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Review article

Epigenetics : History, present status and future perspective

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Abstract

Epigenetics refers to the heritable changes in the pattern of gene expression resulting from the modification of DNA bases, histone proteins and/or non-coding-RNA biogenesis without altering the underlying nucleotide sequence. Genome-wide epigenetic variations are being reported which are often associated with variation in gene expression. Many of these changes occur during developmental processes and stress exposures. Both, the level of gene expression and the epigenetic changes may relapse to the pre-stress state shortly after removal of the stress. One of the common mechanisms involved in epigenetic changes is methylation of 5th carbon by the action of the enzyme DNA methyltransferase. In addition, histone proteins are post-translationally modified which may affect transcription, DNA replication, chromosome segregation/condensation, and/or DNA repair process. Small-RNA (particularly small-interfering RNAs) play a crucial role in DNA methylation via RNA-directed DNA methylation (RdDM) pathway. The epigenetic changes in plants induced by aforesaid processes can be inherited over the generations in the form of epialleles. Epigenetic change in genes caused by DNA methylation and/or histone modifications during plant development often results in phenotypic changes. It is becoming increasingly evident, that epigenetic changes have important roles to play in acclimatization, stress tolerance, adaptation, and evolution processes. With the growing reports on epigenetic changes affecting gene expression, it would be worth investigating the epigenetic machinery of gene regulation in plants, and their possible utilization in crop improvement. This review focuses on the historical development and basics of epigenetics followed by the present status and future prospects in crop improvement.

Key words: DNA methylation, evolution, gene regulation, histone modification, stress memory

Introduction

Ever since the domestication of plants, considerable progress has been made in agriculture due to human behavioral changes from food gathering to farming. Domestication followed by selection of plants with desirable traits, breeding varieties for higher yield, tolerance to abiotic and biotic stresses, better quality and nutrition (Singh et al. 2002; Nandakumar et al. 2008; Parida et al. 2009; Marathi et al. 2012) and the technological advancements (Nandakumar et al. 2004; Kumar et al. 2006; Chikkappa et al. 2011; Kumar 2014) for generating better agricultural inputs enabled more than four times increase in food grain production in India from 50 million tons in 1950 to 273 million tons in 2016 (Kulkarni 2017). While plant breeding aims at developing newer crop varieties with wider adaptation to the changing climatic condition, understanding the adaptation process in plants to the changing environmental conditions is also an interesting phenomenon. Therefore, researchers have been interested in deciphering the underlying mechanisms that plants have evolved to adapt to diverse environments, particularly different types of biotic and abiotic stresses (Joseph et al. 2004; Basavaraj et al. 2010). A French biologist Jean-Baptist Lamark (1744-1829) proposed the theory of 'soft inheritance' or 'inheritance of acquired characters' describing that an organism can pass on the characters to its offspring that it acquires during its lifetime. Later on, Charles Darwin published his book 'On the Origin of Species' in 1859 wherein he proposed the 'theory of evolution by natural selection' and emphasized on the use and

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disuse inheritance, but rejected the Lamark's theory of inheritance of acquired characters. Darwin described the natural selection as the process in which struggle for existence and survival of the fittest has a similar effect to that of artificial selection involved in the selective breeding. Later on, Gregor Johann Mendel (1822-1884) proposed 'Laws of inheritance' which supplanted the notion of inheritance of acquired traits. Despite this abandonment, interest in Lamarckism continued.

Remarkably, Lamark, Darwin, Mendel and other biologists of that time had a little understanding of the molecular mechanisms of the inheritance of traits. Integration of Darwin's theory with the advancing genetic and molecular sciences facilitated the development of a well-supported neo-Darwinian theory of evolution. The current concept of evolution is based on genetics and mutation deploying random DNA sequence alterations in the creation of genetic variation that affects the phenotype and trait. Most of the proposed models in evolutionary biology include the changes in the nucleotide sequence as a primary molecular mechanism behind the heritable phenotypic changes (Laland et al. 2014). One of the mysteries of evolutionary theory had been the extremely low frequency of potentially advantageous genetic mutations. Recent studies demonstrate that the genetic variations are sufficient for evolution, but genetic theory alone faces difficulty in explaining some features of evolution (Ho and Zhang 2014). Explaining genotypic variations with the rapid evolutionary changes under environmental pressure has become difficult using the classical genetics alone. The rate of phenotypic variations and genetic mutations are considerably different, which cannot be explained merely based on genetics as the primary molecular mechanism. Additional mechanisms such as epigenetics can help explaining this enigma (Kumar 2017a). Many traits do not follow normal Mendelian inheritance and are difficult to be explained by the classical genetics. The recently documented molecular mechanisms such as epigenetics can help explaining such genome activity and phenotypic variations (Skinner 2015). If epigenetics is considered as a complementary molecular mechanism, many of the phenotypic variations (e.g. dissimilarity between the clones) can be easily explained.

Plants, being sessile, are exposed to multiple environmental stresses. The knowledge of biochemical, physiological and genetic mechanisms of stress tolerance could enable us to understand several aspects of plant's ability to cope with the stresses. Until the last century, it was thought that isolation of the gene(s) associated with a trait of interest was sufficient to transfer the trait to a crop plant and to achieve the expected phenotype (Kumar et al. 2010; Kumar et al. 2013). Recently, definitive evidence has been gathered for the DNA to provide only part of the genetic information for a trait, and that chromatin changes also contribute to the expression of the trait. DNA (cytosine) methylation, post-translational modifications (acetylation, methylation, phosphorylation, etc.) of histone proteins, and regulatory RNAs (non-coding RNAs or ncRNAs) define distinct chromatin/epigenetic states of the genome (epigenome), which vary with the changing environmental conditions. Thus, chromatin is a highly dynamic structure which carries diverse information: (i) the one encoded by the DNA sequence, and (ii) those provided by the epigenetic states. Since the epigenetic states of chromatin are variable, transfer of a trait from one species to another not only requires the transfer of the gene(s) associated with the trait but also the appropriate chromatin/epigenetic states so as to enable the trait to express. It is, therefore, essential to study the epigenetic states in the donor plant/species and to ensure proper re-establishment of the epigenetic state of the genes in the recipient plant/species for their expression under the appropriate (de)methylation level. However, epigenetic mechanisms of gene regulation are yet to be fully understood and utilized as epialleles (the alleles that are genetically identical but epigenetically different due to the epigenetic modifications, showing variable expression) in crop improvement programs.

