Genes & Genomics (2022) 44:259–266
https://doi.org/10.1007/s13258-021-01189-7

Online ISSN 2092-9293
Print ISSN 1976-9571

PERSPECTIVE

Harnessing epigenetic variability for crop improvement: current status and future prospects

Eun Yu Kim[1] · Kyung Do Kim[2] · Jungnam Cho[1,3,4]

Received: 29 October 2021 / Accepted: 7 November 2021 / Published online: 22 November 2021

Abstract

Background The epigenetic mechanisms play critical roles in a vast diversity of biological processes of plants, including development and response to environmental challenges. Particularly, DNA methylation is a stable epigenetic signature that supplements the genetics-based view of complex life phenomena. In crop breeding, the decrease in genetic diversity due to artificial selection of conventional breeding methods has been a long-standing concern. Therefore, the epigenetic diversity has been proposed as a new resource for future crop breeding, which will be hereinafter referred to as epibreeding.

Discussion The induction of methylome changes has been performed in plants by several methods including chemical drugs treatment and tissue culture. Target-specific epigenetic engineering has been also attempted by exogenous RNAi mediated by virus-induced gene silencing and grafting. Importantly, the new and innovative techniques including the CRISPR–Cas9 system have recently been adopted in epigenetic engineering of plant genomes, facilitating the efforts for epibreeding.

Conclusion In this review, we introduce several examples of natural and induced epigenetic changes impacting on agronomic traits and discuss the methods for generating epigenomic diversity and site-specific epigenetic engineering.

Keywords Epigenetics · Epiallele · DNA methylation · Epibreeding · Epigenetic recombinant inbred line · CRISPR

Introduction

Conventional crop breeding technologies have greatly improved people's life by producing crops with better yield and quality. We have witnessed in the past how crop biotechnology could remarkably improve the yield of rice, for instance, one of the most important crops in the world feeding more than half of the global population. However, crop breeding process caused genetic erosion, i.e. loss of genetic diversity, which in turn limits the crop breeding resources (Springer and Schmitz 2017; Tirnaz and Batley 2019; Dalakouras and Vlachostergios 2021). Moreover, as global population is rapidly growing and farming resources are becoming limited, the food security has become a serious and demanding issue (Garnett et al. 2013; Nelson et al. 2014). This is in principle why future crop breeding field requires unprecedented innovation of the next level.

It has been documented well that plant phenotypes can be determined by non-genetic variations, so called epigenetic diversity. Epigenetics refers to a study of heritable gene expression changes over mitotic and meiotic cell divisions, which do not involve any DNA sequence alterations (Wu and Morris 2001). Epigenetic control involves both DNA methylation (methylated cytosine, mC) and histone modifications, which often crosstalk to each other primarily through small regulatory RNAs. While histone modifications are associated with both transcriptional activation and suppression depending on the types and positions of modifications, DNA methylation is mostly linked with transcriptional suppression (Xiao et al. 2016). It is also important to note that

✉ Eun Yu Kim
eunyukim@cemps.ac.cn

✉ Kyung Do Kim
kyungdokim@mju.ac.kr

✉ Jungnam Cho
jungnamcho@cemps.ac.cn

[1] National Key Laboratory of Plant Molecular Genetics, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China

[2] Myongji University, Yongin 17058, Korea

[3] University of Chinese Academy of Sciences, Beijing 100049, China

[4] CAS-JIC Center of Excellence for Plant and Microbial Science, Shanghai 200032, China

although there are reported evidence of mitotic inheritance of histone modification changes in certain conditions (e.g. priming of heat tolerance) (Kim et al. 2015; Friedrich et al. 2019; Bhadouriya et al. 2021), it is more generally accepted that alteration of DNA methylation can be maintained more strongly over mitotic and meiotic generations. Given that stable heritability is critical in the crop breeding procedures, DNA methylation is considered a more promising epigenetic modification and therefore will be highlighted in this review.

There is accumulating evidence implicating that DNA methylation impacts vastly on a variety of biological processes in plants, including development and stress responses. Several naturally occurring mutants with altered DNA methylation (i.e. epialleles) have been identified to be associated with diverse phenotypic changes of plants. Apart from natural epialleles which are found relatively rarely, epialleles can also be induced artificially, for instance, by generating epigenetic recombinant inbred line (epiRIL) population (Reinders and Paszkowski 2009; Reinders et al. 2009; Johannes et al. 2009; Catoni and Cortijo 2018). EpiRILs are plants with mosaic methylome that are derived from a cross of two genetically identical plants differing in methylation levels. Until now, epiRILs have been generated only in Arabidopsis by two independent research groups who used the *met1* and *ddm1* mutants (Reinders et al. 2009; Johannes et al. 2009). Importantly, these epiRILs revealed that various plant phenotypes such as flowering time and disease resistance are largely attributed to epiallelic changes. Despite the critical biological relevance of DNA methylation and its prevalence in crop genomes, the epigenetic mechanisms gained relatively poor attention in the field of crop breeding. Nonetheless, the recent advances in the epigenomic engineering techniques opened-up the possibility that epiallelic variations can be used as a stable breeding resource. In the next following sections, we will introduce selected major agronomic traits controlled by DNA methylation and summarize the technical approaches for generating epialleles.

