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Review

Chemical Epigenetic Modifiers as Tools to Enhance Alkaloid Biosynthesis in Medicinal Plants

Mini Devi [1], Vasudha Datta [1], Satish Kumar [1], Bunty Sharma [2], Damandeep Kaur [3], Hardeep Singh Tuli [1,4], Shafiul Haque [5,6] and Diwakar Aggarwal [1,*]

[1] Department of Bio-Sciences and Technology, Maharishi Markandeshwar Engineering College, Maharishi Markandeshwar (Deemed to be University), Ambala 133207, India; mininarwal98@gmail.com (M.D.); vasudha.datta27@gmail.com (V.D.); satish.kumar23081994@gmail.com (S.K.); hardeep.biotech@gmail.com (H.S.T.)
[2] Department of Biotechnology, Graphic Era (Deemed to be University), Dehradun 248002, India; sharmabunty097@gmail.com
[3] University Center for Research & Development (UCRD), Chandigarh University, Mohali 140413, India; deepdaman602@yahoo.com
[4] Centre of Excellence in Computational Research and Drug Discovery, Department of Bio-Sciences and Technology, Maharishi Markandeshwar Engineering College, Maharishi Markandeshwar (Deemed to be University), Ambala 133207, India
[5] Department of Nursing, College of Nursing and Health Sciences, Health Research Center, Jazan University, Jazan 45142, Saudi Arabia; shafiul.haque@hotmail.com
[6] School of Medicine, Universidad Espíritu Santo, Samborondón 091952, Ecuador
* Correspondence: diwakaraggarwal@yahoo.co.in

Abstract

Epigenetic regulation plays a significant role in controlling plant secondary metabolism and offers a promising strategy to enhance medicinal alkaloid production without altering the genomic sequence. This review highlights major plant epigenetic mechanisms, including DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs, and their influence on alkaloid biosynthetic pathways. Particular emphasis is placed on chemical epigenetic modulators, such as DNA methyltransferase and histone deacetylase inhibitors, which can activate silent metabolic genes in a rapid, reversible, and non-GMO manner. Evidence from medicinal plants demonstrates tissue-, dose-, and time-dependent enhancement of alkaloid production. The review further discusses emerging approaches including CRISPR-based epigenome editing, multi-omics integration, and advanced bioinformatics for systems-level understanding of epigenetic regulation. Despite challenges, such as limited species-specific datasets, and concerns regarding long-term stability, epigenetic modulation represents a promising tool for sustainable alkaloid production with important pharmaceutical and industrial applications.

Keywords: medicinal plants; epigenetic modifiers; biosynthesis of alkaloids; bioinformatics tools

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1. Introduction

Alkaloids are a major class of nitrogen-containing plant secondary metabolites with diverse chemical structures and significant pharmacological importance. For centuries, alkaloid-containing medicinal plants have been used in traditional medicine, and many alkaloids continue to serve as important pharmaceutical compounds. Medicinal plants such as *Papaver somniferum*, *Catharanthus roseus*, and *Cinchona* produce therapeutically valuable alkaloids with anticancer, antimicrobial, anti-inflammatory, antimalarial, antioxidant,

analgesic, and cardioprotective activities [1,2]. In plants, these metabolites also function as important defense compounds against herbivores and pathogens.

Despite their medicinal value, alkaloids are generally produced in very low quantities, often below 1% of plant dry weight, and their accumulation is strongly influenced by developmental and environmental factors. To overcome low metabolite yields, several biotechnological approaches have been explored, among which modulation using physical, chemical, and biological elicitors has emerged as an effective strategy for enhancing secondary metabolite biosynthesis. Plants exposed to stress signals or modulators often exhibit increased accumulation of alkaloids and other secondary metabolites as part of adaptive responses [3,4].

Recent studies have highlighted the important role of epigenetic regulation in controlling alkaloid biosynthesis. Epigenetics refers to heritable changes in gene expression that occur without alterations in the DNA sequence. Major epigenetic mechanisms in plants include DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs, which collectively regulate plant growth, stress responses, and metabolic pathways. Chemical epigenetic modulators, particularly DNA methylation and histone deacetylase inhibitors, have shown considerable potential in activating silent biosynthetic genes and enhancing alkaloid production. For example, modulation of DNA methylation has been associated with increased metabolite accumulation in medicinal plants, including enhanced alkaloid biosynthesis in *Catharanthus roseus* [3,5].

Compared with conventional metabolic engineering approaches, chemical epigenetic modulation offers several advantages, including reversible regulation, rapid response, non-GMO applicability, and the potential for tissue-specific enhancement of metabolite production. Advances in epigenomics, transcriptomics, metabolomics, and bioinformatics are further improving our understanding of the complex regulatory networks involved in alkaloid biosynthesis. In addition, emerging technologies, such as CRISPR-based epigenome editing, provide new opportunities for precise regulation of biosynthetic pathways in medicinal plants [5,6].

This review summarizes current advances in the epigenetic regulation of alkaloid biosynthesis in medicinal plants, with emphasis on chemical epigenetic modulators, regulatory pathways, transcriptional control, and emerging technologies for improving metabolite production.

2. Biosynthesis and Structure–Activity Relationships

Alkaloids are characterized by the presence of at least one basic nitrogen atom, most commonly embedded within a heterocyclic ring structure, which imparts both alkalinity and pronounced biological activity. They represent a highly diverse class of natural products, encompassing structural groups such as indole, isoquinoline, quinoline, tropane, pyridine, and steroidal alkaloids. Biosynthetically, the nitrogen atom is generally derived from specific amino acid precursors, including L-tryptophan for indole alkaloids, L- tyrosine and L-phenylalanine for benzylisoquinoline alkaloids, L-ornithine for tropane alkaloids, and L-lysine for piperidine alkaloids (Figure 1). These precursor amino acids undergo a series of enzymatically catalyzed transformations, such as decarboxylation, transamination, and condensation, to establish fundamental carbon–nitrogen frameworks [7]. The resulting core structures are then further modified through diverse tailoring reactions, including O- and N-methylation, hydroxylation, oxidative coupling, glycosylation, and acylation, ultimately generating the extensive structural complexity and functional diversity characteristics of alkaloids. The structural attributes of the alkaloid moiety play a decisive role in defining the structure–activity relationships and, consequently, biological performance. Specific features, including ring architecture, stereochemical orientation, extent of nitrogen substitu-

tion, oxidation level, and the spatial arrangement of functional groups, collectively govern receptor recognition, binding affinity, membrane transport, and overall pharmacodynamic behavior [8]. Even subtle modifications can substantially influence activity; for example, the presence of a tertiary versus a quaternary nitrogen atom may significantly affect solubility, bioavailability, and molecular target interactions. Therefore, the stepwise biosynthetic construction and enzymatic tailoring of the alkaloid scaffold are not merely sources of the structural diversity but key determinants of pharmacological efficacy. A detailed understanding of these biosynthetic and structural determinants is essential for strategically optimizing and enhancing the production of therapeutically valuable alkaloids [9,10].

