

EPIGENETIC REGULATION OF GENE EXPRESSION IN PLANTS: MOLECULAR MECHANISMS, ENVIRONMENTAL INTERACTIONS AND APPLICATIONS IN CROP IMPROVEMENT

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ABSTRACT

Plant epigenetic regulation links DNA methylation, histone modifications, small-RNA pathways and chromatin remodeling to transcription, development and environmental response. This review critically evaluates original experimental evidence for these mechanisms and asks when environmentally induced states are causal, persistent and useful for crop improvement. Plant methylomes show that DNA methylation must be interpreted according to sequence context, genomic location and genetic background; promoter, gene-body and transposon-associated methylation are not equivalent. Genetic and targeted-manipulation studies support RNA-directed DNA methylation and histone/chromatin mechanisms, while heat-stress experiments provide strong evidence for short-term somatic memory at selected loci. Evidence for sexual transgenerational inheritance is more mixed: some parental stress experiments report descendant effects, but multigenerational drought work found few conserved methylation changes and limited association with phenotype. Natural epigenomic variation in crops is often linked to local sequence polymorphism, complicating claims of sequence-independent epialleles. We propose an evidence framework that separates molecular association, causal regulation, within-plant or clonal persistence, meiotic inheritance and field-level value. Translation should prioritise matched methylome, transcriptome and phenotype measurements; causal perturbation and rescue; genotype-aware controls; common-garden generation tests; and multi-environment validation. Targeted epigenome editing provides a route to test specific loci, but stability after editor removal, off-target effects, pleiotropy and breeding performance remain central gates. Plant epigenetics is therefore a credible source of mechanistic insight and candidate breeding variation, but its practical value must be established trait by trait rather than inferred from chromatin change alone.

KEYWORDS: DNA methylation; chromatin; stress memory; epialleles; RNA-directed DNA methylation; crop improvement.

INTRODUCTION

Epigenetic regulation is best understood as the set of molecular processes that alter the use of genetic information without requiring a change in the underlying DNA sequence. In plants, this definition spans cytosine methylation, histone modifications, nucleosome dynamics, small-RNA pathways and the regulatory consequences of chromatin folding. These mechanisms are not independent layers placed on top of transcription. They interact with transcription factors, polymerases and DNA sequence features to determine whether a locus is accessible, active, repressed or poised for a later response. Genome-wide methylome maps established that plant methylation must be interpreted by sequence context and genomic location rather than as a uniform repressive mark (Cokus et al., 2008; Lister et al., 2008; Zemach et al., 2010; Feng et al., 2010).

This distinction matters for crop improvement because an epigenetic difference is not automatically a useful trait variant. It can be a cause of altered transcription, a downstream consequence of transcription, a response to tissue composition, or a proxy for a nearby genetic variant. Crop populations often carry transposon insertions and other sequence polymorphisms that influence local methylation, making genotype-aware analysis essential (Eichten et al., 2013; Dubin et al., 2015). At the same time, experimental epigenetic recombinant inbred lines and locus-specific perturbations show that methylation states can contribute to complex traits and gene regulation independently of simple DNA-sequence differences (Johannes et al., 2009; Cortijo et al., 2014).

Environmental epigenomics adds a second source of complexity. Plants can alter expression rapidly during drought, salinity, temperature extremes, nutrient limitation or pathogen attack. Some responses persist during recovery and allow a faster response to recurrence, yet this somatic stress memory should not be conflated with transmission through meiosis.

Heat-stress experiments have resolved histone-associated mechanisms that sustain transcription for days, whereas a carefully controlled multigenerational drought experiment found very little conserved methylation change associated with descendants' phenotypes (Ding et al., 2012; Lämke et al., 2016; Ganguly et al., 2017). The contrast is scientifically useful: it identifies the conditions under which memory claims require more than a methylome difference measured at one time point.

The central question for crop epigenetics is therefore not whether plants exhibit epigenetic variation, which is well established, but when that variation provides reproducible, causal and agronomically valuable control of gene expression. This review integrates molecular pathways with environmental response, persistence and breeding application. Its contribution is an evidence-calibrated framework that separates four levels of claim: molecular association, causal regulation, persistence within an individual or clonal lineage, and stable inheritance with measurable trait value. It uses original experimental research to identify where causal evidence is strong, where results conflict and what must be demonstrated before an epigenetic state can be used as a breeding target.

2. MATERIALS AND METHODS: LITERATURE SEARCH AND EVIDENCE SELECTION

A structured, targeted search was conducted on 12 October 2026 using publisher pages and indexed records discoverable through PubMed, PubMed Central, journal websites and scholarly search. Search combinations included plant DNA methylation, RNA-directed DNA methylation, histone modification, chromatin accessibility, stress memory, transgenerational inheritance, epigenetic variation in crops, epialleles and targeted epigenome editing. The search was extended through reference chaining from experimentally focused papers when a mechanism, stress class or crop application required additional primary evidence. This was a structured narrative review, not a systematic review: a preregistered protocol, duplicate-independent screening, exhaustive database exports and a PRISMA flow diagram were not undertaken, and no exhaustive-search claim is made.

