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Review

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Population epigenomics of plant DNA methylation: perspectives for molecular breeding

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Abstract: DNA methylation, a key epigenetic modification, contributes to regulatory diversity in plants and plays important roles in phenotypic plasticity and environmental adaptation. Recent population epigenomic studies have revealed extensive methylation variation across plant genomes, characterized genomic distribution and genetic architecture, and demonstrated both its dependence on and partial independence from underlying DNA sequence variation. These studies have further linked methylation variation to gene expression regulation, domestication, local adaptation, and complex trait variation through integrative analyses and epigenome-wide association research. Advances in high-throughput sequencing have enabled the generation of population-scale plant methylomes across natural accessions, wild relatives, landraces, and breeding germplasm, providing new avenues to systematically investigate epigenomic diversity and its functional consequences. In parallel, public methylome resources, epigenomic databases, and multi-omics analytical platforms are accelerating comparative and cross-species analyses of plant epigenomic variation. Future efforts that integrate population methylomes with genomics, transcriptomics, chromatin accessibility, three-dimensional genome organization, deep learning-based analytical frameworks, and targeted epigenome editing are expected to facilitate the discovery of epigenetic breeding markers, promoting the application of epigenomic variation in crop improvement.

Key words: DNA methylation; Population-scale epigenomics; Single methylation polymorphism; Gene expression; Epigenome-wide association study; Molecular breeding

1 Introduction

Genetics examines the mechanisms that govern the transmission of traits across generations. Traditional evolutionary theory has largely emphasized that DNA sequence variation is generated by mutation and recombination and shaped over time by genetic drift, migration, and natural selection (Mueller et al., 2025). However, this sequence-based view of inheritance has been increasingly expanded by evidence demonstrating that transgenerational inheritance may also be mediated by epigenetic mechanisms that do not alter the primary DNA sequence (Grossniklaus et al., 2013).

DNA methylation is one of the most extensively studied epigenetic modifications and is catalyzed by DNA methyltransferases, which add a methyl group to the C5 position of cytosine to generate 5-methylcytosine (5mC). 5mC was first reported in bacterial nucleic acids in 1925 (Johnson and Coghill, 1925) and was later detected in plant and animal genomes in 1950 (Wyatt, 1950). DNA methylation is widely observed across diverse organisms, although its sequence contexts, molecular forms, and biological functions differ substantially among bacteria, plants, and animals (Zemach and Zilberman, 2010).

In plants, cytosine methylation occurs in CG, CHG, and CHH contexts, where H represents A, C, or T (Guseinov and Vanyushin, 1975). These methylation states are established, maintained, and removed by coordinated methyltransferase and demethylase

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pathways, including methyltransferase 1 (*MET1*), chromomethylases (*CMT*s), domains rearranged methyltransferase 2 (*DRM2*), DEMETER (*DME*), and repressor of silencing 1 (*ROS1*) (Law and Jacobsen, 2010; Erdmann and Picard, 2020; Xie et al., 2025). CG methylation is generally the most stable across mitotic and meiotic divisions, whereas CHH methylation is often more dynamic and tissue- or development-specific (Bouyer et al., 2017; Kawakatsu et al., 2017). Through these context-dependent patterns, DNA methylation has many regulatory roles, such as in transposon silencing, gene expression, genomic imprinting, developmental transitions, stress responses, and agriculturally important traits. Representative examples of plant genes and traits regulated by DNA methylation are summarized in Table 1, illustrating the broad involvement of methylation in development, stress adaptation, metabolism, and crop domestication.

Despite extensive knowledge of DNA methylation pathways and individual epialleles, it is incompletely understood how naturally occurring methylation variation is distributed, inherited, regulated, and selected at the population scale. Population epigenomics addresses this issue by treating DNA methylation as a quantitative molecular phenotype across diverse germplasms. In this review, we summarize recent progress in plant population methylomics, discuss the genetic and functional architecture of methylation variation, and highlight emerging opportunities for epigenetic marker discovery, functional validation, and molecular breeding applications.

2 Population-scale methylome resources in plants

Population-scale methylome resources provide the foundation for characterizing natural epigenomic diversity across wild accessions, landraces, domestication panels, and breeding germplasms (Fig. 1a). By