Main text

Maintenance and establishment of DNA methylation

In plant genomes, DNA methylation appears in three different sequence contexts; CG, CHG (where H denotes A, C or T) and CHH. The maintenance and establishment of methylation at these cytosines are mediated by distinct mechanisms which have been well characterized (Law and Jacobsen 2010; Henikoff and Greally 2016). In brief, the symmetrical DNA methylation mCG and mCHG is maintained by METHYLTRANSFERASE 1 (MET1) and CHROMOMETHYLASE 3 (CMT3), respectively (Ronemus et al. 1996; Lindroth et al. 2001; Kankel et al. 2003). CHH methylation is established de novo via two independent pathways; the RNA-directed DNA methylation (RdDM) and CHROMOMETHYLASE 2 (CMT2)-mediated pathways (Matzke and Mosher 2014; Wendte and Pikaard 2017). RdDM involves the biogenesis of small interfering RNAs (siRNAs), which are produced by the plant-specific RNA POLYMERASE IV (PolIV) (Li et al. 2015; Zhai et al. 2015; Blevins et al. 2015). These siRNAs, in conjunction with ARGONAUTE 4 (AGO4), interact with the nascent transcripts of PolV, another plant-specific RNA polymerase (Wierzbicki et al. 2009; Wang and Axtell 2017). Importantly, AGO4 interacts with RNA-DIRECTED DNA METHYLATION 1 (RDM1) that is in association with a de novo methyltransferase, DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) (Gao et al. 2010). Recently, it has been also suggested that the PolIV-independent transcripts can also trigger RdDM through the RDR6-mediated pathway (Nuthikattu et al. 2013; Fultz et al. 2015; Kim et al. 2021; Hung and Slotkin 2021). Apart from the RdDM pathway, mCHH can also be mediated by CMT2, which interacts with an ATP-dependent chromatin modifier DECREASE IN DNA METHYLATION 1 (DDM1), particularly around the histone H1-enriched chromatin regions (Zemach et al. 2013).

Epialleles associated with crop traits

Crop yield is one of the major agronomic traits studied in crop science and increasing the crop yield has been an important breeding target. Several previous studies have shown that the epigenetic mechanisms are implicated in yield traits of rice. Zhang et al. identified a QTL, qWS8/ipa1-2D, that is associated with the ideal plant architecture (IPA) (Zhang et al. 2017). The cloned qWS8/ipa1-2D locus turned out to be a large tandemly repeated sequences which attenuated the epigenetic suppression of the *IPA1* promoter (Zhang et al. 2017). Therefore, the qWS8/ipa1-2D locus can serve as a new breeding target for high-yield rice varieties (Zhang et al. 2017). In addition, the epimutation of rice dwarfism (Epi-df) revealed the hypomethylation at *OsFIE1* gene that led to the reduction of histone H3 lysine 9 dimethylation (H3K9me2) and increase of H3K4me3 in the 5' region of *OsFIE1* (Zhang et al. 2012). Importantly, the increased expression of *OsFIE1* resulted in the higher grain yield of rice (Zhang et al. 2012).

Apart from the yield-related epialleles, multiple studies thus far have suggested that the DNA methylation is critical for various quality-related traits of crop plants. Particularly, diversity in flowers and fruits phenotypes is an important breeding resource in the horticultural industry. For example, natural epialleles with hypermethylation in the *Cycloidea* gene of *Linaria vulgaris* exhibit the radial symmetry of flowers (Cubas et al. 1999). In addition, tomato fruit ripening

and skin colors are regulated by DNA methylation at the genes encoding for the ripening-related transcription factors and the enzymes in the carotenoid synthesis pathway (Lang et al. 2017; Tang et al. 2020). These genes are in large part under direct regulation of *SlDML2*, a DEMETER-like DNA demethylase gene (Liu et al. 2015). Similar examples for fruit skin color control by DNA methylation can also be found in apples. The red-skinned "Kidd's D-8" (KID) and yellow-skinned "Blondee" (BLO) are natural apple mutants with different anthocyanin contents in the fruit skins (El-Sharkawy et al. 2015). During the fruit development of BLO, the DNA methylation levels are increased in the *MdMYB10* locus, which activates the anthocyanin production (El-Sharkawy et al. 2015). The hypermethylation of *MdMYB10* in BLO leads to the transcriptional suppression and therefore reduction of the anthocyanin levels (El-Sharkawy et al. 2015). The hypermethylation of *MdMYB10* was in fact observed previously in "Honeycrisp" apples (Telias et al. 2011). The increased DNA methylation was detected in the promoter of *MdMYB10* in green stripes of this apple variety (Telias et al. 2011). Moreover, the fruit size of "Golden Delicious" apples was suggested to be controlled by DNA methylation. The GDDH18 apple, a natural mutant of Golden Delicious, shows small fruit size and has increased DNA methylation in the promoter of *MdACS6*, which controls ethylene production and cell division (Daccord et al. 2017).

It has been well documented that DNA methylation influences plant's fitness under abiotic and biotic stresses. For example, Garg et al. investigated the DNA methylation profiles in three different stress-tolerant rice cultivars (IR64, Nagina 22 and Pokkali) and identified numerous differentially methylated regions (DMRs), some of which were associated with genes involved in the abiotic stress response (Garg et al. 2015). In addition, Akimoto et al. tested the treatment of 5-azacytidine, a chemical inhibitor for DNA methylation, to the stress-susceptible rice plants (Akimoto et al. 2007). The chemical treatment reduced the methylation at the promoter of *Xa21G* and resulted in the stronger resistance to *Xanthomonas oryzae* (Akimoto et al. 2007). Kumar et al. carried out similar experiments using 5-azacytidine in durum wheat and found that the chemical-induced demethylation can increase the resistance of wheat plants to the Fusarium head blight (Kumar et al. 2020). Overall, the natural and induced DNA methylation changes impact vastly on a wide range of agriculturally relevant traits.

Induction of epigenomic changes

We have so far introduced several major agricultural traits determined by DNA methylation. Given the strong potential of the epigenetic approaches in crop breeding, development of robust methods to induce epigenetic changes in crop plants will be an important next step. In this section, several methods triggering DNA methylation changes will be highlighted and discussed (illustrated in Fig. 1).