Eligible evidence consisted of peer-reviewed original research reporting experimental or original population-genomic data on plant epigenetic mechanisms, environmental responses, inheritance, agronomic traits or epigenome manipulation. Reviews were used only to discover candidate primary papers and were not treated as direct experimental evidence in the synthesis. Bibliographic metadata and DOI records were checked against publisher or indexed records where accessible. The screening emphasis was on matching each claim to the actual experimental system and measured outcome, not on citation frequency. The evidential hierarchy distinguished (i) correlation between chromatin and expression, (ii) temporal evidence and within-system co-measurement, (iii) genetic perturbation or targeted manipulation, (iv) restoration or complementation and (v) replicated field-level performance. Inheritance claims were assessed according to the reproductive route, number of generations studied, continued exposure, common-garden controls and separation from sequence variation. Where full text or metadata were not independently accessible, that limitation is recorded in the accompanying audit rather than implying full-text verification.

3. RESULTS: EVIDENCE SYNTHESIS

3.1. DNA methylation is context- and locus-dependent

Plant DNA methylation occurs in CG, CHG and CHH contexts, where H denotes A, C or T. MET1 predominantly maintains CG methylation after DNA replication; CMT3 contributes to CHG methylation through a reinforcing relationship with H3K9 methylation; CMT2 contributes strongly to CHH methylation in deep heterochromatin; and DRM2 catalyses *de novo* methylation under RNA-directed DNA methylation (RdDM). The pathways overlap rather than forming insulated modules. Mutant methylomes show that loss of one component can redistribute methylation across other contexts and genomic compartments (Lindroth et al., 2001; Stroud et al., 2013; Stroud et al., 2014). DDM1-dependent nucleosome remodelling allows methylation machinery to access heterochromatic DNA, while the KYP/SUVH4 histone methyltransferase system contributes to the coupling of H3K9 methylation and CHG methylation (Jackson et al., 2002; Gendrel et al., 2002; Saze et al., 2003). Functional genetics established CMT3-dependent non-CG methylation and showed that DRM enzymes contribute to *de novo* methylation, with partially overlapping roles for the DRM and CMT3 pathways (Bartee et al., 2001; Cao and Jacobsen, 2002; Cao et al., 2003). AGO4-dependent locus-specific small-RNA accumulation and chromatin methylation provide a mechanistic bridge between the small-RNA signal and DNA modification itself (Zilberman et al., 2003; Zhong et al., 2014).

A common but inaccurate shorthand is that methylation always represses transcription. Methylation in promoters or regulatory elements can be associated with reduced transcription-factor access or stable silencing, particularly when it overlaps repeats and transposable elements. In contrast, gene-body CG methylation is frequently found in expressed genes and cannot be interpreted as equivalent to promoter methylation. Comparative plant methylomes and high-resolution Arabidopsis maps documented these different distributions (Cokus et al., 2008; Lister et al., 2008; Zemach et al., 2010; Feng et al., 2010). A credible functional interpretation therefore asks which cytosines changed, whether the change affected a promoter, enhancer-like element, gene body or transposon, whether expression changed in the same tissue, and whether genetic or targeted perturbation establishes direction of effect.

DNA methylation is also actively removed. ROS1-family DNA glycosylases initiate base-excision repair-mediated demethylation, opposing methylation and helping protect selected regulatory regions from inappropriate silencing (Gong et al., 2002). Developmental demethylation is not random: endosperm and fruit-ripening studies found locus- and tissue-specific methylation changes that coincide with reproductive development and gene activation (Hsieh et al., 2009; Liu et al., 2015; Lang et al., 2017; Zhong et al., 2013). These examples are important for crop traits because epigenetic control is often most interpretable when studied in a defined developmental process with known phenotypic endpoints, not only in bulk leaves sampled after stress.

3.2. RNA-directed DNA methylation connects small RNAs to chromatin

In canonical RdDM, Pol IV transcription and RDR2 activity generate double-stranded RNA substrates processed by DCL proteins into small interfering RNAs. AGO4-family complexes use these small RNAs to recognize nascent transcripts associated with Pol V, recruiting DRM2 and associated factors to establish methylation. Genetic experiments support distinct but coordinated contributions of the polymerases, AGO4 and DRM methyltransferases (Chan et al., 2004; Chan et al., 2006; Onodera et al., 2005; Qi et al., 2006; Wierzbicki et al., 2008; Blevins et al., 2011). The pathway is particularly consequential at repeats and transposable elements, where silencing limits ectopic transcription and genome instability. Genetic perturbation of Pol IV clarified its role in plant small-RNA metabolism, while later analyses showed that transposable-element insertions can alter local regulatory landscapes and methylation stability (Zhang et al., 2007; Lippman et al., 2004; Reinders et al., 2009). However, the regulatory outcome depends on target context: RdDM at a promoter can repress a gene, while repeat silencing may indirectly preserve expression of neighbouring genes by limiting transposon-associated disruption.

Mechanistic evidence is strongest where pathway components have been mutated or targeted and both methylation and expression were measured. The targeted-recruitment work of Gallego-Bartolomé et al. (2019) showed that co-targeting the small-RNA-producing and Pol V recruitment arms can improve de novo methylation at selected loci. CRISPR-Cas9 SunTag-based systems extended this principle to programmable locus-specific manipulation in Arabidopsis (Papikian et al., 2019). These are proofs of molecular capability, not yet demonstrations that an edited epigenetic state will deliver durable yield gains across elite crop genotypes. The remaining gap is how to maintain the desired state without continuous editor expression, off-target changes or reversion during breeding and environmental variation.

