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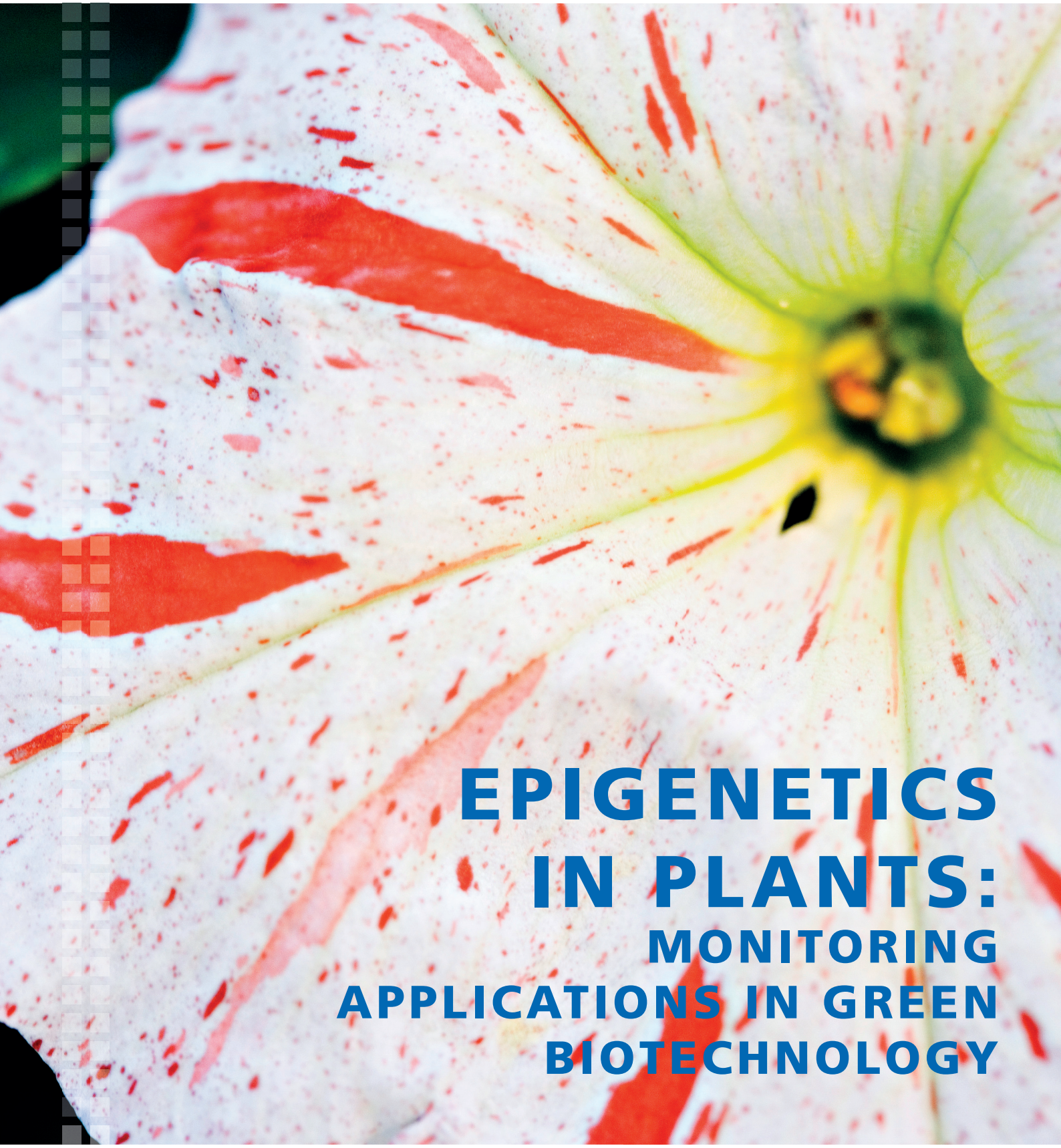

EPIGENETICS IN PLANTS: MONITORING APPLICATIONS IN GREEN BIOTECHNOLOGY

ONAFHANKELIJKE WETENSCHAPPELIJKE ADVISERING EN SIGNALERING VAN DE RIJKSOVERHEID

Epigenetics in plants: monitoring applications in green biotechnology

Are we ready?

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Preface

De ontwikkelingsbioloog Conrad Waddington was de eerste die in 1942 de term Epigenetica gebruikte, een fusie van epigenese en genetica. Hij wilde met dit concept weergeven dat het DNA van een bevruchte cel in staat is verschillende routes van cel-differentiatie te sturen en daarmee verschillende celtypes, organen, en een heel organisme te laten ontstaan. Tevens kan het cellulaire DNA reageren op interne- en ook externe signalen, waaronder pathogenen, temperatuur, licht, etc. Waddington zal zich indertijd niet hebben gerealiseerd hoe groot de impact zou worden van het door hem geïntroduceerde Epigenetica fenomeen en zoals we nu weten, de complexe moleculaire wereld die daaraan ten grondslag ligt. Genetische informatie kan namelijk op een dynamische manier beschikbaar worden gemaakt via een scala aan biochemische mechanismen die op een gecontroleerde manier de RNA- en eiwit-coderende informatie in de chromosomen kan bereiken, sturen, en tot expressie laten komen. In feite is bij elk proces dat zich afspeelt met DNA een epigenetisch mechanisme betrokken. Belangrijk is dus ook de verpakking en 3D structuur van het DNA in chromosomen, herstellen van DNA-schade, genoomstabiliteit, en segregatie van chromosomen. De basis van deze epigenetische mechanismen is de chemische modificatie van DNA en dat van de eiwitten waar het DNA mee geassocieerd is, de zg. histon-eiwitten, en vele andere.

Het eerste rapport dat de COGEM over epigenetica heeft laten schrijven dateert uit 2011. Het geeft een overzicht van de toen bekende chemische modificaties en mechanismen in zowel dier als plant. Sinds dit eerste rapport is de epigenetische kennis explosief toegenomen, met name ook dat van planten. Afhankelijk van het type modificatie en de plaats in het genoom kunnen genen aan- en uitgezet worden. Dit dynamische karakter komt doordat de genoemde modificaties verwijderd en weer aangebracht kunnen worden wat het potentieel mogelijk maakt dergelijke processen biotechnologisch te sturen.

Door de beschikbaarheid van nieuwe technieken, waaronder de CRISPR-cas (epi)genoom-editing technologie, en de gedetailleerde kennis en het dynamische karakter kunnen mogelijk allerlei eigenschappen van voedsel- en siergewassen aangepast en gegenereerd worden zonder de basevolgorde van het genoom te veranderen. De epigenetisch-aangepaste planten vormen dus een andere klasse dan de genetisch aangepaste planten en de verwachting is dat in de nabije toekomst dergelijke epigenetisch aangepaste gewassen op de markt zullen komen.

De mogelijkheden die ‘Epi-breeding’ programma’s potentieel bieden roept vragen op m.b.t. de aard van de veranderde eigenschappen, stabiliteit van fenotypes, overerfbaarheid, veiligheid, en mogelijke gevolgen voor het milieu. De COGEM heeft daartoe dit nieuwe literatuuronderzoek laten uitvoeren, met nadruk op de epigenetica en zijn toepassingen in planten. Het onderzoek is uitgevoerd door Ruud de Maagd waarmee de COGEM-begeleidingscommissie drie bijeenkomsten heeft gehad waarin opzet, inhoud, en wensen van de COGEM zijn besproken. Met het uiteindelijke resultaat is de commissie zeer tevreden. Het biedt de COGEM een overzicht van de mogelijkheden van ‘Epi-breeding’ en een kader voor risicobeoordeling en mogelijke regelgeving, mocht dat nodig blijken.

Dr. Jan Kooter

Voorzitter van de begeleidingscommissie

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Samenvatting

Epigenetica in de biologie bestudeert hoe ontwikkelings- en omgevingsfactoren, waaronder biotische en abiotische factoren, de genexpressie beïnvloeden zonder de onderliggende DNA-sequentie te veranderen. Dit rapport vat de huidige kennis over epigenetische modificaties in planten samen, inclusief de bekende modificatietypen, hun regulatie, toevoeging en verwijdering, en hun effecten op de genexpressie in planten. Hierbij lag de focus specifiek op huidige en toekomstige toepassingen in de groene biotechnologie, de toepassing van moleculaire technieken en instrumenten in de landbouw, plantenproductie en duurzaamheid. De studie richtte zich ook op ontwikkelingen na 2011, het jaar waarin een eerder COGEM-rapport over epigenetische modificaties werd gepubliceerd. Met deze focus in gedachten werden een uitgebreid literatuuronderzoek, een gerichte zoektocht naar patenten en goedkeuringen van GGO-gewassen en contacten met vertegenwoordigers van de internationale veredelingsindustrie uitgevoerd. De lezer zal concluderen dat het vakgebied enorm is gegroeid en dat praktische toepassingen van ten minste enkele veredelingsmethoden in de nabije toekomst mogelijk zijn, zo niet al beschikbaar zijn. Meer geavanceerde, gerichte modificaties zijn pas de afgelopen 15 jaar mogelijk geworden, dankzij de vooruitgang in DNA-sequentie-specifieke stuurmethoden, zoals CRISPR/Cas. De meeste van deze geavanceerde, gerichte modificaties worden nog steeds uitgevoerd in de modelplant *Arabidopsis thaliana* en moeten nog worden bewezen voor gewassen in de praktijk.

Het rapport beschrijft de twee belangrijkste typen epigenetische modificaties of merkers: cytosinemethylering en een veelheid aan histonmodificaties, hoe deze worden behouden of verwijderd, welke eiwitcomponenten daarbij betrokken zijn en hun effect op genexpressie. Kort gezegd betekent het activeren of onderdrukken van genexpressie via epigenetische merkers dat de chromatinecondensatie wordt aangepast naar een meer open, of respectievelijk, meer gesloten staat. Deze condensatietoestand bepaalt of de transcriptiemachinerie, bestaande uit specifieke transcriptiefactoren, algemene transcriptiefactoren en RNA-polymerase II, toegang kan krijgen tot de promotor van een gen en transcriptie kan initiëren. Opzettelijke epigenetische modificatie van een plantengenoom kan op twee algemene benaderingen worden uitgevoerd: ongericht of gericht, waarbij de eerste al verder gevestigd is. Niet-gerichte modificatie treft meestal een zeer groot aantal epigenetische merkers door chemicaliën te gebruiken om cytosinemethylering of histonmodificatie te verstoren, of door mutanten te gebruiken die worden beïnvloed bij het behoud van cytosinemethylering tijdens DNA-replicatie. Producten van deze methoden kunnen al op de markt zijn gebracht, omdat ze waarschijnlijk niet zijn gereguleerd en geen residuen van de gebruikte chemicaliën bevatten. De producten van het veredelen met zogenaamde epiRIL's, inteeltlijnen met verschillende maar stabiele methyleringspatronen, zijn een andere toepassing die mogelijk bijna commercialiseerd wordt, mits hun productie geen transgene methoden hanteert, of, als alternatief, de transgene methoden zijn goedgekeurd door de regelgevende autoriteiten.

Zoals hierboven vermeld, was COGEM vooral geïnteresseerd in toepassingen en eigenschappen die het dichtst bij de introductie in de gewasveredeling stonden. Men kan twee klassen van eigenschappen onderscheiden: één omvat de lijst van gebruikelijke eigenschappen die al het onderwerp zijn van traditionele en GGO-veredeling, zoals opbrengst, kwaliteit, ziektebestendigheid en tolerantie voor omgevingsstress zoals droogte, zout, hitte of kou. De tweede klasse is specifieker (maar niet exclusief) voor epigenetische modificaties en nauwer gedefinieerd: regeneratie of

somatische embryogenese, het elimineren van kruisingsbarrières voor interspecifieke hybridisatie, en het elimineren of verminderen van barrières voor crossovers tijdens meiose.

Zoals bij alle biotechnologie zullen deze ontwikkelingen in de samenleving vragen oproepen over risico's voor voedsel- of voederveiligheid en voor milieuduurzaamheid. In overeenstemming met de relatief jonge staat van het onderzoeksveld werd er geen literatuur gevonden die deze risico's van epigenetische modificatie behandelde, behalve die welke al waren geïdentificeerd bij GGO-veredeling of zelfs traditionele veredeling. Een uitzondering kan de opkomst zijn van nieuwe interspecifieke hybriden die relatief onvoorspelbare effecten hebben door twee verschillende genomen te combineren ("genomische schok"). Dergelijke hybriden kunnen invasiever zijn en wilde verwanten vervangen, hoewel dergelijke schadelijke hybridisatie al is aangetoond bij traditioneel geproduceerde gewassen. Stabiliteit en erfelijkheid van modificaties zijn mogelijk niet gegarandeerd, maar zullen waarschijnlijk worden geselecteerd om te voldoen aan het registratieproces voor de cultivar.

Hoewel het waarschijnlijk minder relevant is voor niet-transgene modificaties, zal de regelgevingsstatus van epigenetische modificatie met transgenen, zoals bij de recentere gerichte benaderingen, afhangen van de ontwikkelingen in de regelgeving, althans in de EU. Er bestaat geen twijfel over dat deze gewassen onder de huidige regelgeving afhankelijk zijn van goedkeuring voor milieuvrijlating of voor voedsel-/voederproductie na een risicobeoordeling door het Europees Agentschap voor Voedselveiligheid, EFSA. De nieuw voorgestelde regelgeving voor gewassen afgeleid van NGT's (New Genomic Technologies) definieert een aparte categorie I met "eenvoudige" mutaties, zonder risicobeoordeling. Deze categorie lijkt echter de NGT "epigenetische modificatie" niet te omvatten en sluit die daarom waarschijnlijk ook uit. In alle gevallen waarin risicobeoordeling niet vereist is maar er wel significante veranderingen in de samenstelling optreden, zullen gewassen waarschijnlijk nog steeds onder de EU-verordening "Nieuwe Voedingsmiddelen" vallen.

Samenvattend vordert het onderzoek naar epigenetische modificatie voor gewasverbetering snel, maar met enkele uitzonderingen blijft de toepassing ervan buiten de modelplant *Arabidopsis* schaars. Toch neemt de ontwikkelingssnelheid toe, en kunnen de toepassingen eerder komen dan we denken. Dit roept de vraag op: zijn we er klaar voor?