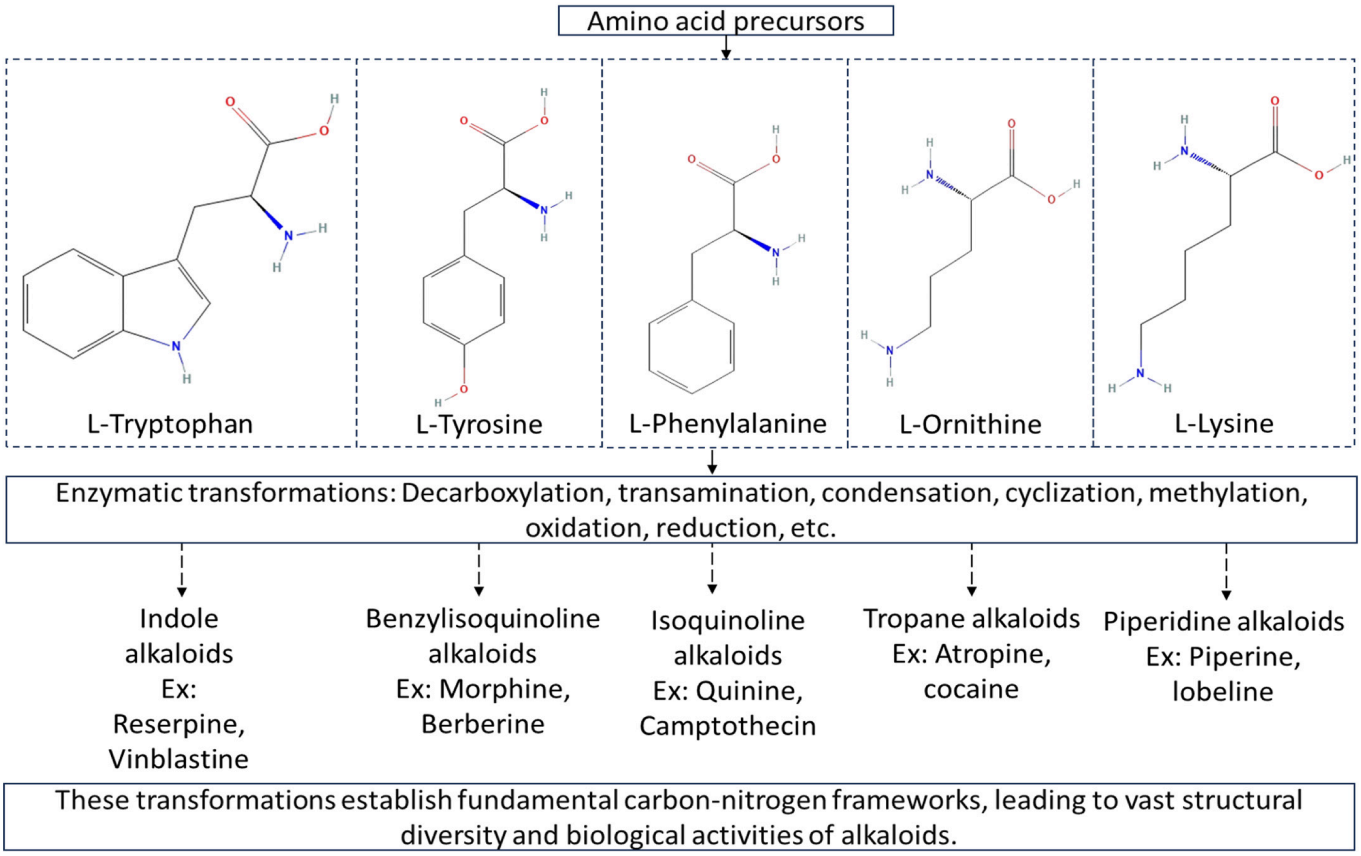


Figure 1. Chemical structures of major amino acid precursors involved in alkaloid biosynthesis [11].

3. Mechanisms of Epigenetic Regulation in Plants

The epigenetic changes (Table 1) in chromatin material have been explored in recent years as a leading contributor to the adjustments in plant fitness under unfavorable environmental situations. The study of changes in gene activity that are heritable during mitosis and/or meiosis and are based on alterations in chromatin organization rather than alterations in DNA sequence is known as epigenetics [12]. Covalent modifications to DNA and histone proteins that impact chromatin's accessibility to transcriptional machinery are known as epigenetic regulation. DNA methylation, histone modification, and short RNA are the three main categories of epigenetic markings or signatures [13].

3.1. DNA Methylation

Gene expression, genomic stability, and gene imprinting are all regulated by DNA methylation, a conserved epigenetic mark. DNA methylation is found in the CG, CHG, and CHH sequence contexts in plants (where H can be A, C, or T). This alteration is highly concentrated in heterochromatic transposable elements (TEs) and repetitive sequences, where it

has a notable impact on their transcriptional silencing, transcriptional gene silencing (TGS). There are contexts in which DNA methylation can be seen to amplify gene expression; recent studies suggest that this can at least be partially mediated by DNA methyl readers, including the SU (VAR) 3-9 homologs of SUVH1 and SUVH3. As a result, they observe numerous instances of both induced and natural epialleles that impact various physiological processes and alter their expression in response to methylation modifications [14]. DNA methyltransferase inhibitors and histone post-translational modulators, like 5-azacytidine and zebularine, have been used in plant systems where they primarily antagonize DNA methyltransferases (like MET1, DRM2) and cause DNA demethylation, which can be caused either directly (like 5-azacytidine, a nucleic acid analog of methyltransferase- sequestering cytidine) or indirectly (like by blocking DNA). As a derivative of cytidine, 5-azacytidine binds to DNA and RNA to capture methyltransferases and causes global hypomethylation, which reactivates genes that had been suppressed during development or stress reactions. It boosts alkaloid production in rice by demethylating biosynthetic promoters. Zebularine provides gentler, reversible inhibition by covalent binding to DRM2/CMT3, decreasing CHG/CHH methylation with less toxicity, and reactivating the transgene more selectively according to comparative in-plant *Arabidopsis* studies [15–17].

Table 1. Types of epigenetic modifications and their functional features.

Epigenetic Mechanism	Molecular Basis	Biological Significance in Plants	Mechanistic Insights
DNA methylation	Addition of methyl groups at CG, CHG, and CHH sites mediated by DNA methyltransferases	Maintains genome stability, regulates transposon silencing, modulates stress-responsive and developmental genes	Altered DNA methylation associated with abiotic stress tolerance and enhanced secondary metabolite biosynthesis in medicinal plants
Histone acetylation	Acetylation of histone lysine residues by histone acetyltransferases (HATs)	Promotes chromatin relaxation and transcriptional activation	Histone acetylation enhances expression of genes related to flavonoid and alkaloid biosynthesis
Histone methylation	Methylation of histone tails at specific lysine/arginine residues	Can activate or repress transcription depending on modification site	H3K4me3 linked with active transcription, whereas H3K27me3 suppresses developmental genes
Chromatin emodeling	ATP-dependent remodeling complexes alter nucleosome positioning	Regulates accessibility of transcription machinery during development and stress responses	SWI/SNF complexes participate in stress-responsive gene regulation
Small interfering RNAs (siRNAs)	Short non-coding RNAs guide RNA-directed DNA methylation (RdDM)	Transcriptional gene silencing and transposon suppression	siRNA-mediated RdDM contributes to genome defense and environmental adaptation
MicroRNAs (miRNAs)	Small endogenous RNAs regulate target mRNA degradation or translation	Fine-tunes developmental pathways and secondary metabolism	miRNAs regulate transcription factors associated with plant growth and metabolite accumulation

Table 1. *Cont.*

Epigenetic Mechanism	Molecular Basis	Biological Significance in Plants	Mechanistic Insights
Long non-coding RNAs (lncRNAs)	Regulatory RNAs influence chromatin organization and transcription	Modulates stress signaling and epigenetic regulation	lncRNAs participate in regulation of flowering and stress-responsive pathways