3.3. Histone modifications, nucleosomes and chromatin accessibility

Histone-tail modifications change the interaction between nucleosomes, chromatin-associated proteins and transcriptional machinery. H3K4me3 is frequently enriched around active promoters, H3K27me3 is a canonical Polycomb-associated repressive mark, and H3K9me2 commonly associates with constitutive heterochromatin. Acetylation is often associated with accessible chromatin, but no mark has a universal meaning independent of locus and protein context. Integrated epigenomic mapping in Arabidopsis identified chromatin states from combinations of DNA methylation, histone marks and genomic features, demonstrating why single-mark interpretations are incomplete (Roudier et al., 2011). Polycomb-associated histone regulation also contributes to developmental transitions, including vernalization-dependent silencing of FLOWERING LOCUS C (FLC) (Bastow et al., 2004; Finnegan et al., 1996).

Nucleosome remodellers can alter DNA accessibility without changing the underlying histone modification pattern. During heat stress, FGT1 interacts with SWI/SNF- and ISWI-related remodelling machinery; loss of FGT1 affects nucleosome dynamics and weakens the sustained expression of heat-responsive genes (Brzezinka et al., 2016). Cold-stressed potato showed increased chromatin accessibility and the co-occurrence of H3K4me3 and H3K27me3 at some active genes, a result that cautions against assuming that a conventionally repressive mark means that a gene is transcriptionally off (Zeng et al., 2019). Such bivalent or mixed-mark states may reflect dynamic regulation or heterogeneous cell populations. Time-resolved sampling and locus-specific perturbation are needed to distinguish these possibilities. Studies that sampled Arabidopsis during drought recovery also found that chromatin status changed after stress relief, reinforcing that recovery is a biological phase and not merely an untreated control condition (Kim et al., 2012).

Histone marks can persist after the initiating signal has disappeared, but persistence must be demonstrated rather than inferred from one post-stress sample. During repeated dehydration, a subset of Arabidopsis genes showed faster or stronger transcriptional re-induction (Ding et al., 2012). In heat-stress memory, H3K4me2 and H3K4me3 remained enriched at memory-related loci after HSFA2 association had declined, and higher marks accompanied stronger expression after a second heat exposure (Lämke et al., 2016). A separate study found that the chromatin remodeller FGT1 was necessary for sustained memory-associated gene expression over several days (Brzezinka et al., 2016). Together these results support short-term somatic chromatin memory under controlled conditions. They do not establish that the marks persist through gametogenesis or improve field yield in descendants.

3.4. Non-coding RNAs, histone variants and three-dimensional organisation

Small RNAs have well-established roles in RdDM and transposon silencing, while other non-coding RNAs can influence transcriptional complexes or chromatin-associated proteins. The best-supported causal plant examples involve genetic perturbation of core RdDM components and targeted methylation rather than broad claims that any stress-responsive small RNA establishes heritable memory. DNA sequence, repeat density and chromatin state provide the target landscape for these pathways; consequently, the same small-RNA signal can yield different outcomes in different genotypes or at different loci. Studies of natural methylation diversity further show that local genetic background contributes substantially to observed epigenomic differences (Dubin et al., 2015; Kawakatsu et al., 2016).

Histone variants and higher-order chromatin organisation add another level of regulation, especially during rapid responses when nucleosome turnover and enhancer/promoter accessibility can change over minutes to hours. However, high-throughput accessibility or conformation data are often descriptive unless linked to matched expression, perturbation and phenotype. For breeding-oriented inference, multi-omic co-measurement should be designed around a stated causal chain: environmental trigger, upstream signal, chromatin event, transcript response and measurable trait. A broad association map can prioritise loci, but it cannot alone distinguish whether chromatin initiates, reinforces or follows transcription.

3.5. Environmental exposure: drought, temperature and salinity

Drought studies provide one of the clearest examples of conflicting evidence. Recurrent dehydration can train the transcriptional response of a subset of *Arabidopsis* genes and alter the rate or amplitude of re-induction (Ding et al., 2012). Yet the study testing slow-onset drought across five successive generations found that conserved differentially methylated regions were negligible, that drought-linked epialleles did not correlate with drought-responsive expression and that only one of six tested traits showed detectable transgenerational memory, persisting one generation beyond exposure (Ganguly et al., 2017). These results are not necessarily contradictory: transcriptional memory within a plant and meiotically inherited methylation are different outcomes, and memory may be encoded by histone marks, transcription-factor feedback or physiological acclimation rather than persistent DNA methylation.

Heat and cold studies show a more direct chromatin connection in certain systems. *Arabidopsis* heat-memory loci accumulate H3K4 methylation and require HSFA2 for the sustained response; FGT1-associated nucleosome remodelling provides complementary evidence that chromatin architecture contributes to memory (Lämke et al., 2016; Brzezinka et al., 2016). In potato, cold exposure was associated with higher accessibility and co-occurring H3K4me3/H3K27me3 at active loci (Zeng et al., 2019). The general lesson is not that temperature stress always leaves a beneficial epigenetic memory, but that time-resolved and perturbation-based experiments can isolate specific mechanisms. Heat duration, recovery interval, plant age, genotype and sampled tissue are plausible sources of divergent results.

Salinity can alter methylation and histone states, but the evidence ranges from association to functional testing. Salt-stressed flax treated with 24-epibrassinolide showed changes in DNA cytosine methylation, illustrating an interaction between stress signalling and the methylome (Amraee et al., 2019). Experiments in *Arabidopsis* and clonal species also show that environmental history can affect offspring phenotype or methylation persistence (Suter and Widmer, 2013; Verhoeven et al., 2010; Verhoeven and van Gurp, 2012). However, changes induced by a demethylating compound such as 5-azacytidine need cautious interpretation because these treatments can have broad effects on genome maintenance, growth and cell division. Site-specific manipulations, matched untreated controls and multiple stress intensities are more informative than global methylation percentages alone.