Table 1 Representative plant genes and traits regulated by DNA methylation

Gene	Reference	Species	Phenotype
ACT1	Song et al., 2025	*Oryza sativa* (rice)	Promoter hypomethylation enhances cold tolerance and adaptability to high-latitude regions
COL2	Song et al., 2017	*Gossypium hirsutum* (cotton)	Methylation dynamics regulate photoperiod sensitivity
FWA	Soppe et al., 2000	*Arabidopsis thaliana* (thale cress)	Epigenetic allele causes a late-flowering phenotype
CMT2	Jiang et al., 2024	*Arabidopsis thaliana* (thale cress)	*CMT2* reduces genome-wide CHH methylation, improving thermotolerance and ultraviolet-B (UV-B) resistance
OsSPL14	Miura et al., 2010	*Oryza sativa* (rice)	Promoter methylation changes are associated with altered chromatin structure, enhanced *OsSPL14* expression, and improved plant architecture and yield
tb1	Xu et al., 2020	*Zea mays* (maize)	DMR methylation regulates tiller number, affecting plant architecture
Lcyc	Cubas et al., 1999	*Linaria vulgaris* (yellow toadflax)	Promoter hypermethylation leads to radial flower symmetry
Karma	Ong-Abdullah et al., 2015	*Elaeis guineensis* (African oil palm)	Loss of methylation causes mantled somaclonal variation, affecting clonal propagation
SlDML2	Lang et al., 2017	*Solanum lycopersicum* (tomato)	Genome-wide demethylation acts as a switch for fruit-ripening initiation
Cnr	Manning et al., 2006	*Solanum lycopersicum* (tomato)	Promoter hypermethylation inhibits ripening
CAMTA2	Song et al., 2024	*Pyrus pyrifolia* (Asian pear)	*CAMTA2*, a gene associated with a hypermethylated DMR, delays fruit ripening when overexpressed in tomato
VTE3	Quadrana et al., 2014	*Solanum lycopersicum* (tomato)	Methylation dynamics of a promoter SINE transposon regulate vitamin E synthesis
lpa1-241	Pilu et al., 2009	*Zea mays* (maize)	DNA methylation-mediated paramutation leads to phytic acid synthesis deficiency

ACT1: acquired cold tolerance 1; *COL2*: constans-like 2; *FWA*: flowering Wageningen; *CMT2*: chromomethylase 2; *OsSPL14*: squamosa promoter binding protein-like 14; *tb1*: teosinte branched 1; DMR: differentially methylated region; *Lcyc*: *Linaria* cycloidea (*cyc*)-like gene; *SlDML2*: *Solanum lycopersicum* DEMETER-like DNA demethylase 2; *Cnr*: colorless non-ripening; *CAMTA2*: calmodulin-binding transcription activator 2; *VTE3*: vitamin E 3; SINE: short interspersed nuclear element; *lpa1-241*: low phytic acid 1-241.

profiling DNA methylation across diverse individuals, these datasets enable the discovery of methylation polymorphisms and their integration with genetic, transcriptomic, environmental, and phenotypic variation (Fig. 1a). Whole-genome bisulfite sequencing remains the dominant approach because it quantifies cytosine methylation at the single-base resolution in CG, CHG, and CHH contexts.