Chemical inhibitors such as 5-azacytidine and zebularine are commonly used reagents to induce DNA demethylation (Baubec et al. 2009; Nowicka et al. 2020). These chemical reagents act as analogues to cytosines and interferes with the maintenance of DNA methylation during DNA replication. This then allows for epigenetic alterations over cycles of cell divisions and might result in certain phenotypic changes in crops. Although the effect of demethylation by these inhibitors is generally transient and the epigenetic mark easily reverts to original status when the chemical treatment is stopped, there are also reports showing that the induced epialleles may persist stably across mitotic development. For example, the 5-azacytidine treatment led to the early flowering phenotype of strawberries (Xu et al. 2016). Such mitotically stable epimutations can be maintained directly through vegetative propagation, giving rise to new crop varieties. It is also worth mentioning that the chemically induced epiallelic changes are particularly useful in polyploid crops as genetic manipulation (through which DNA methylation can be perturbed) is particularly challenging in polyploid genomes.

Tissue culture is a widely used agricultural practice exploited for vegetative clonal propagation of crop plants and has recently been actively employed in the gene editing techniques (Twaij et al. 2020). It is well known that tissue culture causes drastic DNA methylation reduction which is often stable over multiple generations (Tanurdzic et al. 2008; Smulders and de Klerk 2011; Stroud et al. 2013; Azizi et al. 2020). Soma-clonal variation refers to phenotypic divergence observed in regenerated plants from in vitro tissue culture and is strongly associated with certain epiallelic changes (Kaeppler et al. 2000; Ong-Abdullah et al. 2015; Azizi et al. 2020; Masuda et al. 2020). It has been proposed that epigenetic diversity induced by tissue culture can be directly exploited in the crop breeding processes. Particularly, vegetatively propagating plants, where genetic manipulation is technically limited, can benefit more from tissue culture-mediated epiallelic alterations. It is noteworthy, however, that in vitro tissue culture might activate transposon mobilization (Cho et al. 2019; Satheesh et al. 2021), and thereby induces insertional gene mutations, which must be carefully discriminated from bona fide epiallelic difference.

The approaches of chemical treatment and tissue culture described above generate massive genome-wide alterations of DNA methylation in rather stochastic manner. Such complex epigenomic changes make it difficult to assess causal effect of a specific epiallele to certain phenotypic outcomes. In this regard, epiRIL population can be an alternative method to bypass the complexity and uncertainty imposed by massive methylome changes (Reinders