3.6. Biotic stress, immunity and nutrient environments

Nutrient limitation and waterlogging deserve separate treatment because the physiological state they impose differs from simple dehydration or salinity. Nutrient deficiency changes carbon allocation, root architecture and developmental progression, while hypoxia restricts ATP production and can rapidly alter hormone balance and chromatin-associated transcription. In both cases, changes in the relative abundance of root, vascular and senescing tissues can produce apparent methylation differences in bulk samples. A useful design should therefore specify tissue, stage, nutrient concentration or oxygen status, and recovery timing, and should not infer a stable epigenetic state from a single endpoint. The same logic applies to herbivory: defence-associated methylation is most informative when it is paired with herbivore performance, damage, hormone signalling and gene expression in the same treatment series.

Pathogen exposure can be accompanied by widespread dynamic methylation changes, as demonstrated in *Arabidopsis* after biotic challenge (Dowen et al., 2012). This observation establishes that the methylome is responsive, but not that every differentially methylated region is required for immunity. Defence expression, pathogen burden, methylation and small-RNA abundance should be measured together, preferably with mutants in the relevant pathway. A locus that changes methylation only after defence transcription has peaked may be a consequence or stabilising correlate of the response, while a locus changed before transcription and functionally tested is a stronger candidate regulator.

Evidence for herbivory, nutrient limitation, waterlogging and combined stresses remains more fragmented than for single abiotic treatments. A major limitation is ecological mismatch: laboratory treatments are typically uniform and brief, while field plants experience repeated, interacting and spatially heterogeneous stress. Combinations may not be additive. For example, temperature and water availability influence plant developmental rate, tissue composition and transcription, each of which can confound cross-treatment methylome comparisons. Crop-relevant experiments should therefore sample matched developmental stages, use factorial stress designs where feasible and report absolute exposure conditions, not simply label treatments as drought or heat. Table 2 compares the principal stress classes, affected marks and limits of the current evidence.

Table 2. Environmental stress-induced epigenetic responses in plants.

Exposure	System	Epigenetic feature	Gene / expression or phenotype result	Inference and limitation	Primary study
Repeated drought	<i>Arabidopsis thaliana</i>	Transcriptional memory; histone-linked effects studied at selected genes	Faster/stronger re-induction for a subset of genes	Within-plant re-challenge evidence; not proof of meiotic inheritance	Ding et al. (2012)
Multigenerational drought	<i>Arabidopsis thaliana</i>	Genome-wide methylome across five successive stressed generations	Few conserved DMRs; limited trait evidence for descendant memory	Strong negative/limiting evidence for DNA methylation as a general mechanism	Ganguly et al. (2017)

Exposure	System	Epigenetic feature	Gene / expression or phenotype result	Inference and limitation	Primary study
Heat / recurrent heat	Arabidopsis thaliana	H3K4me2/3 at memory-associated genes; HSFA2 dependence	Sustained expression and enhanced re-induction following heat	Mechanistic short-term somatic memory; field value untested	Lämke et al. (2016)
Heat / recurrent heat	Arabidopsis thaliana	FGT1 interactions with nucleosome remodelers	Loss of FGT1 weakens sustained heat-responsive transcription	Supports chromatin remodelling, not inherited yield response	Brzezinka et al. (2016)
Cold	Potato	Chromatin accessibility; H3K4me3 and H3K27me3 co-occurrence	Active genes can carry mixed histone marks under cold	Highlights context-dependent interpretation of marks	Zeng et al. (2019)
Salinity plus hormone treatment	Flax (<i>Linum usitatissimum</i>)	Global/locus DNA cytosine methylation shifts	24-epibrassinolide alters methylation under salt stress	Association; trait causality and inheritance require further tests	Amraee et al. (2019)
Pathogen challenge	Arabidopsis thaliana	Widespread dynamic DNA methylation	Methylation patterns change after biotic stress	Genome response shown; functional significance differs by locus	Dowen et al. (2012)
Heat stress	Clonal duckweed (Lemna minor)	CG and CHG methylation; subset persists across 3–12 generations	Persistent CHG differences in common environment	Clonal persistence, not sexual inheritance; expression consequences unresolved	van Antro et al. (2023)
Salt / heat parental exposure	Arabidopsis thaliana	Epigenetic mechanisms inferred from descendant phenotypes	Phenotypic effects reported in offspring	Parental effects can be real without identifying the causal mark	Suter and Widmer (2013)

3.7. Persistence and inheritance require distinct tests

A stress-induced state can persist for minutes or days within the exposed plant, through mitotic cell divisions, across clonal propagation or through sexual reproduction. These categories have different mechanisms and practical implications. Persistent H3K4 methylation in Arabidopsis heat memory and memory effects among clonal plant ramets support somatic or vegetative persistence (Lämke et al., 2016; Verhoeven et al., 2010). Clonal duckweed exposed to elevated temperature retained a subset of CHG methylation differences for three to twelve generations in a common environment, a relevant demonstration of long-term clonal persistence (van Antro et al., 2023). Vegetative persistence is especially relevant for clonally propagated crops, but should not be used as evidence of meiotic inheritance.