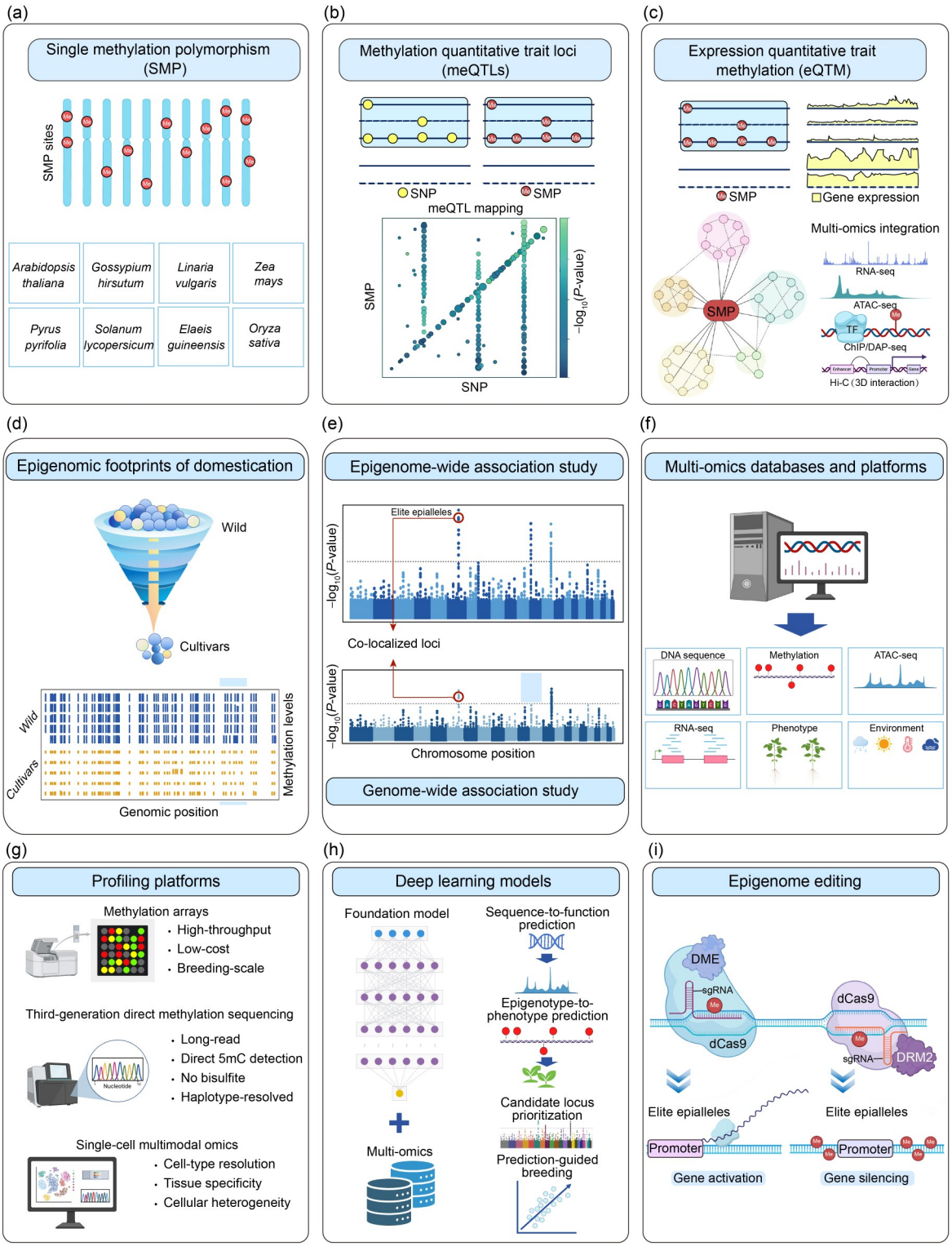

Fig. 1 Integrated framework for population epigenomics, functional validation, and epigenetic breeding in plants. Schematic overview of the key concepts, resources, analytical strategies, and emerging technologies in plant population epigenomics. (a) Single methylation polymorphisms (SMPs) and population-scale methylome resources provide a foundation for characterizing natural epigenomic variation in plants. (b) Genetic dissection of methylation variation, through association analyses between genetic variants and methylation loci, enables the identification of methylation quantitative trait loci (meQTLs) and genetic determinants of epigenetic variation. (c) Expression quantitative trait methylation (eQTM) analysis links methylation variation at individual SMP sites with gene expression variation, facilitating the construction of integrated multi-omics regulatory networks. (d) Comparative methylome analyses between wild accessions and cultivated germplasm reveal domestication-associated epigenomic footprints and methylation shifts shaped by selection. (e) Epigenome-wide association studies (EWAS) associate methylation variation with agronomic traits, enabling the identification of candidate epialleles and trait-associated methylation markers. (f) Multi-omics integration databases and plant epigenomic platforms provide resources for data mining, visualization, and cross-layer regulatory analyses. (g) Methylation arrays, Oxford Nanopore Technologies (ONT) long-read sequencing, and single-cell multi-omics platforms represent emerging approaches for high-throughput, haplotype-resolved, and cell-type-specific methylation profiling. (h) Transformer- and deep learning-based foundation models integrate sequence, epigenomic, transcriptomic, and phenotypic information to support sequence-to-function prediction, epigenotype-to-phenotype prediction, candidate locus prioritization, and prediction-guided breeding. (i) Targeted epigenome editing using dCas9 fused to methylation effector domains enables the functional validation of candidate epialleles. dCas9–DME induces locus-specific demethylation and gene activation, whereas dCas9–DRM2 promotes targeted de novo methylation and transcriptional silencing. The figure was created with BioRender.com, with final layout, labeling, and formatting performed using Adobe Illustrator 2026 and Adobe Photoshop 2021 (Adobe Inc.), as well as Microsoft PowerPoint (Microsoft Corp.). SNP: single nucleotide polymorphism; RNA-seq: RNA sequencing; ATAC-seq: assay for transposase-accessible chromatin sequencing; TF: transcription factor; ChIP: chromatin immunoprecipitation; DAP-seq: DNA affinity purification sequencing; Hi-C: high-throughput chromosome conformation capture; sgRNA: single-guide RNA; Cas9: clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9; dCas9: catalytically dead Cas9; 5mC: 5-methylcytosine; DME: DEMETER; DRM2: domains rearranged methyltransferase 2.

Population-scale methylome resources have been established for the model species *Arabidopsis thaliana* (Schmitz et al., 2013; Kawakatsu et al., 2016), including global natural accessions, and for several major crops, such as rice (Cao et al., 2023), maize (Xu et al., 2020), soybean (Jiang et al., 2026), tomato (Guo et al., 2023), lettuce (Cao et al., 2024), pear (Song et al., 2024), and cotton (Zhao et al., 2024) (Table 2). These datasets represent a wide range of biological materials, including wild and cultivated accessions, domestication panels, diversity collections, and representative breeding materials. In many cases, methylomes are generated from tissues directly relevant to agronomic traits, including seedling leaves, shoot apices, cotton fibers, fruit flesh, and pericarp. Such tissue-specific sampling strategies enhance the utility of population methylomes for linking epigenomic variation to developmental processes and agriculturally important traits. Collectively, these datasets reveal a clear transition from single-reference methylome profiling toward population-level, tissue-informed, and multi-omics epigenomic resources.

3 Origin and architecture of methylation variation

Population-level methylation variation can arise from three major sources (Becker et al., 2011). First, spontaneous epimutations result from the imperfect maintenance, establishment, or removal of DNA methylation and occur at rates substantially higher than DNA sequence mutations (van der Graaf et al., 2015). Second, genetically associated methylation variation can be caused by local or distal variants, such as single nucleotide polymorphisms (SNPs), transposable element (TE) insertions, structural variants, copy-number changes, as well as mutations in methylation-related genes such as *MET1* (Reinders et al., 2009) and *ROS1* (Tang et al., 2024). Third, environmentally or developmentally induced methylation changes may occur in response to stress, biotic interactions, or developmental transitions. Although many such changes are transient, a subset may persist across generations, especially when reinforced by genetic background or recurrent environmental selection.