3.2. Histone Modifications

In medicinal plants, histone modifications, including acetylation, methylation, and phosphorylation, are important epigenetic mechanisms that control gene expression in response to different environmental and developmental cues [5]. Plant histone modifications serve as reversible chemical markers that control gene expression by changing chromatin accessibility. HATs catalyze histone acetylation, which is commonly observed at marks such as H3K9ac and H3K14ac, and increases transcriptional activity by relaxing nucleosome packing and neutralizing the positive charge in lysine residues. HMT-mediated histone methylation can either activate genes (like H3K4me3) or inhibit them (like H3K27me3 deposited by PR2), depending on the residue and degree of methylation. Phosphorylation of residues, including H3S10, influences chromosome condensation during mitosis, as well as gene activation during stress [18–20]. All these modifications interact within the so-called "histone code," which balances heterochromatin and euchromatin to regulate plant growth and stress responses. The transcriptional state of genes involved in the synthesis of secondary metabolites is determined by the constant interaction between acetylation and deacetylation, which has a significant impact on chromatin structure [21]. In *Platycodon grandiflorus*, sodium butyrate, which is an established histone deacetylase (HDAC) inhibitor, significantly increased the level of acetylation of histone H3 in lysine K9, K14, and K27, leading to relaxation of chromatin and transcriptional activation. This epigenetic modification increased the accumulation of polyphenolic compounds like gallic acid and dihydroquercetin by upregulating genes involved in the phenylpropanoid biosynthesis pathway. Enrichment of the resultant metabolites is attributed to the result of the metabolite enrichment process, resulting in a significantly more potent anti-melanogenic effect of the root extracts. The study reveals that secondary metabolite biosynthesis is directly regulated by HDAC inhibition-mediated histone acetylation, underscoring epigenetic modification as an effective way for augmenting the production of medicinal compounds in plants [22].

3.3. Chromatin Remodeling and Non-Coding RNAs

In plants, ATP-independent chromatin remodeling complexes utilize the energy generated by ATP hydrolysis to relocate, remove, or reorganize nucleosomes and include bridging factors of the SNF family and the Snf2 family of proteins. These changes in chromatin accessibility and permits stress-responsive or developmentally regulated gene expression. These remodeling machineries are often cooperative with histone-modifying enzymes and DNA methylation complexes, and thus provide an example of a multi-layered system of epigenetic control [23]. By directing post-transcriptional silencing of target mRNAs and RNA-directed DNA methylation (RdDM) at particular loci, which results in transcriptional gene silencing and chromatin alterations, non-coding RNAs (ncRNAs), especially microRNAs (miRNAs) and small interfering RNAs (siRNAs), mediate epigenetic regulation. There is a closely related crosstalk between DNA methylation, histone marks, and non-coding RNAs, such as small RNAs, by recruiting DNA methyltransferases within transposable elements or genes, strengthening repressive chromatin, and histone modifications can, in turn, regulate the formation and functioning of small RNAs [24]. A well-known illustration of epigenetic control via RNA-mediated methylation and chromatin

state modifications is the RNA-directed DNA methylation (RdDM)-mediated silencing of the *FWA* gene in *Arabidopsis*, where siRNA-guided DNA methylation and modifications in the state of chromatin. Moreover, microRNAs like miR156 document the progression of growth by targeting transcriptional elements that can alter chromatin modifications in the flowering area, and thus the functioning of small RNA in chromatin alteration and transcriptional outputs [25,26] (Figure 2).

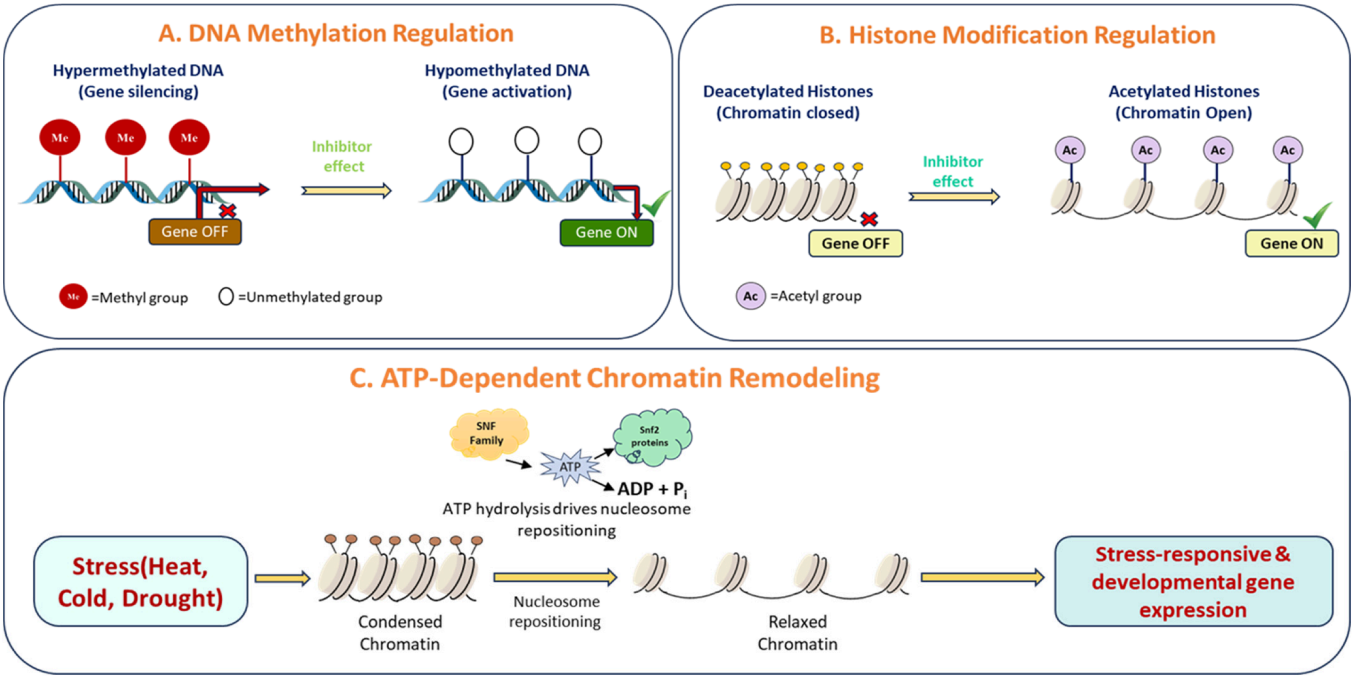


Figure 2. Overview of epigenetic mechanisms regulating plant gene expression. DNA methylation, histone modifications, epigenetic inhibitors, and ATP-dependent chromatin remodelers influence chromatin structure, thereby controlling gene activation or repression during development and stress responses [18,19].