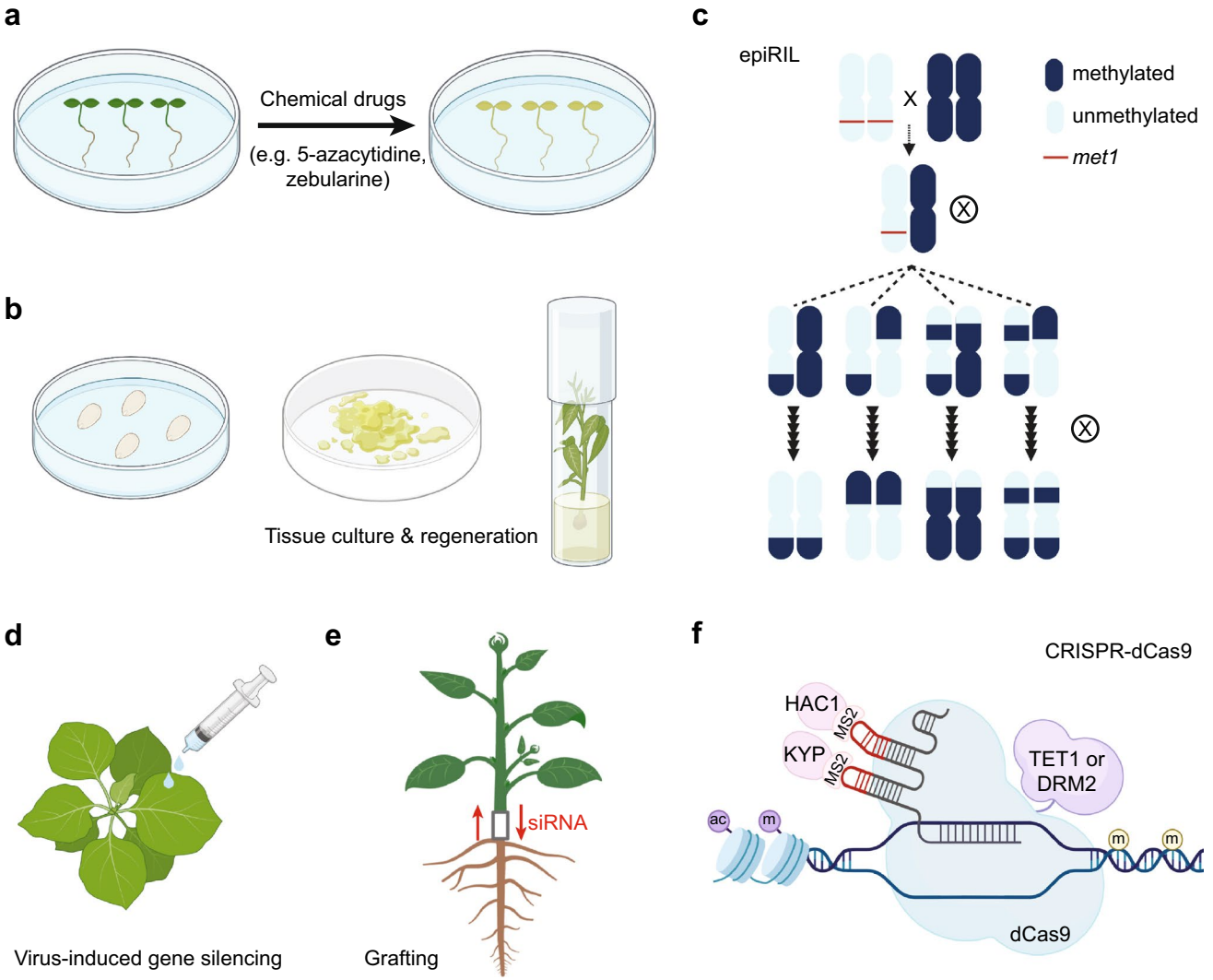

Fig. 1 Experimental methods used to induce epiallelic changes. **a** Chemical reagents such as 5-azacytidine and zebularine are potent DNA methylation inhibitors. **b** In vitro tissue culture and plant regeneration from dedifferentiated cells induce strong DNA demethylation. **c** EpiRILs artificially induce massive epialleles representing random mosaic methylome patterns. **d** Delivery of dsRNAs via viral vectors can trigger PTGS on specific genomic targets. **e** Grafting causes epigenetic alterations in scions and rootstocks, and also can be used to provide siRNAs produced from donor plants. **f** CRISPR–dCas9 is a versatile platform to accommodate epigenetic effector proteins to specific target loci

and Paszkowski 2009; Johannes and Colomé-Tatché 2011; Catoni and Cortijo 2018). The advantages of epiRIL include that epiallelic changes are artificially induced in higher frequency and density, and each epiRIL possesses unique mosaic methylome pattern enabling the mapping of causal loci associated with certain phenotypes (Reinders et al. 2009; Johannes et al. 2009; Zhang et al. 2013; Kooke et al. 2015; Debladis et al. 2017). EpiRIL population can be constructed by crossing two near-isogenic individuals with significant differences in DNA methylation (Reinders et al. 2009; Johannes et al. 2009). Subsequently, repeated self-pollination of single seed descent for at least 6 generations can achieve near homozygosity of methylation status in the individual lines (Reinders et al. 2009; Johannes et al. 2009). Unfortunately, it is still technically challenging in most crop species to generate genetic mutations for DNA methyltransferase and demethylases, and dramatic DNA methylation changes are hardly tolerated (Hu et al. 2014; Yamauchi et al. 2014; Li et al. 2014). For example, the mutants for DNA methyltransferases and demethylases in rice and maize are mostly lethal or sterile (Hu et al. 2014; Yamauchi et al. 2014; Li et al. 2014). Therefore, development of new methods is required to redirect the functions of the epigenetic regulators and induce epigenetic changes transiently in crop genomes, so the plant viability is not compromised by the drastic loss of DNA methylation.

Site-specific epigenetic engineering

Recently, targeted epigenetic engineering has been attempted by utilizing exogenous RNAi system. One example is virus-induced gene silencing (VIGS) system that carries specific double-stranded (ds) RNA inducing mostly post-transcriptional gene silencing (PTGS) or RNAi in the complementary target genes (Baulcombe 2004; Vaucheret 2006). In fact, VIGS has been used extensively in many crop species and demonstrated to work robustly (Torti et al. 2021); however, the downside of this approach particularly for breeding purpose is that its effect is only transient and is scantily transmitted to stable DNA methylation by RdDM. Nonetheless, it has been recently suggested that high pressure spraying of dsRNAs and targeting them to nucleus can trigger stable RdDM (Dalakouras and Papadopoulou 2020; Dalakouras and Ganopoulos 2021).

Grafting is a widely used agricultural practice that connects the root and shoot systems of different plants. In many crop species, it is a useful and advantageous method to increase the disease resistance and crop quality. Interestingly, it has been previously suggested that the DNA methylation status can be changed in the grafts between Solanaceae plants including tomato, eggplant, and pepper (Wu et al. 2013). Similar observation of DNA methylation changes has been made in Cucurbitaceae plants where cucumber and melon was grafted on pumpkin (Avramidou et al. 2015). In addition, several studies reported that siRNAs can transport through grafted junction and establish RdDM in Arabidopsis (Molnar et al. 2010, 2011; Melnyk et al. 2011a, 2011b). Such transmissible RdDM has been tested in potato, demonstrating that the siRNAs produced from transgenic tobacco scion established stable DNA methylation in the potato rootstock (Kasai et al. 2011, 2016). Overall, grafting can contribute to epibreeding by either generating epialleles and transmitting siRNAs from transgenic donor plants.

The gene editing systems such as zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), and CRISPR–Cas are rapidly emerging techniques that are broadening their application beyond gene editing. These systems consist of two modules, endonuclease and gene targeting modules, the latter of which is used for guiding the effector proteins to specific genomic location. For example, ZFN fused with SUVH9, a histone methyltransferase in the RdDM pathway, was tested in Arabidopsis (Johnson et al. 2014). The ZFN-SUVH9 fusion protein successfully targeted the *FWA* locus and stably deposited DNA methylation (Johnson et al. 2014). In addition to SUVH9, a series of RdDM factors was tethered to artificial ZF, confirming the robustness of the system in Arabidopsis (Gallego-Bartolomé et al. 2019). In another study, similar ZF module was also fused with the catalytic domain of the human DNA demethylase TEN-ELEVEN TRANSLOCATION1 (cdTET1), and it caused demethylation at the *FWA* gene and *CACTA1* transposon efficiently (Gallego-Bartolomé et al. 2018).