Epigenetics: A missing link in genetics

William Bateson and Caroline Pellew observed an interesting phenomenon in 1915, i.e. rogue pea passing their "rogue" phenotype to the progenies. Later, R. A. Brink in 1950s noticed interaction between two alleles of *b1* (booster 1) locus (*B–I*: paramutable allele, active; and *B*': paramutagenic allele, inactive). This interaction between the two alleles (possessing the same DNA sequence) of a single locus in maize resulted in a heritable change in one allele that is induced by the other allele (Brink 1956). The *b1* locus codes for a transcription factor (bHLH) which activates the genes of anthocyanin biosynthesis pathway in maize. At that time, Brink could not explain the genetic basis of the observed phenomenon based on the available

knowledge in the field of genetics. Therefore, the phenomenon was termed as paramutation, as this did not fit into the definition of mutation. Brink also reported that the influence of paramutagenic allele persists for several generations. Now, the *B–I* and *B*' alleles in maize are known to posses variation in DNA methylation in the tandem repeats near the coding region of the gene. Such variation in cytosine methylation has been reported to be the feature of the paramutagenic *B'* allele, and when a paramutable *B-I* allele gets converted into paramutagenic, it acquires the same DNA methylation pattern. In order to inherit methylation, RNA-dependent RNA polymerases, as well as other components of RNA-silencing pathways, are required, suggesting that paramutation is mediated through the endogenous RNA-silencing pathway (Alleman et al. 2006). In addition to the variations in DNA methylation, histone modifications in the methylated genic regions have also been observed to mediate paramutation in different cases.

Human genome sequencing could not provide the anticipated answers about the causative mutations in case of many diseases. Over the past decade, it has become clear that merely the knowledge of DNA sequence is not sufficient to explain the aberrations associated with heritable diseases. It is now widely accepted that epigenetic mechanisms can facilitate orchestration of development from embryo to adult animal, explain aging, variations observed in the heritable diseases, clones, paramutations which were difficult to explain based on the principles of genetics.

What is epigenetics?

One of the characteristic features of epigenetics is exhibition of alternative phenotypes by the same genome because of its different epigenetic states. The term epigenetics was first used by Conrad Waddington in early 1940's without having the understanding of its molecular basis of action. The Greek prefix *epi* (means over, outside of, around) in *epi* genetics implies that the features are "on top of", "in addition to" or "from outside of" the classical genetic basis of inheritance. Waddington at that time tried to integrate this new knowledge of genetics in embryology. He provided new insight into the gene and environment interactions in *Drosophila* and demonstrated that temperature-shock after puparium formation caused morphological (cross veinless wings) variation in flies. Definitions of epigenetics have evolved with increasing clarity of the molecular mechanisms involved in it and a better understanding of the genetic phenomena. Considering the current molecular understanding, epigenetics is defined as the studies of molecular processes in and around DNA that control genome activity independent of the DNA nucleotide sequence which may be inherited through mitosis or meiosis (Kumar and Singh 2016). These epigenetic mechanisms include DNA methylation, histone protein modifications and biogenesis of ncRNAs (Kumar 2017a). Environmental factors have also been reported to promote epigenetic variations. A number of researchers have proposed the role of epigenetics in the evolution process, primarily as a sensible and responsive molecular mechanism in the natural selection (Pigliucci 2007; Laland et al. 2014).

Many of the traits of economic importance such as flowering time, yield, abiotic stress tolerance etc. are complex in nature and controlled by the joint action as well as interactions of multiple genes. Recent findings indicate that heritable variations in a trait may also be caused by epigenetic changes such as enzymatic modifications of DNA base or histone proteins that control transcriptional activity of genes, repetitive sequences and transposable elements (Allis et al. 2007; Richards 2011). Transgenerational stability of the epigenetic marks requires their passage through the germline without being erased by the mechanisms that ensure the establishment of cellular totipotency at the time of ontogenesis. The observed transgenerational legacy of the epialleles in different organisms, including plants, indicates that resetting epigenetic changes to default-state is probably a leaky process (Hauser et al. 2011). There is limited evidence for naturally occurring epialleles, and yet we know only a little about phenotypic and ecological consequences of the epigenetic variations (Manning et al. 2006). One of the difficulties in correlating phenotypic effects with the epigenetic variations is that epigenetic and phenotypic variations covary in natural systems, which make it difficult to unravel their effects on phenotype (Richards et al. 2010). However, there is a way which can be used to minimize the difficulty including (i) studies on the natural epialleles, (ii) manipulation of DNA methylation using chemical demethylation agents such as 5-azacytidine, and (iii) studies with epiRILs. EpiRILs are identical for the DNA sequence but carry the difference(s) in the epigenetic mark(s) due to recombination. They are generated by crossing two near-isogenic parental lines (one of them is a mutant for methyltransferase 1, while the other is a wild-type plant) which are variable at epigenetic level but identical for DNA sequence of the gene. Since the methylation-

mutant is deficient in the DNA methylation machinery, it shows a genome-wide decrease in DNA methylation compared with that in the wild-type plant. Molecular analyses have demonstrated that variation in methylation pattern in epiRILs is stable over the generations (Johannes et al. 2009; Reinders et al. 2009); thus, epiRILs may prove to be a powerful tool for epigenetic studies in plants.

Components of epigenetics

Propagation of epigenetic marks in plants takes a much more direct route than that in animals for transmission of cytosine methylation from one generation to the next. Studies on the evolutionary implications of DNA methylation are being carried out in a number of plant species. In addition to DNA methylation, histone modifications and ncRNAs play an important role as epigenetic marks in the expression of genes. For example, IBM1 (a histone demethylase) functions to remove heterochromatic histone marks from genes and limits this mark to transposable elements (TEs). Inheritance of epigenetic marks (e.g. natural variation of DNA methylation associated with the environmental changes) over the generation has been reported (Zheng et al. 2017) and may have a genetic (single nucleotide polymorphisms-driven gene-body methylation) root cause. It has also been reported that the rate of spontaneous epimutations is higher in the CG context because these sites are not retargeted by RdDM. It has also been proposed that CG epimutations can be viewed as a molecular clock (Slotkin 2017).

DNA methylation

DNA is composed of four different bases: adenine (A), cytosine (C), guanine (G) and thymine (T). DNA also contains 5-methylcytosine (5-mC), N^4-methylcytosine and N^6-methylcytosine in small amounts. The 5-mC (now also known as the 5th base of DNA) is common among these. It was identified long before the DNA was recognized as the genetic material (see Kumar and Singh 2016). The genetic information of a gene is concealed in the nucleotide sequence; however, epigenetic changes cause variation in the gene activity without making any change in the nucleotide sequence. DNA methylation indicates the addition of a methyl group at the 5th carbon of the pyrimidine ring of cytosine as a post-replicative event. Cytosine of nuclear DNA may get methylated by the action of an enzyme called DNA methyltransferase, wherein the methyl group is provided by *S*-adenosylmethionine (Fig. 1). Deamination of cytosine residue converts it to uracil,