Population-scale bisulfite sequencing allows methylation variation to be quantified at both regional and single-cytosine resolution. At the regional level, differentially methylated positions and regions (DMPs and DMRs) are used to compare individuals, populations, or domestication groups (Richards, 2006; Xu et al., 2020; Song et al., 2024; Zhao et al., 2024). At the single-cytosine level, single methylation polymorphisms (SMPs) describe methylation-state variation at individual cytosines and can be encoded as methylated, unmethylated, or partially methylated states (Zhao

Table 2 Summary of population-scale DNA methylation datasets in plants

Species	Reference	Sample number	Germplasm	Tissue	Multi-omics integration
Arabidopsis thaliana	Schmitz et al., 2013; Kawakatsu et al., 2016	1107	Natural accessions	Whole seedling	WGBS, RNA-seq
Oryza sativa	Cao et al., 2023	95	Wild rice, cultivated *indica*, cultivated *japonica*, aromatic rice, aus rice, weedy *indica*, weedy *japonica*	Third leaf (30 d)	WGBS
Zea mays	Xu et al., 2020	37	Modern inbreds, landraces, teosintes/wild relatives	Seedling	WGS, WGBS
Glycine max	Shen et al., 2018	45	Wild soybeans, landraces, cultivars	Leaf	WGBS
Solanum lycopersicum	Guo et al., 2023	96	Wild tomato, landraces, cultivars	Pericarp	WGBS, RNA-seq, metabolome
Lactuca sativa	Cao et al., 2024	52	Cultivars, wild relatives	Whole seedling	WGBS, RNA-seq, chromatin accessibility, interaction data
Pyrus pyrifolia	Song et al., 2024	41	Wild pear, landraces, improved cultivars	Fruit flesh	WGBS, RNA-seq, WGS
Gossypium hirsutum	Zhao et al., 2024	207	Cultivars	20-d post-anthesis fibers	WGBS, RNA-seq, WGS
Glycine max	Jiang et al., 2026	1102	Wild soybeans, landraces, and improved cultivars	Second trifoliate leaves	WGBS

WGBS: whole genome bisulfite sequencing; RNA-seq: RNA sequencing; WGS: whole-genome sequencing.

et al., 2024; Jiang et al., 2026). This encoding enables population-genetic analyses of methylation diversity, including minor epiallele frequency, methylation disequilibrium, population structure, and methylation-based epigenotypes or "menotypes" (Zhao et al., 2018).

The persistence of methylation variation in natural populations depends strongly on sequence context. CG methylation is generally the most meiotically stable epigenetic modification and can be transmitted across generations, allowing methylation variants to accumulate over time (van der Graaf et al., 2015). In contrast, CHH methylation is often erased and re-established during reproductive or embryonic development, making it more dynamic and less stably inherited across generations (Hollwey et al., 2023). SMP-based analyses have revealed that methylation polymorphisms differ markedly among sequence contexts. In cotton, CHH-SMPs have shown the highest minor allele frequency, followed by SNPs, CHG-SMPs, and CG-SMPs, suggesting that CHH methylation is more dynamic at the population level (Zhao et al., 2024). Methylation disequilibrium analyses further revealed that CG methylation variation can be correlated across regions of up to approximately 50 bp, whereas CHG methylation and CHH methylation tend to show shorter-range associations (Zhao et al., 2024). These patterns are consistent with the relatively stable inheritance of CG methylation and the more dynamic regulation of non-CG methylation.

Genomic localization provides another important dimension for characterizing population-level methylation variation. SMPs and DMRs are often enriched within or near genes, where they may affect transcriptional regulation and phenotypic variation (Schmitz et al., 2013; Cao et al., 2024; Zhao et al., 2024). However, comparative analyses across genomic compartments indicate that many SMPs originate from TE regions. TE-associated SMPs often show lower minor allele frequencies and higher methylation conservation than SMPs in genes or single-copy regions, consistent with the roles of DNA methylation in transposon silencing and genome defense (Zhao et al., 2024; Jiang et al., 2026). Therefore, the genomic distribution of methylation variants reflects a balance between epigenetic plasticity in regulatory regions and evolutionary constraint in transposon-rich regions.

4 Genetic control vs. epigenetic independence

A central question in plant population epigenomics is how to distinguish methylation differences

driven by underlying DNA sequence variation from methylation variants that arise and persist relatively independently of genotype. For an epigenetic variant to contribute independently to adaptive evolution, it should satisfy several stringent criteria: it must arise independently of genotype, be stably inherited across generations, and induce heritable phenotypic changes that can be subject to natural selection (Mueller et al., 2025).

DNA methylation levels can be treated as quantitative molecular phenotypes that mediate or reflect the effects of genetic variation (Smith et al., 2014) (Fig. 1b). Many DNA methylation polymorphisms are closely associated with local sequence variants, including *cis*-SNPs, TE insertions, structural variants, copy-number variants, and variants in regulatory elements. Mutations in genes involved in methylation establishment, maintenance, or removal may also generate broader methylome changes (Lindroth et al., 2001; Lang et al., 2017; Zhao et al., 2021; He et al., 2022). These observations indicate that a substantial fraction of methylation variation has no independent epigenetic cause but is rather the consequence of genetic differences.