The CRISPR–dCas9 (catalytically inactive Cas9) system is by far the best utilized system for site-specific epigenetic editing owing to its extraordinary targeting efficiency and specificity. The dCas9 protein can be simply fused directly with an epigenetic effector protein. For example, the Arabidopsis histone acetyltransferase 1 (AtHAC1) was fused to dCas9 and targeted to the promoter of a gene encoding for abscisic acid (ABA)-responsive element binding protein 1/ABRE binding factor 2 (AREB1/ABF2) (Roca Paixão et al. 2019). The plants expressing this fusion protein exhibited higher transcriptional activity of the target gene and stronger tolerance to drought stress (Roca Paixão et al. 2019). In addition, epigenetic effector proteins can be tethered to dCas9 through the MS2 system (Moradpour and Abdulah 2020; Pan et al. 2021). In this system, the short guide (sg) RNA is modified to incorporate a short RNA hairpin aptamer that can accommodate the binding of MS2. In the work of Lee et al., the MS2 fusion was attempted to KRYPTONITE (KYP), G9a (H3K9 methyltransferases), and p300 (histone acetyltransferase) targeting the *FT* gene, a floral activator, in Arabidopsis (Lee et al. 2019). These plants exhibited divergent flowering phenotypes, likely caused by the chromatin modifications at the *FT* locus (Lee et al. 2019). The SunTag system is another efficient approach to tether proteins to dCas9 that contains the tandem GCN4 peptide repeats fused to dCas9 and a single chain variable fragment (scFv) GCN4 antibody anchored with epigenetic effector proteins (Tanenbaum et al. 2014; Pan et al. 2021). Such method was tested in DNA demethylase effector protein cdTET1 in Arabidopsis, which exhibited high efficiency of demethylation at the targeted *FWA* locus (Gallego-Bartolomé et al. 2018). Moreover, the tethering of CRISPR–dCas9-SunTag to DRM2, a de novo DNA methyltransferase, was also demonstrated to function efficiently in *FWA* of Arabidopsis (Papikian et al. 2019). Although all these known examples of epigenetic editing were demonstrated only in Arabidopsis, given the versatility and robustness of CRISPR–Cas systems in crop plants, it appears very promising that site-specific epigenetic modification will be more feasible in crop models in the near future.

Conclusion

In this review, we have introduced several examples of the epiallelic diversity influencing on relevant agronomic traits of crops. Most importantly, the epibreeding approach provides several advantages in crop breeding. Firstly, epiallelic variations can supplement the currently limited genetic diversity, broadening the crop breeding resource. Secondly,

unlike traditional crop breeding methods, epibreeding does not require any costly and time-consuming selection processes, which can accelerate the breeding procedures. Thirdly, epibreeding is not subject to the issues regarding genetically modified organisms since epigenetic variability is genuinely non-genetic. Nonetheless, despite the vast impact of epimutations on crop traits, the stability and heritability of epigenetic variation is an important consideration for potential application to breeding strategies. In other words, the stability of newly created epialleles over meiotic divisions and generations is a critical issue, so that the epigenetic variability in crop genomes can be stably perpetuated during breeding process. Overall, the techniques for epigenetic engineering in crops are maturing for better precision and applicability, and such improvement will eventually facilitate crop breeding process.

Acknowledgements We are grateful to Wuqian Wang for assisting with literature search. This work was supported by grants from the National Natural Science Foundation of China (31970518, to JC), Strategic Priority Research Program of the Chinese Academy of Sciences (XDB27030209, to JC), 2020 Research Fund of Myongji University (to KDK), National Research Foundation of Korea (2020R1G1A1100757, to KDK) and General Program of Natural Science Foundation of Shanghai (21ZR1470700, to EYK). EYK is the recipient of a President's International Fellowship Initiative (PIFI) young staff fellowship (2021FYB0001) from the Chinese Academy of Sciences.

Author contributions EYK drafted the manuscript. KDK and JC edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Akimoto K, Katakami H, Kim H-J et al (2007) Epigenetic inheritance in rice plants. Ann Bot 100:205–217. https://doi.org/10.1093/aob/mcm110

Avramidou E, Kapazoglou A, Aravanopoulos FA et al (2015) Global DNA methylation changes in Cucurbitaceae inter-species grafting. Crop Breed Appl Biotechnol 15:112–116. https://doi.org/10.1590/1984-70332015v15n2n20

Azizi P, Hanafi MM, Sahebi M et al (2020) Epigenetic changes and their relationship to somaclonal variation: a need to monitor the micropropagation of plantation crops. Funct Plant Biol 47:508. https://doi.org/10.1071/FP19077

Baubec T, Pecinka A, Rozhon W, Mittelsten Scheid O (2009) Effective, homogeneous and transient interference with cytosine methylation in plant genomic DNA by zebularine. Plant J 57:542–554. https://doi.org/10.1111/j.1365-313X.2008.03699.x

Baulcombe D (2004) RNA silencing in plants. Nature 431:356–363. https://doi.org/10.1038/nature02874

Bhadouriya SL, Mehrotra S, Basantani MK et al (2021) Role of chromatin architecture in plant stress responses: an update. Front Plant Sci 11:2131. https://doi.org/10.3389/fpls.2020.603380

Blevins T, Podicheti R, Mishra V et al (2015) Identification of Pol IV and RDR2-dependent precursors of 24 nt siRNAs guiding de novo DNA methylation in Arabidopsis. Elife 4:1–22. https://doi.org/10.7554/eLife.09591

Catoni M, Cortijo S (2018) EpiRILs: lessons from Arabidopsis. In: Mirouze M, Bucher E, Gallusci P (eds) Advances in botanical research, vol 88. Elsevier, Netherlands, pp 87–116. https://doi.org/10.1016/bs.abr.2018.08.002

Cho J, Benoit M, Catoni M et al (2019) Sensitive detection of pre-integration intermediates of long terminal repeat retrotransposons in crop plants. Nat Plants 5:26–33. https://doi.org/10.1038/s41477-018-0320-9