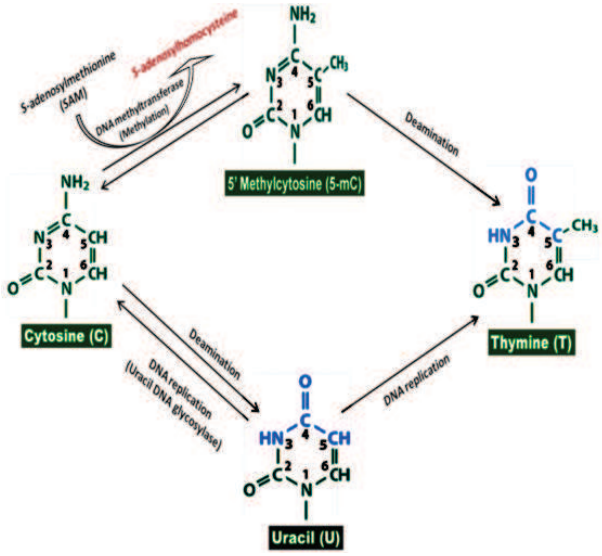

Fig. 1. Modification of pyrimidine bases by (de)amination, and their replacement during DNA repair and replication processes

which either gets repaired by the action of Uracil DNA glycosylase enzyme in due course of DNA repair process or gets replaced by thymine during DNA replication. Thus, the absence of U in DNA is very much essential; otherwise, in due course of time all the cytosines will be replaced by uracils and C will disappear from DNA over the time. As soon as U appears in DNA (due to deamination of C), it is recognized as a foreign base and removed by the glycosylation process. Since DNA is the carrier of genetic information, such changes/substitutions in nucleotide sequence would lead to instability of the genome.

In plants, cytosine methylation occurs in CG, CHG and CHH contexts (where H = A, C or T), while in somatic cells of animals/vertebrates, cytosine methylation is limited to CG context (Wang et al. 2016). In plants, CG methylation is maintained by MET1 (Methyltransferase 1) and by VIM (Variation in Methylation) family proteins. CHG methylation is maintained primarily by plant-specific DNA methyltransferase CMT3 (Chromomethylase 3), which acts in conjunction with histone methyltransferase KYP (Kryptonite/SUVH4) (Jackson et al. 2002; Malagnac et al. 2002).

On the other hand, CHH methylation is maintained by DRM2 (Domains Rearranged Methyltransferase 2). Interestingly, in *Arabidopsis* DRM2 is responsible for *de novo* methylation in all the contexts of cytosine (Cao et al. 2003). DRM2 is

recruited to the target loci by a specialized 24 nucleotide small interfering RNA (RNA-directed DNA methylation pathway) (Pikaard et al. 2008; Wierzbicki et al. 2009; Law and Jacobsen 2010). On genome level, DNA methylation is controlled by the position and composition of nucleosomes and associated histone modifications (Chodavarapu et al. 2010). The chromatin remodelers DDM1 (Decrease in DNA Methylation 1) and DRD1 (Defective in RNA-mediated DNA Methylation 1) are important for CG methylation and non-CG methylation, respectively (Lippman et al. 2004). Approximately 5% of Arabidopsis genes show DNA methylation within promoter regions, which adversely affects transcription of the genes (Zhang et al. 2006; Cokus et al. 2008). Nearly one-third of Arabidopsis loci exhibit CG methylation within transcribed regions (gene-body methylation controlled by MET1) with a bias towards the 3′ half of the transcription unit (Zhang et al. 2006; Cokus et al. 2008). DNA methylation is enzymatically reversible by the action of bifunctional DNA glycosylases and AP lyases (Zhu 2009). ROS1 (Repressor of Silencing 1), DML2, and DML3 (Demeter-Like 2 and 3) act in somatic cells, and function in fine-tuning the methylation level at specific loci (Penterman et al. 2007). Demeter (DME) functions in the endosperm (extra-embryonic tissue of seeds) where it causes genome-wide hypomethylation resulting in imprinting of the maternal genome (Hsieh et al. 2009; Gehring et al. 2009). Cytosine methylation homeostasis is determined by the DNA methylation and demethylation processes. Promiscuous methylation is pruned by demethylases to create the desired methylation pattern. Demethylation of the promoter and/or coding region may also be required to activate expression of specific genes under the changing environmental conditions or during the developmental stages of plant (Li et al. 2017).

Histone modifications

In eukaryotes, DNA is densely packed into a chromatin structure. A nucleosome core particle is composed of protein octamer consisting of pairs of histone proteins *viz*., H2A, H2B, H3 and H4. A 146 base pair of DNA is wrapped in almost two turns around the protein complex. N-terminal tail of these histone proteins protrudes from the nucleosome core. Histone protein H1 binds at the DNA entry-exit site of the nucleosome and organizes the linker DNA in the formation of chromatin structure. The N-terminal histone tails are subjected to various post-translational modifications, which affect DNA-associated processes, such as chromosome condensation/segregation, replication, DNA repair, and transcription. Histone proteins have numerous evolutionary conserved lysine (K) residues that are subjected to acetylation (ac), methylation (me), ubiquitylation (ub) etc. (Miller and Grunstein 2006). Lysine can be either monomethylated (me1), dimethylated (me2) or trimethylated (me3) which may have different functional consequences (Sims et al. 2003). A variety of histone modifications and their possible combinations (such as H3K4me3 & H3K27Ac: activation marks, and H3K9me3 & H3K27me3: repressive marks) regulate transcriptional potential of a gene (Kouzarides 2007). The level of histone acetylation is controlled by histone acetyltransferases (HAT) and histone deacetylases (HDAC) (Pandey et al. 2002). Histone lysine (K) methylation is catalyzed by SET domain of histone lysine methyltransferases (HKMT) (Pontvianne et al. 2010). Histone lysine methylations have differential effects on transcriptional activity, depending on the site (K4, K9, K27) and mode (me1, me2, me3) of modifications (Liu et al. 2010). Histone lysine methylation can also be reversed by the action of two different types of histone demethylases.

Non-coding RNA biogenesis

Study of epigenetics must expand from DNA methylation and histone modifications to non-coding RNAs (ncRNAs) as they help to maintain chromatin state, chromatin-mediated gene silencing and mediate epigenetic modifications. Genetic analysis of the Arabidopsis mutants impaired for the genes involved in small interfering RNA (siRNA) biogenesis revealed the involvement of siRNAs in RNA-directed DNA methylation (RdDM). *De novo* DNA methylation in plants is mediated by a canonical *RNA-directed DNA methylation* (*RdDM*) pathway (Law and Jacobsen 2010), in which the actions of plant-specific RNA polymerases IV (Pol IV), RNA-dependent RNA polymerase 2 (RDR2) and Dicer-Like 3 (DCL3) produce 24-nt siRNAs. These siRNAs form complex with Argonaute 4 (AGO4) in the cytoplasm and get imported into the nucleus. Another plant-specific RNA polymerase V (Pol V) transcribes long scaffold transcripts, which, through base-pairing, recruit the siRNA/AGO4 complex and DRM2 to RdDM target loci. In addition to the canonical RdDM pathway, several non-canonical RdDM pathways (such as Pol II, RDR6, DCL2, DCL4 and the pathway involving Dicer-independent siRNAs) have been identified to contribute to the establishment of DNA methylation (Cuerda-Gil and Slotkin 2016; Ye et al. 2016).