3.4. Classes of Chemical Epigenetic Modulators (CEMs)

Chemical epigenetic modulators (CEMs) are small molecules that can alter chromatin structure and DNA methylation patterns, thereby modulating transcriptional regulation and gene expression. These agents influence epigenetic processes by either blocking or activating enzymes that remodel chromatin [27]. For example, DNA methyltransferase (DNMT) inhibitors, such as 5-azacytidine and zebularine, inhibit DNMT activity and lead to cytosine hypomethylation, transcriptional reactivation of silenced loci, and loosening of heterochromatin in plant cells. It is empirically proven that zebularine decreases the level of DNA methylation and enhances chromatin decondensation in plants and other systems [28]. Histone acetylation profiles in *Arabidopsis* and other plants are impacted by HDAC inhibitors, such as sodium butyrate and trichostatin A (TSA), which inhibit histone deacetylases, increasing histone acetylation, relaxing chromatin, and enhancing transcriptional activity. These agents regulate the histone acetylation in both *Arabidopsis thaliana* and various plant species [29]. Histone methyltransferase and demethyltransferase modulators can influence whether certain genes are switched on or off by adding or removing specific chemical marks on histones. However, the exact ways these modulators work and their effects in plant systems are not fully understood. Chemical epigenetic modulators (CEMs) have several benefits, such as allowing for reversible changes in gene expression and the activation of previously inactive biosynthetic pathways. However, their use can also lead to various unintended consequences, affecting plant growth and showing different reactions

depending on the species. This highlights the importance of careful optimization when applying these modulators in different systems [30]. In plants, a complex interplay of DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs works together as regulatory layers that manage how genes are expressed. These epigenetic mechanisms change chromatin accessibility at promoter and regulatory regions of transcription factors and biosynthetic genes, thus affecting recruitment of RNA polymerase II and associated transcriptional machinery [31]. By activating or repressing specific families of transcription factors like, WRKY, MYB, Bhlh, and AP2/ERF, these epigenetic changes indirectly influence the expression of metabolic enzymes involved in the production of specialized metabolites. Thus, these coordinated changes at the chromatin level can reprogram metabolic flux to increase or decrease the production of secondary metabolites, including pharmaceutically relevant alkaloids. This intricate regulatory system underscores how essential epigenetic control is in connecting environmental and developmental cues with the ability of plants to adapt their metabolism [32,33].

4. Impact of Epigenetic Modulators on Alkaloid Biosynthesis

The effects of the epigenetic modulators on the alkaloid biosynthesis are central to the chromatin remodeling that does not interfere with the actual sequence of the DNA. Variations in the accumulation of metabolites are influenced by changes in chromatin accessibility that occur through epigenetic mechanisms. These changes can regulate the activity of transcription factors and the expression of biosynthetic enzymes, which in turn affects the overall flow of chemical pathways and, ultimately, impacts the levels of alkaloids produced (Table 2).

Table 2. Overview of chemical epigenetic modulators and chromatin-associated regulatory mechanisms affecting alkaloid biosynthesis in medicinal plants.

S. No.	Epigenetic Modulator/ Regulatory Approach	Mechanism & Molecular Targets	Plant System	Target Metabolites	Effect on Alkaloid Production	Limitations & Challenges	Reference
1.	5-Azacytidine (5-Aza)	Competitive inhibition of DNA methyltransferase (DNMT) leads to global DNA hypomethylation by inhibiting DNMT activity and thereby reducing overall DNA methylation levels.	*Salvia miltiorrhiza* (hairy roots)	Tanshinones	Activating important biosynthetic genes like SmDXS and SmKSL significantly boosts the accumulation of tanshinone I & IIA.	At high doses, plants may experience phytotoxicity, stunted growth, and chromosomal abnormalities, but these effects are usually temporary and not passed on to the next generation.	[34]
2.	Chromatin remodeling-associated regulation	Blocking histone methyltransferase lowers the levels of H3K9me2, an important marker of tightly packed DNA.	*Catharanthus roseus*	Ajmalicine, vincristine, and vinblastine	Unlocking silent gene clusters can trigger the production of complex alkaloids like TIAs.	Because the pathway is so complex, plants naturally produce only small amounts of these alkaloids.	[35]
3.	Hairy root-mediated metabolic activation	Jasmonate signals can loosen DNA packaging at gene promoters, indirectly turning on certain genes.	*Rauwolfia serpentina*	Reserpine	Treating root cultures with modulators and loosening DNA structures work together to boost alkaloid production.	Metabolite levels often vary in hairy root cultures, and we still do not fully understand why this happens.	[36]
4.	Stress-induced epigenetic regulation	Environmental stress can change DNA packaging and small RNA patterns, shifting how genes are turned on or off.	*Withania somnifera*	Withanolides	Drought or salt stress primes plants to produce more protective compounds.	Results can vary between plant types, making it hard to get consistent outcomes.	[37]
5.	Genome-scale biosynthetic regulation	Finding epigenetically sensitive genes can help control how plants make valuable compounds.	*Tinospora cordifolia*	Isoquinoline alkaloids	Mapping epigenetic controls helps guide genetic improvements to boost valuable metabolites.	Limited direct proof that epigenetic changes actually drive these effects.	[38]
6.	Biosynthetic pathway regulation	Combining transcription factors with epigenetic tools can enhance alkaloid production.	*Papaver somniferum*	Morphinan alkaloids (morphine, codeine, noscapine)	Pathway engineering can help plants keep making alkaloids, even when their levels are already high.	Channeling excess carbon into alkaloid production can upset the plant's balance and reduce its supply of vital nutrients.	[39]
7.	Genome & chromatin-associated pathway regulation	Controlling how chromatin is packed within a large gene cluster helps regulate specialized metabolism in plants.	*Camptotheca acuminata*	Camptothecin	Gaining insight into camptothecin biosynthetic regulation can increase the activity of nearby genes in the quinoline alkaloid pathway.	Validating these regulatory mechanisms is still a challenge because keeping chromatin open long-term without constant stimulation is difficult.	[40]

Experimental results in medicinal species include those obtained from plants, which demonstrate that alkaloid medications are regulated through epigenetic activity in terms of tissue and developmental regulation. The chemical epigenetic modulators, therefore, represent a non-genetic approach to activate quiescent genes and promote alkaloid productivity in medicinal plants, which is reversible [39,41].

4.1. Epigenetic Activation or Silencing of Biosynthetic Genes

The epigenetic regulation of biosynthetic genes has become one of the most crucial modulators of alkaloid pathway activity in medicinal plants, via the regulation of chromatin accessibility and resultant transcriptional activity. Strictosidine synthase (STR), strictosidine β-glucosidase (SGD), and downstream vindoline pathway genes are among the key genes in the monoterpenoid indole alkaloid (MIA) biosynthesis of *Catharanthus roseus* that are activated by epigenetic marks like histone acetylation, while repressive epigenetic marks can constrain their tissue-specific expression of clustered genes, including Norcoclaurine synthase (NCS), Coclaurine N-methyltransferase (CNMT), and berberine bridge enzyme (BBE), in benzylisoquinoline alkaloid (BIA) pathways of *Papaver somniferum*, resulting in cultivar-specific alkaloid profiles. Similarly, organ-specific accumulation of atropine and scopolamine in plants that produce tropane alkaloids (Atropa, Hyoscyamus) is determined by epigenetic regulation of putrescine N-methyltransferase (PMT) and tropinone reductase (TRI/TRII). Therefore, environmental and developmental cues trigger reversible activation or silencing of alkaloid biosynthetic genes by epigenetic mechanisms [42–44] (Figures 3 and 4).