Cubas P, Vincent C, Coen E (1999) An epigenetic mutation responsible for natural variation in floral symmetry. Nature 401:157–161. https://doi.org/10.1038/43657

Daccord N, Celton J-M, Linsmith G et al (2017) High-quality de novo assembly of the apple genome and methylome dynamics of early fruit development. Nat Genet 49:1099–1106. https://doi.org/10.1038/ng.3886

Dalakouras A, Ganopoulos I (2021) Induction of promoter DNA methylation upon high-pressure spraying of double-stranded RNA in plants. Agronomy 11:789. https://doi.org/10.3390/agronomy11040789

Dalakouras A, Papadopoulou KK (2020) Epigenetic modifications: an unexplored facet of exogenous RNA application in plants. Plants 9:673. https://doi.org/10.3390/plants9060673

Dalakouras A, Vlachostergios D (2021) Epigenetic approaches to crop breeding: current status and perspectives. J Exp Bot 72:5356–5371. https://doi.org/10.1093/jxb/erab227

Debladis E, Llauro C, Carpentier MC et al (2017) Detection of active transposable elements in *Arabidopsis thaliana* using Oxford Nanopore Sequencing technology. BMC Genomics 18:1–8. https://doi.org/10.1186/s12864-017-3753-z

El-Sharkawy I, Liang D, Xu K (2015) Transcriptome analysis of an apple (Malus × domestica) yellow fruit somatic mutation identifies a gene network module highly associated with anthocyanin and epigenetic regulation. J Exp Bot 66:7359–7376. https://doi.org/10.1093/jxb/erv433

Friedrich T, Faivre L, Bäurle I, Schubert D (2019) Chromatin-based mechanisms of temperature memory in plants. Plant Cell Environ 42:762–770. https://doi.org/10.1111/pce.13373

Fultz D, Choudury SG, Slotkin RK (2015) Silencing of active transposable elements in plants. Curr Opin Plant Biol 27:67–76. https://doi.org/10.1016/j.pbi.2015.05.027

Gallego-Bartolomé J, Gardiner J, Liu W et al (2018) Targeted DNA demethylation of the Arabidopsis genome using the human TET1 catalytic domain. Proc Natl Acad Sci 115:E2125–E2134. https://doi.org/10.1073/pnas.1716945115

Gallego-Bartolomé J, Liu W, Kuo PH et al (2019) Co-targeting RNA polymerases IV and V promotes efficient de novo DNA methylation in Arabidopsis. Cell 176:1068-1082.e19. https://doi.org/10.1016/j.cell.2019.01.029

Gao Z, Liu H-L, Daxinger L et al (2010) An RNA polymerase II- and AGO4-associated protein acts in RNA-directed DNA methylation. Nature 465:106–109. https://doi.org/10.1038/nature09025

Garg R, Narayana Chevala V, Shankar R, Jain M (2015) Divergent DNA methylation patterns associated with gene expression in rice cultivars with contrasting drought and salinity stress response. Sci Rep 5:14922. https://doi.org/10.1038/srep14922

Garnett T, Appleby MC, Balmford A et al (2013) Sustainable intensification in agriculture: premises and policies. Science (80-) 341:33–34. https://doi.org/10.1126/science.1234485

Henikoff S, Greally JM (2016) Epigenetics, cellular memory and gene regulation. Curr Biol 26:R644–R648. https://doi.org/10.1016/j.cub.2016.06.011

Hu L, Li N, Xu C et al (2014) Mutation of a major CG methylase in rice causes genome-wide hypomethylation, dysregulated genome expression, and seedling lethality. Proc Natl Acad Sci USA 111:10642–10647. https://doi.org/10.1073/pnas.1410761111

Hung Y-H, Slotkin RK (2021) The initiation of RNA interference (RNAi) in plants. Curr Opin Plant Biol 61:102014. https://doi.org/10.1016/j.pbi.2021.102014

Johannes F, Colomé-Tatché M (2011) Concerning epigenetics and inbreeding. Nat Rev Genet 12:376. https://doi.org/10.1038/nrg2664-c3

Johannes F, Porcher E, Teixeira FK et al (2009) Assessing the impact of transgenerational epigenetic variation on complex traits. PLoS Genet 5:e1000530. https://doi.org/10.1371/journal.pgen.1000530

Johnson LM, Du J, Hale CJ et al (2014) SRA- and SET-domain-containing proteins link RNA polymerase V occupancy to DNA methylation. Nature 507:124–128. https://doi.org/10.1038/nature12931

Kaeppler SM, Kaeppler HF, Rhee Y (2000) Epigenetic aspects of somaclonal variation in plants. Plant Mol Biol 43:179–188. https://doi.org/10.1023/a:1006423110134

Kankel MW, Ramsey DE, Stokes TL et al (2003) Arabidopsis MET1 cytosine methyltransferase mutants. Genetics 163:1109–1122. https://doi.org/10.1093/genetics/163.3.1109

Kasai A, Bai S, Li T, Harada T (2011) Graft-transmitted siRNA signal from the root induces visual manifestation of endogenous post-transcriptional gene silencing in the scion. PLoS ONE 6:e16895. https://doi.org/10.1371/journal.pone.0016895

Kasai A, Bai S, Hojo H, Harada T (2016) Epigenome editing of potato by grafting using transgenic tobacco as siRNA donor. PLoS ONE 11:e0161729. https://doi.org/10.1371/journal.pone.0161729

Kim J-M, Sasaki T, Ueda M et al (2015) Chromatin changes in response to drought, salinity, heat, and cold stresses in plants. Front Plant Sci 6:114. https://doi.org/10.3389/fpls.2015.00114