Summary

Epigenetics in biology is the study of how developmental and environmental factors, including biotic and abiotic factors, affect gene expression without altering the underlying DNA sequence. This report summarizes current knowledge of epigenetic modifications in plant genomes, including the types of modifications known, their regulation, addition, and removal, and their effects on plant gene expression. This was done with a particular focus on current and future applications in green biotechnology, the application of molecular techniques and tools to agriculture, plant production, and sustainability. The study also focused on developments after 2011, the year of publication of a previous COGEM report on epigenetic modifications. With this focus in mind, the study conducted a comprehensive literature search, a dedicated search of patents and GMO crop approvals, and communications with representatives of the international breeding industry. The reader will conclude that the field has expanded enormously and that practical applications of at least some breeding approaches are possible in the near future, if not already among us. More advanced, targeted modifications have become possible only in the last 15 years, thanks to advances in DNA sequence-specific guiding tools, such as CRISPR/Cas. Most of the latter advanced targeted modifications are still being achieved in the model plant *Arabidopsis thaliana* and still need to be proven for actual crops.

The report describes the two major types of epigenetic modifications or markers, cytosine methylation and a multitude of histone modifications, how they are maintained or removed, which protein components are involved, and their effect on gene expression. Briefly, activating or repressing gene expression through epigenetic markers means altering the chromatin condensation to a more open or more closed state, respectively. This condensation state determines whether the transcription machinery, comprising specific transcription factors, general transcription factors, and RNA polymerase II, can access a gene's promoter and initiate transcription. Intentional epigenetic modification of a plant genome can be carried out in two general approaches: untargeted or targeted, with the former being longer established. Untargeted modification usually affects a very large number of epigenetic marks by using chemicals to interfere with cytosine methylation or histone modification, or by using mutants that are affected in cytosine methylation maintenance during DNA replication. Products of these approaches may already have been marketed, as they are likely not regulated and do not contain residues of the employed chemicals. The products of breeding with so-called epiRILs, inbreeding lines with different but stable methylation patterns, are another application that may be nearing commercialization, provided that their production does not use transgenic methods, or, alternatively, that the transgenic methods have been approved by the regulatory authorities.

As stated above, COGEM was especially interested in applications and traits that are closest to the introduction in crop breeding. One can distinguish two classes of traits: one comprises the list of regular traits that are already the focus of traditional and GMO breeding, such as yield, quality, disease resistance, and tolerance to environmental stresses such as drought, salt, heat, or cold. The second class is more specific (but not exclusive) to epigenetic modifications and more narrowly defined: regeneration or somatic embryogenesis, eliminating crossing barriers for interspecific hybridization, and eliminating or reducing barriers to crossovers in meiosis.

As with all biotechnology, these developments will raise questions in society about risks for food or feed safety and for environmental sustainability. Mirroring the relatively young state of the research field, no literature was found addressing these risks arising from epigenetic modification beyond those already identified for GMO breeding or even traditional breeding. One exception may be the appearance of new interspecific hybrids that have relatively unpredictable effects of combining two disparate genomes (“genomic shock”). Such hybrids may be more invasive and replace wild relatives,

although such harmful hybridization has already been shown to occur with traditionally bred crops. Stability and heritability of modifications may not be assured, but will likely be selected for to conform to the cultivar registration process.

While probably less relevant for non-transgenic modifications, the regulatory status of epigenetic modification using transgenes, as in the more recent targeted approaches, will depend on the developments in regulations, at least in the EU. There is no doubt that, under the current regulation, these crops depend on approval for environmental release or for food/feed production following a risk assessment by the European Food Security Agency, EFSA. The newly proposed regulations for crops derived from NGTs (New Genomic Technologies), define a separate category I with “simple” mutations, not requiring risk assessment. However, this category does not seem to include, and therefore probably excludes, the NGT “epigenetic modification.” In all cases where risk assessment is not required but significant changes in composition occur, crops will likely still be subject to the EU “Novel Foods” regulation.

In conclusion, research into epigenetic modification for crop improvement is rapidly advancing, but, with some exceptions, its application beyond the model plant Arabidopsis remains scarce. Yet the speed of development is picking up, and applications may come sooner than we think. This presses the question: Are we ready?

1 Introduction

COGEM has expressed the need for an up-to-date inventory of developments and possible applications of epigenetics in “green biotechnology”. Although the term green biotechnology may encompass more than crops alone, this report focuses on crops. There are several definitions for epigenetics, such as those in the "Explanatory Note on New Techniques in Agricultural Biotechnology (new breeding techniques)" of the High Level Group of Scientific Advisors (2017): chromosome-encoded information, not in the DNA base order, which, together with genotype and environmental factors, contributes to the determination of a stable, inherited phenotype. “Inherited” in this context may refer to mitotic or meiotic inheritance. All definitions agree that no changes to the DNA base sequence are involved, which raises the question of whether epigenetic modification falls under current European GMO regulations. The above mentioned "Note" suggests that it should. Such modification raises questions about the risks, which need to be continuously updated in view of the rapid developments in the field.

This report continues where a previous report commissioned by COGEM covered developments up to 2011 (Nap & Geurts van Kessel, 2011). While the 2011 report covered animals and plants, the field has since expanded greatly, warranting a separate treatment of plants, including crops. This report also attempts to identify crop traits that can already be modified or are likely to be successfully modified in the near future, while noting that the actual commercialization of such crops remains some time away.

The report examines existing methods for the *untargeted* manipulation of plant or crop epigenetics. However, since the 2011 report, the discovery of CRISPR/Cas and derived technologies, together with our expanded knowledge of epigenetic modification types and of the effector proteins that mediate them, has opened and made relatively accessible a wide array of *targeted* modification strategies. In addition to discussing targeted and untargeted epigenetic modification techniques, the report also covers applications in crops, effects on phenotypes, stability and heritability, potential environmental risks, and food safety.

2 Methods

2.1 Literature search strategies

Three approaches were used to gather literature that was considered, at least superficially, relevant for writing this report.

1. A fairly low-stringency internet search using the keywords "plant epigenetic modification", supplemented by in-paper citations from the first results, particularly in review papers. Furthermore, I scanned the abstracts of two recent conferences:
    a. Keystone symposium "Plant Epigenetics and Epigenome Engineering," held in Fort Collins, Colorado, October 13-16, 2025, and
    b. EpiSeedLink International Conference "Priming, Stress & Epigenetic Memory", held in Amsterdam, February 9-10, 2026

    for relevant citations, or if unavailable, searched the internet for publications from the authors of relevant abstracts.
2. To systematically explore the literature, I optimized PubMed search terms with ChatGPT in the following steps (see Annex 3). After each step, I ran a PubMed search using the terms, recorded the number of hits, and extracted references.
3. I used two fully artificial intelligence approaches to collect references and construct a review with those: A. using Elicit.com, I produced a literature review report by screening 500 sources with the promp: "make a literature search report, with references, on the subject of epigenetic modification in plants or crops for improvement of traits". The resulting report used 25 references, all but one after 2011. B. using ChatGPT.com, I received 44 hits with the same prompt. Both approaches yielded many slightly "off-topic" results, as they referred to "passive" epigenetic modification, i.e., the selection and use of non-targeted natural epigenetic variation for trait improvement. These references were cross-checked with the hits from the first two search approaches. This action added 23 references. A further refinement of the Elicit prompt by adding the word "targeted" before "epigenetic modification" and adding "targeted is essential" at the end yielded 25 references, all but two after 2020, of which 9 were new and I considered relevant to the subject.

Furthermore:

4. While writing this report, I conducted a few *ad hoc* Google searches for publications on subjects that were not, or were only partially, covered in the first three search steps.
5. References appearing in the papers produced by approaches 1-4 and not yet in the list of references were checked and, where appropriate, added to a library of possibly relevant publications, from which this report cites.

Altogether, this resulted in 505 relevant literature references from which a selection was used in this study.

2.2 Patent searches

To get an impression of the upcoming practical applications of epigenetic modification in crop breeding, I performed patent searches in two databases: Google Patents (https://patents.google.com, accessed May 4, 2026) and the European Patent Office database, Espacenet (https://worldwide.espacenet.com, accessed May 4, 2026). The search terms used and

the results are listed in Annex 4. The raw output from all searches was manually curated to remove duplicates and for relevance to plants.

2.3 Interviews with breeding companies

Employees of crop breeding companies may have deeper insight into epigenetic modifications relevant to crop traits, as well as the nature of those traits and their priority for application.

Therefore, in addition to searching the scientific literature, I also contacted professionals who work, or have previously worked, in various international plant breeding companies. The insights from those contacted, of which seven responded, are summarized in section 6.6.

3 Types of epigenetic modifications in plants

Epigenetic modifications can be divided into two broad classes: DNA methylation (mostly of cytosine residues) and posttranslational histone protein modifications in the nucleosomes around which the DNA is wrapped. Dynamic changes in both types can occur in response to developmental and environmental factors, and both can interact synergistically, reinforcing each other's modifications and creating a "memory" of that response. This chapter describes the various modifications and the general effects they may have on gene expression.

3.1 DNA methylation

The oldest known and most studied epigenetic modification, including in plants, is DNA methylation, particularly methylation at the 5th position of the cytosine ring (5mC, m5C), sometimes called the 5th base (Mattei et al., 2022). Other, more recently discovered modifications in plants include 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), 5-carboxylcytosine (5caC), all products of 5mC oxidation, and N6-methyladenosine (m6A, m6A) (S. Kumar & Mohapatra, 2021). Although these may all have (some yet to be discovered) functions in epigenetic regulation in plants (Liang et al., 2020), here I focus on the most prevalent modification, 5-methylcytosine.

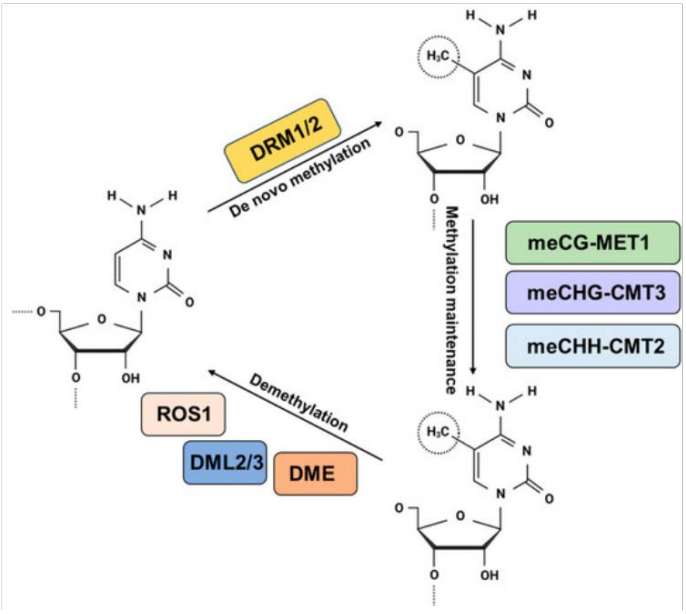

Fig. 1. Cytosine methylation and demethylation pathways in plant cells.

In contrast to animal systems, where 5mCG is predominant, 5mC in plants occurs in three contexts: CG, CHG, and CHH (where H=A, T, or C). 5mCG is generally associated with transcribed genes, whereas methylation across all contexts is often linked to transcriptional silencing, including that of transposable elements and repeats. In flowering plant genomes, CGs are most frequently methylated (80-100%), followed by CHG and, to a much lesser extent, CHH, though variation exists between species. For example, CHH methylation in the well-studied *Arabidopsis thaliana* and related Brassicaceae is lower than in most other studied species (Niederhuth et al., 2016).

Because DNA replication leads to loss of methylation on newly synthesized strands, maintenance methylation is required to restore it (**Fig. 1**). In Arabidopsis, this maintenance is controlled by several pathways: CG methylation by DNA METHYLTRANSFERASE 1 (MET1); CHG methylation by

CHROMOMETHYLASE (CMT2 or CMT3); and CHH methylation maintained by DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2), a homolog of animal DNMT3, through the RNA-directed DNA methylation (RdDM) pathway (see section 3.2). RdDM also controls de novo methylation in all three contexts. Mutations in genes encoding maintenance pathways have been used to induce untargeted epigenetic modifications and create "epialleles" (see section 6.2.3). Enzymes in this pathway have also been deployed in targeted methylation experiments in plants or plant cells (see section 6.3).

DNA methylation is dynamically maintained, including by demethylation. Passive, non-enzymatic demethylation can occur by reduced methylase activity during DNA replication (see also section 6.2.3). Active erasure of DNA methylation in plants is mediated by so-called bifunctional DNA glycosylases, which in Arabidopsis are REPRESSOR OF SILENCING 1 (ROS1 or DML1), DEMETER (DME), DEMETER-LIKE PROTEIN 2, and DML3 (Zhu et al., 2025) **(Fig. 1**). In targeted DNA demethylation experiments, the mammalian TEN-ELEVEN TRANSLOCATION 1 (TET1) or its catalytic domain (TET1cd) is the enzyme of choice (see section 6.3.2).

3.2 Histone- and nucleosome variants, and posttranslational histone modifications

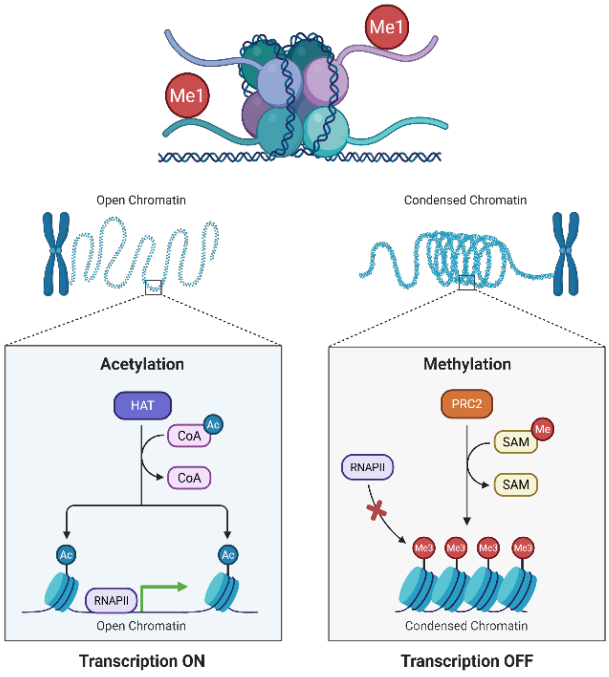

Fig. 2. Chromatin architecture. A. Nucleosome structure with H3/H4 dimers holding DNA strands. The Histone tails that can be posttranslationally modified are indicated with methyl groups (not true to scale). B. Effects of activating (left) and repressing (right) histone modifications and their effect on chromatin condensation and availability for transcription.

