oryzae* appeared. Furthermore, one of the proposed ways for inducing agronomically valuable epigenetic variation is the MSH1 system that is described in further detail by Raju et al. (2018). Use of MSH1 system is exemplified in experiments with *Glycine max* (Raju et al., 2018) and *Solanum lycopersicon* (Yang et al., 2015). In *G. max* an increase in yield and its stability was achieved in comparison to the wild type. In *S. lycopersicon* an enhanced heritable vigor was achieved.

Possible future uses of epigenetics in plant breeding

Existence of epialleles has been demonstrated in natural populations (Spinger and Schmitz, 2017). Even if they show lower levels of transgenerational heritability, but are stable enough to be passed on mitotically, they still have the potential to be used in crop improvement. Epigenetic marks can be passed on in cultures that are clonally propagated or regenerated via cell culture (Spinger, 2013). What remains contended is whether epigenetic markers can be transmitted meiotically. There is evidence that in some cases they cannot be inherited, while in others they are at least partially inherited (Gallusci et al., 2017).

Epialleles could provide a valuable source of morphological variation. But identification of such

epialleles experimentally is a slow process mostly relying on spontaneous epimutations (Spinger and Schmitz, 2017). Discovering new epialleles might be efficiently achieved using comparative epigenome studies. Epialleles induced in epiRILs could prove to be a new way of creating variation in complex traits, but there are challenges due to usually large genomes of crop plants and their long generation times. Inducing epigenetic shocks could still provide us with invaluable heritable phenotypic variation (Gallusci et al., 2017). Novel methods proposed by Paszkowski (2015) outline the possibility of controlled use of retrotransposition to unlock genetic, epigenetic and finally phenotypic variation that might be valuable for crop improvement. From several studies, it has been gathered that environmental stimuli invoke epigenetic change brought on by stress conditions, such as high salinity, drought, heat, and UV radiation. Some of these changes are potentially stable, but most last as long as the stress lasts (Zhang and Hsieh, 2013). During environmental stress, being biotic or abiotic, research has shown significant epigenetic changes in certain plants. Due to stress in some cases a form of 'memory' is created that can 'prime' the plant for the next time it encounters that stress (Spinger, 2013). There is evidence that plants are able to transmit it to their progeny as a form of transgenerational memory that leads to a rise in genome instability, higher tolerance of the progeny to the stress experienced by their progenitors, and a cross-tolerance to different stresses (Bilichak and Kovalchuk, 2015). But there is little evidence that improved genotypes can be created in this fashion (Spinger, 2013). However, recently discovered technique of engineered DNA-binding domains could enable precise epigenome engineering at specific loci. If epigenetic marks associated with specific stressors could be identified, they could allow the creation of stress tolerant plants (Bilichak and Kovalchuk, 2015). One of the biggest challenges in use of epigenetic modification for crop improvement will be the predictability of the impact of those variations on the phenotype and its performance. Attempts have been made in creation of statistical models that are able to predict the epigenetic impact on trait behavior in *Arabidopsis* and *Mimulus guttatus*. Statistical

models are flawed since they are context specific, while process-based models are able to simulate the impact of epigenetic marks on gene expression and predict the outcome using mathematical equations. They are difficult to develop, but they were successfully created to predict lycopene production during maturation of *S. lycopersicum* (Gallusci et al., 2017).

CONCLUSIONS

Understanding mechanisms that stabilize epigenetic states, interactions between sRNAs and DNA methylation, histone modification, and transcription would allow us to use the concept of epigenetic breeding (like in the case of EUE epigenetic component) to further improve even top-performing hybrids that could potentially outperform heterosis. Also, it could assist in creation of cultivars better prepared for biotic and abiotic stress through transgenerational memory, fine tune their traits via epialleles to fit specific environments, and induce epimutations for new sources of variability. It is clear that the complexity of epigenetic mechanisms and pathways, and their interaction with existing genetic and physiological structures, will make it challenging to harness epigenetics and implement them in standard crop improvement. Future of breeding might indeed have an epigenetic component that is regularly used alongside other tools, in order to ensure a high quality, stable food supply to a growing global population.

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