Kim EY, Wang L, Lei Z et al (2021) Ribosome stalling and SGS3 phase separation prime the epigenetic silencing of transposons. Nat Plants 7:303–309. https://doi.org/10.1038/s41477-021-00867-4

Kooke R, Johannes F, Wardenaar R et al (2015) Epigenetic basis of morphological variation and phenotypic plasticity in *Arabidopsis thaliana*. Plant Cell 27:337–348. https://doi.org/10.1105/tpc.114.133025

Kumar J, Rai KM, Pirseyedi S et al (2020) Epigenetic regulation of gene expression improves Fusarium head blight resistance in durum wheat. Sci Rep 10:17610. https://doi.org/10.1038/s41598-020-73521-2

Lang Z, Wang Y, Tang K et al (2017) Critical roles of DNA demethylation in the activation of ripening-induced genes and inhibition of ripening-repressed genes in tomato fruit. Proc Natl Acad Sci 114:E4511–E4519. https://doi.org/10.1073/pnas.1705233114

Law JA, Jacobsen SE (2010) Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat Rev Genet 11:204–220. https://doi.org/10.1038/nrg2719

Lee JE, Neumann M, Duro DI, Schmid M (2019) CRISPR-based tools for targeted transcriptional and epigenetic regulation in plants. PLoS ONE 14:e0222778. https://doi.org/10.1371/journal.pone.0222778

Li Q, Eichten SR, Hermanson PJ et al (2014) genetic perturbation of the maize methylome. Plant Cell 26:4602–4616. https://doi.org/10.1105/tpc.114.133140

Li S, Vandivier LE, Tu B et al (2015) Detection of pol IV/RDR2-dependent transcripts at the genomic scale in Arabidopsis reveals features and regulation of sirna biogenesis. Genome Res 25:235–245. https://doi.org/10.1101/gr.182238.114

Lindroth AM, Cao X, Jackson JP et al (2001) Requirement of CHROMOMETHYLASE3 for maintenance of CpXpG methylation. Science (80-) 292:2077–2080. https://doi.org/10.1126/science.1059745

Liu R, How-kit A, Stammitti L et al (2015) A DEMETER-like DNA demethylase governs tomato fruit ripening. Proc Natl Acad Sci 112:10804–10809. https://doi.org/10.1073/pnas.1503362112

Masuda K, Akagi T, Esumi T, Tao R (2020) Epigenetic flexibility underlies somaclonal sex conversions in hexaploid persimmon. Plant Cell Physiol 61:393–402. https://doi.org/10.1093/pcp/pcz207

Matzke MA, Mosher RA (2014) RNA-directed DNA methylation: an epigenetic pathway of increasing complexity. Nat Rev Genet 15:394–408. https://doi.org/10.1038/nrg3683

Melnyk CW, Molnar A, Bassett A, Baulcombe DC (2011a) Mobile 24 nt small RNAs direct transcriptional gene silencing in the root meristems of *Arabidopsis thaliana*. Curr Biol 21:1678–1683. https://doi.org/10.1016/j.cub.2011.08.065

Melnyk CW, Molnar A, Baulcombe DC (2011b) Intercellular and systemic movement of RNA silencing signals. EMBO J 30:3553–3563. https://doi.org/10.1038/emboj.2011.274

Molnar A, Melnyk CW, Bassett A et al (2010) Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. Science (80-) 328:872–875. https://doi.org/10.1126/science.1187959

Molnar A, Melnyk C, Baulcombe DC (2011) Silencing signals in plants: a long journey for small RNAs. Genome Biol 12:215. https://doi.org/10.1186/gb-2010-11-12-219

Moradpour M, Abdulah SNA (2020) CRISPR/dCas9 platforms in plants: strategies and applications beyond genome editing. Plant Biotechnol J 18:32–44. https://doi.org/10.1111/pbi.13232

Nelson GC, Valin H, Sands RD et al (2014) Climate change effects on agriculture: economic responses to biophysical shocks. Proc Natl Acad Sci 111:3274–3279. https://doi.org/10.1073/pnas.1222465110

Nowicka A, Tokarz B, Zwyrtková J et al (2020) Comparative analysis of epigenetic inhibitors reveals different degrees of interference with transcriptional gene silencing and induction of DNA damage. Plant J 102:68–84. https://doi.org/10.1111/tpj.14612

Nuthikattu S, McCue AD, Panda K et al (2013) The initiation of epigenetic silencing of active transposable elements is triggered by RDR6 and 21–22 nucleotide small interfering RNAs. Plant Physiol 162:116–131. https://doi.org/10.1104/pp.113.216481

Ong-Abdullah M, Ordway JM, Jiang N et al (2015) Loss of Karma transposon methylation underlies the mantled somaclonal variant of oil palm. Nature 525:533–537. https://doi.org/10.1038/nature15365

Pan C, Sretenovic S, Qi Y (2021) CRISPR/dCas-mediated transcriptional and epigenetic regulation in plants. Curr Opin Plant Biol 60:101980. https://doi.org/10.1016/j.pbi.2020.101980

Papikian A, Liu W, Gallego-Bartolomé J, Jacobsen SE (2019) Site-specific manipulation of Arabidopsis loci using CRISPR–Cas9 SunTag systems. Nat Commun 10:729. https://doi.org/10.1038/s41467-019-08736-7

Reinders J, Paszkowski J (2009) Unlocking the Arabidopsis epigenome. Epigenetics 4:557–563. https://doi.org/10.4161/epi.4.8.10347

Reinders J, Wulff BBH, Mirouze M et al (2009) Compromised stability of DNA methylation and transposon immobilization in mosaic Arabidopsis epigenomes. Genes Dev 23:939–950. https://doi.org/10.1101/gad.524609