Several recent studies indicate that genome-wide hypomethylation due to mutations in DDM1 or MET1 induces biogenesis of 24-nt siRNAs, and activates the *de novo* methylation pathways (Blevins et al. 2009; Teixeira et al. 2009). The 24-nt siRNAs in endosperm may be translocated to the embryo and help to silence TEs in the embryonic genome. Mosher et al. (2009) reported that the 24-nt siRNAs are highly expressed during early embryogenesis and their accumulation continues during seed development. Because the endosperm genome is not passed on to the next generation, reactivation of TEs in endosperm may not be deleterious but may help to restrict the TEs in the egg cell and later in the embryo, thus contributing to the genome integrity in offspring. It is speculated that the maternal Pol IV-dependent siRNAs are the "messenger" that communicate between the endosperm and embryo; however, the siRNAs were identified only in the endosperm but not in the embryo (Mosher et al. 2009). In-depth studies would be necessary to understand the role of RdDM pathway in the epigenetic regulation of genes and its deployment in epigenetic manipulation.

Epigenetic regulation of plant development

Cytosine methylation was initially considered as a part of the host defense systems in prokaryotes. Later, it was also found to be operational in eukaryotes performing different roles, mostly as a genomic-defense mechanism for silencing TEs and maintaining genome integrity over the generations (Zhang et al. 2011). Now, DNA methylation is considered to be crucial for a wide range of cellular functions in plants. Significant epigenetic changes in plants during the developmental processes and interaction with the environment have been recognized over the last two decades. DNA methylation in promoter region was reported to suppress transcription of the gene directly by interfering with the binding of the transcriptional activators, and indirectly by favoring the formation of repressive-chromatin due to interaction with methylated DNA-binding proteins (Bird 2002). The genome of higher plants possesses many TEs that may disrupt genome stability. For integrity and stability of the genome over the generations, TEs and repetitive elements must remain silent in reproductive cells. DNA methylation is one of the mechanisms that help plants in maintaining genome integrity. Large-scale methylation reprogramming may be necessary for non-germ line reproductive cells to reinforce silencing of TEs. In Arabidopsis, at least four bifunctional DNA glycosylases and AP lyases, namely DME (Demeter), DML2 (Demeter-Like 2), DML3 (Demeter-Like 3) and ROS1 (Repressor of Silencing 1) are known to recognize and remove methyl-cytosine. Genomic studies suggest that ROS1, DML2, and DML3 mainly function in vegetative tissues and demethylate specific loci across the genome (Law and Jacobsen 2010). However, triple knock-out mutant for all these three genes did not show any remarkable effect on the global DNA methylation level in the Arabidopsis genome (Penterman et al. 2007; Lister et al. 2008). Therefore, these enzymes appear to counterbalance the RdDM pathway to fine-tune the methylation levels at particular genomic locations.

Recent data indicate that apomictic seed development in plants is associated with dynamic transcriptional activity in ovule probably regulated through epigenetic mechanisms. Epigenetic model of regulation of apomixis indicates that reversible changes in chromatin configuration might alter the expression of key genes of the apomictic pathway at the different developmental stage or in different cell types (Garcia-Aguilar et al. 2010; Podio et al. 2014). It has been observed that in many apomictic species developmental program is not tightly conserved, and initiation of apomixis in response to environmental conditions/stresses support the view that apomixis is epigenetically regulated. Such regulatory flexibility is a characteristic feature of epigenetic mechanism. Since the discovery of imprinted *R* gene in maize, dozens of imprinted genes have been identified in plants, and epigenetics has been found to play a crucial role in this process. The imprinted gene refers to the gene/allele that is preferentially expressed coming either from the maternal or paternal genome. In Arabidopsis, the well-characterized maternally expressed imprinted genes (MEGs) include *FWA* (*flowering Wageningen*), *MEA* (*MEDEA*), *FIS2* (*fertilization independent seed 2*), *AtFH5*, *AGL36* (Agamous-like36) and *NUWA* (Kinoshita et al. 2004; Fitz Gerald et al. 2009; Shirazadi et al. 2011; He et al. 2017). The imprinted genes are maintained in the silenced state by DNA methylation and/or repressive histone modifications. Demethylation of maternal genome in the endosperm and activation of MEGs have been reported in rice (Luo et al. 2011; Rodrigues et al. 2013) and maize (Waters et al. 2011; Zhang et al. 2011). Thus, demethylation-dependent gene imprinting appears to be a conserved feature in the flowering plants. Maternal alleles of MEGs in the central cells of female gametophyte and TEs of the

vegetative cell but not of the sperm cell, in pollen grains get activated by DME-mediated active DNA demethylation. Silencing of TEs in the male gametes is essential for genome stability and integrity (Slotkin et al. 2009).

Decrease in methylation in pericarp on ripening of tomato suggests the involvement of DNA demethylation in fruit ripening (Teyssier et al. 2008; Lang et al. 2017). Gliadins (low-molecular-weight glutenin subunits, LMWgs) and high-molecular-weight glutenin subunits (HMWgs), the storage proteins in wheat and barley endosperm, require *TaDME* for their expression. RNAi-mediated suppression of *DME* resulted in significant reduction in gliadins and LMWgs, but HMWgs remained unchanged (Wen et al. 2012). In a recent study, it was revealed that *MtDME* gets strongly induced in *Medicago truncatula* during nodule differentiation, and knockdown of *MtDME* resulted in morphological and functional alterations in the nodule (Satge et al. 2016). Variation in DNA methylation and its effect on expression of high-affinity potassium transporter under salt stress was reported to provide salt tolerance in wheat (Kumar et al. 2017a). There are increasing evidence for the involvement of epigenetic regulations in various developmental processes in plants (see Li et al. 2017). Thus, understanding epigenetic regulation and functions of the machinery involved would be very much essential for epigenetic manipulation of plants for the trait of interest.