In eukaryotic nuclei, DNA appears as chromatin, a complex of proteins and RNA that is more or less densely packed, depending on the specific processes occurring at a given genomic location. Chromatin can occur as loosely arranged euchromatin, in which most active transcription and, during meiosis, crossovers happen. Condensed heterochromatin is less active and in plants comprises mostly silenced transposable elements. The DNA in chromatin is complexed in so-called nucleosomes (**Fig. 2**).

Nucleosomes comprise approximately 147 bp of DNA wrapped around an octamer of four histone types, with a core of two H3-H4 heterodimers flanked by two H2A-H2B dimers. H1 is a linker histone that interacts with the nucleosome core and the linker DNA and plays a role in nucleosome interactions and chromatin condensation. The canonical histones H2A, H3B, H3, and H4 are highly expressed during the cell cycle S phase to facilitate nucleosome assembly of newly synthesized DNA. Additionally, there are histone variants (indicated by the histone family name followed by a period and a letter or number to indicate the specific variant). The properties and (putative) functions of these histone variants were reviewed by Foroozani et al. (2022) and Hari-Sundar Gandhivel & Shivaprasad (2026). Summarizing, flowering plant-specific H2A.W is involved in tight chromatin condensation in heterochromatin, among others in transposons. H2A.Z promotes transcription when incorporated into the nucleosome proximal to the transcriptional start site and is also located in stress-responsive genes. H2A.X and H3.3 are associated with euchromatin. H1, with three variants in Arabidopsis, is involved in chromatin condensation.

Histone variants, their posttranslational modifications, and their impact on chromatin density and gene expression were reviewed by Foroozani et al. (2022), Jamge et al. (2023), and Le et al. (2025).

Table 1. Common posttranslational modifications of histones H2, H3, and H4. The modified amino acids and their positions are indicated as K(lysine) or R(arginine) with a number, ending with the type and number of modifications (me: 1,2, or 3 methylations; ac: acetylation; ub: ubiquination)

Histone modification	Typical effect on transcription	Common plant context or function
H3K4me3	Activating	Strongly associated with active promoters and gene expression
H3K4me2	Activating	Found in actively transcribed genes
H3K36me3	Activating	Enriched in the gene bodies of actively transcribed genes
H3K36me2	Activating	Linked to transcription elongation
H3K9ac	Activating	Opens chromatin and promotes transcription
H3K14ac	Activating	Associated with active chromatin
H3K27ac	Activating	Marks active enhancers/promoters in plants
H4K5ac	Activating	Correlates with active transcription
H4K8ac	Activating	Promotes relaxed chromatin structure
H4K12ac	Activating	Associated with transcriptional activation
H4K16ac	Activating	Facilitates chromatin accessibility
H2Bub1	Activating	Supports transcription elongation and crosstalk with H3K4me3
H3K27me3	Repressing	Polycomb-mediated silencing; developmental gene repression
H3K9me2	Repressing	Major heterochromatin and transposon silencing mark in plants
H3K9me1	Repressing	Often linked with silent chromatin
H3K27me1	Repressing	Associated with constitutive heterochromatin
H4R3me2s	Repressing	Linked to transcriptional repression
H2Aub1	Repressing	Polycomb-associated repression
Histone deacetylation (e.g., low H3/H4 acetylation)	Repressing	Chromatin condensation and reduced transcription

Chromatin dynamics that affect gene expression are mediated by posttranslational modifications (PTMs) of histone tails, collectively known as the histone code. These modifications include methylation, acetylation, phosphorylation, and ubiquitylation, and are listed in Table 1. These, along with more recently discovered acylation modes, were reviewed by (Le et al., 2025) and (Wei et al., 2026). Most active genes are depleted of nucleosomes near the transcription start site and are characterized by the presence of the histone variant H2A.Z. nearby. Active genes are mostly located in euchromatin, with histone modifications clearly distinguishing them from heterochromatin (Table 1; (Roudier et al., 2009). Further analysis of 12 different chromatin marks identified four main chromatin states: active genes, repressed genes, silenced transposons and repeats, and intergenic regions (Roudier et al., 2011). A distinction can be made between modifications associated with gene silencing and those associated with gene activation, with further distinction in their relative position with respect to features such as gene bodies, promoters, and so on. Silencing of expression is associated with modifications of H3 histone tails at specific lysine (K) residues: H3K9me1 or -2 at repetitive DNA, H3K27me1 or 2 in constitutive heterochromatin where they silence transposons and repetitive elements, and H3K27me3 at developmentally silenced genes. Histone deacetylation of H3 or H4 is also associated with repression. Activation of expression is associated with methylation (in all three forms) at lysine residues 4 and 36: H3K4me and H3K36me, as well as with acetylation of 7 or 5 lysine residues in H3ac or H4ac, respectively (Le et al., 2025). The epigenetic marks that have so far been modified by intentional, targeted modifications using effector fusions to Zinc Finger or dCas9 will be discussed in section 6.3 and are listed in the accompanying Table 2.

Each of the modifications mentioned above is associated with a cluster of effector proteins: Writers, Readers, and Erasers of PTMs. While I will not discuss the number and variations of them here, H3K29me deserves special attention because of its cross-talk with non-CG DNA methylation: binding of methylated non-CG DNA by SU(VAR)3–9 HOMOLOG 4 (SUVH4), also called KRYPTONITE (KYP), is the main determinant of H3K9me2 modification. At the same time, recognition of H3K9me by CHROMOMETHYLASES CMT2 and CMT3 leads to targeted non-CG methylation, closing the feedback loop. Histone acetylation by Histone Acetyltransferases (HATs) in general increases gene transcription. HATs (12 in Arabidopsis) have no DNA-binding specificity and reside in histone-modifying complexes that are recruited to promoters and enhancers by DNA-binding transcription factors. Erasers of histone acetylation are the Histone deacetylases (HDACs), whose activity leads to gene silencing. The SUPERMAN REPRESSION DOMAIN X (SRDX) or ETHYLENE-RESPONSIVE ELEMENT-BINDING FACTOR-ASSOCIATED AMPHIPHILIC REPRESSION (EAR) domain, which are common motifs in several repressive transcription factor families, are used to render other TFs repressive, recruit HDACs using a bridging cofactor (Causier et al., 2012; Krogan et al., 2012). Arabidopsis has 18 known HDACs, distributed across three main families: the RPD3/HDA1 family (further subdivided into three classes), the Sir2 family, and the HD family (plant-specific HDACs) (Luo et al., 2017).

Several of the histone modifier proteins mentioned above are used as effectors in targeted histone modification experiments, either as stand-alone fusions to dCas9 or as cofactors in targeted DNA methylation and demethylation experiments (see section 5.3.3).

3.3 Chromatin remodeling and modifying complexes

Chromatin remodeling, the changes that occur in nucleosome occupancy of the DNA, and condensation of the chromatin are dynamically regulated by two types of protein complexes with, broadly speaking, opposite effects on gene expression: Polycomb Repressive Complexes (PRCs) and the SWI/SNF (switch defective/sucrose non-fermentable) multiprotein chromatin-remodeling ATPases.

PRCs in plants are multi-component epigenetic regulators of plant development, flowering time, and environmental responses. They provide transcriptional memory to cell lineages and maintain cell identity. They act sequentially: PRC2 deposits the repressive mark H3K27me3, which is subsequently read by PRC1, an H3K27me3 reader, to catalyze H2A ubiquitination, resulting in chromatin condensation and silencing of gene expression. PRC2 recruitment to target genes works at least partially by sequence-specific transcription factors (Godwin & Farrona, 2022; Molitor & Shen, 2013).

Silencing of gene expression by PRCs in plants is often counteracted by the SWI/SNF multiprotein chromatin-remodeling ATPases. Recruited by either histone modifications, transcription factors, or non-coding RNAs, their ATPase activity moves or evicts nucleosomes, opening chromatin in the process, and exchanges canonical histones for histone variants. In Arabidopsis, as well as in rice, there are three types of SWI/SNF ATPases: BRAHMA (BRM/CHR2), SPLAYED (SYD/CHR3), and the two closely related MINUSCULE1 (MINU1/CHR12) and MINUSCULE2 (MINU2/CHR23). These ATPases form three subtypes of multiprotein SWI/SNF complexes: the BRM-associated SWI/SNF (BAS) complex, the SYD-associated SWI/SNF (SAS) complex, and the MINU1/2-associated SWI/SNF (MAS) complexes, which enhance chromatin accessibility at the transcriptional start site to enhance transcription factor binding. Other SNF2 family-related ATPases participate in the imitation switch (ISWI, regulates nucleosome spacing), inositol requiring 80 (INO80, regulates H2A/H2A.Z exchange), and SWI2/SNF2-related 1 (SWR1, regulates H2A.Z deposition) complexes (Guo & He, 2024; Han et al., 2015). These complexes regulate distinct gene expression patterns and developmental programs, including flowering time and the development of flowers, leaves, and embryos. In contrast to the generally activating action of SWI/SNF ATPases, the subunit SWI3B interacts with long non-coding RNAs (lncRNAs; >200 nt)) through the lncRNA-binding protein INVOLVED IN DE NOVO 2 (IDN2)/RNA-DIRECTED DNA METHYLATION 12 (RDM12) to repress gene expression (Zhu et al., 2013).

3.4 RNA-mediated mechanisms of gene regulation (lncRNA and siRNA)

lncRNAs in plants have several functions, among which are some that are relevant to the subject of this report. They act as epigenetic regulators by guiding Polycomb Repressive Complexes (PRC1 and 2; see section 3.3) to specific genomic loci, thereby controlling gene expression and, as a result, affecting environmental stress responses and development (Chanwala et al., 2026; Gaggi et al., 2025; Gonzales et al., 2024).

Key examples include COLDAIR and COOLAIR (vernalization, (Ormancey & Qüesta, 2026)), MAS (flowering), and DRIR (drought/salt), which regulate histone methylation. The lncRNA *COLDAIR* recruits PRC2 for silencing of FLOWERING LOCUS C during vernalization in Arabidopsis. This recruitment catalyzes the deposition of the repressive H3K27me3 mark, but histone acetylation is also implicated. In this and other examples, the lncRNA originates in *cis* with its target gene, likely contributing to its specificity, as seen with COLDAIR, which originates from the first intron of *FLC*. The lncRNAs discovered so far are disproportionately involved in environmental sensing (cold, drought, immunity, seed dormancy).

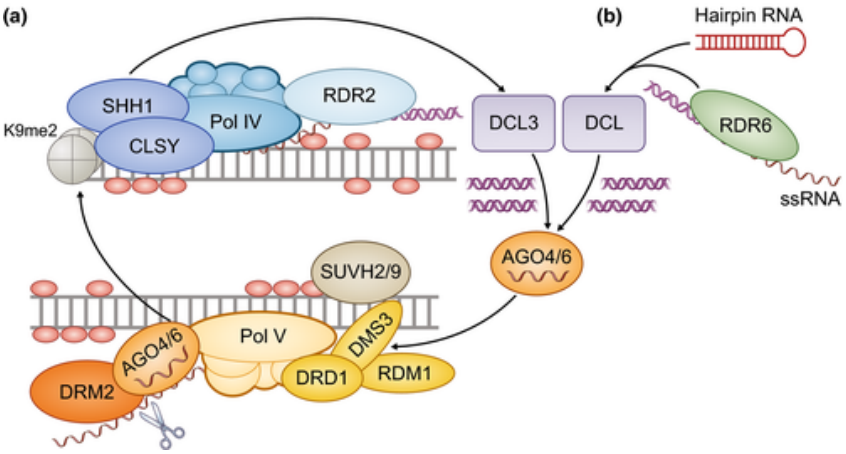

Fig. 3. A current model of the two pathways of RNA-dependent DNA methylation (Gallego-Bartolomé, 2020).

24 nt small interfering RNA (siRNA) is involved in de novo DNA methylation as well as in maintenance methylation at CHH sites through RdDM, reviewed in (Xie & Yu, 2015). At target loci, RNA polymerase IV (Pol IV) produces single-stranded RNAs, which are converted to double-stranded RNA (dsRNA) by RNA-dependent RNA polymerase 2 (RDR2) and subsequently processed to siRNA by the RNAse DICER-LIKE 3 (DCL3). The siRNAs are subsequently loaded into the protein ARGONAUTE 4 (AGO4). The latter complex then recruits DRM2 to catalyze DNA methylation by recognizing scaffold transcripts produced by DNA-directed RNA Polymerase V (Pol V). DNA methylation is additionally reinforced by H3K9 methylation, which overlaps with the RdDM target, via the KRYPTONITE (SUV4), SUV5, and SUV6 methyltransferases, which are specific for H3K9. This mechanism is also active in the 21/22 nt sRNA-mediated, RdDM-dependent de novo transcriptional silencing of FWA using a viral vector containing a homologous sequence (Bond & Baulcombe, 2015).

3.5 RNA modification

Chemical modifications similar to those in DNA (6mA, 5mC; see section 3.1) also occur in plant mRNA, with a growing range of identified functions, reviewed in (Dong et al., 2026; Song et al., 2026; Yao et al., 2025). These can also be actively targeted, albeit transiently, using dCas13 (Shi et al., 2024; Yu et al., 2024). Although this is considered an epigenetic modification by some, it will not be covered in this report.

3.6 Conclusions

Since the previous COGEM report (Nap & Geurts van Kessel, 2011) the number of publications on the epigenetic regulation of gene expression in plants in all its facets has exploded, providing novel techniques for their detection (section 4), and a dazzling array of options for modifying this regulation (section 5). In particular, the complexity of posttranslational modification variation, with its associated writers, readers, and erasers, underscores its involvement in plant developmental processes such as seed maturation and dormancy, stem cell transitions, floral transition and maintenance, and responses to environmental stresses (Le et al., 2025). Many of these regulated histone modifications also interact with DNA cytosine methylation and involve lncRNAs and siRNAs as intermediaries.