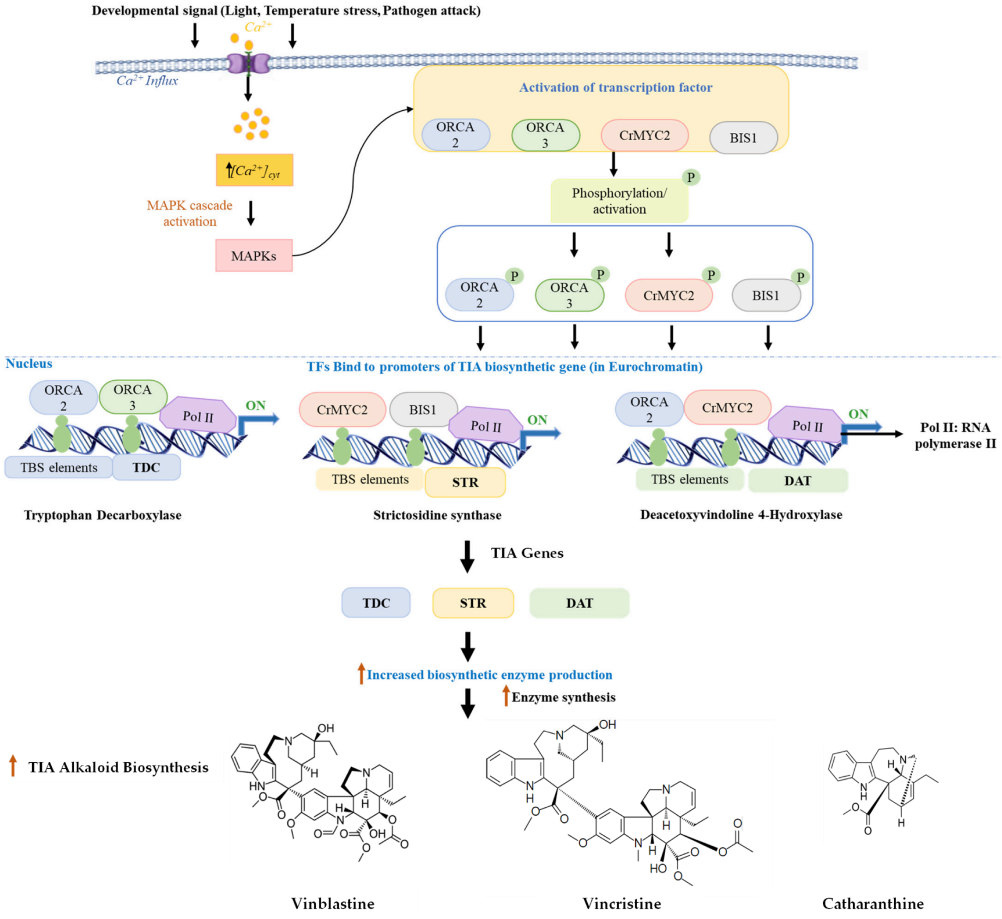


Figure 3. Transcriptional regulation of terpenoid indole alkaloid (TIA) biosynthesis in *Catharanthus roseus*. Calcium-mediated signaling activates transcription factors that regulate biosynthetic genes involved in alkaloid production [45,46].

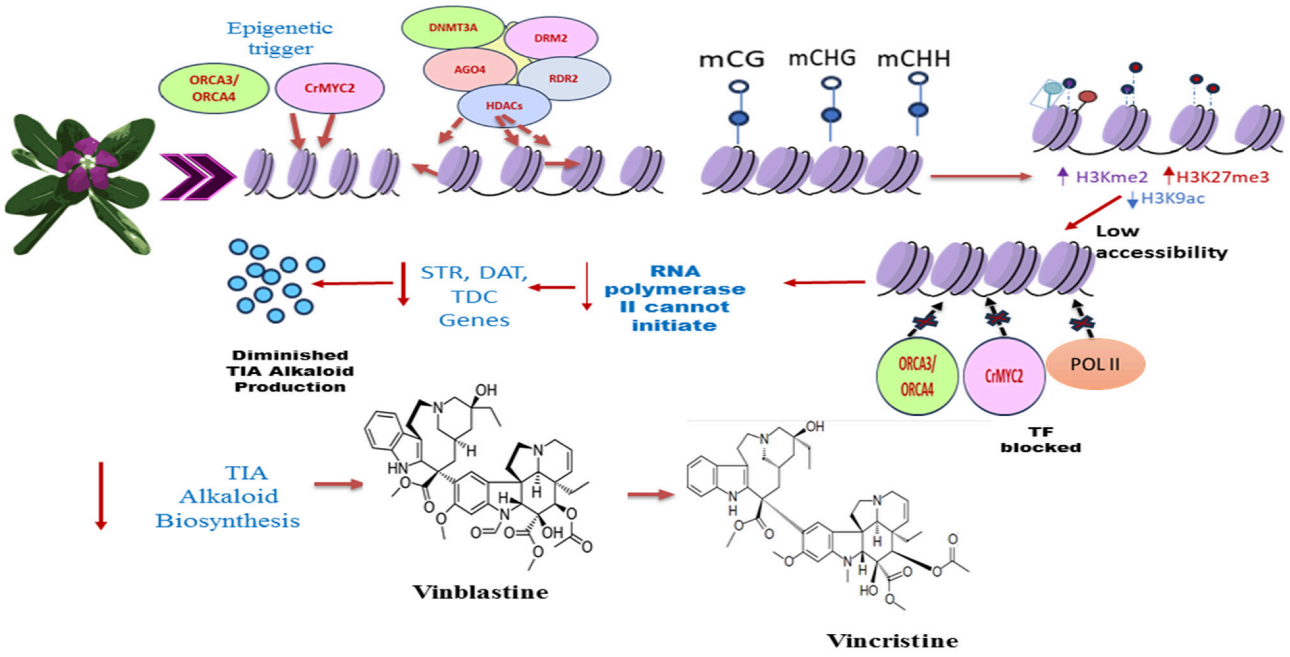


Figure 4. Epigenetic regulation of secondary metabolite biosynthesis through RNA-directed DNA methylation (RdDM) and histone modifications. Repressive chromatin marks reduce transcription factor and RNA polymerase II accessibility, leading to gene silencing [47,48].

4.2. Regulation of Transcription Factors

Plants tightly control the production of specialized alkaloids through key transcription factor families such as WRKY, MYB, bHLH, and AP2/ERF, which function as molecular switches that turn on biosynthetic pathways when needed. While AP2/ERF factors like ORCA proteins bridge jasmonate signaling with activation of multiple monoterpenoid indole alkaloid genes for coordinated response. WRKY proteins control defense and stress-linked alkaloid genes by binding conserved W-box motifs [49,50]. By coordinating the spatial and developmental expression of biosynthetic enzymes, frequently through cooperative transcription factor complexes, MYB and bHLH transcription factors contribute to pathway specificity. When biosynthetic enzymes are activated, they often form cooperative complexes for fine-tuned control. Their activity gets dialed up or down through epigenetic tweaks: histone acetylation opens up access to boost transcription factor gene expression and promoter binding, while DNA methylation and repressive histone marks clamp things shut to limit expression and DNA access. This dynamic epigenetic layer lets plants flexibly ramp alkaloid output in response to environmental stresses or growth stages, ensuring efficient adaptation without constant overproduction [51,52].