Applications in crop improvement

Epigenetic changes in DNA methylation, histone modifications, and ncRNA expression cause important biochemical, physiological and molecular consequences in plants. The epigenetic-phenotypes are now being explained based on the fundamental discoveries such as activation, excision and translocation of TEs, allelic interactions, transgene silencing and epialleles of the endogenous genes. Recent studies on epiRIL in *Arabidopsis* demonstrate that epigenetics of QTL can explain the heritability of the complex traits. The examples include fruit ripening in tomato (Manning et al. 2006) and somaclonal variation in oil palm (Rival et al. 2000). Since epigenetic variations can affect important traits in crop plants, creation/manipulation of stably inherited epigenetic variation could be a powerful tool in plant breeding. It can enable modification of traits in plant without altering DNA sequence of the gene. Similarly, understanding the basis of phenotypic plasticity (the ability of a single genotype to express multiple phenotypes in response to environment or instability of the trait) is crucial for crop breeding. Reports on phenotypic plasticity have increased considerably in the recent years, particularly on abiotic and biotic stresses under the global climate change. The rapid appearance of a new phenotype in response to environmental stimuli cannot be explained by genetic mutations, because of the very low rate of mutation. Instead, growing body of evidence suggests that the processes of phenotypic plasticity involve epigenetic mechanisms. Therefore, epigenetic mechanisms, particularly DNA methylation, which is a generator of epialleles, would have important implications for plant breeders.

Once it is established that an epigenetic mark is having causal effects on gene expression, its effect can be epigenetically manipulated with the help of targeted DNA/chromatin-modifying enzyme. A number of chromatin-modifying enzymes have already been used to demonstrate successful addition or removal of chromatin/epigenetic marks at the target site (Table 1). Pioneering studies have proved that catalytic-domain of a chromatin-modifying enzyme can be sufficient to provoke transcriptional changes when targeted to a specific site. For example, demethylation at several sites in the promoter of *RHOXF2* gene resulted in transcriptional up-regulation of the gene (Maeder et al. 2013). In another recent example, a dCas9-p300 histone acetyltransferase fusion was used to activate transcription of *MYOD* and *OCT4* genes from proximal promoters and distal enhancers (Hilton et al. 2015). Thus, chromatin modifiers can be used for up-regulating/down-regulating gene transcription. However, the observed effect is exclusively mediated by the epigenetic mark or due to local modifications of other chromatin proteins is yet to be established. Moreover, it is important to note that effects on transcription are observed on modification of some, but not all, targeted sites (Stricker et al. 2017).

Zheng et al. (2017) reported accumulation of transgenerational epimutations in rice due to drought stress over 11 successive generations. They observed multi-generational drought stress to improve the adaptability of the offspring in the field, which can be correlated with the view proposed by Lamark about 200 years ago. Zheng et al. (2017) also reported that the genes of stress-responsive pathways showed accumulation of transgenerational epimutations, and the DNA methylation patterns in the drought-responsive genes were affected by multi-generational drought. They reported that about 30% of the changes in

Table 1. Chromatin-modifying enzymes (a component of epigenetic machinery) successfully tested for epigenetic modification

Enzyme	Function	Targeting protein	Locus targeted	Observed modification	Effect on transcription/ phenotype	Reference
Repression of gene						
LSD1	Histone H3K4 demethylase	TALE	Enhancer of stem cell leukemia, and 40 additional enhancers	65% loss of H3K4me2 and 60% loss of H3K27ac	Up to 50% decrease in RNA level	Mendenhall et al. 2013
LSD1	Histone H3K4 demethylase	dCas9	*Oct4* distal enhancer, 8 enhancers regulating pluripotency in embryonic stem cells, and *Tbx3*	Up to 85% loss of H3K4me2, and >90% loss of H3K27ac	>90% loss of mRNA, changes in morphology of embryonic stem cells	Kearns et al. 2015
DNMT 3A	DNA methyltransferase	ZF	Promoters (*MASPIN* and *SOX2*)	Increased DNA methylation	60% down-regulation of RNA, reduced breast cancer colony formation, reduced proliferation	Rivenbark et al. 2012
DNMT 3A	DNA methyltransferase	dCas9	Promoters (*IL6ST* and *BACH2*)	Increased DNA methylation	40-50% down-regulation	Vojta et al. 2016
Combination of DNMT 3A, DNMT 3L and KRAB	DNA methyltransferase	dCas9 and TALE	Promoter (*IFNAR1*, *VEGFA*), Promoter and enhancer (B2M-tdTomato)	Up to 100% DNA methylation, loss of H3K4me3, increased H3K9me3 (B2M)	500 fold down-regulation of B2M mRNA, and 80% less mRNA of *IFNAR1* and *VEGFA*	Amabile et al. 2016
Activation of gene						
p300	Histone acetyltransferase	dCas9, ZF and TALE	Promoters (*IL1RN*, *MYOD*, *OCT4*, β-globin and *ICAM1*), enhancers (*MYOD*, *OCT4* and β-globin)	Increased H3K27ac	Increase in transcription	Hilton et al. 2015
TET1	DNA demethylase	dCas9	Promoter (*BRCA1*)	DNA demethylation, 10-50% decrease in methylation levels	Increase in transcription up to 2.5 fold, reduction in cell proliferation	Choudhury et al. 2016
TET1	DNA demethylase	dCas9	Promoter (*Bdnf*), enhancer (*MyoD*)	Up to 60% demethylation at reporter locus, *MyoD*, and up to 35% at *Bdnf* promoter	3 fold increase in *Bdnf IV* and *MyoD* mRNA	Liu et al. 2016
PRDM9	K4 methylase	dCas9 and ZF	Promoters (*ICAM1*, *RASSF1A*, *EPCAM,* and *PLOD2*)	Up to 60% increase in H3K4me3	Up to 8 fold up-regulation of *EPCAM*	Cano-Rodriguez et al. 2016
TET1	DNA demethylase	dCas9	Promoters (*RANKL*, *MAGEB2*, and *MMP2*)	Variation in DNA demethylation	Up to 10 fold increase in transcription, reduction in cell proliferation	Xu et al. 2016

dCas9 = Nuclease dead-Cas9, DNMT3A = DNA methyl-transferase 3A, DNMT3L = DNA methyl-transferase 3L, ES = Embryonic stem, H3K4 = Histone 3 lysine 4, H3K27ac = Histone 3 lysine 27-acetylated, H3K4me2 = Histone 3 lysine 4-dimethylated, H3K9me3 = Histone 3 lysine 4-trimethylated, HAT = Histone acetyltransferase, KRAB = Kruppel-associated box, LSD1 = Lysine-specific histone demethylase 1, PRDM9 = PR domain containing methyltransferase 9, TALE = Transcription activator-like effector, TET1 = Ten-eleven translocation methylcytosine dioxygenase 1, ZF = Zinc finger

methylation were stable and heritable, which corroborated with the earlier findings of Wang et al. (2011) who reported that 29% of the drought-induced DNA methylation is maintained even after recovery to the normal condition, and that of Kumar and Singh (2016) who recorded that 25% of the increased methylation is retained in a rice genotype IR-64-DTY$_{1.1}$ even after removal of drought stress. Thus, epigenetics can be considered as an important regulatory mechanism in plant's long-term adaptation and evolution under adverse environments. Demeter (DME, a DNA glycosylase) preferentially targets short, AT-rich and nucleosome-free euchromatic TEs (Ibara et al. 2012) for active DNA demethylation which leads to the activation of TEs (Mosher et al. 2009). In *Arabidopsis*, DNA demethylases target promoter TEs to regulate stress-responsive genes (Le et al. 2014). The TEs (accounting for ~35% of the genome in rice) are usually suppressed by DNA methylation, and contribute to the activation of plant responses to abiotic stress (Jiao and Deng 2007). Therefore, manipulating DNA methylation of TEs in the promoter region (by recruiting DRM2 to the target loci) could be considered for epigenetic manipulation of stress tolerance in plants (Liu et al. 2015; Xu et al. 2017).

