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HETEROCHROMATIN IN PLANTS - STRUCTURE, FUNCTION AND EPIGENETIC REGULATION

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Abstract. Heterochromatin is a highly condensed form of chromatin, essential for genomic organization and gene regulation in eukaryotes. In plants, its importance is amplified by the complexity of their genomes, which are rich in transposable elements and often result from polyploidy events. This review addresses the structure, functions and epigenetic regulation of plant heterochromatin. Structurally, it is characterized by densely packed nucleosomes, repressive histone modifications (H3K9me, H3K27me) and specific DNA methylation patterns (CG, CHG, CHH), established and maintained by reader proteins and chromatin remodellers. Their functions include silencing transposable elements, maintaining chromosome stability and regulating development and response to environmental stresses. Epigenetic regulation, notably the RNA-directed DNA methylation (RdDM) pathway, demonstrates the plasticity and interconnectedness of molecular mechanisms that allow plants to adapt to dynamic environments. An in-depth understanding of heterochromatin offers vast opportunities for crop improvement and agricultural biotechnology.
Keywords: heterochromatin; plants; epigenetics; DNA

INTRODUCTION

Life, in all its complexity and diversity, rests on the ability of cells to store and express their genetic information. In the vast and intricate universe of eukaryotic cells, which range from fungi and plants to animals and human beings, DNA (deoxyribonucleic acid) is not just the molecule of heredity; it is the complete instruction manual that dictates the structure, function and regulation of every biological process. In order for this manual to be read, copied and protected inside the tiny nuclear compartment, it needs an extremely sophisticated organization. DNA can stretch for metres, it is packaged in a complex way in a dynamic and highly organized structure known as chromatin. This organization is fundamental for two main reasons: first, it efficiently condenses the vast expanses of DNA in the confined space of the nucleus, and second, it meticulously regulates gene expression while protecting the stability of the genome (Kornberg & Lorch, 1999; Rando & Winston, 2012). Chromatin itself is a sophisticated composite of DNA, histone proteins (which form the fundamental nucleosomes) and various non-histone proteins. Together, these components interact in complex ways, continuously modulating the accessibility and functionality of the genetic material (Du et al., 2015).

Within this chromatin architecture, two major states can be distinguished: euchromatin and heterochromatin. Euchromatin, generally found in regions rich in genes, adopts a more relaxed and open conformation, making it easily accessible for active transcription. In contrast, heterochromatin is characterized by its highly condensed and compact nature, typically located at the nuclear periphery or in specific chromosomal domains, such as centromeres and telomeres (Guerra, 2000). For a long time, heterochromatin was seen as a "silent" or "inactive" part of the genome, mainly because of its strong association with transcriptional repression of genes and, notably, transposable elements (TEs) (Allshire & Madhani, 2018). However, contemporary research has drastically transformed this perception. Today, we understand heterochromatin as a dynamic and functionally diverse structure. Its roles go far beyond mere gene silencing, encompassing crucial contributions to nuclear organization, chromosomal stability and the complex regulation of gene expression in response to both developmental signals and environmental stimuli (Grewal & Jia, 2007; Feng & Jacobsen, 2011; Liu & Wendel, 2020).

The importance of heterochromatin is particularly pronounced in plants, given the unique complexity and remarkable plasticity of their genomes. Plant genomes exhibit extraordinary variation in size, often containing an exceptionally high proportion of TEs and repetitive sequences. This genomic configuration is often shaped by ancient polyploidy events and gene duplications, leading to massive genomic expansions (Bennetzen, 2000; Schnable et al., 2009). In this context, heterochromatin acts as a primary defense mechanism, preventing the proliferation and uncontrolled movement of TEs, which could otherwise introduce deleterious mutations and compromise genomic integrity (Slotkin & Martienssen, 2007; Lippman & Martienssen, 2004). In addition, the remarkable ability of plants to adapt and thrive in a wide range of challenging environmental conditions, such as drought, salinity, heat stress and pathogen attacks, is deeply linked to their genomic and epigenetic flexibility, where heterochromatin plays a vital regulatory role, allowing rapid and precise adjustments in gene activity, crucial for survival (Mirouze & Paszkowski, 2011; Sani et al., 2013).