Claims of sexual transgenerational inheritance require descendants grown without the original exposure, documentation of the generations tested and, where possible, reciprocal crosses and common-garden designs. In Arabidopsis, stress-associated transcriptional states can be reset by DDM1 and MOM1; stable inheritance in the tested system required their combined disruption (Iwasaki and Paszkowski, 2014). In other experiments, parental heat or salt exposure altered offspring phenotypes (Suter and Widmer, 2013), and progeny of stressed plants showed changes in methylation and homologous recombination frequency (Boyko et al., 2010). These results establish that transgenerational effects can occur, but do not make them universal. Long-term methylation variation can also arise spontaneously, and inherited methylation may track local genetic variants rather than a purely environment-induced state (Becker et al., 2011; Schmitz et al., 2011; Eichten et al., 2013).

An adequate inheritance study should genotype parents and descendants, sequence candidate loci, sample more than one tissue and use enough replicate lineages to estimate stochastic variation. It should test whether the epigenetic difference remains after the phenotype appears and whether targeted restoration or disruption changes that phenotype. Without these controls, parent-offspring resemblance can reflect maternal provisioning, seed size, microbiome inheritance, persistent environmental carryover, DNA sequence change or selection among survivors. This level of caution is central if the aim is a deployable crop allele rather than a mechanistic description of stress response. Table 3 distinguishes persistence within an organism or clonal lineage from demonstrated sexual inheritance.

Table 3. Evidence for epigenetic memory and inheritance in plants.

System exposure /	Duration or generation	Observed persistence	Transmission route	Causal strength	Interpretation
Arabidopsis heat memory	Hours to days after first heat	H3K4 methylation and expression persist after initial signal	Somatic / mitotic	HSFA2 mutant and re-challenge support mechanism	Strong evidence for short-term memory; no conclusion on meiotic inheritance (Lämke et al., 2016)
Arabidopsis heat memory	Several days	FGT1-dependent expression and nucleosome dynamics	Somatic / mitotic	Mutant and chromatin assays	Mechanism supported within plant (Brzezinka et al., 2016)
Arabidopsis multigenerational drought	Five successive exposed generations plus descendants	Negligible conserved drought-associated DMRs	Sexual generations	Methylome and six traits measured	Does not support methylation as a universal cause of inherited drought memory (Ganguly et al., 2017)
Arabidopsis stress-induced silencing	Progeny of stressed seedlings	Transmission evident only in ddm1 mom1 double mutant background	Sexual generations	Genetic perturbation identified resetting machinery	Shows active resetting; not a general inheritance rule (Iwasaki and Paszkowski, 2014)
Dandelion (asexual lineages)	Clonal offspring / common garden	Some stress-linked methylation changes persisted	Vegetative	Methylation and phenotype measured	Relevant to clonal crops; distinguish from meiosis (Verhoeven et al., 2010)
Lemna minor temperature stress	3–12 clonal generations	Subset of CHG methylation differences retained	Vegetative	epiGBS and common-garden propagation	Evidence for long-term clonal methylome memory; direct trait causality remains open (PMID 36324253)
Spontaneous Arabidopsis methylation variation	30 generations single-seed descent	New methylation polymorphisms arise without imposed stress	Sexual; baseline epigenome drift	Longitudinal lineage design	Stress-linked claims need comparison with spontaneous and stochastic drift (Schmitz et al., 2011; Becker et al., 2011)
Maize natural DMRs	Recombinant inbred lines	Many DMRs inherited; nearly half common DMRs associated with local SNPs	Sexual	Population methylome and genetic association	Inherited methylation is often genotype-linked, limiting claims of sequence-independent inheritance (Eichten et al., 2013)

3.8. Epigenetic diversity, agronomic traits and crop improvement

Natural epigenetic diversity is extensive, but its use in breeding depends on whether it adds information beyond genotype. Arabidopsis accessions and maize inbred lines show substantial methylation variation; in maize, a large proportion of common DMRs were associated with local genetic variants, and many DMRs were transmitted in recombinant inbred lines (Eichten et al., 2013; Kawakatsu et al., 2016). Comparative work across angiosperms further shows that methylation patterns differ with genome composition and transposon landscape (Niederhuth et al., 2016). Thus, epigenomic association studies should include local haplotypes, structural variants and transposon insertions, and should test incremental predictive value against genomic models.

Evidence that epigenetic variation can affect complex traits is stronger in controlled Arabidopsis epigenetic recombinant inbred lines than in elite crop breeding populations. Johannes et al. (2009) and Cortijo et al. (2014) demonstrated approaches for estimating epigenetic contributions to quantitative traits. The core advantage is that sequence variation can be reduced or controlled, allowing selected epialleles to be tested. The challenge is that chemically or genetically induced epigenetic lines may contain off-target methylation differences, linked sequence variants or growth penalties. The crop-level examples and their validation status are compared in Table 4. A candidate epiallele becomes useful only when its

molecular state is reproducible, its effect survives relevant crossing and propagation, and the associated agronomic outcome is useful under multiple environments.

Table 4. Epigenetic regulation of agronomically important traits.