The proportion of methylation variation explained by genetic factors varies markedly among species, methylation contexts, genomic regions, and analytical methods. In cotton, *cis*-acting methylation quantitative trait loci (meQTLs) account for 5.82% of CG sites, 2.64% of CHG sites, and 1.52% of CHH sites (Zhao et al., 2024). In maize, fewer than 40% of DMRs are associated with nearby SNPs, suggesting that much of the observed methylation variation cannot be explained by local sequence variants alone (Xu et al., 2019). Similarly, local sequence variants explain approximately 22.5% of DMRs in soybean (Shen et al., 2018) and roughly 20% of methylation variation in *A. thaliana* (Schmitz et al., 2013). These estimates suggest that genetic effects are widespread but incomplete. The broad range of reported values likely reflects true biological differences among species, as well as differences in sample size, sequencing depth, methylation context, statistical model, marker density, and definitions of *cis*-regulatory windows. Moreover, estimates based only on nearby SNPs may underestimate the contribution of structural variants, TE polymorphisms, rare variants, and distal *trans*-acting loci.

In contrast, independent epialleles generally refer to methylation states that show no obvious association with local DNA sequence variation but can be stably maintained across cell divisions or generations. Such epialleles may arise from spontaneous epimutations, environmentally induced methylation changes, TE silencing, RNA-directed DNA methylation, developmental epigenetic reprogramming, or stochastic failures in methylation maintenance and demethylation pathways. If these methylation states are meiotically stable and alter gene expression or agronomic traits, they may represent a novel class of heritable variation that is potentially useful for crop improvement.

When a methylation polymorphism is controlled by nearby or distal genetic variants, it may function as an intermediate molecular phenotype through which genetic variation influences gene expression and downstream traits. Alternatively, it may simply be a linked passenger signal that is correlated with the causal genetic variant but has no direct functional effect. Therefore, associations between methylation and traits should not be automatically interpreted as evidence for an independent epigenetic effect. Without accounting for genotype, population structure, and linkage disequilibrium, methylation–trait associations may overestimate the causal contribution of DNA methylation to phenotypic variation.

5 Functional interpretation through expression quantitative trait methylation and multi-omics

Expression quantitative trait methylation (eQTM) analysis provides a population-scale framework for linking DNA methylation variation with gene expression variation (Fig. 1c). By associating methylation states or methylation levels with transcript abundance, it can identify methylation loci whose variation is correlated with expression changes in nearby or distal genes. This suggests that methylation variation, particularly CG methylation in upstream regulatory regions, can contribute substantially to transcriptional diversity. The subsequent sections elaborate on the mechanistic basis of these associations, starting with eQTM.

Joint multi-omics analysis is essential for filtering out noise and anchoring core methylation-sensitive regulatory genes (Fig. 1c). Large-scale paired methylomic and transcriptomic datasets allow researchers to quantify how methylation state fluctuations at specific regulatory elements, including promoters, enhancers, silencers, and TE-derived regulatory regions, affect

target gene expression. When combined with chromatin accessibility data, transcription factor (TF) binding profiles, and three-dimensional (3D) chromatin interaction maps, these datasets can reveal whether methylation variation alters regulatory element activity, TF occupancy, or enhancer–promoter communication (Fig. 1c).

The construction of methylation-mediated gene regulatory networks further facilitates the integration of population-scale methylomes, transcriptomes, chromatin accessibility profiles, genotypes, and phenotypes. Such networks can identify key regulatory elements, hub TFs, and downstream genes through which methylation variation contributes to complex quantitative traits, including stress resistance, metabolite synthesis, fiber development, and fruit quality. This integrative framework bridges the mechanistic gap between epigenetic markers and complex phenotypes and provides a more precise basis for evaluating the functional contribution of methylation variation in plant populations.

Promoter methylation is often associated with transcriptional repression in both plants and animals (Zhang et al., 2018), although its impact depends on the type, proportion, genomic position, and density of methylation (Yu et al., 2013). Alterations in methylation can also reshape chromatin states, modifying the 3D genome architecture (Zhong et al., 2021). In an in vitro *Arabidopsis*-binding assay, more than 75% (248/327) of tested TFs showed methylation-sensitive DNA binding preferences (O'Malley et al., 2016; Héberlé and Bardet, 2019). In maize, trait-associated DMRs such as *vgt1*-DMR and *tb1*-DMR form chromatin loops with their downstream target genes, as demonstrated by high-throughput chromosome conformation capture (Hi-C) and chromatin immunoprecipitation (ChIP) analyses in the B73 reference genome (Xu et al., 2020).

eQTM creates statistical associations between methylation variation and gene expression (Fig. 1c). Compared with single-genome analyses, eQTM provides a more comprehensive and accurate understanding of the relationship between DNA methylation and gene expression. Studies within individual populations typically identify hundreds of genes subject to methylation-mediated regulation, often enriched in specific functional categories. Moreover, increasing the sample size markedly expands the number of detectable eQTMs (Meng et al., 2016). For instance, a large-scale cotton population study identified over 5000 *cis*-acting eQTMs among tens of thousands of protein-coding genes (PCGs) and long non-coding RNAs (lncRNAs). These eQTMs regulate approximately 5.7% of expressed PCGs and up to 29.0% of expressed lncRNAs, suggesting that lncRNA expression is particularly sensitive to methylation variation (Zhao et al., 2024). The relationship between DNA methylation and gene expression is influenced by both sequence context and the spatial proximity of DMRs to transcription start sites (TSSs). In maize and cotton, CG and CHG methylation levels are typically negatively correlated with gene expression, whereas CHH methylation can show positive correlations (Xu et al., 2019; Zhao et al., 2024).