4.3. Epigenetic Modulation of Secondary Metabolism and Alkaloid Production in Plants

Plants encounter significant variations in light, temperature, weather conditions, and numerous other environmental elements over their lifespan. Unlike animals, they are sessile organisms that cannot move, hide, or even flee. As a result, interactions with the outside world play a major role in how plants grow and develop. The degree of phenotypic plasticity, which is primarily influenced by epigenetic control, determines the success of this interaction. Epigenetic regulation determines how genetic expression changes throughout the differentiation of one cell type into another and how patterns of gene expression are transferred from one cell to progeny, in addition to how environmental stimuli can alter gene expression patterns [53]. The changes in the histone variants, histone N-tail post-translational modifications, and DNA methylation, which are a result of stress, have been found to regulate the expression of stress-responsive genes and plant development when

the environment is not favorable. The acclimatization response is mediated by transient chromatin changes. Within generational and transgenerational stress, memory may be provided via heritable, epigenetic changes. Since little is known about the mitotic or meiotic heredity of stress induced histone and DNA modification alterations, it is unknown to what extent these changes may be epigenetic in origin. It is not yet clear how far the histone and DNA modification changes currently available are truly epigenetic [54]. Therefore, the adaptive meaning of the epigenetic alterations induced by abiotic stress is not entirely understood. The epigenetic changes that result due to abiotic stress may have adaptive benefits, but the stress memory may have a negative effect on plant production by not allowing the plant to develop to its full potential. Stress memory hence affects breeding for stress settings, in situ conservation of plant species, and farmers using seeds from stressed crops to raise subsequent crops. It is now feasible to critically examine epigenetic stress memory and use it for crop management of DNA methylation and demethylation, histone changes, short RNAs, and the development of strong and adaptable instruments to research these epigenetic processes [55]. *Catharanthus roseus* is one of the most extensively analyzed models used to understand the mechanisms of chromatin regulation of alkaloid biosynthesis, and especially of monoterpenoid indole alkaloids (MIAs) like vinblastine and vincristine. Genomic interrogation of neural biosynthetic plasticity, via experimental involvement of DNA methyltransferase (DNA methyltransferase or 5-azacytidine) and histone deacetylase (HDAC) (HDAC or WRN) inhibitors, has shown consistent superior activity in upregulating essential biosynthetic genes (e.g., TDC, STR, and DAT) and WRKY. These epigenetic disturbances loosen chromatin compaction so that transcriptional activation of pathway genes with low expression levels eventually peak with a sharp increase in alkaloid levels [34,35]. Prolific stylist (*Papaver somniferum*) has shown a crossroad between the effect of epigenetic control and the control of genes involved in the BIA (Benzylisoquinoline alkaloid) pathway (e.g., TYDC and COR), particularly. Dynamics of histone acetylation, together with specific patterns of DNA methylation, have been associated with tissue-specific expression patterns of the alkaloid biosynthetic genes in the tissues that secrete latex. It has been demonstrated that chemical epigenetic modulators can strengthen pathway flux without reducing specificity, settling their potential future application in the controlled synthesis of alkaloids [39]. *Tinospora cordifolia* is a pharmacologically significant species, rich in isoquinoline alkaloids, which is a nascent paradigm of the epigenetic study. Preliminary results show that manipulation of epigenetics alters the transcriptional activity of metabolically and stress-responsive pathway-related genes, which then realigns the alkaloid profiles. However, these data indicate a central role in the regulation of metabolite biosynthesis in this taxon that is played by chromatin remodeling and development in DNA methylation without full atlases of epigenomics [38]. The production of indole alkaloids, including ajmalicine and reserpine, by *Rauwolfia serpentina* has been reported to be epigenetically regulated at several vital enzymatic nodes of its production pathway. The presence of distinct histone signature alterations and transcriptional aspects of reprogramming are associated with greater alkaloid accumulation in cell cultures, which suggests that epigenetic modulators are capable of providing stability to metabolite synthesis under in vitro conditions [36]. Lastly, *Nicotiana tabacum* is a clear-cut model to study the epigenetic regulation of nicotine production. Both the transcriptional activity of nicotine pathway genes and the members of the ERF transcription factor family are moderated by the modification of DNA methylation and histone acetylation. Epigenetic perturbations have proven to adjust nicotine levels in a concerted way with developmental patterns and environmental hassles, hence fabricating the vast universalizability of the epigenetic process in alkaloid regulation [56] (Table 3). *Camptotheca acuminata* is the key player in elucidating the epigenetic processes that oversee indole alkaloid production,

especially camptothecin production. Empirical studies have shown that alterations in DNA methylation level and histone acetylation patterns suppress or activate transcription of key biosynthetic genes, such as TDC-like genes, HMGR, and related transcription factors. These epigenetic modifications contribute to relaxation of chromatin and, consequently, to increased expression of pathway genes and enhanced camptothecin levels, which in turn highlights chromatin-based regulation as a determining factor of the indole alkaloid metabolic flux [40]. Similarly, *Withania somnifera*, a medicinal plant that endows a high density of withanolides, shows high levels of epigenetic responsiveness to abiotic stress. Stress-induced changes in DNA methylation and histone modification dynamics have been reported to restructure the expression of stress response and secondary metabolism-related genes, which translate into withanolide biosynthesis. These data underscore the central role of chromatin remodeling in granting metabolic plasticity and adaptive control of steroidal alkaloid production of *W. somnifera* [37]. The epigenetic regulation of alkaloid biosynthesis operates via a concerted multilayered mechanism involving chromatin remodeling, transcriptional regulation, enzymatic activity, and metabolic pathway flux. Specifically, modifications to DNA and histones can influence how accessible certain regions of the DNA are, especially at the promoters of genes coding for transcription factors like WRKY, MYB, bHLH, and AP2/ERF. These factors play a critical role in turning on or off the genes that are essential for producing alkaloids [32]. These transcription factors subsequently regulate the expression of essential alkaloid biosynthetic enzymes including TDC, STR, DAT, PMT, and BBE. Increased transcription of these enzymes increases their catalytic efficiency and also diverts the use of precursors into specialized alkaloid pathways, ultimately changing metabolic flux. Consequently, when the biosynthetic pathways are activated epigenetically, there is a greater accumulation of valuable alkaloids like vinblastine, vincristine, nicotine, morphine, and camptothecin [57]. On the other side, when certain repressive epigenetic markers are present, they inhibit these pathways, leading to less production of these metabolites. This intricate regulatory system demonstrates how epigenetic mechanisms play a vital role in shaping secondary metabolism, allowing plants to adapt their alkaloid production in response to changes in their development and environmental conditions [58].

Table 3. Role of epigenetic regulation in alkaloid biosynthesis across medicinal plants.

Plant Species	Major Alkaloids	Epigenetic Mechanisms Involved	Key Genes/ Regulators Affected	Major Outcomes	References
Catharanthus roseus	Vinblastine and vincristine (MIAs)	DNA methylation, histone acetylation/deacetylation.	TDC, STR, DAT, and WRKY transcription factors	The chromatin structure was relaxed, biosynthetic genes were significantly increased, and alkaloid accumulation was increased by epigenetic modulators, such as DNA methyltransferase and HDAC inhibitors.	[34,35]
Papaver somniferum	Benzylisoquinoline alkaloids (BIA)	Histone acetylation dynamics, DNA methylation	TYDC and COR	Histone acetylation and methylation limited and controlled by tissue-specific patterns regulate alkaloid gene expression in the tissues that produce latex. The use of epigenetic modulators enhances the flux of pathways but does not diminish specificity.	[39]