Crop / species	Trait or process	Mechanism	Evidence reported	Validation status	Primary research
Arabidopsis thaliana	Vernalization / flowering time	FLC silencing associated with histone methylation and Polycomb state	Cold exposure produces a stable developmental transition in FLC regulation	Mechanistically developed model system; crop transfer is trait-specific	Bastow et al. (2004); Finnegan et al. (1996)
Tomato (Solanum lycopersicum)	Fruit ripening	DNA demethylation at ripening-associated regions; SIDML2	Methylation dynamics and demethylase perturbation affect ripening-associated expression	Causal developmental evidence; quality trade-offs need cultivar/field testing	Zhong et al. (2013); Liu et al. (2015); Lang et al. (2017)
Potato (Solanum tuberosum)	Cold-response transcription	Chromatin accessibility and H3K4me3/H3K27me3	Mixed marks at active genes under cold stress	Molecular profiling; agronomic benefit not established	Zeng et al. (2019)
Maize (Zea mays)	Natural methylome variation, candidate gene regulation	DMRs influenced by local genetic variation and transposons	Heritable DMRs and methylation-expression associations	Strong population evidence; causal trait validation is locus-specific	Eichten et al. (2013)
Flax (Linum usitatissimum)	Salinity response	Hormone-associated DNA methylation changes	24-epibrassinolide altered methylation under salt stress	Methylation association; useful tolerance allele not demonstrated	Amraee et al. (2019)
Arabidopsis thaliana	Complex quantitative traits	Epigenetic recombinant inbred lines / epialleles	Epigenetic variation explained part of trait variation in controlled lines	Proof-of-principle; transfer to elite crop populations limited	Johannes et al. (2009); Cortijo et al. (2014)
Arabidopsis thaliana	Transposon control and local regulation	Methylation / silencing around mobile elements	Transposon polymorphisms influence genome-wide gene regulation	Foundational mechanism; needs trait-level validation per crop	Quadrana et al. (2016); Miura et al. (2001)

Flowering and fruit ripening illustrate the potential for developmental epigenetics. Vernalization-associated FLC repression involves a defined chromatin transition, and studies of tomato ripening identified extensive methylation dynamics around ripening-responsive genes (Bastow et al., 2004; Zhong et al., 2013; Liu et al., 2015; Lang et al., 2017). These are mechanistically anchored processes with relatively clear phenotypes. They should not be assumed to prove that epigenetic breeding can easily increase yield or stress stability, since the regulatory circuitry, developmental timing and fitness consequences are trait-specific. Breeding value must be measured rather than inferred from a molecular mark. Epigenome editing is a promising experimental route for testing causal loci and potentially generating epialleles without changing the DNA sequence at the targeted site. CRISPR/dCas9 recruitment and engineered RdDM have shown that locus-specific methylation can be directed in Arabidopsis (Papikian et al., 2019; Gallego-Bartolomé et al., 2019). Before deployment, several questions remain. Does the mark persist after editor segregation? Does it survive meiosis and commercial seed increase? Can the effect be reproduced across elite genetic backgrounds? Are nearby genes or transposons affected? Does an intended benefit under stress carry a yield cost under normal conditions? Until these questions are tested in crop lines and multi-location trials, epigenome editing should be described as an emerging proof-of-principle rather than a mature breeding platform. Table 5 compares the translational options and their main gates. Figure 1 integrates the environmental, chromatin, inheritance and breeding relationships discussed here. Table 1 summarises the mechanisms and the corresponding strength of causal evidence.

Table 5. Epigenetics-based approaches for crop improvement.

Approach	Principle	Evidence	Potential advantage	Principal limitation / breeding gate
Epiallele discovery	Identify stable differential methylation with expression/trait association	Natural methylome diversity in Arabidopsis and maize	May identify regulatory variation missed by SNP-only scans	Must control for local SNPs, structural variants, transposons and tissue composition
Epigenetic diversity analysis	Measure population-level methylation variation	Genome-wide accession/inbred panels	Maps natural variation and possible stress-linked loci	Association can be genetic, stochastic or developmental; replication required
Epigenetic QTL / epiRILs	Segregate epigenetic states under controlled genetic backgrounds	Arabidopsis epiRIL and mapping studies	Tests heritability and quantitative trait effects	Generation, background and off-target epigenetic changes can complicate inference
Epigenomic selection	Add methylome features to genomic/phenotypic prediction	Promising concept supported by methylome association datasets	Could add dynamic state information for stress-response prediction	Must outperform genomic/phenotypic baselines in held-out populations and environments
Targeted epigenome editing	Recruit dCas or RdDM factors to a selected locus	SunTag and engineered RdDM studies in Arabidopsis	Allows causal tests and potentially sequence-preserving trait changes	Off-target activity, reversion, editor dependence, pleiotropy and crop validation
Seed priming / stress conditioning	Use a controlled pre-exposure to alter later response	Strong practical physiological basis; epigenetic mechanism varies	Low-cost existing route to stress preparedness	Benefit is not proof of heritable epigenetic change; optimise for crop and environment
Field deployment	Validate epigenetic state and trait across seasons, sites and elite genotypes	Limited compared with molecular proof-of-principle work	Establishes value in real production systems	Requires genotype-by-environment testing, seed-system fit, cost and regulatory review

Table 1. Molecular mechanisms of epigenetic regulation in plants.