The identity and maintenance of heterochromatin are carefully regulated by a complex set of epigenetic modifications. These are chemical changes in DNA or histone proteins that do not modify the underlying DNA sequence, but profoundly influence chromatin structure and, consequently, gene activity (Berger, 2007; Du et al., 2015). In plants, the main epigenetic markers that define heterochromatin include DNA methylation, histone modifications and small RNAs.

DNA methylation, which consists of the addition of methyl groups to cytosine residues, is undoubtedly one of the most robust and widely studied epigenetic marks of heterochromatin, correlating strongly with the stable silencing of TEs and repetitive sequences. Plants exhibit unique DNA methylation patterns, notably in CHG and CHH contexts (where H stands for A, T or C), which are distinct from those observed in animals. These patterns are often established and maintained by complex enzymatic pathways, such as RNA-directed DNA methylation (RdDM), standing out as a plant-specific epigenetic regulatory component (Law & Jacobsen, 2010; Zemach et al., 2010; Matzke et al., 2009; Feng et al., 2020).

In histone modifications, the N-terminal tails of histone proteins are subject to a wide range of post-translational modifications, including acetylation, methylation, phosphorylation and ubiquitination. Heterochromatin is characteristically marked by specific repressive modifications, such as trimethylation of lysine 9 of histone H3 (H3K9me3) and dimethylation or trimethylation of lysine 27 of histone H3 (H3K27me2/3). These marks serve as crucial signals, recruiting specialized proteins that drive chromatin condensation and reinforce gene silencing (Du et al., 2015; Zhang et al., 2018).

Small RNAs in plants, particularly small interfering RNAs (siRNAs), are indispensable components of the RdDM pathway. These sRNAs guide the DNA and histone methylation machinery to specific heterochromatic loci, effectively establishing and maintaining the silenced state of TEs and other repetitive sequences. This sRNA-mediated targeting system represents a central pillar of heterochromatin formation and adaptive gene regulation in plants (Vaucheret, 2008; Matzke & Mosher, 2014).

A comprehensive and detailed understanding of heterochromatin structure, its diverse functions and its intricate epigenetic regulation in plants offers more than just fundamental insights for basic biology. It has significant practical implications for the advancement of agriculture and biotechnology. The poten-

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tial to precisely manipulate heterochromatin opens up exciting new avenues for crop improvement. This could include stable silencing of undesirable genes, increasing genomic stability in polyploid crop varieties, modulating gene expression to boost productivity and increasing tolerance to a wide range of biotic (pathogens) and abiotic (drought, salinity) stresses (Lusser et al., 2011; Springer & Schmitz, 2017).

This review aims to synthesize and critically evaluate current knowledge about heterochromatin in plants. We will address its main structural and molecular features, explore the multiple roles it plays in genomic organization and gene regulation, and analyze the complex epigenetic mechanisms that govern its formation and maintenance. In addition, this review will discuss recent advances in understanding the remarkable plasticity of heterochromatin in response to environmental challenges while pointing to promising avenues for future research and possible biotechnological applications, reinforcing its central role in plant genome biology.

MATERIAL AND METHODS

This study consists of a comprehensive and systematic review of scientific literature focused on the structure, function and epigenetic regulation of heterochromatin in plants. In order to ensure transparency, scientific rigor and reproducibility, we detail below the methodology used in the selection and analysis of sources. The bibliographic search was conducted in various electronic databases and scientific content platforms recognized for their scope and relevance in the field of biological and agricultural sciences, including (PubMed/MEDLINE, Scopus, Web of Science, Google Scholar, ScienceDirect and SpringerLink).

We used a combination of key terms in Portuguese and English, using Boolean operators ("AND", "OR") to optimize the results. The main terms and their combinations were: "heterochromatin AND plants"; "epigenetics AND plants"; "DNA methylation AND plants"; "histone modification AND plants"; "transposable elements AND plants"; "gene silencing AND plants"; and "chromatin structure AND plants".

We refined the search strategy progressively, adding more specific terms related to mechanisms and model organisms as necessary. For the selection of sources, we established strict inclusion and exclusion criteria. We included original research articles and review articles published in peer-reviewed scientific journals, as well as book chapters and monographs from academic publishers.