Table 3. *Cont.*

Plant Species	Major Alkaloids	Epigenetic Mechanisms Involved	Key Genes/ Regulators Affected	Major Outcomes	References
Tinospora cordifolia	Isoquinoline alkaloids	DNA methylation changes, chromatin remodeling	Stress- and metabolism-related pathway genes	By modulating transcriptional activity and turning around alkaloid profiles, changes in epigenetic marks influence chromatin remodeling as an important factor, despite epigenomic mapping.	[38]
Rauwolfia serpentina	Ajmalicine and reserpine	Histone modification, transcriptional reprogramming	Key enzymatic nodes of alkaloid pathway	Distinct histone signatures correlated with enhanced alkaloid accumulation, and epigenetic modulators stabilized metabolite production in in vitro cultures.	[36]
Nicotiana tabacum	Nicotine	DNA methylation, histone acetylation	Nicotine biosynthetic genes and ERF transcription factors	Coordinated epigenetic regulation fine-tuned nicotine biosynthesis in response to developmental cues and environmental stress.	[56]
Camptotheca acuminata	Camptothecin	DNA methylation, histone acetylation	TDC-like genes and HMGR transcription factors	Epigenetic modulation influenced camptothecin biosynthetic gene expression and metabolic accumulation, indicating chromatin-level control of the indole alkaloid pathway.	[40]
Withania somnifera	Withanolides	Histone modification, DNA methylation	Stress-responsive and secondary metabolism genes	Epigenetic reprogramming under stress conditions altered the biosynthesis of withanolides, suggesting a regulatory role of chromatin remodeling in metabolic plasticity.	[37]

5. Rationale Behind Using Epigenetic Modulators

These epigenetic modulators are aimed at chromatin alteration to liberate silent biosynthetic gene clusters (BGCs) in plants, leading to improved production of secondary metabolites like alkaloids without causing any lasting change in the DNA sequence. Because of repressive markers like H3K9me3 or high DNA methylation, these BGCs, which are frequently buried in heterochromatin regions close to telomeres or subtelomeric zones, remain transcriptionally silent under normal conditions, restricting the finding of natural products [59]. This non-GMO approach maintains genomic integrity while causing reversible epigenetic changes for metabolic activation, such as DNA demethylation (using DNMT inhibitors like 5-AZA) or histone acetylation (using HDAC inhibitors like Trichostatin A (TSA) or Suberoylanilide hydroxamic acid (SAHA)) [60].

5.1. Non-GMO Approach for Metabolic Activation

Unlike transgenic genetically modified organisms (GMOs) techniques that insert foreign DNA, epigenetic alterations modify gene expression by different processes, such as DNA methylation, histone acetylation, and non-coding RNAs, without altering the underlying DNA sequence. This approach does not involve the use of invasive methods to avoid strict GMO regulations that are normally applicable to genetic modification of crops, because regulatory authorities tend to focus on sequence change as opposed to the change in gene expression. Non-transgenic edits, including those from CRISPR/Cas9 SDN-1 (indels) and SDN-2 (precise replacements utilizing plant templates), are categorized as non-GMO in nations like the US, Argentina, and Brazil, similar to spontaneous mutations or traditional breeding outcomes [61]. In plant breeding, the genetic integrity of the plant remains intact, allowing the modulation of specific traits to be desired (such as the production of secondary metabolites), including through the activation of specific transcription factors with minimal regulatory control and compliance costs, which is possible via foreign DNA insertions. This makes epigenetics a tool for sustainable metabolite engineering that can be used at the commercial level [62,63]. Plant-derived goods are ideally aligned with popular consumer desires for "natural" foods thanks to their non-GMO status, which is achieved through epigenetic modification. This allows them to avoid the ongoing stigma associated with GMOs, which is linked to health hazards and environmental concerns. The results in transgene-free plants also increase marketability significantly because, when tested on a survey of consumers, it was proven that the receptivity towards precision edits that reflect typical breeding activities, instead of immediate genetic engineering methods, was significantly higher. This strategy can improve overall commercial viability and ensure sustainable market penetration for alkaloid-rich plants in particular by opening the door to wider acceptance in regions that are naturally wary of GM labels [64].

5.2. Enhancement of Secondary Metabolite Yield

By providing a quicker, easier, and more useful alternative to traditional methods, epigenetic modulators are revolutionizing the way secondary metabolite production is increased in plants. Silent biosynthetic gene clusters can be rapidly activated, frequently in a matter of days, by substances like DNA methyltransferase inhibitors (DNMTis) and histone deacetylase inhibitors (HDACis). This rapid induction process speeds up pathways related to alkaloid, flavonoid, and terpenoid plants without altering the genomic DNA of the plant [6]. The reversibility of those effects and dependence on chromatin remodeling make the effects significantly faster than the processes associated with genetic engineering or the classical approach to modification. These effects operate far more quickly than genetic engineering or traditional modifiers because they are reversible and depend on chromatin remodeling. Cost-effectiveness is yet another significant benefit. Instead of using costly transgenic techniques or complicated infrastructure, epigenetic treatments rely on inexpensive chemicals and simple growth conditions [65]. They work well in tissue cultures, bioreactors, and field systems, and are very simple to scale up. In many instances, advantageous epigenetic modifications are preserved throughout plant generations, facilitating steady metabolite production. Epigenetic manipulation is a potentially sustainable approach for the commercial manufacture of bioactive chemical compounds obtained from plants because of its speed, affordability, and scalability [66].

5.3. Industrial and Pharmaceutical Applications, Advantages over Conventional Modulators

By improving the dependability, scalability, and control of bioactive chemical production, epigenetic modulators are transforming the industrial and pharmaceutical applications of medicinal plants. In contrast to traditional modifiers, they have a higher level of

specificity, directly including selected biosynthetic pathways, usually including silent or weakly expressed genes, resulting in the production of compounds such as alkaloids and flavonoids [67]. A focused control makes it possible to produce standard metabolite profiles, without which pharmaceutical purity and consistency cannot be maintained. Stress-based modulators like jasmonic or salicylic acid cause transient and unpredictable reactions, but epigenetic agents like HDAC and DNMT inhibitors promote steady and sustained metabolite production in plant cell cultures and bioreactor systems. Generally, epigenetic modulation provides a non-GMO approach to transforming plant-based therapeutics into high-value and reliable industrial products [68].

6. Future Directions and Emerging Technologies

6.1. CRISPR-Based Epigenome Editing

Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) technology have made it possible to regulate and specifically modify genome activity and locus function in recent years. Understanding chromatin activity in numerous cellular processes has already shown promising results from the recent applications of this paradigm to epigenome modifications. The adaptive immune response system uses non-coding RNA to direct the Cas9 nuclease to cleave viral DNA sequences in particular, which are the source of the most widely used CRISPR type II systems. A single-guided RNA (sgRNA) is all that is needed in a CRISPR-mediated genome editing experiment to direct the Cas9's cleavage of DNA at a specific genomic site and produce mutations (knock out) or insertions (knock-in) via DNA repair [69]. Mutations in Cas9's catalytic sites D10A and H840A prevent DNA cleavage while maintaining target sequence recognition. Therefore, this catalytically dead Cas9 (dCas9) is employed as a synthetic platform in several applications, such as epigenome engineering. Direct epigenome editing, which uses dCas9 to recruit a chromatin modifier, disrupts locus-specific epigenetic regulation and transcriptional engineering which aims a trans-acting factor to a gene regulatory region and destabilizes or initiates a shift in the local chromatin state. De novo and maintenance DNA methyltransferases as well as demethylases regulate cytosine methylation in plants, which occurs in the CG, CHG, and CHH sequence contexts (Where H is adenine, cytosine, or thymine) [70]. DNA methylation is found in several gene regulatory sites and is substantially enriched over repetitive sequences, where it is linked to transcriptional silence. In plants, RNA-directed DNA methylation (RdDM) processes play a major role in establishing DNA methylation. *Nicotiana tabacum* DRM methyltransferase catalytic domain (NtDRMcd) was simultaneously recruited via the SunTag method linked to dCas9 in a groundbreaking attempt to focus DNA methylation to the *Arabidopsis FWA* locus (Figure 5) [71]. To use numerous sgRNA as landing pads and sustain the expression of the system throughout two successive generations, it is necessary to reach a significant level of DNA methylation to establish stable silencing. Moreover, CG methylation levels at the (*FLOWERING WAGENINGEN*) *FWA* gene affected methylation heredity [72].