Mechanism	Components / context	Regulatory interpretation	Evidence class and example	Key primary studies
CG maintenance methylation	MET1; CG sites across replication	Maintains inherited methylation; promoter versus gene-body location changes interpretation	Mutant and methylome evidence; CG methylation stabilizes some epigenetic states	Mathieu et al. (2007); Stroud et al. (2013)
CHG methylation	CMT3 coupled to H3K9 methylation	Reinforcing heterochromatin pathway; often repeat-associated silencing	Genetic disruption changes non-CG methylation and silencing	Lindroth et al. (2001); Jackson et al. (2002)
CHH methylation in heterochromatin	CMT2; DDM1-dependent access	Targets repeat-rich heterochromatin; distribution depends on chromatin accessibility	Mutant methylomes and chromatin genetics	Stroud et al. (2013); Stroud et al. (2014)
RNA-directed DNA methylation	Pol IV, RDR2, DCLs, AGO4, Pol V, DRM2	siRNA-guided de novo methylation; silences transposons or selected regulatory loci	Genetic loss-of-function and targeted recruitment	Chan et al. (2004); Onodera et al. (2005); Qi et al. (2006); Gallego-Bartolomé et al. (2019)
Active DNA demethylation	ROS1-family glycosylases / base-excision repair	Removes methylation from selected loci, opposing inappropriate silencing	Genetic perturbation and developmental evidence	Gong et al. (2002); Liu et al. (2015)

Mechanism	Components / context	Regulatory interpretation	Evidence class and example	Key primary studies
H3K4me3 / H3K4me2	Promoters and stress-memory loci	Often associated with active or re-inducible transcription; not sufficient alone to prove memory	Time course, mutant and heat re-challenge evidence	Lämke et al. (2016); Zeng et al. (2019)
H3K27me3 / Polycomb	PRC2-associated loci; developmental silencing	Supports repression or poised states; co-occurrence with active marks is context-dependent	Developmental and chromatin profiling	Bastow et al. (2004); Roudier et al. (2011); Zeng et al. (2019)
Nucleosome remodeling	DDM1, FGT1, SWI/SNF and ISWI families	Changes DNA accessibility and can sustain transcriptional competence	Mutants, interaction assays and nucleosome mapping	Saze et al. (2003); Brzezinka et al. (2016)
Transposon-associated epigenetic control	DNA methylation, H3K9me2, small RNAs	Constrains transposon activity; insertions can also alter nearby gene regulation	Population and perturbation evidence	Miura et al. (2001); Quadrana et al. (2016); Eichten et al. (2013)

4. DISCUSSION

The strongest conclusion from the primary literature is that plant epigenetic regulation is mechanistically real, deeply integrated with DNA sequence and transcription, and capable of influencing development and stress response. A second conclusion is equally important: the evidence does not support a general claim that environmentally induced DNA methylation is commonly inherited or that inherited changes routinely improve crop performance. The difference between these statements explains much of the apparent disagreement in the field. It is possible to demonstrate chromatin memory over days in one plant while observing little conserved methylation after several sexual generations. Both results may be correct because they assay different molecular marks, different persistence windows and different biological processes.

First, epigenetic association is vulnerable to genetic confounding. Transposon insertions can recruit methylation and influence neighbouring genes; DNA polymorphisms can alter transcription-factor motifs and chromatin recruitment; and local sequence variants can explain a substantial share of methylation differences in crops (Eichten et al., 2013; Dubin et al., 2015). The term epiallele should therefore be reserved for a stable epigenetic state that is not fully explained by DNA sequence variation, unless the author explicitly defines a sequence-associated epiallele. Genomic controls and allele-specific measurements are needed to avoid treating genetic regulation of methylation as sequence-independent epigenetic inheritance.

Second, causality requires temporal order and perturbation. If expression changes before the methylation difference, the methylation event may consolidate rather than initiate transcription. A methylation inhibitor that alters a stress phenotype does not isolate a locus-specific mechanism. The most convincing studies combine time courses, the epigenetic mark and transcript in the same tissue, mutants or targeted editing, and restoration or rescue. These criteria matter particularly for stress-induced marks, because stress can change development rate, cell-type composition and the abundance of a few highly expressed transcripts, all of which can shift bulk epigenome profiles.

Third, inheritance is not a binary property. CG methylation has robust maintenance systems, while other marks may turn over during development or be reset in reproductive lineages. Arabidopsis experiments identified resetting mechanisms, and the multigenerational drought study found that methylome differences were not a convincing general explanation for descendant phenotypes (Iwasaki and Paszkowski, 2014; Ganguly et al., 2017). Conversely, some clonal systems maintain particular stress-associated marks for many vegetative generations. Therefore, an epigenetic breeding approach should specify the reproductive biology of the crop. A stable state in potato tubers, cassava cuttings or other vegetatively propagated material may be useful even if it is not transmitted through sexual reproduction, provided it persists through propagation and does not erode field performance.

Fourth, agronomic effect size and stability are more important than the mere existence of epigenetic variation. A methylation marker that predicts expression in one accession may fail in a genetically different background. Stress tolerance is multigenic and often involves trade-offs between growth and defence. Enhancing an acute stress response may reduce biomass or seed production when the stress is absent; constitutive silencing may also affect developmental timing. Epigenetic selection should therefore be evaluated with genotype-by-environment models and compared against genomic selection and conventional phenotypic selection. Its value should be shown as improved prediction, lower breeding-cycle cost or a validated causal handle not available through DNA-sequence variation alone.

A further interpretive issue is that developmental silencing can be robust and reproducible without being an adaptation to the environment that induced it. Imprinting, vernalization and fruit ripening show how methylation or histone states can be integrated into developmental programmes, but their direction of effect depends on the locus and stage (Kinoshita et al., 2004; Kinoshita et al., 2007; Liu et al., 2015; Lang et al., 2017). Likewise, population-level epigenetic diversity may affect plant performance under particular experimental conditions, yet population effects do not automatically identify a single transferable epiallele (Latzel et al., 2013). These distinctions should be reflected in manuscript language: “associated with” is appropriate for profiling results, “required for” should be reserved for perturbation evidence, and “improves crop performance” requires agronomic data.