We prioritized publications with a publication date between 2015 and 2025 to ensure that knowledge was up-to-date, but we also included seminal and classic articles that established fundamental concepts in the field, regardless of the year. We only considered articles available in full and in English or Portuguese. We excluded conference papers (abstracts or proceedings without full publication), non-peer-reviewed preprints, studies focused exclusively on heterochromatin in non-plant organisms, articles not directly related to the central themes of the review, and duplicate publications or those not available in full text.

The article selection process took place in multiple stages: an initial screening by reading titles and abstracts to identify relevance, followed by a complete reading of the pre-selected articles for an in-depth evaluation. Relevant information from each selected article was extracted and organized, covering main concepts, structural and molecular characteristics, biological functions, epigenetic regulation mechanisms, gaps in knowledge and future research directions.

The data was synthesized thematically, grouping information by similarity and relevance to the topics of the review (structure, function and regulation). We used a critical approach to compare findings, identify consensus, controversies and trends in research, contextualizing the knowledge extracted to build a coherent and up-to-date narrative on heterochromatin in plants. As this is a literature review study, based exclusively on published information, this research is exempt from evaluation by Ethics Committees.

LITERATURE REVIEW

HETEROCHROMATIN STRUCTURE

Heterochromatin in plants represents one of the most compacted and least accessible forms of chromatin, in contrast to euchromatin, which is more open and conducive to transcription. This structural organization is not static, but rather a dynamic state maintained by a complex network of molecular interactions and epigenetic modifications (Barros e Silva & Guerra, 2010; Allshire & Madhani, 2018; Du et al., 2015). Understanding its structure is fundamental to unraveling how it performs its functions of silencing and maintaining genomic stability.

The basis of the structure of heterochromatin, as well as all chromatin, lies in the fundamental unit of the nucleosome. The DNA is wrapped in approximately 1.65 turns around an octamer of histone proteins (two copies of H2A, H2B, H3 and H4), forming a nucleosome particle (Kornberg & Lorch, 1999). In heterochromatin, these nucleosomes are packed more densely, resulting in higher-order structures. Although the traditional model of the 30 nm chromatin fiber has been widely accepted for decades, recent studies question its existence in vivo. Research indicates that in both active and repressed regions of the genome, chromatin is composed of densely packed 10 nm fibers, with no clear evidence of the formation of regular 30 nm structures. Despite these uncertainties, it is widely recognized that heterochromatin organizes itself into highly compacted domains, restricting access to DNA and playing a crucial role in genomic stability and the regulation of gene expression. Marcelo Guerra (2000) described these structural characteristics of heterochromatin in detail, highlighting its importance in the chromosomal organization of plants. In addition, the molecular composition of heterochromatin is defined by a specific set of epigenetic modifications and associated proteins, such as histone methylation and the presence of HP1 proteins, which contribute to its maintenance and functionality

Histone modifications play a central role in coding heterochromatic identity. Trimethylation of lysine 9 of histone H3 (H3K9me3) is a classic and conserved marker of constitutive heterochromatin, establishing a "histone code" that recruits specific binding proteins and promotes condensation (Zhang et al., 2018; Du et al., 2015). Another important repressive mark is the methylation of lysine 27 of histone H3 (H3K27me3), often associated with facultative heterochromatin and developmental gene silencing (Zhang et al., 2007; Köhler & Hennig, 2010). Histone acetylation, on the other hand, is typically excluded from heterochromatic regions, and deacetylation by histone deacetylases (HDACs) contributes to the condensed state of chromatin (Earley et al., 2007).

DNA methylation is one of the structural pillars of heterochromatin in plants, showing greater diversity than in animals. In plants, methylation can occur in all three possible cytosine configurations: **CG**, **CHG** and **CHH** (where H = A, C or T) (Law & Jacobsen, 2010; Zemach et al., 2010).

CG methylation is mainly maintained by DNA methyltransferase **MET1**, a homologue of mammalian DNMT1, ensuring the inhe-

ritance of methylation marks during DNA replication (Du et al., 2015). **Methylation in CHG**, on the other hand, is established and maintained by **CMT3 (Chromomethylase 3)**, and this maintenance is often aided by histone methylation **H3K9me2**, establishing a mutually reinforcing cycle between DNA methylation and histone modifications (Du et al., 2015).