In cotton, over 90% of eQTMs have been significantly associated with CG methylation, underscoring its predominant role in transcriptional regulation. Approximately 31% of PCGs and nearly 60% of lncRNAs are concurrently influenced by all three methylation contexts. Notably, 90% of *cis*-eQTMs are positioned upstream of their target genes (Zhao et al., 2024). While eQTM mapping successfully establishes widespread statistical associations between methylation and transcription, deciphering the exact underlying molecular mechanisms requires multi-omics integration. Population-scale assay for transposase-accessible chromatin using sequencing (ATAC-seq) and cistrome studies have already been reported for some crops (Engelhorn et al., 2025; Zhu et al., 2025). Furthermore, integrating ATAC-seq with DNA affinity purification sequencing (DAP-seq) in future research could provide mechanistic insights into how DNA methylation regulates gene expression.

6 Roles in domestication, adaptation, and complex traits

DNA methylation can contribute to phenotypic variation by modulating gene expression (Zhang et al., 2018), TE activity (Xu et al., 2020), TF binding (O'Malley et al., 2016; Héberlé and Bardet, 2019), and chromatin states (Zhong et al., 2021), thereby linking genetic background and environmental cues to complex traits.

In natural plant populations, methylation variation is frequently associated with geography, climate, and ecological gradients. For example, the *Arabidopsis*

1001 Epigenomes Project revealed strong associations between methylation patterns and environmental factors, with TE methylation positively correlated with latitude and precipitation but negatively correlated with elevated temperature and gene body methylation associated with colder winter conditions (Kawakatsu et al., 2016). Studies along elevation gradients and artificial selection experiments further suggest that environmental pressures can reshape population-level methylation profiles (Singh et al., 2024). In rice, cold-acclimation-related methylation sites in the acquired cold tolerance (*ACT*) promoter exhibited a "higher in the south, lower in the north" gradient, reflecting methylation differentiation under distinct climatic conditions (Song et al., 2025).

Domestication and crop improvement are further factors that reshape methylation landscapes (Fig. 1d). The overall methylation levels tend to decrease in rice (Cao et al., 2023) and tomato (Guo et al., 2023) but increase in pear (Song et al., 2024) and lettuce (Cao et al., 2024), suggesting that domestication-associated methylation changes are species-specific and may depend on genome architecture, selected traits, tissue type, and breeding history. Such changes may generate or fix stable epialleles affecting agronomic traits, including fruit ripening, metabolite accumulation, stress responses, and developmental transitions.

Evidence from epigenetic recombinant inbred lines (epiRILs) demonstrates that heritable methylation variation can directly contribute to quantitative traits (Trerotola et al., 2015). For example, in *Arabidopsis*, epiRILs derived from parents with wild-type and mutant decreases in DNA methylation 1 (*DDM1*) showed substantial variation in flowering time and plant height, with some epialleles stably maintained for more than eight generations (Johannes et al., 2009). In natural populations, epigenome-wide association studies (EWAS) have identified methylation loci associated with metabolic traits, fiber yield and quality, and metabolite accumulation in maize (Xu et al., 2019), cotton (Zhao et al., 2024), and tomato (Guo et al., 2023) (Fig. 1e). These findings suggest that DNA methylation can reveal trait-associated regulatory variation missed by sequence-based mapping. Nevertheless, methylation–trait associations require cautious interpretation because methylation marks may be causal, genetically driven intermediate phenotypes or linked passenger signals.

Network-based methods may further identify core regulatory genes, methylation-sensitive TFs, and downstream pathways involved in complex trait regulation. Such frameworks are essential for bridging the mechanistic gap between epigenetic markers and breeding-relevant phenotypes; integrating EWAS with meQTL, eQTM, transcriptomics, environmental data, and functional validation will be essential for clarifying the true contribution of methylation variation to adaptation, domestication, and agronomic trait diversity.

7 Resources and analytical platforms

The accumulation and public release of plant methylome datasets have provided important resources for epigenomic research (Fig. 1f). Existing databases and analytical platforms, such as *A. thaliana* Multi-omics Association Database (AtMAD) (Lan et al., 2021), cross-species angiosperm methylome resources, and MethBank 4.0, have enabled the organization, visualization, and comparative analysis of DNA methylation profiles across species, tissues, and developmental stages (Niederhuth et al., 2016; Zhang et al., 2023) (Fig. 1f). These resources have greatly facilitated the identification of conserved methylation features, differentially methylated regions, and candidate regulatory loci.

