



Epigenomic Insights for Crop Improvement- Bridging Genotype and Phenotype Gaps: A Review

Samilla Sheershika¹, G. Prasanna¹, Gopal Narkhede¹, Deepak Kumar², Rajneesh Kumar³

10.18805/IJArE.A-6515

ABSTRACT

Epigenomics has already become the disruptive methodology in crop science nowadays, which presents new possibilities to fill the gap between genotype and phenotype that had a long history of its existence. In addition to small RNA mediated mechanisms, histone modifications and DNA methylation, epigenomics has an effect on gene expression that does not result in a change in the underlying DNA sequence to create heritable and reversible phenotypic variation. It grew to its current state as a result of a series of major historic findings that have influenced our perception of the concept of gene regulation. The groundwork of the field was set with the innovative ideas like the epigenetic landscape introduced by Waddington who conceptualized the idea of gene regulation through developmental pathways. As time went by, epialleles had been discovered and epigenetic recombinant inbred lines (*epiRILs*) were established, this indicated that epigenetic variation could have a great impact on agronomic traits, including yield potential, stress resilience and developmental flexibility. Recent technological breakthroughs in high-resolution technologies including bisulfite sequencing and ATAC-seq, ChIP-seq and single-cell epigenomics have facilitated the extensive mapping of crop regulatory landscapes and this has identified new loci in relation to complex phenotypes. New technologies, including reprogramming using MSH1 and paramutations, have the potential to narrow-tune gene expression in order to modify crops. Combining epigenomic knowledge with models of breeding genomic prediction improves the precision of breeding by improving the elements of missing heritability. However, there are still the problems of epigenetics, environmental impact and adaptation to polyploid plants that are still a major issue. Epigenomics provides a powerful framework for developing resilient and high yielding crop varieties suited to future climate demands.

Key words: CRISPR/dCas, Crop improvement, DNA methylation, Epialleles, Epigenome editing, Epigenomics, Histone modification, Small RNA.

The fundamental concept of crop enhancement is trying to understand how genetic information can be expressed in plants. The focus on classical breeding is largely on the differences in DNA, SNP, insertions, deletions and large-scale changes, as they lead to different appearances and a different outcome (Ahmar *et al.*, 2020). But it is not the characteristics just being hard coded within the DNA complex of a plant. Plants do not move, they have to adjust to the surrounding and they can do so without breaking or altering their genes permanently. The epigenetic tricks that enable genes to be turned on and off *via* a reversible process of changes to DNA and histone proteins provide that flexibility (Seem *et al.*, 2024). About these levels, looking at them allows us to fill the gap between what the data says and what you really read, particularly when an inherited trait cannot be solely explained as a result of genetics.

This grand change thing the epigenetic research the way it thinks of Waddington, but now in a connection with concrete stuff such as DNA methylation, histone finger fiddling and small RNAs, have altered the perception of inherited variation in plants. The initial research on maize paramutation and *Arabidopsis epialleles* indicated that inheritance does not necessarily require a change in the DNA but may occur with significant phenotypes (Tripathi *et al.*, 2024). MTS moved the discipline to the modern era of epigenomics as it enabled researchers to map chromatin condition and controlling elements across the genome.

¹Department of Genetics and Plant Breeding, School of Agriculture, SR University, Warangal-506 371, Telangana, India.

²Department of Genetics and Plant Breeding, School of Agricultural Sciences, K.R. Mangalam University, Gurugram-122 103, Haryana, India.

³Division of Genetics and Plant Breeding, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Wadura-193 201, Jammu and Kashmir, India.

Corresponding Authors: Gopal Narkhede, Rajneesh Kumar; Department of Genetics and Plant Breeding, School of Agriculture, SR University, Warangal-506 371, Telangana, India; Division of Genetics and Plant Breeding, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Wadura-193 201, Jammu and Kashmir, India.

Email: n.gopalwasudeo@sru.edu.in; rajneesh_phd@skuastkashmir.ac.in

How to cite this article: Sheershika, S., Prasanna, G., Narkhede, G., Kumar, D. and Kumar, R. (2026). Epigenomic Insights for Crop Improvement- Bridging Genotype and Phenotype Gaps: A Review. *Indian Journal of Agricultural Research*. **60(9)**: 1318-1327. doi: 10.18805/IJArE.A-6515.

Submitted: 27-01-2026 **Accepted:** 24-03-2026 **Online:** 07-04-2026

Recently we have heard that the genome is under tight regulation by carefully regulated epigenetic marks to maintain its stability and regulate gene activity. These are some of the reasons opposed to epigenetic engineering and as a result, critics highlight such studies as the MSH 1

(Yang *et al.*, 2020) which demonstrated that the epigenetic reprogramming could be regularly transmitted and applied to breeding, which is evidence of the potential of the field to make new and hereditary varieties (Varotto *et al.*, 2020).