Methylation in CHH is often mediated by the RNA-directed DNA methylation (RdDM) pathway. This pathway is a crucial mechanism in plants that involves small RNAs (siRNAs) and RNA polymerases IV (Pol IV) and V (Pol V) to direct the methylation and silencing of transposable elements (TEs) and repetitive regions (Matzke & Mosher, 2014; Onodera et al., 2005). More recent studies have detailed the intricate network of molecular interactions in the RdDM pathway, including the function of several accessory proteins that ensure the specificity and efficiency of the process (Yang et al., 2021).

These methylation marks are recognized by binding proteins, such as MBD (Methyl--CpG Binding Domain) proteins, which are able to bind specifically to methylated DNA regions. The binding of these proteins, together with other chromatin modulating proteins, promotes condensation and gene silencing, contributing to genome integrity and the regulation of gene expression in plants (Du et al., 2015; Zhang et al., 2024). Recent advances have focused on understanding the dynamic interactions between MBD proteins and other epigenetic modifications, revealing how they orchestrate the formation and maintenance of heterochromatin (Zhang et al., 2024).

In addition to epigenetic modifications, several non-histone proteins are essential components of the heterochromatic structure. **HP1-type** proteins (Heterochromatin Protein 1), characterized by their *chromo* domains, bind specifically to H3K9me. They act as "readers" of this repressive mark, which leads to chromatin compaction and the formation of heterochromatic domains (Richards & Elgin, 2002; Reuter & Jenuwein, 2010).

In plants, proteins such as VIM (Variant in Methylation) also play important roles in maintaining DNA methylation and, consequently, heterochromatin structure (Woo et al., 2008). Complexes such as Polycomb Repressive Complex 2 (PRC2), which catalyzes the methylation of H3K27, are crucial in the formation of facultative heterochromatin, regulating developmental genes and response to stresses (Kaufmann et al., 2009; Mozgova & Hennig, 2015).

Heterochromatin is not uniform in its structure and function, and is generally divided into constitutive heterochromatin and facultative heterochromatin. Constitutive heterochromatin, typically found in centromeres, telomeres and pericentromeric regions, is permanently condensed and rich in repetitive sequences and TEs, and is marked by H3K9me2/3 and robust DNA methylation in all cytosine sequences (CG, CHG, CHH). Facultative, on the other hand, can switch between condensed and open states in response to developmental or environmental signals, and is often marked by H3K27me3 and associated with reversible gene silencing (Feng & Jacobsen, 2011; Mirouze & Paszkowski, 2011).

Thus, the nuclear location of heterochromatin contributes to its structure and function. In many eukaryotes, including plants, heterochromatin is often associated with the nuclear periphery and the nuclear lamina (in animals), or with analogous structures in plants, such as the nuclear matrix. This anchoring may contribute to its condensed state and to the functional compartmentalization of the genome, although the exact mechanisms of this spatial organization in plant nuclei are still under investigation (Fransz & de Jong, 2002; Köhler & Hennig, 2010. Thus, the struc-

ture of heterochromatin in plants is the result of a complex interaction between the organization of nucleosomes, the profile of epigenetic modifications in DNA and histones, and the action of various proteins and complexes that promote their condensation and stability

FUNCTION OF HETEROCHROMATIN

Heterochromatin, traditionally recognized for its condensed state and its association with gene silencing, performs a multifaceted range of crucial functions for plant biology. Far from being an inert region of the genome, it is a dynamic regulatory center, essential for genomic integrity, development and the adaptation of plants to the environment.

One of the best-characterized functions of heterochromatin is to silence and restrict the mobility of transposable elements (TEs) and other repetitive sequences (Slotkin & Martienssen, 2007). TEs, also known as "jumping genes", are DNA sequences capable of moving and replicating within the genome. Their unregulated activity can cause mutations, chromosomal structural alterations and genomic instability, and is a constant threat to the integrity of the genome (Bennetzen, 2000). Heterochromatin acts as a physical and epigenetic barrier, tightly packing these sequences and preventing their transcription and transposition. In plants, this is strongly mediated by DNA methylation in all its forms (CG, CHG, CHH) and by H3K9 methylation, often established by the RdDM (RNA-directed DNA Methylation) pathway (Law & Jacobsen, 2010; Matzke & Mosher, 2014). This mechanism is vital for maintaining genomic stability, especially in large, repeat-rich genomes such as those of many plant species (Schnable et al., 2009).