Despite the above progress, most current methylome databases still have limited population-scale coverage and insufficient integration with other omics layers (Kawakatsu et al., 2016). In practical research, particularly for polyploid crops or species with large and structurally complex genomes, a standalone epigenomic database has limited interpretive power. DNA methylation variation must be analyzed in the context of pangenome variation (Tao et al., 2019; Song et al., 2020), structural variants (Alonge et al., 2020), TE polymorphisms, transcriptomic diversity, chromatin accessibility, and phenotypic variation (Lu et al., 2017; Maher et al., 2018). Without these layers, it is difficult to determine whether a methylation variant is genetically driven, functionally associated with gene expression, or causally linked to complex traits.

In view of the above, the current trend is shifting toward comprehensive multi-omics databases and interactive visualization platforms that integrate pangenomes, variomes, transcriptomes, epigenomes,

regulatory annotations, and phenomes (Springer and Schmitz, 2017; Gui et al., 2020; Cui et al., 2023). Such platforms should support deep integrative analyses, including meQTL mapping, eQTM analysis, EWAS, methylation-mediated regulatory network construction, and the pangenome-aware visualization of methylation variation (Rakyan et al., 2011; Dubin et al., 2015; Kremling et al., 2019). These integrative resources will help filter noise, prioritize core regulatory genes, distinguish genetically dependent methylation changes from candidate independent epialleles, and bridge epigenetic markers with complex phenotypes. Building such resources will be essential for advancing plant population epigenomics and incorporating epigenetic information into precision breeding.

8 Advancing toward epigenetic breeding

The spontaneous formation of epialleles and their potential transmission across generations make plants an attractive system for studying epigenetic inheritance and exploring the use of epigenetic variation in crop improvement. Epigenomic modifications can strengthen, weaken, or generate regulatory interactions, thereby influencing gene expression, chromatin organization, and phenotypic diversity (Liu et al., 2025).

8.1 Scalable methylation profiling for breeding applications

A major prerequisite for epigenetic breeding is the ability to profile DNA methylation accurately and cost-effectively across large numbers of samples. However, accurate methylation quantification typically requires bisulfite sequencing at substantially higher coverage than conventional whole-genome sequencing used for SNP detection, making population-scale methylome profiling expensive and technically demanding.

However, targeted methylation profiling platforms may provide a more practical solution. Methylation arrays or targeted methylation sequencing panels can be designed to focus on informative differentially methylated regions, single methylation polymorphisms, methylation-sensitive regulatory elements, and trait-associated epigenetic markers (Fig. 1g). However, most currently available methylation arrays are optimized for human and animal studies, and crop-specific methylation arrays remain largely unavailable. In this regard, the accumulation of plant methylome datasets provides an important foundation for designing crop-adapted methylation platforms. For breeding applications, these markers must remain informative across genetic backgrounds, tissues, developmental stages, environments, and management conditions.

Third-generation sequencing technologies, such as Oxford Nanopore Technologies (ONT), provide important advantages for methylation analysis, including long reads, rapid sequencing, and direct detection of base modifications such as 5mC and N^6-methyladenine (6mA) without bisulfite conversion (Liu and Conesa, 2025) (Fig. 1g). These platforms enable the simultaneous acquisition of genetic and epigenetic information and are particularly useful for crops with complex genomes, abundant TEs, or extensive structural variation. Nevertheless, sequencing cost, base-modification calling accuracy, analytical standardization, and throughput remain major limitations for routine breeding-scale applications.

8.2 Deep learning and pre-trained models for epigenotype-to-phenotype prediction

As methylomes, transcriptomes, chromatin accessibility profiles, pangenomes, and phenomes continue to accumulate, epigenetic breeding faces a challenge not only in data generation but also in data interpretation. Traditional statistical models remain essential for EWAS, meQTL, eQTM, and genomic prediction, but they often have limited capacity to capture nonlinear, context-dependent, and multi-layer regulatory relationships (Albert and Kruglyak, 2015). This limitation is particularly relevant to DNA methylation because its regulatory effect depends on sequence context, methylation context, genomic position, chromatin accessibility, TF binding, tissue identity, developmental stage, and environmental exposure. Therefore, computational frameworks that can jointly model sequence features, epigenomic states, regulatory annotations, and phenotypic variation are increasingly needed for epigenotype-to-phenotype prediction.

Deep learning provides one such framework by learning regulatory patterns directly from genomic and epigenomic data. Early sequence-to-function models, such as Basset (Kelley et al., 2016) and DeepSEA (Zhou and Troyanskaya, 2015), have demonstrated that DNA sequence can be used to predict regulatory

features, including chromatin accessibility and TF binding. These models have established the concept that regulatory grammar is learnable from sequence and provided a foundation for predicting the functional effects of non-coding variation. Although these early models were not designed specifically for plant DNA methylation, they introduced a general strategy for linking sequence variation to regulatory activity.

More recently, pre-trained genomic language models have expanded this strategy by learning generalizable representations from large-scale genome sequences. Models, such as DNABERT (Ji et al., 2021), Nucleotide Transformer (Dalla-Torre et al., 2025), AgroNT (Mendoza-Revilla et al., 2024), and PlantCaduceus (Zhai et al., 2025), use Transformer or related architectures to capture sequence patterns across broader genomic contexts. These models are particularly useful for regulatory annotation, variant prioritization, and sequence-to-function prediction. Plant-oriented models, including AgroNT (Mendoza-Revilla et al., 2024) and PlantCaduceus (Zhai et al., 2025), further increase the relevance of foundation models for crop genomics by incorporating plant genome diversity and longer-range sequence dependencies.