Roca Paixão JF, Gillet F-X, Ribeiro TP et al (2019) Improved drought stress tolerance in Arabidopsis by CRISPR/dCas9 fusion with a histone acetyltransferase. Sci Rep 9:8080. https://doi.org/10.1038/s41598-019-44571-y

Ronemus MJ, Galbiati M, Ticknor C et al (1996) demethylation-induced developmental pleiotropy in Arabidopsis. Science (80-) 273:654–657. https://doi.org/10.1126/science.273.5275.654

Satheesh V, Fan W, Chu J, Cho J (2021) Recent advancement of NGS technologies to detect active transposable elements in plants. Genes Genom 43:289–294. https://doi.org/10.1007/s13258-021-01040-z

Smulders MJM, de Klerk GJ (2011) Epigenetics in plant tissue culture. Plant Growth Regul 63:137–146. https://doi.org/10.1007/s10725-010-9531-4

Springer NM, Schmitz RJ (2017) Exploiting induced and natural epigenetic variation for crop improvement. Nat Rev Genet 18:563–575. https://doi.org/10.1038/nrg.2017.45

Stroud H, Ding B, Simon SA et al (2013) Plants regenerated from tissue culture contain stable epigenome changes in rice. Elife 2:1–14. https://doi.org/10.7554/eLife.00354

Tanenbaum ME, Gilbert LA, Qi LS et al (2014) A protein-tagging system for signal amplification in gene expression and fluorescence imaging. Cell 159:635–646. https://doi.org/10.1016/j.cell.2014.09.039

Tang D, Gallusci P, Lang Z (2020) Fruit development and epigenetic modifications. New Phytol 228:839–844. https://doi.org/10.1111/nph.16724

Tanurdzic M, Vaughn MW, Jiang H et al (2008) Epigenomic consequences of immortalized plant cell suspension culture. PLoS Biol 6:e302. https://doi.org/10.1371/journal.pbio.0060302

Telias A, Lin-Wang K, Stevenson DE et al (2011) Apple skin patterning is associated with differential expression of MYB10. BMC Plant Biol 11:93. https://doi.org/10.1186/1471-2229-11-93

Tirnaz S, Batley J (2019) Epigenetics: potentials and challenges in crop breeding. Mol Plant 12:1309–1311. https://doi.org/10.1016/j.molp.2019.09.006

Torti S, Schlesier R, Thümmler A et al (2021) Transient reprogramming of crop plants for agronomic performance. Nat Plants 7:159–171. https://doi.org/10.1038/s41477-021-00851-y

Twaij BM, Jazar ZH, Hasan MN (2020) Trends in the use of tissue culture, applications and future aspects. Int J Plant Biol. https://doi.org/10.4081/pb.2020.8385

Vaucheret H (2006) Post-transcriptional small RNA pathways in plants: mechanisms and regulations. Genes Dev 20:759–771. https://doi.org/10.1101/gad.1410506

Wang F, Axtell MJ (2017) AGO4 is specifically required for heterochromatic siRNA accumulation at Pol V-dependent loci in *Arabidopsis thaliana*. Plant J 90:37–47. https://doi.org/10.1111/tpj.13463

Wendte JM, Pikaard CS (2017) The RNAs of RNA-directed DNA methylation. Biochim Biophys Acta Gene Regul Mech 1860:140–148. https://doi.org/10.1016/j.bbagrm.2016.08.004

Wierzbicki AT, Ream TS, Haag JR, Pikaard CS (2009) RNA polymerase V transcription guides ARGONAUTE4 to chromatin. Nat Genet 41:630–634. https://doi.org/10.1038/ng.365

Wu C-T, Morris JR (2001) Genes, genetics, and epigenetics: a correspondence. Science 293:1103–1105. https://doi.org/10.1126/science.293.5532.1103

Wu R, Wang X, Lin Y et al (2013) Inter-species grafting caused extensive and heritable alterations of DNA methylation in Solanaceae plants. PLoS ONE 8:e61995. https://doi.org/10.1371/journal.pone.0061995

Xiao J, Lee U-S, Wagner D (2016) Tug of war: adding and removing histone lysine methylation in Arabidopsis. Curr Opin Plant Biol 34:41–53. https://doi.org/10.1016/j.pbi.2016.08.002

Xu J, Tanino KK, Robinson SJ (2016) Stable epigenetic variants selected from an induced hypomethylated *Fragaria vesca* population. Front Plant Sci 7:1768. https://doi.org/10.3389/fpls.2016.01768

Yamauchi T, Johzuka-Hisatomi Y, Terada R et al (2014) The MET1b gene encoding a maintenance DNA methyltransferase is indispensable for normal development in rice. Plant Mol Biol 85:219–232. https://doi.org/10.1007/s11103-014-0178-9

Zemach A, Kim MY, Hsieh PH et al (2013) The arabidopsis nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. Cell 153:193–205. https://doi.org/10.1016/j.cell.2013.02.033

Zhai J, Bischof S, Wang H et al (2015) A one precursor one siRNA model for pol IV-dependent siRNA biogenesis. Cell 163:445–455. https://doi.org/10.1016/j.cell.2015.09.032

Zhang L, Cheng Z, Qin R et al (2012) Identification and characterization of an epi-allele of FIE1 reveals a regulatory linkage between two epigenetic marks in rice. Plant Cell 24:4407–4421. https://doi.org/10.1105/tpc.112.102269

Zhang Y, Fischer M, Colot V, Bossdorf O (2013) Epigenetic variation creates potential for evolution of plant phenotypic plasticity. New Phytol 197:314–322. https://doi.org/10.1111/nph.12010

Zhang L, Yu H, Ma B et al (2017) A natural tandem array alleviates epigenetic repression of IPA1 and leads to superior yielding rice. Nat Commun 8:14789. https://doi.org/10.1038/ncomms14789