4 Epigenetic mutations, somaclonal variation, and hybridization effects on DNA methylation

4.1 Spontaneous epigenetic mutations

Changes in cytosine methylation or demethylation may occur stochastically, and these so-called epimutations are sometimes (very) stably maintained through subsequent generations. The majority of such epimutations likely go unnoticed because few have a readily distinguishable phenotype. Monitoring methylation variation across multiple generations derived from the selfed progeny of a single inbred individual allows estimation of this frequency per generation, as reviewed by Johannes & Schmitz ((Johannes & Schmitz, 2019). The epigenetic mutation rate per generation was much higher than the (base) mutation rate (7 x 10^-9 base substitutions per site per generation), with thousands of CG methylation differences accumulating over 30 generations. Changes at CHG and CHH sites were detectable but do not appear to accumulate over generations.

Notable examples of striking phenotypes include the hypermethylated *clark kent* alleles of the Arabidopsis *SUPERMAN* gene, which cause flowers with supernumerary stamens (Jacobsen & Meyerowitz, 1997), the Colorless non-ripening mutation in tomato (Manning et al., 2006), flower morphology in Linaria vulgaris (Cubas et al., 1999), and fruit shape in clonally propagated African oil palm, the latter with considerable economic impact (Ong-Abdullah et al., 2015). A more extensive list of phenotypes associated with spontaneous, induced, or developmentally regulated epigenetic changes can be found in (Tonosaki et al., 2022).

The Arabidopsis *FLOWERING LOCUS WAGENINGEN* (*FWA*) gene is a recurring focus of epigenetic studies. It was originally identified in *fwa* mutants, late-flowering gain-of-function alleles obtained by chemical mutagenesis (Koornneef et al., 1991). Wild-type *FWA* encodes a transcription factor that represses flowering and contains two highly methylated direct repeats in its promoter. In *fwa* mutants, these repeats are hypomethylated, leading to activated expression and late flowering (Soppe et al., 2000). Early or late flowering resulting from targeted (de)methylation at *FWA* is a readily observable phenotype for studying the function of genes involved in expression silencing or for testing epigenetic modification strategies: the number of rosette leaves formed before bolting is a measure of flowering time (Gallego-Bartolomé et al., 2018; Papikian et al., 2019; Wang et al., 2025). It is unknown whether *FWA* or *FWA*-like genes with similar functions occur outside the Brassicaceae family (to which Arabidopsis belongs), so broad applicability remains uncertain.

4.2 Somaclonal variation

Somaclonal variation occurs during *in vitro* tissue culture, likely due to stress from culture conditions. In *in vitro* plant multiplication practices, it produces so-called “off-types” with altered, mostly undesired phenotypic variation in material that was originally genetically homogenous. The causes may be genetic changes, but it is widely assumed that epigenetic changes also play a role (Smulders & de Klerk, 2011). An example is the mantled flower phenotype in clonally propagated oil palm (see the previous section), and stable epigenetic changes also occur in rice tissue culture (Stroud et al., 2013).

4.3 Hybridization and genomic shock

Interspecific hybridization, which brings together the genomes of two (relatively) unrelated parents, results in disruptions of epigenetic regulatory mechanisms that, for example, silence transposons.

This led to the hypothesis of “genomic shock” (de Tomás & Vicient, 2024). At a less drastic scale, “hybridization” between Arabidopsis ecotypes changes 24-nt siRNA levels, which might contribute to hybrid vigor (Groszmann et al., 2011). Similar events occur after hybridization of Arabidopsis epiRILs (Bucher et al., 2012; Reinders et al., 2009)

5 Detection and characterization of epigenetic modifications

For the detection of 5mC (or other cytosine modifications) at base resolution, bisulfite sequencing has been the gold standard since the 1990's. This method relies on the conversion of unmodified cytosine to uracil, which, after PCR amplification, will be read as thymidine by conventional sequencing technologies. Depending on the treatment after bisulfite conversion, this may either be targeted to specific regions or be genome-wide (WGBS, Whole Genome Bisulfite Sequencing). Other, more global methylation assays rely on methods that enrich or deplete high-mC-containing genomic DNA, followed by sequencing. More recently, next-generation long-read sequencing methods such as Oxford Nanopore and PacBio sequencing, and particularly their base-calling algorithms, have improved to the point that the identification of modified cytosines is possible. These methods and their comparison were reviewed by Agius et al. (2023).

Chromatin immunoprecipitation (ChIP) followed by deep sequencing (ChIP-Seq) is the method of choice for studying the distribution and dynamics of histone modifications. ChIP is based on the enrichment of DNA using a specific antibody against the DNA-bound modified histones. The captured DNA may be identified and quantified by (q)PCR, microarray hybridization, or, most commonly nowadays, deep sequencing. Since the focus of research is often on the effects of DNA methylation or histone modifications on the accessibility of the chromatin that determines gene expression ("open" or "closed" chromatin), techniques that determine this chromatin state, such as DNase I hypersensitivity profiling or, more recently, ATAC-seq (Wang et al., 2021) are also commonly used.

6 Techniques for targeted or untargeted epigenetic modification

6.1 Introduction

Although untargeted manipulation of epigenetic marks may seem a crude method to achieve a phenotype, the fact that induced epialleles can segregate independently in subsequent generations and are, at least partially, stably maintained helps identify the underlying gene(s), opening the way for targeted modification and reduction of pleiotropic effects.

6.2 Untargeted modifications

6.2.1 Use of chemicals to modify or block DNA methylation – 5-Aza, zebularine

Chemicals that affect DNA methylation are always blocking DNA (cytosine) methylation and can be divided into two categories: those that inhibit DNA methyltransferases (DNMTs) and those that disrupt the methyl supply for methyltransfer reactions, as reviewed by Zhang et al., 2013) The first category comprises the cytidine analogs 5-Azacytidine (5-Aza, 5-AzaC), 5-aza-2'-deoxycytidine (aza-dC), and Zebularine, where Zebularine is more stable under physiological conditions. Yet the former two have been used more extensively, and Zebularine treatment has been associated with major chromosomal rearrangements, making it less suitable for epigenetic studies (Finnegan et al., 2018, 2025), although DNA damage is likely an integral part of the mode of action of all these inhibitors (Nowicka et al., 2020). 5-Aza and aza-dC have been used in many experiments aimed at decreasing methylation in an untargeted manner, producing novel phenotypes, such as promoting shoot regeneration during Agrobacterium-mediated transformation (Zhao et al., 2019). A phenotype resulting from 5-Aza treatment and of considerable promise in plant breeding is the bypassing of the so-called triploid block that prevents interspecies hybridization (Huc et al., 2022). Alternatively, 5-Aza treatment was used to demonstrate the involvement of epigenetic mechanisms in incompletely characterized mutagenesis experiments, such as in *msh1* reprogramming (see section 6.2.4) and in cold acclimation in *Brassica rapa* (Liu et al., 2017). Treatment with zebularin also activated the expression and extrachromosomal DNA of retrotransposon ONSEN in Arabidopsis and adzuki bean, which are the first required steps for transposition (Boonjing et al., 2020).

6.2.2 Use of chemicals to change or block histone modifications

A range of chemicals that affect histone modifications has been discovered either through direct screening of small-molecule libraries (Otsuka et al., 2025) or through their demonstrated activity in animal systems (Lepri et al., 2023; Dai et al., 2024). The majority of these chemicals affect histone acetylation by either inhibiting or activating HATs and HDACs. Only a few chemicals affect histone lysine methyltransferases, but it will be a matter of time before more are identified and widely used. A frequently used HDAC inhibitor is TSA (Trichostatin A), although more inhibitors of other histone modifiers have been discovered and tested more recently (Patanun et al., 2017; Sako et al., 2016; Valero-Rubira et al., 2024). Trichostatin is particularly used to induce somatic embryogenesis from microspores or to stimulate regeneration in tissue culture, yet HDAC inhibitors have broader potential, such as in the induction of stress tolerance.

6.2.3 Epialleles, stress-induced epialleles, or epialleles created in (de)methylation mutants (*ddm, dme*)

By crossing stable lines with distinct methylation patterns and phenotypic variation, it becomes possible to identify epigenetic quantitative trait loci (epiQTLs) and the causal epigenetic modifications

underlying them. This identification was, for example, achieved for a spontaneous expression QTL determining vitamin E content in tomato (Quadrana et al., 2014). Other such spontaneous epialleles were identified in maize (Eichten et al., 2011), and rice (Schmidt et al., 2018; Zeng et al., 2023).

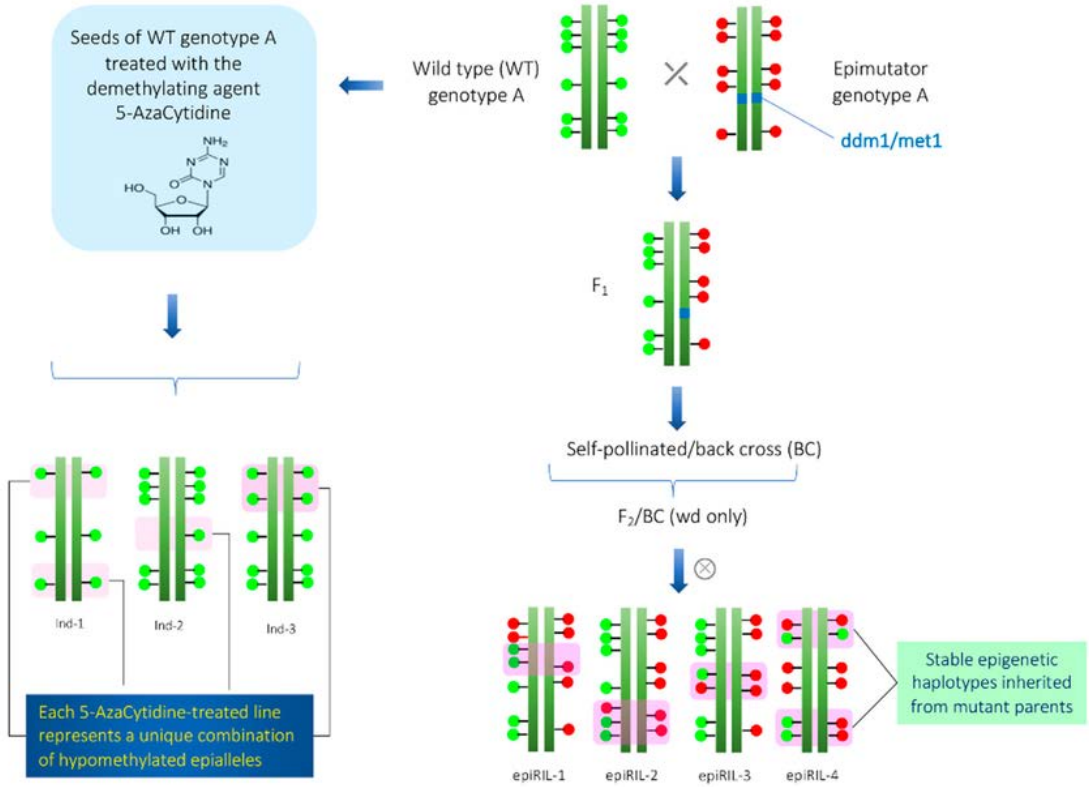

Fig. 4. Creation of epiRILs starting from chemical demethylation (left) or using methylation mutants and backcrossing with wild-type (WT), followed by multiple generations of selfing.

Rather than relying on *natural* variation, cytosine methylation variation can also be induced by 5-Aza treatment (Amoah et al., 2012) or by using mutants affecting the establishment and maintenance of cytosine methylation, such as *dme* and *ddm* mutants (see section 3.1), which cause global hypomethylation (Corem et al., 2018; Hu et al., 2014; Li et al., 2014). Subsequent backcrossing with the wild type and followed by inbreeding of individual F2 lines produces a collection of homozygous recombinant inbred lines (RILs) with different hypomethylated genomic patches and (virtually) no primary sequence variations (epiRILs, **Fig. 4**). In combination with phenotyping experiments, these can be used to identify epiQTLs and, subsequently, the underlying causal epigenetic variation. This approach has been applied, for example, to identify several epiQTLs that contribute to transgenerational resistance against *Hyaloperonospora arabidopsidis* (Furci et al., 2019). Identification of the underlying hypomethylated cytosines may eventually lead to *targeted* hypomethylation, as described in section 6.3. Although most research on the epiRIL approach has focused on Arabidopsis, epiRILs have been developed, or are being developed, for crops, including maize (Li et al., 2014), tomato (Corem et al., 2018; Kumar et al., 2024; Kuswandi, 2019; Singh et al., 2021), canola (Verkest et al., 2015), and rice (Schmidt et al., 2018). A problem is that mutations in maintenance methylation genes to induce hypomethylation may be lethal in the long term, as seen with the *mut1-2* mutation in rice (Hu et al., 2014). Transient inducible overexpression of RNAi constructs or genes that suppress maintenance methylation may prevent such problems.

A variation on the above approach is the induction of cytosine hypomethylation, as observed in *ddm1* and other epigenetic modifier mutants, sometimes in combination with stress, to activate transposon

transcription and mobility (Bucher et al., 2012). Increasing mobility and de novo insertions of retrotransposons generate genetic variation via Transposon Insertion Polymorphisms (TIPs). Induction of Arabidopsis retrotransposon *ONSEN* mobility by heat stress treatment led to developmental changes and increased drought tolerance in some lines, although the (semi-)random nature of transposition will make the approach difficult to steer (Thieme et al., 2017, 2022). Although the approach has been described multiple times in Arabidopsis and proposed for crop breeding, examples of positive phenotypic effects in crops remain lacking.

6.2.4 *Msh1* RNAi or knockout mutations with or without grafting

In several plant species, RNAi-mediated downregulation of the nuclear gene encoding the chloroplast and mitochondrial protein MutS Homolog1 (MSH1) induces variable phenotypes that remain heritable in the absence of the transgene responsible for the initial downregulation (Xu et al., 2011, 2012). It was subsequently demonstrated that MSH1 depletion triggers methylation of various genomic loci (Yang et al., 2020). Crossing or grafting msh1 mutants onto wild-type plants results in heritable enhanced vigor (Virdi et al., 2015; Yang et al., 2020). In Arabidopsis, the DNA methylation inhibitor 5-Aza-C reduces this phenotype, indicating that the differential DNA methylation patterns are responsible. The *msh1* reprogramming requires the epigenetic modifiers HDA6 and MET1, whereas the transition to memory requires the RNA-dependent DNA methylation pathway (Yang et al., 2020).

Plants using the MSH1 technology are nearing commercialization. EpiCrop Technologies, co-founded by Dr. Sally Mackenzie, is advancing epigenetically enhanced crops such as tomato and soybean, with potential commercial availability within the next 5 years, according to the company website.