Nowadays, individuals believe that epigenomics belongs to crop science and have discovered certain genome segments that influence how the crops respond to drought, salinity and flowering periods. In whole-methylome studies of maize and rice, regions that vary in their state of methylation and are also connected to growth and stress resistance were identified. Conventional DNA markers are deprived of these alterations. The better news is that the new tech has the ability to modify the epigenetic conditions. CRISPR epigenome editing enables scientists to activate or silence genes by directing enzymes at them, modifying gene activity (Fal *et al.*, 2025), but not the DNA sequence itself, an adaptable means of enhancing crop characteristics (Khan and Shahwar, 2023). The process may assist crops to cope better with climatic adversities by means of a process known as epigenetic priming that pre-programs a plant to react to stress. There is also an insight provided about the control of complex genomes such as wheat, cotton and canola through Epigenomics (Furci *et al.*, 2023). Such epigenetic modifications assist in equalizing various versions of genes, establish dominant traits and enhance the hybrid performance. This new form of hybrid the researchers believe forms a new form of hybrid advantage besides historical crossbreeding. Epigenomic data are now being combined with genomic selection by scientists. Conventional methods of selection utilize DNA differences alone, however, by incorporating methylation patterns and DNA openness, it is now possible to target a better prediction of environment-driven traits, such as in rice and maize (Behrens *et al.*, 2025).

Combined with multi-layer biological datasets (genomics, transcriptomics, epigenomics, proteomics and metabolomics) and advanced machine learning (ML) based predictive modelling such as deep neural networks, graph neural networks (GNNs), transformer-based sequence models and ensemble learning approaches for gene expression prediction and regulatory network inference new long-read and single-cell sequencing technologies enable scientists to comprehensively resolve gene regulation across diverse developmental stages, environmental conditions and stress responses. On big crops such as rice, maize and tomatoes, using epigenome editing allows researchers to transcript highly concentrative, inheritable manipulation that is so substantial that it possibly contrasts the ancient mutagenic or transgenic breeding (Gogolev *et al.*, 2021). This provides an alternative to non-GM crops that provide high, drought resistant and efficient use. Research on epigenetic memory could also provide the plant with a memory of previous stress and offer them a better reaction the next time around. To put it briefly, there is something novel and refreshing about using the epigenomics information combined with the traditional

and molecular breeding in order to forge the modern agriculture. Discovering reversible, hereditary modifications that dictate the traits, epigenomics bridges the gap between DNA and the real world and promises the following generation of high-performers, strong-powered crops designed down to the ground.

Core molecular mechanisms underlying plant epigenomic regulation

The epigenome is a malleable coating that the gene regulated by without any DNA modifications. This system aids plants to adapt to various environments, adjust to stress and enhance yield in crops. It relates the genetic composition of the plant to its appearance and its operation (Han *et al.*, 2022). The key pathways that are affected are DNA methylation, histones modifications, chromatin structure and small RNA. These systems co-operate to regulate the control of gene activity, ensure the stability of the genome and regulate the expression of genes in a manner that is hereditary. DNA methylation can repress genes, silence elements of the genes such as transposons and regulate the expression of genes that can be passed down heritability (Talarico *et al.*, 2024). In plants, stress has the capacity to induce stress-related modifications in methylation that is likely to assist the next generations in future stress management, which is named epigenetic memory. Histone changes are also implicated, where the marks assist to open up DNA to allow it to be active and some are made more compact and inactive. Small RNAs assist with regulating gene expression once messenger RNA has been produced and also with DNA methylation that helps maintain the genome in plants with high levels of repetitive DNA. These processes enable the regulatory proteins, enhancers and promoters to communicate in a growth as well as under adverse conditions that the plant faces. Recent technological developments have enabled the study of all these layers simultaneously. Different technologies, including scATAC-seq, Hi-C, long-read sequencing of DNA-methylation and cut and tag can assist researchers in identifying regions of gene control, stress response areas and consistent modification of the DNA that can be used to enhance crops, including techniques such as dCas9-TET and dCas9-KRAB. With the intensification of climate change-induced stresses, epigenomic profiling provides a robust framework to uncover cryptic regulatory variation that conventional breeding and disease-resistance screening approaches may fail to detect. High-resolution whole-genome bisulfite sequencing (WGBS) for DNA methylation mapping, ATAC-seq for chromatin accessibility profiling, small RNA sequencing for miRNA and siRNA network characterization and ChIP-seq for histone modification analysis (e.g., H3K4me3, H3K27me3) collectively enable precise delineation of stress-responsive regulatory landscapes. The integration of these multi-layer epigenomic datasets facilitates mechanistic understanding of how plants perceive abiotic and biotic cues, modulate transcriptional programs and

establish adaptive or transgenerational stress memory. The integration of maps of methylation, chromatin access, small RNA networks and histone changes can inform scientists to understand how plants sense and respond to their environment. This combined method accelerates the production of crops which are more resistant and productive to satisfy future agriculture demands (Jabeen *et al.*, 2026).