A source of heritable variation

Certain epigenetic changes in plants persist even after withdrawal of the stress and might be inherited over the generation in the form of epigenetic alleles (Kakutani 2002). These heritable epigenetic alleles (epialleles) are now considered as another source of polymorphism which may be utilized in the breeding program. Epialleles result as a genome-response to environmental stress and enable plants to tolerate the stress (Tsaftaris and Polidoros 2000; Steward et al. 2002). Interestingly, the emergence of epialleles is much faster and the rate is higher than that of mutations which give rise to the new alleles. However, reversion rate of epiallele is also high and its emergence is affected by the growth conditions of the plant. Nevertheless, epigenetic changes create more heritable variations (epialleles) and help in the evolution process. Somaclonal variations are considered as the tissue-culture-induced, heritable genetic changes. However, distinguishing the somaclonal variation from the preexisting genetic/epigenetic variations is difficult unless the exact genetic/epigenetic makeup of individual cells in the explant is known prior to tissue culture. It is now apparent that somatically-acquired epigenetic changes in plants may be mitotically stable and meiotically heritable, hence emphasis is given to the variations in DNA methylation as a source of variation. First such evidence of DNA methylation was presented about two decades ago from crown-gall tumor culture showing phenotypic variation in the regenerated plant (John and Amasino 1989). Evidence suggests that a major part of somaclonal variation might arise because of the preexisting mitotically and meiotically stable epigenetic variations in the individual somatic cells (Neuhuber et al. 1994). Kaeppler et al. (2000) also reported that changes in methylation level and methylation of specific sites occur during tissue culture process. However, they reported a decrease in methylation level during tissue culture. Liu et al. (2004) reported that retrotransposon Tos17 is transcriptionally activated in rice by tissue culture, but it gets repressed upon plant regeneration. Several recent studies demonstrated that activation of the transcription process, as well as transposition of Tos17 in tissue cultured calli, is associated with DNA hypomethylation (Cheng et al. 2006; Ding et al. 2007). Lately, La et al. (2011) reported that 5-methylcytosine DNA glycosylase/lyase demethylates retrotransposons and promotes their transposition during tissue culture in rice. A better understanding of the role and significance of this new source of genetic and phenotypic diversity in plants would be achieved as more data accumulates about the role of DNA methylation in plant evolution, domestication, and breeding.

Identification and assessment of the importance of epialleles in plant breeding require determination of (i) the extent of variation in epigenetic marks among the individuals, (ii) the degree to which the epimarks affect phenotype, and (iii) the extent to which the epimark-linked superior phenotypes are stably inherited. Although there are several challenging tasks, the technical potential to assess epigenetic variations between individuals and estimation of the levels of epimark-associated phenotypic diversity does exist. With the increasing understating of epigenetic phenomena, it is expected that our potential to exploit epigenetics in crop improvement would get better, and will have significant implications for plant breeding.

Manifestation of heterosis and hybrid vigour

Data indicates that F_1 hybrids are, in general, less methylated than their parental inbreds. The possible role of DNA methylation in regulating expression of genes and performance of hybrids under different growth conditions have been examined in experiments with maize inbreds and their hybrids (Tsaftaris and

Kafka 1998; Tani et al. 2005). Quantitative variations in gene expression are responsible for the variations observed in different biochemical and physiological processes which are essential for the phenomena linked with heterosis/hybrid vigour. Variability in gene expression can be estimated using individual RNA amount polymorphism (RAP) and individual protein amount polymorphism (PAP). A significant correlation between PAP indices and the hybrid vigour for agronomic traits in maize has been reported earlier (Leonardi et al. 1991). The results suggested that individual RAP was similar to that of PAP. In a RAP analysis, heterotic hybrids showed a significant number of genes to over-express compared to that in the better parent at three developmental stages (Tsaftaris and Polidoros 1993). Data of PAP and RAP also indicated that quantitative variations in the expression of certain loci might be important in the manifestation of hybrid vigour, which underlines the significance of regulatory mechanisms involved in the quantitative modulation of gene expression. Therefore, DNA methylation can be considered as a regulatory mechanism that affects the expression of several genes important for the manifestation of heterosis (Tsaftaris and Kafka 1998). Results of coupling of CRED-RA and RLGS (restriction enzyme and aleatory amplification) (Tani et al. 2005) and (restriction landmark genome scanning) analyses (Kovacevic et al. 2005) suggested that (i) in general, hybrids are less methylated than their parental inbreds, (ii) heterotic hybrids are less methylated than related non-heterotic hybrids, (iii) old and low-yielding inbreds are highly methylated, (iv) new inbreds, especially those selected for high and stable yield, have lower methylation level in comparison to their progenitors. Repeated selfing carried out during the development of inbreds, with more emphasis on combining ability of the inbreds, leads to gradual accumulation of methylated loci, which is released and/or repatterned when the inbreds are crossed to develop hybrids. The stressful growth conditions during the development of inbreds result into more methylated DNA, and these stress-induced methylations and the linked suppression of genome activity could be at the core of higher yield of the hybrid.

Manipulation of genome imprinting

Genome imprinting is widespread in flowering plants. *FWA*, *MEA* and *FIS2* are some of the well-characterized imprinted genes which are expressed from the maternal genome in the endosperm, while the alleles coming from the paternal genome are silenced (Gehring et al. 2006; Jullien et al. 2006). Manipulation of parental imprinting by epigenetic manipulations may lead to the development of a superior endosperm, which has become a necessity for the improvement of seed crops (Berger 2003). Understanding the epigenetic regulation of seed development would eventually uncover the mysteries behind apomixis, the asexual mode of reproduction through seeds wherein embryo develops without meiosis and double-fertilization leading to the production of progenies genetically identical to the mother plant (Koltunow et al. 2003; Yadav et al. 2012; Dwivedi et al. 2015). If this mechanism could be deployed successfully in the commercial seed crops, hybrid vigour can be maintained indefinitely which may help to overcome the current limitations of plant breeders in maintaining hybrid vigour for more than one generation (Kumar et al. 2015). *DME* is expressed in the central cell prior to the fertilization process which leads to extensive hypomethylation of the maternal genome (Gehring et al. 2009; Hsieh et al. 2009). This difference in methylation between the maternal and paternal genomes in the endosperm causes differential expression of a number of genes. Based on the methylation-sensitive amplified polymorphism marker, Zhang et al. (2011) reported a significant decrease in methylation level in the endosperm of *Sorghum bicolor* primarily because of the mCG-demethylation. Moreover, TEs were also reported to be extensively demethylated in endosperms at the whole-genome level (Gehring et al. 2009; Hsieh et al. 2009). These data suggested that imprinting in plants might have evolved because of targeted methylation of TEs inserted near the genic regulatory elements. Genome-wide demethylation in the endosperms has been found to be accompanied by extensive non-CG hypermethylation of siRNA-targeted TEs.