6.3 Targeted modifications

6.3.1 Introduction

Targeted epigenome modification involves using a guidance system for sequence-specific targeting, either Zinc Finger (ZF) proteins or dead Cas9 (dCas9), in combination with one or more guide RNAs (gRNAs) (Leech et al., 2025). Single examples of successful targeting for demethylation and gene activation with fusions to dead LbCas12a and to the hypercompact Cas12j2 have also been published (Liu et al., 2022; Zheng & He, 2023). While targeting by transcription activator-like effectors (TALEs) is an obvious third alternative, has been used in animal systems and may be more effective for targeting heterochromatin in plants (Jain et al., 2021), I could not find examples of its use for epigenome editing in plants. Effector proteins for DNA (de)methylation or Histone protein modification may be covalently attached by encoding them as a fusion gene, or non-covalently tethered effector proteins (**Fig. 5**). Non-covalent tethering amplifies effector activity by genetically fusing them to the bacteriophage MS2 coat protein. MS2 dimers binding to each of two guideRNA hairpin aptamers (Lee et al., 2019) or of single-chain variable fragment GCN4 antibody-effector fusions binding to an adjustable number of tandem GCN4 repeats on the dCas9 protein, the SunTag system (Papikian et al., 2019), or the plant-optimized MoonTag system (Casas-Mollano et al., 2023). An effector that may or may not be categorized as an epigenetic modifier is VP64, a tetrameric repeat of the viral activation domain VP16. VP16/64 activates transcription generally by recruiting the general transcription machinery (Mediator complex and general Transcription Factors), but has also been implicated in chromatin opening in mammalian systems. No evidence for the latter mechanism in plants exists to date, but the observation that targeted VP64-mediated activation of the Arabidopsis *FWA* gene reduced CG methylation specifically in its promoter indicates that activating a silenced gene in this manner reduces promoter methylation (Papikian et al., 2019).

Table 2. Types of targeted DNA- or histone modifications reported for plants. Derived from (Gardiner, 2025).

Epigenetic modification	#Studies
Methylation	16
De-methylation	7
Histone modifications:	
H3K4me3	6
H3K27ac	4
H3K9ac	1
H4K16ac	1
H3K27me3	3

6.3.2 Targeted DNA (de)methylation

Although effector proteins from plants themselves could be used for targeted (de)methylation, following prior research on targeted epigenetic modifications in mammalian systems, mammalian or bacterial effectors have also been employed. Examples are the DNA (CYTOSINE-5)-METHYLTRANSFERASE 3 alpha and 3-like (DNMT3A-3L) catalytic domains, Arabidopsis DNA methyltransferase (DRM2), the tobacco DNA methyltransferase catalytic domain (NtDRMcd), and the bacterial DNA methyltransferase variant M.Sss1 (also called MQ1, and a more specific variant, MQ1v) (He et al., 2025; Leech et al., 2026). DNA methyltransferase DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) targeting is in vivo regulated by two arms of the RdDM pathway. ZF fusions with several of the proteins involved in RdDM (SUVH9, NRPD1, RDR2, DMS3, RDM1, MORC1, and MORC6) trigger DNA methylation in Arabidopsis when targeting the FWA promoter (Gallego-Bartolomé et al., 2019).

For targeted DNA demethylation, Arabidopsis DNA demethylase ROS1 (REPRESSOR OF SILENCING 1) or its catalytic domain (ROS1cd), as well as human DNA demethylase TET1 or its catalytic domain (TET1cd), are commonly used effectors. Although most of these applications are restricted to Arabidopsis, some successes have been achieved in other species, most notably the activation of heritable cold tolerance by *ACT1* promoter demethylation in rice (X. Song et al., 2025). Delivery of the gRNAs (as a phloem-mobile tRNA-gRNA fusion) by Tobacco Rattle Virus (TRV) infection of (dCas9)SunTag-VP64 expressing plants also resulted in demethylation and activation of the *FWA* promoter in the next generation, indicating how alternatives for transgenesis could be developed (although here the host plant was still transgenic for part of the machinery (Ghoshal et al., 2020). Targeted methylation of TAL-effector binding element in the effector-target promoter of the host gene (Cassava *SWEET10*) using a ZF-DMS3 fusion prevented effector binding, rendering the host resistant to bacterial leaf blight (Veley et al., 2023). The inheritance of resistance in the absence of the transgene still has to be determined, and a similar experiment with a susceptibility gene for Cassava Brown Streak Virus showed that binding of the transgene-encoded effector (albeit a different one) was already sufficient to block expression (CRISPR interference) (Lin et al., 2026). Thus, questions remain about the exact mechanism of blocking effector binding and its heritability in the absence of the transgene.

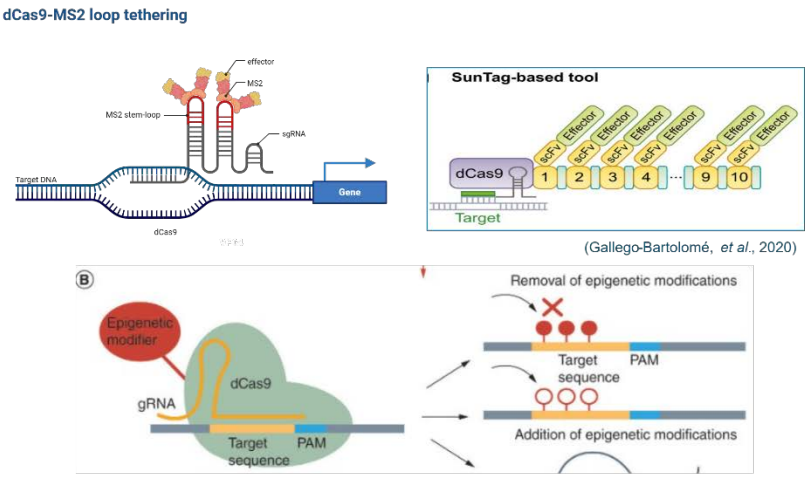

Fig. 5. Modes of targeted delivery of DNA or histone modifiers. A. Tethering by MS2 binding epitopes on the gRNA. B. Tethering by SunTag repeats on dCas9. C. Covalent fusion of the modifier protein to dCas9.

Table 3. Examples of *targeted* epigenetic modifications, the crop, the target gene, and the targeted traits (if any). Adapted from (Gardiner, 2025).

Species	Genes	#Studies	Trait
Arabidopsis	*FWA*	20	Flowering time
	CACTA1	2	Transposon expression
	AREB1	2	Drought tolerance
	EVD	1	Transposon expression
	AP3	1	Flower morphology
	CLV3	1	Meristem maintenance
	SUP	1	Stamen number
	FT	1	Flowering time
	APX2	1	Heat stress memory
	CUC3	1	Plant Development
	LRCen3	1	Recombination in heterochromatin
	SNC1	1	Disease resistance
Chrysanthemum	*MYB6*	2	Flower color
Tomato	*PR-1*	1	Disease resistance
Cassava	*SWEET10*	1	Disease resistance
Rice	*ACT1*	1	Adaptive cold tolerance
	FIE1	1	Dwarfing
	GBSS1	1	Starch
	Tos17	1	Transposon expression

6.3.3 Targeted histone modification

Many epigenetic editors targeting histone modifications in plants are inspired by effectors from animal systems (Thakore et al., 2016) and have been reviewed by Gardiner (2025) and Leech et al. (2026). In general, the epigenetic histone editors are aimed at either adding active marks: acetylation and methylation at H3K4, H3K27, H4K16, or removing repressive ones at H3K27me3 (see Table 3).

Categorized by their effect on gene expression, these are:

Activating effectors: Animal p300 is a histone acetyltransferase rendering chromatin more open through H3K27ac and H3K18ac. Histone Acetyltransferase (HAT1): acetylating newly synthesized histone H4K5 and H4K12 (Roca Paixão et al., 2019). SET DOMAIN GROUP 2 (SDG2) deposits H3K4me3 and activates *FWA,* as well as disease resistance gene *SUPPRESSOR OF NPR1-1, CONSTITUTIVE 1* (*SNC1*) gene expression as a SunTag-SDG2 fusion combined with dCas9-mediated targeting. Additionally, this also increased pericentromeric crossovers in Arabidopsis. Replacing SDG2 with the PR Domain Zinc Finger protein 9 (PRDM9) catalytic domain, which catalyzes the trimethylation of H3K4, has similar effects (see also section 6.3.5) (Binenbaum et al., 2025).

Repressing effectors: KRYPTONITE (KYP or SUVH4) is a SET domain-containing histone methyltransferase. It catalyzes the formation of H3K9me2 (dimethylation of histone H3 lysine 9), a repressing heterochromatin mark. Indirect targeting of H3K27ac deacetylases (HDACs) through recruitment of a mammalian transcriptional repressor domain was recently tested in Arabidopsis (see below, KRAB). JUMONJI C-domain protein 18 (JMJ) H3K4 demethylase was used to reduce gene expression in a heat-inducible manner (Oberkofler & Bäurle, 2022)

Given the known cross-talk between DNA methylation and histone modifications in gene expression regulation, and the progress made with combined effectors in mammalian systems, combining both mechanisms in plant epigenetic editing is a logical next step. While the review by Gardiner et al. (Gardiner, 2025) summarized mostly, if not exclusively, a multitude of studies using single effectors for one of the two epigenetic marker types. DNA (de-)methylation and histone modification were used together and tested in different combinations of effectors and cofactors, which were fused to either side (N- or C-terminal) of dCas9, hoping to achieve synergy and were not included in the above review (He et al., 2025).

Demethylation of the FWA promoter or methylation of an FT enhancer, and their effects on flowering time in Arabidopsis, were used as a measure of successful epigenetic modification. MQ1v was the only effective methylation effector, and for *FWA* activation by demethylation TET1cd, as reported earlier, was effective, whereas ROS1(cd) or IBM1 was only modestly so. The expected synergy from co-expression of histone modifiers had, disappointingly, little effect in most cases. Given the variation in off-target effects across effector/cofactor combinations, this led to recommendations for different editing scenarios, with a clear trade-off between efficacy and specificity (He et al., 2025).

6.3.4 Spray-induced Gene Silencing (SIGS) or Spray-Induced Epigenetic Modification (SPIEM) and similar applications

Considering that de novo DNA methylation through RdDM requires siRNA function, it is imaginable that the application of siRNAs could result in methylation and thus silencing of homologous loci (Xie & Yu, 2015) (see section 3.8). It has been demonstrated that these siRNAs can be used to induce *de novo* silencing of an endogenous promoter by Virus-Induced Gene Silencing (VIGS) (Bond & Baulcombe, 2015). Whereas in the latter approach siRNAs are produced from a viral vector containing a fragment of the target gene's promoter and gene body, smaller, even synthetically produced siRNAs may be used as well, as shown below.

Reducing gene expression by Post-Transcriptional Gene Silencing (PTGS) through spraying plants with dsRNA, hairpin RNA (hpRNA), siRNA, or microRNA (miRNA) is widely explored for modifying plant traits or combating plant pathogens and pests, but involves transcript degradation and therefore is not an epigenetic modification method (Dubrovina et al., 2025; Jha et al., 2025). Considering that de novo DNA methylation through RdDM requires siRNA function, it is imaginable that the application of siRNAs could result in methylation and thus silencing of homologous loci (Xie

& Yu, 2015). Many putative examples exist, but distinguishing RNAi-like effects from true promoter silencing by cytosine methylation is difficult. High-pressure spraying of dsRNA (Dalakouras & Ganopoulos, 2021) or of 24-nucleotide (nt) siRNAs targeting the CaMV 35S promoter (Walz et al., 2022) both successfully induced RdDM and transcriptional gene silencing (TGS) in *Nicotiana benthamiana*. This technique, acting as spray-induced epigenetic modification (SPIEM), resulted in reduced transgene expression and site-specific methylation. Clearly, the application is still in its infancy, and modification of crop traits has not yet been demonstrated.

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6.4 Overlap with more conventional transgenic strategies for ectopic expression or silencing of endogenous crop genes

So far, descriptions of epigenetic modification strategies have focused on induced cytosine (de)methylation and histone posttranslational modifications that render chromatin more compacted and inaccessible (closed) or, conversely, less compacted and accessible (open) to the transcription apparatus. Sequence-specific transcription factors binding to upstream activating (or repressing) promoter sequences may activate or repress transcription in an (almost) direct manner by interacting with RNA Polymerase II through the multiprotein Mediator complex, together forming the PreInitiation Complex (PIC), as reviewed by Freytes et al. (2024). However, it has become increasingly clear that some transcription factors can, by binding to promoter DNA, make it accessible or inaccessible to other transcription factors, thereby allowing or preventing transcription initiation. Thus, the concept of pioneer transcription factors emerged, as reviewed by Strader et al. (Strader et al., 2022). Pioneer transcription factors can bind their targets even in compacted, closed chromatin and, by evicting histone H1 and recruiting the SWI/SNF chromatin remodeler (see section 3.3), open the chromatin for binding by other transcription factors. Examples of such pioneer transcription factors include Leafy (LFY), which opens and activates the *APETALA1* (*AP1*) gene to initiate flowering, and MADS-box transcription factors. During ethylene signaling, the TF ETHYLENE INSENSITIVE 3 (EIN3) recruits EIN2 to target loci, promoting histone H3 acetylation (H3K14ac, H3K23ac) to activate gene expression. Similarly, other plant hormones affect chromatin accessibility through signaling via transcription factors (Shao et al., 2024). Plant TFs can modify chromatin through direct interaction with histone-modifying complexes, indirect recruitment of co-regulators, or interaction with reader-writer complexes (Strader et al., 2022). Examples of repressive chromatin remodelers include EAR- or SRDX-domain-containing repressors (see section 3.2). Although these chromatin remodeling effects continue to define cell lineage identity, they are reset during meiosis. Ectopic expression of the mentioned transcription factors probably mimics these effects, but it is also not known to what extent these effects are heritable after segregation of the transgene in the progeny.

6.5 Traits of interest

6.5.1 Introduction

When surveying the literature on the traits of interest that might be modified by targeted or untargeted epigenetic modification, it became instantly clear that the technology is still in its infancy, particularly in crop applications, let alone commercialization. Much literature exists that speculates about the possibilities and opportunities, but few tangible examples are available. Furthermore, most of the examples listed in the following sections have been achieved in Arabidopsis and are awaiting translation into actual crops. As explained in section 6.4, many, if not all, of the observed phenotypes resulting from ectopic expression of transcription factors in transgenic plants involve epigenetic modifications of the targeted promoters and are therefore epigenetic modifiers. However, the

heritability of the phenotype in the absence of the transgene in the next generation is questionable. Currently approved transgenic crops and their traits are listed in Table 4.