Heterochromatin is essential for maintaining the stability and integrity of chromosomes, particularly in the centromere and telomere regions. In centromeres, pericentromeric heterochromatin (flanking the centromere itself) is crucial for the formation of the kinetochore, a protein structure that allows chromosomes to segregate correctly during cell division (mitosis and meiosis) (Jiang et al., 2003; Allshire & Madhani, 2018). Defects in centromeric heterochromatin can lead to aneuploidy and chromosome loss. In telomeres, the ends of chromosomes, heterochromatin acts to protect DNA from degradation and chromosome fusion, essential functions for maintaining genomic integrity (Shay & Wright, 2000).

Although heterochromatin is mostly associated with silencing, its influence on the regulation of gene expression is more nuanced and complex. It can silence genes transcribing directly inside it, but it can also influence the expression of genes located in its vicinity, through a phenomenon known as the "position effect" (Elgin & Reuter, 2013). In addition, facultative heterochromatin, controlled by Polycomb complexes (PRCs) and marked by H3K27me3, plays a critical role in regulating genes involved in plant development processes (such as flowering, embryogenesis and organ formation), as well as in cell differentiation (Mozgova & Hennig, 2015; Köhler & Hennig, 2010). The reversible dynamics of facultative heterochromatin allow genes to be activated or silenced at specific moments in development or in response to stimuli, conferring regulatory plasticity.

An increasingly interesting function of heterochromatin in plants is its participation in the response to environmental stresses (abiotic and biotic). Heterochromatin structure can be rapidly modulated in response to conditions such as drought, salinity, extreme temperatures and pathogen attack (Mirouze & Paszkowski, 2011; Sani et al., 2013). These epigenetic changes can lead to the transient activation of TEs, generating variability that can be adaptive in stressful environments, or

to the silencing of genes that could be harmful under certain conditions (Pérez-Figueroa et al., 2021). Heterochromatin plasticity, mediated by epigenetic modifications, allows plants to adjust their gene expression profile to optimize survival and resilience in challenging environments

EPIGENETIC REGULATION

The formation and maintenance of heterochromatin in plants are processes intrinsically linked to a complex network of **epigenetic regulation** mechanisms. These modifications, which alter the accessibility of chromatin without changing the underlying DNA sequence, ensure the stable silencing of unwanted genomic sequences and the modulation of gene expression in response to various stimuli. The orchestration of heterochromatin involves DNA, histones and the proteins that modify and read them, in a highly interconnected system (Law & Jacobsen, 2010; Matzke & Mosher, 2014).

DNA methylation is a pillar of heterochromatic identity in plants, occurring in three cytosine contexts (CG, CHG and CHH) and being maintained by different classes of DNA methyltransferases. CG methylation is mainly maintained by *METHYLTRANSFERASE 1* (MET1), which inherits the methylation pattern during DNA replication, ensuring that the marks are passed on to daughter cells (Zemach et al., 2010). This pattern is crucial for the stability of many heterochromatic loci. CHG methylation is mediated by chromomethyltransferase 3 (CMT3). CMT3 activity is interdependent with H3K9 histone methylation, creating a positive *feedback* where H3K9 methylation recruits CMT3 and CHG methylation, in turn, can facilitate H3K9 methylation (Du et al., 2015; Jackson et al., 2002). Finally, CHH methylation is primarily established and maintained by the RNA-directed DNA methylation (RdDM) pathway, a unique and highly characteristic mechanism in plants (Law & Jacobsen, 2010; Matzke & Mosher, 2014). RdDM involves multiple steps and components: RNA polymerase IV (Pol IV) transcribes transposable elements (TEs) and repeats into precursor RNAs, which are then processed into small interference RNAs (siRNAs) of 24 nucleotides by RNA- dependent RNA polymerase 2 (RDR2) and Dicer-like 3 (DCL3) (Matzke & Mosher, 2014; Onodera et al., 2005). These siRNAs are loaded by ARGONAUTE proteins (mainly AGO4) and guide RNA polymerase V (Pol V) to transcribe the target region. Pol V transcripts interact with the RdDM complex, including the DNA methyltransferase DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2), which establishes *de novo* methylation at CHH, in addition to methylating CG and CHG (Zemach et al., 2010).