A number of imprinted genes in monocot species are activated by selective demethylation of maternal alleles (Gutiérrez-Marcos et al. 2006; Haun et al. 2007; Jahnke and Scholten 2009), but the lack of monocot *DME* (Zemach et al. 2010) implies that another member of this gene family or a different biochemical mechanism is responsible for monocot imprinting. However, the earlier study indicated conservation of DNA glycosylase domain, cysteine cluster, and lysine-rich region of the Arabidopsis *DME* in rice also (Choi et al. 2002). Isolation of full-length *DME* gene from barley and wheat has also been reported (von Wettstein 2009). Functional analysis demonstrated that *DME* possesses the glycosylase activity. Therefore, it is quite possible that DME

proteins might exist and be responsible for active demethylation of the maternal endosperm genome in monocots (Zhang et al. 2011).

Tissue-specific gene expression and transgene silencing

Variation in DNA methylation has been correlated with tissue- and developmental stage-specific changes in plants (Messeguer et al. 1991; Tsaftaris and Polidoros 2000). Gene imprinting is one of the well-studied examples of differential expression of genes in tissues due to differential methylation levels. Zhang et al. (2011) reported tissue-specific differentially methylated regions in sorghum and suggested that DNA methylation play an important role in regulating tissue-specific expression of genes. Polycomb group (PcG) proteins are involved in controlling the expression of homeotic genes that are essential for proper developmental processes in plants. The main component of the PcG complex in plants is methyltransferase (e.g. MEA in Arabidopsis) that methylate histones to regulate expression of homeotic genes for development of plant (Reyes and Grossniklaus 2003). In most of the plants, embryogenesis starts with asymmetric cell division which gives rise to a polar embryo having a larger basal cell and a smaller apical cell. Cell division and differentiation during these processes are tightly regulated that are influenced by epigenetic mechanisms (Kumar 2017a). Xiao et al. (2006) demonstrated that MET1 influences gene expression during embryogenesis in Arabidopsis, and is crucial for normal seed development and viability of the seed. It is well-established now that cytosine methylation plays a crucial role in the expression of imprinted genes in endosperm and normal embryo development in plant. Demethylation of the promoter of the gliadins and LMWgs encoding genes in barley was reported to be important for accumulation of gliadins and LMWgs (Van Herpen et al. 2008). However, regulation of HMWgs expression was found to be independent of DNA (de)methylation (Bethune and Khosla 2012). Due to the differential regulation of gliadin/LMWg and HMWg expression in wheat and barley, suppression of *TaDME* and *HvDME* has been proposed to be a potential strategy to eliminate gliadins and LMWgs that cannot be digested/tolerated by many people suffering from *celiac disease* (Wen et al. 2012).

In plants, transcriptional and post-transcriptional silencing of transgene have been correlated with its methylation level. Methylation of promoter attached with the transgene correlates with the transcriptional gene silencing (Park et al. 1996), while methylation of the coding region has been, in general, associated with post-transcriptional gene silencing (Ingelbrecht et al. 1994). Silencing of the transgene has frequently been observed as a major commercial risk of the transgenic technology, creating hindrance in the economic exploitation of transgenic plants. With the increasing knowledge of the mechanisms of epigenetic transgene silencing, it is expected that it would be possible for us to solve this problem in the near future. One of the most efficient strategies suggested to avoid transgene silencing has been the careful designing of the transgene and thorough analyses of transformants at the molecular level (De Wilde et al. 2000). A spontaneous mutant Epi-d1 in rice was found to be metastable dwarf phenotype which was reported to be mitotically and meiotically heritable and corresponding to the metastable epigenetic silencing of the *DWARF1* (*D1*) gene (Miura et al. 2009). The silenced state of the gene was correlated with DNA hypermethylation in the promoter region *D1*, and the epigenetic state was bidirectionally mutable from active to repressed and from repressed to active. This indicates that epigenetic regulation of *D1* is mediated by *de novo* DNA methylation, and this provides a mechanism for rapid adaptation to the changing environmental conditions (Miura et al. 2009).

Improving tolerance to environmental stresses

Epigenetic modifications are reported to play role in acquiring stress tolerance in plants (Kumar 2017b). Abiotic and biotic stresses may cause alterations in DNA methylation in both plants and animals (Richards 2006). The changes in DNA methylation induced by abiotic stress play a functional role in plant's stress tolerance (Richards 2006; Karan et al. 2012). Most of the stress-induced epigenetic variations are reset to the basal level once the stress disappears, but part of the modifications might be stable and carried forward as epigenetic stress memory (Chinnusamy and Zhu 2009). Under osmotic stress, *P5CS* and γ-*OAT* genes were found to show DNA demethylation in mother plants, but it disappeared in the next generation, suggesting that DNA demethylation regulated expression of the genes (Zhang et al. 2013). Recently, Kumar et al. (2017a) reported investigations on variations in cytosine methylation and its effect on the expression of high-affinity potassium transporter (*HKT*) genes in contrasting wheat genotypes under salt stress. They observed a genotype- and tissue-specific increase in cytosine methylation induced by

NaCl stress which was found to downregulate the expression of *TaHKT2;1* and *TaHKT2;3* in the shoot as well as the root of Kharchia-65, thereby contributing to its better salt-tolerance ability. *TaHKT1;4* was reported to be root-specific and downregulated in salt-tolerant genotype under salt stress. However, it was not found to be regulated through variations in cytosine methylation for its differential expression in the contrasting wheat genotypes. Instead, the differential expression of the gene was reported due to genetic factors (Kumar et al. 2017b).

Kumar and Singh (2016) reported a considerable increase (20% in Nagina-22 and 37% in IR-64-DTY$_{1.1}$) in global-methylation of the genome of drought-tolerant rice genotypes under drought stress. On the other hand, IR-64 (a drought-sensitive rice genotype) showed a decrease in cytosine methylation on drought stress imposition. Even after ten days of withdrawal of the stress, drought-induced hypermethylation did not return to the basal level. About 60% of the increased methylation was retained in N-22 and 25% was retained in case of IR-64-DTY$_{1.1}$, and this was suggested to be involved in the epigenetic stress memory. However, transgenerational inheritance of epigenetic marks is important for improving stress tolerance in crop plants. Transgenerational inheritance of epigenetic variations requires the passage of epigenetic marks, like DNA methylation, through the germline without being erased by the surveillance mechanisms which ensure the establishment of cellular totipotency at the onset of ontogenesis (Lange and Schneider 2010). However, its relevance in the developmental decisions during plant embryogenesis is not fully understood (Jullien and Berger 2010). Nevertheless, the observation that epialleles are stably inherited over the generations indicates that resetting of the stress-induced epigenetic changes to 'default-state' is a leaky process.