Table 4. Approved transgenic crops expressing a transcription factor (source: ISAAA GM approval database, https://www.isaaa.org/gmapprovaldatabase accessed May 5, 2026)

Transcription factor	In Crop:	Trait
HaHB-4	Soybean, wheat	Drought stress tolerance
AtBBX32	Soybean	Enhanced yield
AtHB17	Maize	Ear biomass
ZMM28	Maize	Enhanced yield
AmDelila	Tomato	Flower/Fruit color
AmROSEA1	Tomato	Flower/Fruit color

The examples listed below are those that, unlike the above, do not involve ectopic expression of a transcription factor but rather more direct epigenetic modification, although their heritability after segregation of the transgene has not always been demonstrated.

6.5.2 Yield and Vigor

Although it could be argued that epigenetic modification for stress resilience or for developmental traits (branching, flowering time) will improve yield and plant vigor, a few examples of yield improvement without a demonstrable reliance on stress resilience exist. Most notably, the untargeted epigenetic modifications induced in *msh1* mutants or RNAi plants, and their progeny have been claimed to improve these traits in a heritable manner (see section 5.2.4) (Ketumile et al., 2022; Kundariya et al., 2020; Raju et al., 2018; Virdi et al., 2015; Yang et al., 2015). Modifying epigenetic repression of *IDEAL PLANT ARCHITECTURE* (*IPA1*) in rice changes plant architecture and thereby indirectly affects yield (Zhang et al., 2017).

6.5.3 Eliminating crossing barriers to produce viable hybrids

Plant breeding can benefit from heterosis, or hybrid vigor, in the offspring of two different inbred parents, which is the basis of modern commercial hybrid breeding. Polyploidization can also lead to improved traits, as evidenced by the many cultivated polyploid crop species. The creation of new polyploids or interspecific hybrids is hindered by low fertility or reproductive barriers. Such barriers are often the result of the so-called triploid block in the endosperm (Bischof, 2022). Decreasing the triploid block by inhibiting DNA methylation with 5-Aza in diploid pollen enables the creation of triploid Arabidopsis and the successful production of interspecific hybrids of two *Capsella* species, which are the only known examples to date (Huc et al., 2022).

6.5.4 Disease resistance or priming

Enhancing disease resistance is a major focus of epigenetic modification and, importantly, in several crops (reviewed by Abdulraheem et al. (2024), Deng et al. (2017), Xie & Duan (2023), and Xu et al., (Abdulraheem et al., 2024; Deng et al., 2017; S.-S. Xie & Duan, 2023; Q. Xu et al., 2025). This epigenetic modification may come in many forms. Knocking out *S* (Susceptibility) genes to render plants resistant to pathogens that exploit those genes for infection is a proven strategy (Thakur et al., 2025). However, it may suffer from pleiotropic effects, as *S* genes have functions beyond rendering the plant disease susceptible. Thus, more specific interference in the interaction between the pathogen and an *S* gene may be a more viable strategy. For example, Cassava bacterial blight, caused by *Xanthomonas phaseoli* pv. *Manihotis* involves the binding of a transcriptional activator

(TAL20) to induce expression of the *S* gene *MeSWEET10a*. Blocking the binding of TAL20 by targeted methylation of its binding site, using an artificial Zinc Finger fused to DEFECTIVE IN MERISTEM SILENCING 3 (DMS3), a component of the RdDM pathway, decreased disease symptoms without affecting normal growth and development (Veley et al., 2023).

Targeted epigenetic modification may restore the expression of silenced disease-resistance genes. dCas9-SunTag-SDG2 targeted H3K4me3 deposition at *SNC1*, a disease resistance gene encoding a nucleotide-binding leucine-rich repeat protein, within an epigenetically silenced gene cluster, thereby activating resistance to *Pseudomonas syringae* pv. *tomato* (Binenbaum et al., 2025).

Also, memory of jasmonic acid-dependent immunity in Arabidopsis requires DNA demethylation (Wilkinson et al., 2023). Immune priming is the use of a priming stimulus to activate defense responses, thereby developing a long-lasting memory that enables a faster or stronger defense response (Cooper & Ton, 2022; Harris et al., 2023).

Besides histone methylation and acetylation, several other modifications are involved in gene regulation. An example of such a modification is the short-chain histone acylation, 2-hydroxyisobutyrylation (Khib), an active mark at H4K8 (Dai et al., 2014). It was shown in rice that, in synergy with H4K8Ac, it is associated with active expression of, among others, defense genes resulting in pathogen resistance. This effect was even more pronounced when Khib levels were kept high by a treatment with the HDAC inhibitor TSA (Xu et al., 2025).

6.5.5 Stress tolerance (drought, salt, cold, heat) and priming for stress responses

As for biotic stress, epigenetic changes during exposure to biotic stress receive considerable attention, including those related to priming. Several studies report epigenetic changes following stress exposure, but the evidence remains circumstantial and does not identify genes that play a causal role in tolerance or priming, as reviewed in (Harris et al., 2023). Nonetheless, if stable inbred lines are available, one might be able to (epi)genetically map the responsible epigenetic markers. In this context, *targeted*, candidate-gene-specific epigenetic modification ("reverse epigenetics"; see the last paragraph of this section) holds great promise.

Selection for drought tolerance in canola epilines led to distinct changes in gene expression and histone modifications in drought-responsive genes, making it an example of untargeted epigenetic modification for drought tolerance (Verkest et al., 2015). Although no inheritance of drought tolerance was reported, drought-priming-induced differential gene expression and 5mC levels were heritable in rice (Kumar et al., 2023).

The cold stress response in Arabidopsis is known to include rapid changes in histone modifications. Cold stress priming also affects the expression of nearly 10,000 genes, of which 500 were considered cold-stress memory genes because their expression responded more strongly to cold after priming treatment. Simultaneously, genome-wide changes in cytosine methylation were observed. Cold-stress memory genes were marked with lower cytosine methylation and open chromatin states, which are altered in *met1* mutants (Sadykova & Saze, 2025). For many of the identified gene expression changes in these examples, it is not yet clear which genes are causal or required for priming or stress tolerance, making translation into targeted manipulation difficult.

The latter does not apply to an example of *targeted* manipulation of drought stress tolerance in Arabidopsis, where dCas9-HAT1 (CRISPRa) targeting the abscisic acid (ABA)-responsive element binding protein 1/ABRE binding factor (AREB1/ABF2) was used to increase its expression (de Melo et al., 2020; Roca Paixão et al., 2019).

6.5.6 Quality traits

Epigenetic marks affecting crop quality are particularly well known in horticultural crops, such as during ripening and cold damage in tomato and strawberry, as reviewed by Liu et al. (2025). One example that stands out is the silencing of CmMYB6 transcription factor expression in clonally propagated pink Chrysanthemums, leading to yellow flowers. The causal gene promoter hypermethylation was confirmed by targeted demethylation using dCas9-TET1cd (Tang et al., 2022) and subsequently supported by targeted methylation (Li et al., 2025). Additional applications, such as improved shelf life and juice viscosity in tomato, and non-browning in lettuce, are suggested by "Am I regulated?" inquiries (see Table. in section 6.6).

6.5.7 Eliminating barriers to crossovers

Decreased meiotic crossover in pericentromeric heterochromatin is a persistent limitation in plant breeding, as it hampers the easy combination of favorable alleles in proximity on the genome from different origins or the uncoupling of unfavorable alleles that cause linkage drag. Thus, methods to increase crossover frequency are highly sought after. Although specific histone variants and posttranslational histone modifications are differentially associated with heterochromatin and euchromatin, respectively, the causal relationship with crossover frequency remains weak. However, reducing H3K9me2 and non-CG DNA methylation via mutation of the H3K9 methyltransferase genes *KYP/SUVH4,5,6*, or the CHG DNA methyltransferase gene *CMT3*, respectively, increases meiotic recombination near centromeres in Arabidopsis (Underwood et al., 2018). CRISPR/Cas targeting H3K4me3 (with SDG2 or PRDM9) to the centromeric region in Arabidopsis unlocked centromere-proximal crossovers (Binenbaum et al., 2025). Conversely, reducing H3K4me3 by recruiting histone demethylase JMJ14 to defined genomic loci using a dCas9-JMJ14 fusion reduced transcription of an lncRNA and, importantly, reduced crossovers. Activating transcription by recruiting a dCas9-VP64 fusion had the opposite effects on H3K4me3 level, transcription, and crossover frequency (Szymanska-Lejman et al., 2026). Recombination-repressed centromeric regions in Arabidopsis chromosomes are much smaller (relative to chromosome length) than those in the larger genomes of most crops, so the utility of this approach for crops awaits further studies.

6.5.8 Developmental traits

Several developmental traits in crops are of particular interest in the context of epigenetic modification; only a few examples are mentioned here. Flowering time in some Arabidopsis ecotypes and in many crops is regulated by vernalization, the need for plants to be exposed to prolonged cold (winter) to transition from vegetative growth to flowering, which makes those crops, for example, sugar beet, biannual. Similarly, seed germination may also require vernalization, and both are adaptations to temperate climates with strong seasonal temperature variation. Vernalization in crop species other than the Brassicaceae is widespread but may be regulated differently from FLC, yet it still involves epigenetic modifications. Arabidopsis flowering is also regulated by ambient temperature other than vernalization, through FLC and FLOWERING LOCUS M, involving H3K27me3 (Poza-Viejo et al., 2025).

6.6 The epigenetic modification patent landscape and other predictors of future applications

Upcoming practical and commercial applications of epigenetic modification in crop breeding may, in part, be predicted by screening patent applications or issued patents for relevant examples. I conducted searches on Google Patents and the European Patent Office database, Espacenet. The search terms used and the ensuing results are listed in Annex 3. The raw output from all searches

was manually curated to remove duplicates and to ensure relevance to plants, based on titles and summaries or abstracts. Screening the list confirms the impressions elsewhere in this report: Applications of *targeted* modification (mentioning a gene and a trait) are very rare in the published patents, except the large number of applications that describe producing the tools to be used without an associated and demonstrated application to a trait (listed as "generic" in Annex 3). The majority of applications that do describe altered traits are either using chemical modifiers of epigenetic marks in general, like 5-Aza or TSA, or describe methods to produce epigenetically modified plant lines or epiRILs, that may be used to screen for enhanced traits like yield, nitrogen utilization, energy efficiency, drought or salt stress, disease resistance, etcetera: the traits mentioned in section 6.5.

An additional predictor of future applications in crops can come from surveying the "Am I regulated?" inquiries to the USDA Animal and Plant Health Inspection Service (APHIS) regarding the putative regulatory status of a particular crop. Although confidential business information is deleted before publication on the internet, some indications can be derived from those applications listed in Table 5.

Table 5. Extracted from the USAD/APHIS "Am I regulated?" database of inquiries (Regulated Article Letters of Inquiry | APHIS; accessed May 5, 2026). All subjects and methods available from the database are listed.

Applicant	Date	Subject	**Method** (if not deleted)
Decibel Bio	12/22/2025	epigenetically modified improved yield corn lines	Target gene promoter methylation
Decibel Bio	6/3/2025	epigenetically modified tomato lines with increased shelf-life	Increased methylation of *SlFSR* promoter region
Decibel Bio	5/7/2025	Epigenetically modified tomato line with thicker juice	
Decibel Bio	3/13/2025	epigenetically modified lettuce lines with reduced expression of *PAL1* and *PAL2* genes (browning)	
EpiCrop Technologies	4/7/2017	Null segregants from soybean msh1 RNAi parent	

Finally, I interviewed, by email, representatives of seven international breeding companies regarding their views on future applications of epigenetic editing in crops. Although this approach was not exhaustive and may, of course, have missed specialists with other views, the image that emerges confirms the above. Some mention that, after internal discussion, their company has not yet given the subject much thought and is not yet ready to mention traits of interest. Others mention the generic subjects that already receive attention in regular breeding programs: disease and pest resistance, stress tolerance, yield, fruit quality (flavor and shelf life), and flowering induction. Yet others mention creating phenocopies where knockout mutations have to severe pleiotropic effects or are lethal. One mentioned upregulating disease resistance expression above the natural level. One mentioned the use of upregulation of gene expression as an alternative to copy number variation, which was shown to be unstable during breeding, or tissue-specific control of gene expression, where differential expression is desired but not yet available. Finally, one mentioned the role of the seed endosperm, which can affect development long after germination and may underlie differences in the progeny of reciprocal isogenic hybrids with different phenotypes. The latter may have a mechanism partially or fully overlapping with the lifting of interspecific hybridization barriers.

7 Consequences and risks for the environment and for human health

7.1.1 Effects of non-targeted, global perturbations of epigenetic markers

No literature specifically addresses the environmental or human health consequences of epigenetic modifications in crops, other than those already discussed for more conventional genetic modification methods. Two issues that may be more relevant to epigenetic modifications are considered in the following two paragraphs. It could be argued that any introduced epigenetic modifications *within* a species are insignificant in light of the epigenetic variation already present within the species, between breeding lines, or during development and in the life cycle (see section 7.1.4, UK's Precision Breeding Act). However, one application, so far specific to epigenetic modification, may need scrutiny: new, previously unobtainable interspecific hybrids produced by breaking the triploid block in seed development (section 6.5.3). Although not a result of epigenetic modification or chemical treatment per se, the combination of two previously uncombined parental genomes may impact the hybrid's behavior in unforeseen ways. For example, when fertile, this combination may make the hybrid particularly competitive in the wild and prone to becoming weedy (Lozada-Gobilard et al., 2020).