Post-translational modifications of histones are crucial for heterochromatin architecture and interact synergistically with DNA methylation. Trimethylation of lysine 9 of histone H3 (H3K9me) is a repressive marker that is established by histone lysine methyltransferases (KMTs), such as KRYPTONITE (KYP)/SUVH4, SUVH5 and SUVH6 (Jackson et al., 2002; Du et al., 2015). These methyltransferases contain methylated DNA reading domains, which allows a close interaction between DNA methylation and histone methylation, creating a *feedback* loop that reinforces silencing. Another important modification is the methylation of lysine 27 of histone H3 (H3K27me3), the main mark associated with facultative heterochromatin, catalyzed by the Polycomb Repressive Complex 2 (PRC2) (Mozgova & Hennig, 2015). Although less directly linked to TE silencing than H3K9me, H3K27me3 is vital for the regulation of developmental genes in response to internal and environmental signals, allowing the transition between active and inactive chromatin states

(Köhler & Hennig, 2010). In addition, histone deacetylation, carried out by histone deacetylases (HDACs), promotes chromatin compaction, contributing to the condensed state of heterochromatin (Earley et al., 2007).

In addition to modifying enzymes, ATP--dependent chromatin remodelers and epigenetic mark reader proteins are essential for regulating heterochromatin. Remodelers such as DDM1 (*DECREASE IN DNA METHYLATION 1*) act by altering the position and composition of nucleosomes, facilitating access for DNA methyltransferases and histone modification enzymes, or promoting chromatin condensation itself (Zemach et al., 2017). Reader proteins, such as chromo domain proteins (e.g. HP1 proteins in animals and their counterparts in plants) and MBD domain proteins, recognize and bind to histone and DNA methylation marks, respectively, recruiting other proteins and complexes that reinforce the heterochromatic state and silencing (Richards & Elgin, 2002; Woo et al., 2008).

Heterochromatin regulation is characterized by extensive interaction and *crosstalk* between different epigenetic pathways. DNA methylation and histone modifications do not act in isolation, but rather in an interconnected *feedback* system, where one mark can recruit or be established in conjunction with another (Du et al., 2015). For example, the RdDM pathway not only establishes DNA methylation at CHH, but can also influence H3K9 methylation and Pol V-mediated transcription. This complexity endows heterochromatin with remarkable plasticity, allowing its state to be dynamically adjusted in response to developmental signals and, crucially, environmental stresses (Mirouze & Paszkowski, 2011; Sani et al., 2013). Plants can rapidly modulate these epigenetic patterns to activate or silence stress response genes, adjusting their physiology and resilience to adverse conditions (Pérez-Figueroa et al., 2021; Springer & Schmitz, 2017).

In short, the epigenetic regulation of heterochromatin in plants is a highly intricate and multifaceted process, involving the precise coordination of DNA methylation, histone modifications, small RNAs, chromatin remodelers and reader proteins. This regulatory network not only maintains genomic integrity and the silencing of selfish elements, but also gives the plant genome the adaptive flexibility essential for survival in dynamic and challenging environments.

RESULTS AND DISCUSSION

Heterochromatin, for a long time relegated to the role of "junk DNA" or mere genome compactor, has emerged in recent decades of research as a dynamic and multifaceted genomic component with crucial functions in plant biology. The intricate interconnection between its **highly condensed structure**, its **diverse** biological functions and the precise epigenetic regulation that governs it, as detailed in this review, highlights its fundamental importance for the stability and plasticity of plant genomes.