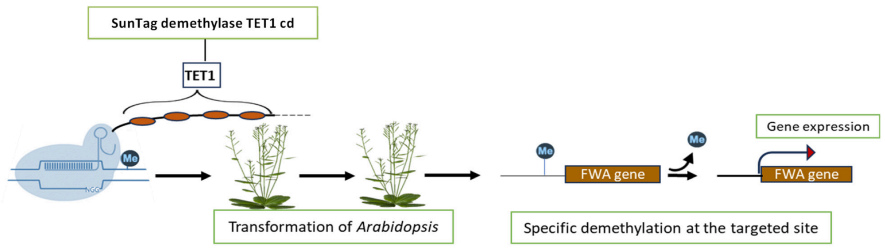


Figure 5. CRISPR/dCas9-TET1-mediated targeted DNA demethylation in *Arabidopsis*. Site-specific demethylation of the FWA locus activates gene expression and alters plant phenotype [71].

6.2. Multi-Omics Integration

Integrating multi-omics data has become a cornerstone of plant systems biology and transformed the scientific paradigm from single observations of biological networks to more comprehensive insights into these biological networks. Through harmonization of the epigenomic, transcriptomic, and metabolomic layers, investigators may successfully explain an organism's inherited genetic potential to its ultimate phenotype. For example, metabolomics offers a functional readout of the biological state, whereas transcriptomics and epigenomics provide the regulatory blueprint and mRNA expression levels. Research on medicinal plants such as *Bidens alba* shows that decoding organ-specific biosynthesis requires integrating these levels, particularly through transcript–metabolite correlation [58]. This approach has successfully discovered how the differential expression of important genes, such as CHS for flavonoids and HMGR for terpenoids, directly results in the unique chemical profiles found in roots versus aerial parts of the plants. To tackle this complexity, systems biology utilizes three unique levels of integration. Level 1 (element-based) focuses on statistical correlations, employing correlation coefficients and clustering to find co-expressed genes and metabolites. By mapping data to well-known biological frameworks, KEGG or MapMan, Level 2 (pathway-based) improves this and enables researchers to observe metabolic fluxes inside specific pathways, such as the citrate cycle or secondary metabolism. Lastly, Level 3 (mathematical-based) includes genome-scale modeling (GSM), which uses differential equations and flux balance analysis to simulate and predict the response of a plant system to perturbations, whether environmental or genetic [73]. The disadvantages of single-omics investigations, such as the common lack of correlation between mRNA and protein/metabolite levels due to post-translational modifications, are addressed by the synergy of these techniques. By employing universal frameworks like KBCommons for data administration and powerful computational models for analysis, systematic multi-omics integration (MOI) provides the essential resolution to comprehend the complicated regulatory processes of plants. This all-encompassing "systems" perspective is essential for fundamental biological research as well as real-world uses in crop improvement, molecular breeding, and the pharmaceutical optimization of therapeutic plant chemicals [74].

6.3. Bioinformatics Tools and Databases

These days, functional genomics, epigenomics, and bioinformatics collaborate to advance plant science. Bioinformatics tools and databases, which enable the management and interpretation of massive datasets, are key to this integration. Resources such as PlantCADB that aggregate millions of accessible chromatin regions across various plant species, which are crucial for identifying loci where gene expression is likely to be activated or repressed, collect data using such high-throughput sequencing approaches as ATAC-sequence and DNase-sequence, which identify open chromatin regions where transcription factors can bind to and regulate the activity of genes. A significant long-term objective in science is building comprehensive plant epigenome maps, often referred to as 'reading the second genetic code' [75]. The goal of international initiatives like the epigenomics of Plants International Consortium (EPIC) is to map out how epigenetic characteristics influence gene accessibility and direct plant growth and stress reactions. When paired with functional analyses in gene co-expression networks, this structural perspective of the genome becomes particularly potent [76]. Using a 'guilt by association' strategy, these networks link genes that behave similarly across situations, showing common regulatory mechanisms and biological roles. All these components are closely related to each other [77]. Co-expression networks describe how genes interact, epigenome maps show the regulatory landscape, and bioinformatics platforms arrange chromatin accessibility data. The combination of these

resources allows the researchers to have more accurate results of connecting the phenotypes with the genotypes and to unravel the plant responses to abiotic stresses like drought and thermal extremes [78]. Importantly, this paradigm also helps transfer knowledge from well-studied model plants, such as *Arabidopsis*, to complex crop species. When taken as a whole, these developments are setting the stage for more intelligent, long-term crop improvement strategies [79,80].

6.4. Knowledge Gaps and Limitations

Even though epigenetic regulation has created intriguing new opportunities in enhancing the synthesis of secondary metabolites in plants, there are still a number of significant gaps and conditions that must be managed. The first is the lack of species and tissue-scale epigenetic data, especially of medicinal flora, which are vast sources of alkaloids. Mostly, the epigenomic evidence is in model organisms, like *Arabidopsis*; thus, it has limited translation ability to non-model taxa with more complex genomic architecture [67]. Furthermore, the long-term stability and heredity of chemically induced epigenetic modifications are correctly poorly understood, even though epigenetic effects are highly reliant on variables like plant age, tissue type, and environmental conditions. The following concerns raise questions about the dependability and consistency of such methods for breeding programs or the mass production [6]. Another drawback is the potential for chemical epigenetic modulators to have off-target effects, leading to widespread chromatin structural alterations rather than targeting particular pathways. These unintended effects can affect plant growth, stress, and metabolic balance as a whole. The diversity in the sensitivity of plant taxa, as well as difficulties in dosage optimization, also makes the practical implementation of these agents more difficult [81]. Although multi-omics tools have enhanced our understanding of the relationship between epigenetics and metabolism, gaps in the integration of data and the low number of predictive models with high predictive abilities continue to limit the ability to predict the outcomes. Also, epigenetic regulation by RNAs, the role of miRNAs and siRNAs in the regulation of alkaloid biosynthesis, has not been well described. The processes involved bioinformatics infrastructure and species-specific epigenome mapping. These attempts are necessary to implement epigenetic interventions as credible and enduring tools in plant biotechnology [82].

7. Conclusions

In conclusion, chemical epigenetic modifiers have emerged as promising tools for enhancing alkaloid biosynthesis in medicinal plants by regulating gene expression without altering the underlying DNA sequence. This review highlights the coordinated involvement of multiple epigenetic mechanisms, including DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs, in controlling the expression of key biosynthetic genes and transcription factors associated with alkaloid production. Among these approaches, chemical epigenetic modulators such as DNA methyltransferase inhibitors and histone deacetylase inhibitors provide a rapid, reversible, and non-transgenic strategy for reactivating silenced or downregulated metabolic pathways in plants. Compared with conventional methods, these modulators offer improved specificity, consistency and metabolite yield. Furthermore, recent advances in CRISPR-based epigenome editing and multi-omics technologies have strengthened the potential of epigenetic interventions by enabling precise and predictive manipulation of plant metabolic networks. Despite existing challenges, including limited species-specific knowledge and concerns regarding long-term stability, epigenetic modulation represents a sustainable and innovative approach for improving the production of pharmaceutically important plant-derived alkaloids and supporting future industrial applications.

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