7.1.2 Specificity of targeted epigenetic modifications

Targeted approaches using epigenetic editors employ dCas9 with one or more enzymatically active effectors that act on or deposit cytosine methylation or histone modifications. These have been shown, in many cases, to modify their intended target sites successfully, but in a growing number of studies, they also appear to have off-target effects, i.e., to modify (sometimes many) other genomic sites. In a study of dCas9 fusions with DNMT3A and 3B in human cells, in addition to the targeted sites, many other sites were methylated, and gene expression was affected (Lin et al., 2018). Less is known about off-target modifications in plants, but it is expected that these are no different from those in mammalian cells. A comparison of the efficacy and specificity of various combinations in Arabidopsis suggests that they may be inversely correlated: a higher frequency of targeted modification seems to be accompanied by a higher frequency of off-target modification He et al., 2025). Also, modifying bacterial MQ1 methyltransferase to a more specific version reduced its activity, but at least it became much more specific (Ghoshal et al., 2021). Non-specific modifications may result from the epigenetic modifier protein acting on the genome independently from the targeting module (such as dCas9), especially when the modifier is non-covalently attached to the targeting module, such as in tethering to MS2 binding sites in the gRNA stem-loop or to multiple GCN4 epitopes as in the SunTag approach. While control of dCas9 targeting by choosing a specific gRNA is an obvious approach, the off-target activity of the epigenetic modifier enzyme may be more difficult to control and will depend on multiple factors (expression, site of tethering if any). Possible improvements to specificity are split-effector domains, transient (versus constitutive) delivery, and controlled concentration of the editors. Thorough comparisons of multiple approaches are missing in plant literature. In light of many untargeted genome-wide epigenetic modifications, for example, induced by chemicals such as 5-Aza and TSA, one could argue that there is ample evidence against undesirable, non-specific modifications that are not already removed or reversed in subsequent breeding (see previous section).

7.1.3 Stability and heritability

One could argue that the lack of stability or heritability of epigenetic modifications in commercialized crops has no deleterious impact on their safety as long as it means a reversal to their original state.

However, such instability is detrimental to breeding new cultivars, because their phenotypic stability and heritability are key to their registration as a new cultivar. Thus, such instability would only be acceptable for transient modification via spraying the modifying agent, where producing a new variety is not the objective. Examples of stability or epigenetic memory surviving the lifetime of an individual plant exist, but this mitotic stability does not predict transgenerational stability, particularly when the inducing epigenetic modifier is segregated out in subsequent generations.

Notwithstanding the above, the occurrence of stable epigenetic mutations or alleles in crops as well as in the model plant Arabidopsis provides evidence that epialleles can be stably inherited through meiosis over many generations (see section 4). The stability of epialleles have been observed particularly in Arabidopsis by virtue of its relatively short life cycle. Crops usually have a much longer life cycle, which makes studying epiallele stability over many sexual reproduction cycles impractical. In Arabidopsis, epialleles created from natural variation between populations or by the use of chemicals or cytosine methylation mutants are stable enough to be used for the identification of epiQTLs (see section 6).

8 Expectations of the regulation of epigenetically modified crops (in Europe).

The following remarks on regulations are strictly my own interpretation and are subject to change or differing interpretations. Since there are, as far as I know, no intentionally epigenetically modified crops currently marketed, unless we consider the crops listed in Table 4, I can only speculate about their future regulation in Europe. As with all biotechnological innovations, current regulations often do not explicitly address such new developments. Under the current regulations (Directive 2001/18/EC), all applications of epigenetic modification involving the use of recombinant DNA (targeted knockouts of cytosine (de)methylases, targeted modification using transgenes (including Table 3), targeted modification of histone modifier genes) are very likely to be subject to risk assessment for environmental impact and food or feed safety. Modifications induced by stress or chemical treatments, such as 5-Aza, do not involve transgenes or the use of recombinant DNA and result in changes that could occur in nature or even within the lifetime of a single plant as a result of environmental or developmental changes. Thus, such applications are unlikely to be subject to Directive 2001/18/EC, which currently regulates plant genetic modification and the required risk assessment (if any) in the European Union. Heritable epigenetic modifications induced by siRNAs (delivered as a spray or introduced into tissue culture) fall into a gray area because siRNA is not considered recombinant DNA. If the proposal for new regulations on NGT (New Genomic Technologies) is *not* adopted by the European Parliament in its final vote (expected July 2026), the existing regulations will remain in place. It is important to note that, in that event, removing an epigenetically modifying transgene by segregation during meiosis will not likely exempt the plant or crop from risk assessment.

The status of crops derived from epigenetic modification in the newly proposed, and almost adopted, EU regulations for crops produced using NGT are open to interpretation were epigenetic modifications are concerned, because these are not explicitly mentioned in that proposal (*17037/25 Proposal REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on Plants Obtained by Certain New Genomic Techniques and Their Products, and Amending Regulation (EU) 2017/625*, 2026). At first glance, the lack of any genetic changes would seem to let epigenetically modified crops remain below the maximum number of edits allowed for Category I plants (and hence no risk assessment required). However, the definition of Category I in the proposed regulation Article 3 point 9 reads: "'NGT plant' means a genetically modified plant obtained *by targeted mutagenesis or cisgenesis*" and General provisions, Article 3, Definition 10: "'targeted mutagenesis' means mutagenesis techniques resulting in one or more modifications of the *DNA sequence* at targeted locations in the genome of an organism". Since epigenetically modified plants are *not* derived from either of those particular NGTs, one could also construe that, therefore, they are not NGT category I and must, by default, be NGT II. On the other hand, they may be seen as "NGT plants that could also occur naturally or be produced by conventional breeding". As said, as epigenetically modified plants are not explicitly mentioned, this may become a matter of interpretation and debate.

In contrast, the UK's Genetic Technology (Precision Breeding) Act 2023 (accessed May 1st, 2026) explicitly includes epigenetic modifications. Under these regulations, precision-bred organisms (PBOs) are defined as plants with alterations to their nuclear or non-nuclear genome, including genetic and epigenetic changes, provided they could have occurred naturally or through traditional breeding. In essence, if an epigenetic change can be deemed equivalent to changes that might occur through traditional breeding techniques, it is treated under the new, simpler UK regulatory framework rather than as a traditional GMO. It seems the proposed EU regulation has missed an opportunity to provide regulatory clarity here.

9 Concluding remarks and outlook

To the best of my knowledge, there are no commercially available crop varieties on the market today that have been (actively) epigenetically modified, barring the examples of ectopic TF expression listed in section 6.1. This leaves open the possibility that some commercial hybrids depend on epigenetic effects without having been specifically bred with this mechanism in mind, or with the breeder being aware but without disclosing this strategy. The technology for rational, targeted modification is still in the research phase and has not yet been applied to commercial agriculture. Most genetically modified crops on the market have been altered through direct genetic modification techniques, such as the insertion of specific genes to confer traits like pest resistance or herbicide tolerance. However, by studying the literature and discussing with fellow scientists and representatives of breeding companies, I could make an educated guess about which traits are more likely to be targeted first (see above, section 6.1).

Nonetheless, the prediction is hampered by the large variety of targeted and untargeted modification methods, and by the distinction between transient and heritable epigenetic modifications. For targeted modifications, most (but not all) published literature is on the model plant Arabidopsis, and while some of the demonstrated effects can probably be applied in actual crop species throughout at least most Angiosperms, others are probably very specific for Arabidopsis or closely related crops such as *Brassica oleracea* varieties (cabbages) or rapeseed, mustard, and canola.

The first applications of targeted modifications would likely be in crops with relatively simple, easily tractable genomes, such as maize, rice, tomato, and lettuce. Polyploid crop species such as potato, wheat, and strawberry may pose similar challenges to those in conventional breeding: complex segregation and inheritance patterns. Before commercialization, the products of targeted technologies will also have to face questions of specificity (off-target modifications and their consequences), stability, safety, and, importantly, their regulatory status and public acceptance, given the almost inevitable use of transgenes at some stage.

While research on targeted epigenetic modification has particularly taken off since the availability of targeting vehicles such as dCas9, untargeted modification, in some cases followed by selection on phenotype, has been ongoing for (much) longer. Only in a subset of these examples have transgenes been involved. Other methods, such as the use of spontaneous epialleles, or treatments that are transient, such as the use of chemicals to modify overall cytosine methylation or histone acetylation levels, are, by nature, much less specific than targeted modifications but may be exempt from regulatory oversight (at least, concerning GMO rules; they may still be subject to the Novel Foods regulation). Therefore, some of these applications may see commercialization much sooner than those of targeted modifications.

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Annex 1: List of Abbreviations/Glossary

5caC 5-carboxylcytosine

5fC 5-formylcytosine

5hmC 5-hydroxymethylcytosine

5mC 5-methylcytosine

6mA N6-methyldeoxyadenosine

Ac acetylation

ATAC-seq Assay for Transposase-accessible Chromatin using sequencing

ChIP chromatin immunoprecipitation

CNV Copy Number Variation

CRISPR/Cas ….

cv. cultivar

COGEM Netherlands Commission on Genetic Modification

dCas9 dead CAS9

dsRNA double-stranded RNA

DNMT DNA methyltransferase

epiRIL epigenetic recombinant inbred line

GMO genetic modification or genetically modified organism

gRNA guide RNA

H3K9 histone 3, lysine 9

hpRNA hairpin RNA

HAT histone acetyltransferase

HDAC histone deacetylase

HMT histone methyl transferase

lncRNA long non-coding RNA

MBD methyl DNA binding

miRNA microRNA

NGT new genomic technique

PTGS post-transcriptional gene silencing

PRC Polycomb Repressive Complex ??

pv. pathovar

QTL quantitative trait locus

RdDM RNA-dependent DNA methylation

RNAi RNA interference

siRNA small interfering RNA; repeat-associated = rasi; transacting = tasi

sRNA small RNA

TALE transcriptional activator-like effector

TE transposable element

ZF zinc finger

Annex 2: Companies and research projects active in the epigenetic modification of plants and plant traits

Chromatin Bioscience (chromatinbio.com) North Berwick, UK. Works on synthetic promoters.

Epibreed (epibreed.com), TEgenesis® breeding method to accelerate natural adaptation based on TE mobilization, PCT/EP2016/079276, and (Thieme et al., 2017)

EpiCrop Technologies (epicrop.com), Lincoln, Nebraska. Work with MSH1. Claim improved yield in tomato, soybean, sorghum (Cold tolerance and performance on marginal soil), and canola. Transfer of siRNAs from rootstock. Claim up to four generations of heritability. https://worldwide.espacenet.com/patent/search?q=pn%3DWO2018125749A1

Sound Agriculture (www.sound.ag) claims to achieve fast, high-quality results using chemicals (?). No GMO's. Technology unclear; no publications

Decibel Bio (decibelbio.com), Berkeley, California. Claim fast epigenetic modification using sprays or seed treatment in order to switch off genes by promoter hypermethylation.

Inari (Cambridge, MA) was co-founded by Steven Jacobsen and took licenses on UCLA patents regarding Jacobsen's discoveries or technologies. Work on corn, soybean, wheat

Cann10 EpiGen is a joint venture between the Israeli medical cannabis company **Cann10** and the biotech firm **Epigenetics**. The partnership was formed to research and develop advanced epigenetic breeding technology for cannabis plants.

Ongoing EU projects from the search of cordis.europa.eu using search term 'PLANT' AND 'CROP' AND 'EPIGEN*' starting after March 1st 2020

H2020 MCSA **EpiSeedLink** "From seed to seedling: Epigenetic mechanisms of priming to design strategies for crop improvement" 2022-2026

H2020 MCSA **mC-EVOLVE** "The evolutionary biology of crop plant DNA methylation" 2025-2028

ERC Project **PlantMemo** "Decoding the Internal and External Drivers of Plant Defence Memory" 2026-2030

ERC project **MagicSpore** "Mechanisms and biological functions of H3K27me3 reprogramming in plant microspores" 2024-2029

ERC project **3Dwheat 3Dwheat**, "A 3 Dimensional functional genomics approach to identify hidden targets controlling heat stress and priming in wheat" 2022-2028

H2020 MCSA Project **STEM** "Elucidating mechanisms underlying trait instability in maize" 2025-2027

H2020 MCSA Project Project **REASONING** "The moleculaR mEchAnismS Of dNa methylatIon reprogrammiNg in Arabidopsis sexual lineaGe" 2024-2026

ERC tuneCHILL, an ERC consolidator Grant for Julia Questa at CRAG. "tuneCHILL - Regulatory mechanisms underlying chilling responses: towards the design of climate-smart plants"

Cost Action CA24152 **EPICROPS** "Epitranscriptomics and ncRNAs for climate-change-resilient and sustainable crops" 2025-2029

Annex 3: Search queries for PubMed, produced with ChatGTP

ChatGPT generated query ("high sensitivity", v2):

("epigenome editing"[tiab] OR "epigenetic editing"[tiab] OR (epigenom*[tiab] AND edit*[tiab]) OR (epigenetic*[tiab] AND edit*[tiab]) OR (target*[tiab] AND epigenetic*[tiab]) OR (target*[tiab] AND epigenom*[tiab]) OR ("targeted DNA methylation"[tiab]) OR ("DNA methylation editing"[tiab]) OR ((dCas9[tiab] OR "dead Cas9"[tiab] OR "CRISPR/dCas9"[tiab] OR "CRISPRi"[tiab] OR "CRISPRa"[tiab] OR "TALE"[tiab] OR "TALEN"[tiab] OR "transcription activator-like effector*"[tiab] OR "zinc finger"[tiab] OR "zinc-finger"[tiab] OR SunTag[tiab]) AND (epigenet*[tiab] OR epigenom*[tiab] OR methylat*[tiab] OR acetylat*[tiab] OR demethylat*[tiab] OR "histone"[tiab] OR chromatin[tiab]))) AND (plant*[tiab] OR crop*[tiab] OR Arabidopsis[tiab] OR rice[tiab] OR wheat[tiab] OR maize[tiab] OR corn[tiab] OR barley[tiab] OR soybean[tiab] OR tomato[tiab]) AND ("2011/01/01"[Date - Publication] : "3000"[Date - Publication])

Resulted in 2065 hits, including many very general references for epigenetic regulation

A higher specificity version (v3) focused on actual editing:

("epigenome editing"[tiab] OR "epigenetic editing"[tiab] OR "targeted epigenetic"[tiab] OR "targeted epigenome"[tiab] OR "targeted DNA methylation"[tiab] OR "DNA methylation editing"[tiab] OR ((dCas9[tiab] OR "dead Cas9"[tiab] OR "CRISPR/dCas9"[tiab]) AND (epigenome[tiab] OR epigenetic*[tiab] OR methylation[tiab] OR demethylation[tiab] OR histone[tiab] OR chromatin[tiab]) AND (target*[tiab] OR locus[tiab] OR "site-specific"[tiab]))) AND (plant*[tiab] OR crop*[tiab] OR Arabidopsis[tiab] OR rice[tiab] OR wheat[tiab] OR maize[tiab] OR barley[tiab] OR soybean[tiab] OR tomato[tiab]) AND ("2011/01/01"[Date - Publication] : "3000"[Date - Publication])