The organization of heterochromatin, mediated by densely packed nucleosomes and the interaction with specific proteins and RNAs (Allshire & Madhani, 2018; Du et al., 2015), is the basis of its functionality. Histone modifications (such as H3K9me and H3K27me) and DNA methylation (in CG, CHG and CHH contexts), acting together, form an epigenetic code that not only marks heterochromatic regions, but also recruits the molecular machinery needed to maintain their condensed and silent state (Law & Jacobsen, 2010; Zemach et al., 2010). This structural complexity is particularly critical in plants, whose genomes are often vast and full of transposable elements (TEs) and repetitive sequences (Bennetzen, 2000; Schnable et al., 2009). Heterochromatin acts as the sentinel of the genome, silencing these mobile elements and preventing genomic instability that would be catastrophic for the cell (Slotkin & Martienssen, 2007).

In addition to silencing TEs, the functions of heterochromatin in plants extend to maintaining chromosome stability at centromeres and telomeres (Jiang et al., 2003; Shay & Wright, 2000), and to a more subtle but vital role in regulating gene expression and development. Facultative heterochromatin, controlled by Polycomb complexes (PRCs) and marked by H3K27me3, illustrates the plasticity of the system, allowing reversible silencing of key developmental genes at specific moments in the plant's life cycle (Mozgova & Hennig, 2015). This duality - of inflexible guardian of the genome and adaptable modulator of gene expression - is a defining characteristic of plant heterochromatin.

The epigenetic regulation of heterochromatin is a highly dynamic process, where DNA methylation pathways, histone modifications and small RNAs (sRNAs) interconnect in a sophisticated *feedback* network. The RdDM (RNA-directed DNA methylation) pathway is a prominent example of this interconnection and a distinctive feature of plants, where sRNAs guide the methylation machinery to specific loci, establishing *de novo* methylation and silencing (Matzke & Mosher, 2014; Onodera et al., 2005). This intricate molecular orchestration not only ensures the inheritance of heterochromatic patterns across cell divisions, but also gives the genome the flexibility to respond to environmental stresses (Mirouze & Paszkowski, 2011; Pérez-Figueroa et al., 2021). The ability of plants to adjust their heterochromatic patterns in response to conditions such as drought or salinity demonstrates how epigenetics is a crucial link between the genome and the environment, enabling rapid adaptations and enhancing resilience (Sani et al., 2013).

Despite substantial advances in the understanding of heterochromatin in plants, especially with the use of model organisms such as *Arabidopsis thaliana* and next-generation sequencing technologies (Du et al., 2015; Zemach et al., 2010), there are still significant gaps in knowledge. High-resolution three-dimensional models of heterochromatin organization *in vivo* and in different cell types are still being refined (Roudier et al., 2011). The exact dynamics of how heterochromatin "opens" or "closes" in response to subtle stimuli and the role of less studied histone variants in these processes are areas of active research (Zhang et al., 2018). In addition, fully understanding the interaction between genotype and environment in the epigenetic modulation of heterochromatin, and the heritability of epigenetic traits in subsequent generations, are open challenges with major implications.

In-depth knowledge of heterochromatin and its epigenetic regulation in plants opens promising doors for biotechnological applications and crop improvement. The ability to precisely manipulate the state of chromatin offers the opportunity to silence undesirable genes (for example, genes that confer susceptibility to diseases), stabilize genomes of polyploid species (common in agriculture) or activate latent genes to confer new traits, such as greater productivity or tolerance to stresses (Lusser et al., 2011; Springer & Schmitz, 2017). "Epigenetic engineering" has emerged as a powerful tool for developing more resilient and productive crops, contributing to global food security in a climate change scenario (IPCC, 2023).

Heterochromatin in plants represents much more than condensed genomic regions; it is a vital epigenetic command center that guarantees genome integrity and confers adaptive plasticity.

CONCLUSIONS

Heterochromatin in plants is a highly condensed and dynamically regulated chromatin structure, showing specific DNA methylation patterns (CG, CHG, CHH) and a characteristic profile of histone modifications (notably H3K9me and H3K27me), which are essential for its identity and stability.

The functions of heterochromatin in plants are complex and crucial for genomic integrity and plant adaptation.

Heterochromatin regulation is orchestrated by a highly interconnected epigenetic network with great plasticity, such as the RNA-directed DNA methylation (RdDM) pathway, the action of Polycomb complexes and the coordinated activity of chromatin remodelers and reader proteins interacting in continuous *feedback*.

Advances in knowledge about heterochromatin and its epigenetic regulation in plants have profound implications for basic and applied environmental biology.

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