For population epigenomics, methylation-aware prediction models are especially important. Targeted models such as PlantDeepMeth have been developed to predict CG, CHG, and CHH methylation states from plant DNA sequences, enabling genome-wide methylation imputation and the identification of sequence determinants of context-specific methylation (Guo et al., 2025). Such models can help distinguish methylation variants that are likely to be sequence-driven from those that may arise independently of local genetic variation. When combined with meQTL and eQTM analyses, methylation prediction models may therefore improve the prioritization of candidate functional epialleles (Fig. 1h).

8.3 Targeted epigenome editing for epiallele creation and validation

Targeted epigenome editing offers a powerful strategy for testing the causal effects of methylation variation and creating novel epialleles with potential breeding value (Fig. 1i). Catalytically inactive clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9), or catalytically dead (dCas9), retains single-guide RNA (sgRNA)-directed DNA binding activity but lacks nuclease function. By fusing dCas9 to DNA methyltransferases, demethylases, transcriptional repressors, or chromatin-modifying domains, researchers can induce site-specific epigenetic modifications at target loci without altering the underlying DNA sequence (Sung and Yim, 2020; Park et al., 2022; Shi et al., 2025; Zhang and Zhu, 2025). This approach enables the precise methylation or demethylation of promoters, enhancers, silencers, TE-derived regulatory regions, and other non-coding regulatory elements.

8.4 Remaining challenges and future outlook

Despite the opportunities detailed in this review, several conceptual and technical challenges must be addressed before population-level DNA methylation variation can be routinely used in molecular breeding.

Tissue specificity and cellular heterogeneity remain major challenges for applying DNA methylation markers in breeding. Since DNA methylation is highly dependent on tissue type, developmental stage, environmental condition, and cellular composition, methylation markers identified from bulk tissues may not necessarily reflect regulatory variation occurring in the most relevant cell types or developmental windows. This issue is particularly relevant to plants possessing many agronomic traits, such as seed development, fruit ripening, fiber elongation, stress response, root architecture, and reproductive transition, that are controlled by highly specialized tissues or transient cell states. As a result, epigenetic signals detected in whole seedlings, mature leaves, or mixed tissues may be diluted, masked, or even confounded by cell-type heterogeneity. For breeding-oriented population epigenomics, future studies should therefore incorporate multi-tissue, multi-developmental-stage, and environment-aware methylome profiling. Candidate epigenetic markers should be evaluated not only for their statistical association with traits but also for their biological stability across tissues, developmental stages, genetic backgrounds, and environmental conditions. In this context, single-cell methylome sequencing, single-cell multi-omics, spatial transcriptomics, and spatial epigenomics provide powerful opportunities to overcome the limitations of bulk methylome profiling. These technologies can resolve cell-type-specific methylation landscapes, identify regulatory methylation changes in rare or transient cell populations, and link spatially restricted epigenetic states with local gene

expression programs. For example, single-cell and spatially resolved approaches may help distinguish true regulatory methylation variation from apparent methylation differences caused by changes in tissue composition. They may also reveal whether trait-associated methylation loci act broadly across tissues or specifically within key developmental domains. The integration of bulk population methylomes with single-cell and spatial omics will be particularly valuable for complex plant tissues, such as developing seeds, shoot apices, root tips, vascular tissues, fruit pericarp, and cotton fibers, as it can provide a hierarchical view of epigenetic regulation, from population-level methylation polymorphisms to tissue-specific regulatory modules and cell-type-resolved methylation states. This will improve the precision of epigenetic marker discovery and help identify methylation variants that are more likely to have direct functional relevance for molecular breeding.

9 Conclusions and perspectives

Population epigenomics has revealed extensive DNA methylation variation across plant populations and has begun to clarify its genetic basis, regulatory consequences, and potential contribution to domestication, adaptation, and complex traits. However, translating methylation variation into breeding applications requires distinguishing causal epialleles from genetically linked or environmentally induced passenger marks. Future progress will depend on larger and better-designed population methylome datasets, multi-tissue and single-cell profiling, pangenome-aware multi-omics integration, robust statistical and deep learning models, and targeted epigenome editing for functional validation. Together, these advances can provide a solid foundation for incorporating epigenetic variation into precision breeding and crop improvement.

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Author contributions

Ting ZHAO, Xi LONG, and Juyun ZHENG drafted the manuscript. Lei FANG coordinated the research and revised the manuscript. Ting ZHAO and Lei FANG conceived the study and designed the overall framework. Xi LONG and Juyun ZHENG performed data visualization, including the construction of the integrative conceptual diagrams. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Xi LONG, Juyun ZHENG, Lei FANG, and Ting ZHAO declare that they have no conflicts of interest.

This review does not contain any studies with human or animal subjects performed by any of the authors.

Declaration on the use of generative AI tools

During the preparation of the revised manuscript and related submission materials, the authors consulted ChatGPT (OpenAI) for suggestions on improving language, formatting, and readability. The authors critically reviewed and revised all AI-assisted content as appropriate and take full responsibility for the final content of the publication.

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