Resulted in only 136 hits

Restricting the high sensitivity (v2, see above) query to "crop or trait improvement":

(/* --- EPIGENETIC MANIPULATION (HIGH SENSITIVITY CORE) --- */ "epigenome editing"[tiab] OR "epigenetic editing"[tiab] OR (epigenom*[tiab] AND edit*[tiab]) OR (epigenetic*[tiab] AND edit*[tiab]) OR (target*[tiab] AND epigenet*[tiab]) OR(target*[tiab] AND epigenom*[tiab]) OR "targeted DNA methylation"[tiab] OR "DNA methylation editing"[tiab] OR "epigenetic reprogramming"[tiab] OR ((dCas9[tiab] OR "dead Cas9"[tiab] OR "CRISPR/dCas9"[tiab] OR CRISPRi[tiab] OR CRISPRa[tiab] OR "CRISPR interference"[tiab] OR "CRISPR activation"[tiab] OR TALE[tiab] OR TALEN[tiab] OR "transcription activator-like effector*"[tiab] OR "zinc finger"[tiab] OR "zinc-finger"[tiab] OR SunTag[tiab]) AND (epigenet*[tiab] OR epigenom*[tiab] OR methylat*[tiab] OR demethylat*[tiab] OR acetylat*[tiab] OR histone*[tiab] OR chromatin[tiab]))) AND (/* --- PLANTS / CROPS --- */ plant*[tiab] OR crop*[tiab] OR Arabidopsis[tiab] OR rice[tiab] OR wheat[tiab] OR maize[tiab] OR corn[tiab] OR barley[tiab] OR soybean[tiab] OR tomato[tiab]) AND (/* --- TRAIT OR CROP IMPROVEMENT FILTER --- */ "trait improvement"[tiab] OR improvement[tiab] OR yield[tiab] OR productivity[tiab] OR biomass[tiab] OR "stress tolerance"[tiab] OR drought[tiab] OR salinity[tiab] OR heat[tiab] OR cold[tiab] OR resistance[tiab] OR adaptation[tiab] OR breeding[tiab] OR agronomic[tiab] OR "crop improvement"[tiab])

Resulted in 588 hits. Therefore, I restricted the search to "from 2020" onward, which only reduced the number of hits to 422, indicating the steep growth in literature references over the past six years. A large part of these hits were reviews, and given the number of already-downloaded reviews,

including recent ones, I mostly ignored them. The approach resulted in 25 new references that were not downloaded earlier, indicating that, even before this described systematic search, the coverage was already quite comprehensive.

Since I noticed that the search focused on *editing* applications rather than, for example, other experimental modifications, I performed an additional search for chemical treatments for epigenetic modification.

This approach yielded 728 hits, which were further narrowed to papers focused on plants or crops and on crop trait improvement.

(/* --- CHEMICAL EPIGENETIC MANIPULATION --- */(chemical*[tiab] OR compound*[tiab] OR inhibitor*[tiab] OR treatment*[tiab] OR agent*[tiab] OR application[tiab]) AND ("DNA methylation"[tiab] OR "cytosine methylation"[tiab] OR demethylation[tiab] OR "histone modification"[tiab] OR "histone acetylation"[tiab] OR "histone methylation"[tiab] OR "histone deacetylation"[tiab] OR chromatin[tiab])) OR (/* --- COMMON CHEMICALS USED IN PLANT EPIGENETICS --- */"5-azacytidine"[tiab] OR "5-aza-2'-deoxycytidine"[tiab] OR zebularine[tiab] OR "trichostatin A"[tiab] OR "histone deacetylase inhibitor"[tiab] OR sodium butyrate[tiab] OR nicotinamide[tiab]) AND (/* --- PLANTS / CROPS --- */ plant*[tiab] OR crop*[tiab] OR Arabidopsis[tiab] OR rice[tiab] OR wheat[tiab] OR maize[tiab] OR corn[tiab] OR barley[tiab] OR soybean[tiab] OR tomato[tiab]) AND (/* --- TRAIT IMPROVEMENT / AGRONOMIC OUTCOMES --- */ "trait improvement"[tiab] OR improvement[tiab] OR yield[tiab] OR productivity[tiab] OR growth[tiab] OR biomass[tiab] OR "stress tolerance"[tiab] OR drought[tiab] OR salinity[tiab] OR heat[tiab] OR cold[tiab] OR resistance[tiab] OR adaptation[tiab] OR breeding[tiab] OR agronomic[tiab]) NOT (mouse[tiab] OR mice[tiab] OR rat[tiab] OR rats[tiab] OR human[tiab] OR humans[tiab] OR cancer[tiab] OR tumor[tiab] OR tumour[tiab])

This resulted in 250 hits, of which I considered only 28 relevant after screening.

Annex 4 Description and curated results of patent searches

1. Google Patents was searched with a query generated by ChatGTP; Espacenet was searched with the query: epigen* and crops; identical or

Title	Trait or generic	Patent ID
Programmable epigenetic control of gene expression in plants	generic	BR112021018472A2
Modified plants produced by modulating cytosine methylation in the plant genome	interspecific hybridization	CA2380627A1
Application of gene methylation in gene expression regulation	cold tolerance	CN104673803A
Rice plant height epigenetic regulation site and Application thereof	plant height	CN104805083A
Rice leaf intersection Angle epigenetic regulation site and Application thereof	leaf intersection angle	CN104805084A
A kind of method that utilization DNA methylation inhibitor builds plant population	mutant library generation with 5-Aza	CN107114235A
A method of improving duckweed total starch yield using methylation inhibitor	mutant generation with zebularine	CN108782204A
An epigenetic Approach to manipulate plant phenotypic plasticity traits	degenerate codon base substitution at methylation sites	CN110637087B
Application of rice histone deacetylase gene HDA710 in delaying leaf senescence	decreased leaf senescence	CN110885813A
Gene for regulating and controlling seed development of solanaceae plant and Application thereof	seedless fruits	CN111394362A
Carrier system for methylation/demethylation of rice epigenome	generic	CN111560397A
Application of A Plant Epigenetic Factor And Its Encoding Gene in Efficient Utilization of Nitrogen	nitrogen use efficiency	CN111825753A
Application of OsHDA710 epigenetic regulatory factor gene in rice development and stress resistance	drought and salt stress	CN112322645A
Technology for cultivating drought-tolerant wheat by utilizing Adversity stress distant symbiosis and epigenetic inheritance	drought tolerance	CN112931090A
Technology for cultivating long-ear wheat by utilizing Adversity stress distant symbiosis and epigenetic inheritance	ear length	CN112931091A
A kind of fusion protein and its Application	generic	CN113307878A
A method for targeted epigenome genetic regulation in marine Nannochloropsis	generic	CN113846019A
Plant epigenetic modification editor for targeted removal of DNA methylation	generic	CN115627272A
Application of epigenetic factor in optimizing gene editing tool in eukaryotic cell	generic	CN115678913A
Optimizing the Application of epigenetic factors in eukaryotic cells and gene editing tools	generic	CN115678913B
CRISPR-dCas9-based gene-targeted demethylation method and its Application	generic	CN115960900A
A recombinant Cas12a protein and its use in preparing gene editing and/or epigenetic editing products	generic	CN118562765B
Application of CMT3 gene knockout tomato rootstock in improvement of tomato biomass, drought resistance, and TYLCV resistance	epimutant library generation	CN118685419A
Engineered DNA methyltransferases and their Applications in epigenetic editing	generic	CN118792270A

An epigenetic gene editing system based on CRISPR/dCas9 and its Application	generic	CN118931878B
Epigenetic gene editing system based on CRISPR/dCAS12a And Application thereof	generic	CN118931879B
ZmMETTL3 gene And Application of related biological material thereof in regulation and control of drought resistance of plants	drought resistance	CN119320795B
Application of tumor cell inhibitor in improving genetic transformation efficiency of wheat	generic	CN120077954B
An epigenetic editing system based on CRISPR/dCas9 targeting histone citrullination modification and its Application	generic	CN120157770A
A new gene involved in the DNA methylation pathway and its Application in plant epigenetic regulation	generic	CN120290589A
Application of histone demethylase gene Osjmj719 and the encoded protein thereof in regulation And control of rice plant height	plant height	CN120424976A
New gene HWS1/HWS2 for determining rice interspecific hybrid recession	interspecific hybridization	CN120424989A
Method for improving European spruce somatic embryo development synchronism	embryogenesis	CN120436059A; CN120436059 B
A method for promoting tillering and rooting of Ash by spraying 5-azacytidine	tillering and rooting	CN120615530A
Application of GW5 gene promoter methylation in regulation and control of rice grain length based on multiple-genetics gene mining technology	rice grain size	CN120738394A
Application of DNA methylation inhibitor in regulation and control of synthesis of ginkgo terpene lactones	terpene production with 5-aza	CN120918183A
Application of GhHAT18 gene in regulation and control of drought stress resistance of plants	drought stress	CN120924587A
A method for constructing rice epigenetic recombinant inbred lines	epimutant library generation	CN120982411A
A CRISPR-Cas protein for epigenetic editing and its Applications	generic	CN121006343A
Histone demethylase gene OsJMJ708 for regulating and controlling rice salt stress toughness, and Application of histone demethylase gene OsJMJ708	salt stress	CN121160782A
Application of HDA108 in regulation and control of plant C4 photosynthetic metabolism	photosynthesis	CN121182855A
Application of histone demethylase GmJD5L in regulation and control of soybean flowering time and plant height	flowering time and plant height	CN121182893A; CN121182893 B
IbROS1, A DNA demethylase related to sweet potato tuber development, and its Application	yield	CN121204018A
Histone demethylase gene HOVUSG0811000 related to powdery mildew resistance	disease resistance	CN121204089A
Application of epigenetic regulatory factor genes in regulating plant heat tolerance	heat tolerance	CN121227747A
Application of related gene for postponing soybean flowering and/or increasing fat content	flowering time and seed fat content	CN121227767A
DNA methylated transferase ItCMT2 related to sweet potato tuberous root development, as well as coding gene and Application of DNA methylated transferase ItCMT2	yield	CN121249716A
Method for inducing embryogenic callus of medium-grain coffee	embryogenesis	CN121264387A
Method for improving wheat plant height character	plant height	CN121271944A
Method for efficiently inducing hybrid tulip tree somatic embryogenesis	somatic embryogenesis	CN121369230A

A method for removing H3K27me3 in plants using the SunTag system	generic	CN121406692A
Method for improving embryogenic Ability of cunninghamia lanceolata based on 5-azacytidine	embryogenesis	CN121511876A
Breeding Method for Rice Varieties Resistant to Southern Rice Black-Streaked Dwarf Disease	disease resistance and drought tolerance	CN121549266A
Engineered gene transcription repression tool and uses thereof	generic	CN121605133A
Method for improving cadmium tolerance of gynostemma pentaphylla and reducing cadmium Accumulation by 5-azacytidine And Application	abiotic stress tolerance with 5-aza	CN121647168A
MODIFIED PLANTS.	interspecific hybridization	ES2265956T3
Plants with useful traits And related methods	msh1 technology for epimutant lines	ES2731638T3
IMPROVEMENT OF YIELD IN CROP PLANTS THROUGH SELECTION OF EPIGENETICALLY MODIFIED POPULATIONS	epiRILs for diverse traits	IN420DEN2012A
Quinoline derivatives for regulating DNA methylation	DNMT inhibitors	JP2010506856A
Sustained epigenetic gene silencing	generic	JP2022068875A
Drm??methyltransferase, A novel target gene of microrna-396 molecules isolated from capsicum annuum And uses thereof	generic	KR101363412B1
Compositions And methods for epigenetic regulation of B2M expression	generic	KR20250068591A
PROGRAMMABLE EPIGENETIC CONTROL OF GENE EXPRESSION IN PLANTS	generic	MX2025000661A
MODULATING PLANT RESPONSES TO STRESS AND GROWTH WITH CHEMICALS	HDAC inhibitors	MX2026003097A
Method for inducing of epigenetic variability of soft wheat	induction of epigenetic variation	RU2322801C1
METHOD OF SELECTION OF PLANTS OR SEEDS WITH THE USE OF EPIGENETICALLY MODIFIED POPULATIONS	creating epiRILs for diverse traits	UA108851C2
Methods for determining fitness in plants	epiRILs for high energy use efficiency	US10793919B2
Methods and compositions for reducing gene expression in plants	generic	US2015150156A1
Methods and compositions for obtaining useful epigenetic traits	generic	US2016032310A1
Site-specific epigenetic editing	generic	US2017014449A1
Similar performance from seeds with epigenetic traits	generic	US2017223914A1
STABLE EPIGENETIC PLANT VARIANTS	epiRILs	US2018066271A1
Increase of yield in crop plants through selection of epigenetically modified populations	epiRILs	US2018235165A1
Epigenome editing systems and methods	generic	US2019256827A1
Modified tomato plants with extended shelf life	shelf life	US2023058857A1
Targeted gene demethylation in plants	generic	US2024141367A1
Engineering disease resistance by editing the epigenome	disease resistance	US2025230463A1
COMPOSITIONS AND METHODS COMPRISING PLANTS WITH MODIFIED ORGAN SIZE AND/OR PROTEIN COMPOSITION	increased organ size/big seeds	US20260002170A1

Modified plants And seeds with enhanced physiological performance And environmental stress resistance	enhanced performance and stress responses	US2026026445A1
A systemic small rna signaling system in plants	small RNAs trafficking	WO2005010197A3
Modulation of fertility in monocots	reduced fertility	WO2007055826A1
Use of A split dcas fusion protein system for epigenetic editing	generic	WO2022225978A1
Composition employed in producing parental line of epigenome-modified plant/animal, and usage thereof	generic	WO2022244544A1
Method for epigenetic editing target and use thereof	epiRILs	WO2024032676A1
Method and use of epigenetic editing target	generic	WO2024032680A1
Suntag system, vector And editing method for editing histone h3k4me3 in plant	generic	WO2024168464A1
Suntag system And vector for editing histone h3k27me3 in plants, And editing method	generic	WO2024168465A1
Highly efficient self-assembling programmable genome And epigenome editing tools	generic	WO2024191816A1
Fusion protein for epigenetic editing And use thereof	generic	WO2025237394A1
Epigenetic modulation system	generic	WO2026064556A1