Finally, methods for targeted epigenome editing create an opportunity to turn observational epigenomics into experimental genetics. Artificial recruitment of methylation machinery can test whether a candidate region is sufficient to alter transcription, but the induced state may be local, transient or dependent on continued effector expression. Repeated independent guide designs, whole-genome off-target assessment, editor segregation, untreated and non-targeting controls, multiple elite genetic backgrounds and field-scale phenotyping should be considered minimum steps for translation. If target methylation does not persist, the result remains valuable mechanistic evidence but should not be marketed as a breeding-ready epiallele.

5. RESEARCH PRIORITIES AND FUTURE DIRECTIONS

Priority 1: establish causal chains rather than cataloguing marks. Select a small number of loci supported by differential accessibility or methylation and matched expression, then perturb the chromatin pathway and rescue the phenotype. Measure the same tissue across stress, recovery and re-challenge. The primary endpoint should be a defined expression or trait response, not the number of differentially methylated regions.

Priority 2: design inheritance experiments around reproductive route. For seed-propagated crops, measure parents and at least two stress-free descendant generations where feasible, genotype lineages and include reciprocal crosses or maternal-effect controls. For clonal crops, track marks across repeated propagation cycles. Report loss as well as persistence. These designs can distinguish stable memory from transient carryover and spontaneous epigenomic drift.

Priority 3: test crop and environment generalisability. Compare multiple genotypes, matched developmental stages and realistic stress sequences. Use at least two seasons or locations for candidate traits and include untreated environments to identify growth penalties. Integrate methylome, transcriptome, chromatin accessibility, genotype and yield/quality observations only when samples are matched and the sampling design permits causal interpretation.

Priority 3b: distinguish regulatory methylation from transposon tagging. Because repeat insertions and local structural variation can influence methylation, candidate DMRs should be tested against suitable structural-variant calls where feasible. If a methylation marker tags a nearby insertion, it may still be useful predictively, but should not be described as an independent epigenetic cause. This distinction affects portability: a marker coupled to a haplotype may be reliable within one breeding pool but fail in unrelated germplasm. Joint models should compare sequence-only, methylation-only and combined predictors on held-out families or locations rather than random individuals from the same population.

Priority 4: benchmark the breeding value explicitly. Compare epigenomic predictors with genomic and phenotype-only baselines using held-out families or locations. For edited states, quantify off-target activity, state persistence after editor segregation, reversibility, pleiotropy and regulatory/seed-system feasibility. Promote a candidate only when its added predictive or causal value is reproducible and economically meaningful. The evidence gaps and recommended tests are summarised in Table 6.

Table 6. Critical evidence gaps and research priorities.

Gap	What is known	Key uncertainty	Recommended experiment / analysis	Measurable outcome
Causality of stress DMRs	Stress can alter methylation; expression associations vary by locus	Does methylation drive transcription or follow it?	Time course + targeted editing / mutant + rescue + matched RNA-seq	Direction of effect and phenotype change at a specific locus
Sexual inheritance	Some descendant effects occur; multigenerational drought methylomes can be stable or reset	Which marks survive gametogenesis and why?	Genotyped lineages, stress-free G1/G2+, reciprocal crosses, common garden	Persistence rate and proportion independent of sequence / maternal effect
Clonal crop persistence	Some clonal plants retain CHG methylation across many vegetative generations	Does the mark change useful crop traits?	Repeated propagation; locus methylation, expression, biomass/yield and stability	Mark retention and trait effect over commercial propagation cycles
Combined stress	Most mechanisms were studied under single-factor treatments	Do combinations reinforce, mask or reset epigenetic states?	Factorial drought × heat/salinity treatment at matched stages	Interaction effects on chromatin, transcript and phenotype
Genotype dependence	Crop DMRs frequently associate with local sequence variation	Will a candidate work across elite backgrounds?	Panel-based validation and near-isogenic / segregating populations	Effect-size distribution and genotype-by-environment variance
Epigenome editing	Targeted methylation is possible in Arabidopsis	Can an edited state be stable, specific and useful in a crop?	Multiple guides, whole-genome off-target screen, editor segregation and field trial	Target retention, off-target burden, yield/quality and fitness costs

Gap	What is known	Key uncertainty	Recommended experiment / analysis	Measurable outcome
Predictive breeding value	Epigenomic datasets capture variation beyond standard phenotype snapshots	Does methylome information add prediction after genomic data?	Cross-validation by family, location and year against genomic baseline	Incremental prediction accuracy and cost-benefit under deployment conditions

6. CONCLUSIONS

Plant epigenetic regulation emerges from interacting DNA methylation, histone, small-RNA and nucleosome-remodelling pathways whose effects depend on locus, tissue, genotype and developmental context. Primary research provides strong evidence for specific chromatin mechanisms in transcriptional regulation, flowering and short-term heat memory. Evidence for environmentally induced sexual inheritance is more mixed, and durable agronomic benefit is not established simply by finding a persistent methylation mark. The practical route to crop improvement is to treat epigenetic states as candidate causal variants: measure them with matched molecular and phenotypic data, separate them from genetic effects, manipulate them where possible, test stability through the crop's reproductive system and validate their value in field environments.

ACKNOWLEDGMENTS

Conflict of interest

Author contributions

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