One of the ways for plants to adapt to environmental stress is to remember a stress episode and to react more efficiently (faster and more strongly) upon subsequent exposures to the stress. At molecular level, short-term memory results from a combination of mechanisms, including modification of the levels of stress-associated receptors, signalling components and transcription factors. Multiple lines of evidence indicate that both short-term and transgenerational memories largely rely on epigenetic modifications, and it can be exploited in developing tolerant crop plants. However, fundamental investigations are required to understand whether stress-induced epialleles can be stabilized over several generations and consequently be utilized in crop breeding programs.

Will epigenetics help in developing climate-resilient crops?

Crop domestication and selection of plants has been successful in breeding varieties for the changing environmental conditions, and genotypic variability offers an additional advantage in efficiency of crop breeding. However, the last century has witnessed considerable genetic erosion leading to genetic bottlenecks in crop breeding. Newer approaches supported by high throughput sequencing, genotyping, analyzing population structure and marker-assisted breeding have helped improving breeding efficiency (Nandakumar et al. 2004; Chikkappa et al. 2011). However, these approaches fail to recognize the contribution of epigenetics to genetic diversity and crop breeding. Advances in high throughput bisulfate sequence (methylome analysis) allows generation of single-base resolution methylome maps describing the DNA methylation landscape in the coding and non-coding regions of genes, intergenic regions, TEs and other repetitive elements. Now the challenges are to compile methylome maps for specific cells, tissues, and organs in different growing environments, along with the equivalent information for histone marks and accounts of siRNA behaviour. The research challenges ahead include improving our understanding of the stability, reversibility, and heritability of epialleles. Epigenetic manipulation may become a valuable strategy in the near future for crop improvement, as the approaches are available for stochastic modulation of DNA methylation using chemical (Amoah et al. 2012) or genetic (Xu et al. 2016) means, followed by the forward or reverse selection of epialleles. Analysis of epiRILs in Arabidopsis revealed that they segregate for the induced DNA methylation at hundreds of loci across the genome. Several of these differentially methylated regions were found to be the bonafide epigenetic quantitative trait loci (QTL^{epi}), accounting for 60 to 90% of the heritability for complex traits like flowering time and primary root length. The QTL^{epi} were found to be reproducible and could be subjected to artificial selection (Cortijo et al. 2014). However, we need to devise strategies to ensure stable retention of desirable epialleles within breeding materials, and to develop techniques for targeted epigenetic manipulation. Eukaryotic genomes are complex in nature, and genome complexity of many crop plants

increases further because of their polyploid origins, which makes gene interaction networks complicated, and difficult to modulate for improved plasticity with inbuilt gene redundancy. Understanding how epigenetic changes are superimposed on the multiple gene copies to confer plasticity may provide a framework for the development of desirable crop variety enabled to cope up with the harsh multiple-stresses the crops are facing now due to the global climate changes. Currently, it is difficult to control epigenetic variations, mobilization of stress-responsive epigenetically-silenced TEs may contribute to the stable inheritance of stress-induced epigenetic changes.

Over the last century, genetic improvement of crops and the modern agronomic practices have underpinned a massive increase in crop yield and productivity. However, most of these gains have been achieved by utilizing the 'Green Revolution' technologies in a period of relative climate stability (Kumar and Singh 2015), compared to the current period of increased climate change and variability. According to an estimate, we need to increase global food production by 70% in order to keep pace with the growing demands of food (Kumar 2013). Moreover, we need to produce more food and livelihood opportunities from reducing per capita arable land and water (Kumar 2015). To facilitate climate resilient agriculture in the future, we need to understand the molecular and mechanistic basis of genotype x environment interactions (G x E) and the emergent property of crop plant plasticity facilitated by epigenetic mechanisms. Epigenetic manipulation may provide a way to achieve the desired variations and adaptive advantages without manipulating DNA sequence (Rodrigues and Koltnow 2005). Importantly, epialleles may alter the expression of the gene(s) controlling cellular/physiological processes during plant development. Stable inheritance of such adaptive epialleles may provide increased fitness/adaptability to the plant in the changing environmental conditions.

Future perspectives

The recent years have witnessed much progress in our understanding of epigenetic regulation of gene expression in plants, particularly in *Arabidopsis*. Proteins involved in DNA (de)methylation, mechanisms of histone modification and role of ncRNA in regulation of developmental processes in plants are becoming clear day-by-day. Different groups of scientist are currently working the world over to identify the gene(s) involved in epigenetic changes to establish proof of the concept of epigenetic manipulation in plant. However, many areas of epigenetics remain to be explored. We still know only a little about the factors that regulate targeting of active DNA demethylation during developmental stages. Does DNA (de)methylation interplay with other epigenetic features or chromatin features? Future research should aim at identifying more developmental processes in different species that involve epigenetic regulation. Assessing the contribution of transgenerational epimarks to heritable phenotypic variation has been a major challenge as many of the chromatin (DNA methylation and histone modification) changes and gene expression variants co-segregate with DNA sequence polymorphisms. Nonetheless, there is evidence that plants possess heritable epiallelic variations that can be associated with the trait of interest and utilized for crop improvement. However, we are still at the beginning of understanding the transgenerational stability of epigenetic variations. Only a little is known to us about the role of environment in creation of induced epialleles. Although it had been difficult to alter DNA methylation and chromatin states in a locus-specific manner, the situation is changing rapidly with the advances in genome editing tools like CRISPR-Cas9 system. For example, a catalytically inactive SpdCas9 can be fused with methylases and/or demethylases, as it has already been demonstrated in mammalian cells using SpdCas9-Tet1 and SpdCas9-Dnmt3a to manipulate DNA methylation in a site-specific manner (Liu et al. 2016). Thus, we can anticipate that soon epigenome editing will provide a means to assess the role of a QTL in epiallelic variations which may provide an interesting new route for the improvement of crop plants. With the modern tools and techniques in molecular biology and biotechnology, it is expected that soon we may achieve a comprehensive understanding of this amazing biological phenomenon, and we might be able to use it for the development of climate-resilient crops for the benefit of human kind. However, this will need a deeper understanding of the interactions between crop genomes and how their genomic regulatory networks contribute to the plasticity of phenotype.

Declaration

The authors declare no conflict of interest.

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