DNA methylation: A stable yet flexible epigenetic system

A highly prevalent genre of control change in plants is DNA methylation and assists in the development of the expression of genes (Liu and He, 2020). In contrast to animals, plants are able to introduce methyl groups to cytosines at CHG and CHH repetitions not only at the CG position, but also at the CH location, where H may be A, T or C. This wider system assists plants in regulatory a variety of activities particularly in defending their genome and fitment to the pressure which counts in complicated crop genomes. During the occurrence of methylation at the site prior to the gene, the entrance of the transcription factors may become impossible and making it difficult to read the gene, the gene may be silenced. On the flip side the presence of some CG methylation within genes is also commonly associated with constantly active genes such as the ones required to help the body perform simple tasks. Under such circumstances, methylation may be useful to maintain the gene expression rather than inhibiting it. The main aspect of plant genomes, particularly with a large number of mobile genetic elements such as in maize, wheat and sugarcane is the fact that methylation has a significant role in the regulation of these elements (Kumar *et al.*, 2024). But other activities such as environmental stress, growing plants in a laboratory and mixing genomes may alter the methylations of these factors and discover new methods of regulating genes and influencing plant traits. As an illustration, responses of stress to methylation have been linked to drought resistance in rice and adaptations of methylation have aided in making soybean plants superior in artificial hybrid lines (Kaya *et al.*, 2024). Notably, some patterns of methylation are capable of remaining by generating gametes with resultant changes in the expression of the genes hereditary without being related to real alterations in the DNA. One example of this is the FWA gene of Arabidopsis, whereby the methylation can be used as a method to provide new stable variation of a plant characteristic. The results inform the development of new breeding techniques such as epigenetic selection and epi mutagenesis the foundational one on which individuals can modify the DNA in a regulated fashion as a matter of developing useful phenotypes without altering the actual bases (Yang *et al.*, 2025).

Histone modifications and chromatin remodelling: Establishing and reverse gene expression

The DNA in plant cells is coiled tightly around histone proteins and any modification of the tail of such proteins is used to regulate the engagement of genes into or out of

activity. Major epigenetic layers, their functions and crop examples tabulated in Table 1. The events that are known as post-translational changes have an influence on the DNA packaging and this response determines either which portions of the genome is active or not. Others notable histone modifications are as follows: -H3K27me3: This is a developmental gene silencing mark, which takes part in the stature off particular genes. H3K9me2: These involve in the areas of DNA that do not vary to a large extent that enable the keeping of transposable regions at check-up points. H3K4me3 and H3K36me3: These are marks that occur on active genes in the cell that is being read and utilized. Histone acetylation, such as H3K27ac: These changes allow the DNA to be more accessible most so that genes are expressed. These modifications allow the introduction of special proteins that can move, remove, or rearrange the DNA stitching around histones. The process alters the composition of DNA rendering it easier or more difficult to utilize genes depending upon the requirements of the cell. The control that is based on histones is particularly significant in the properties of crops. As an example, the H3K27me3 assists the regulation of plant responses to cold in a process known as vernalization in wheat and barley. The H3K9me2 insulates the genome in maize to prevent the undesirable changes in DNA. Even more than DNA packaging histone modifications to environmental changes, special forms of histones such as H2A.Z are also important to the heat perception and drought responses of plants. It is a complicated mechanism that receives cues on the environment, the development level of the plant and mechanisms that maintain the genome stable. The combination of these changes in a network shapes the availability of DNA, its ability to be utilized in the synthesis of proteins and even produces long-term memories as epigenetic alterations that impact the growth and development of plants under stress.

Expanded dimensions of histone modifications

Histone deacetylation involving HDAC where, the histone deacetylases remove acetyl groups attached to specific amino acids on histones and result in the chromatin becoming smaller, preventing the gene expression. As an example, OsHDA710 HDAC gene regulates grain size in rice, eliminating acetyl groups of target regions associated with grain related growth in genes. Here is an example of how histone deacetylation at the chromatin level serves as a switch to regulate characteristics that influence crop yield (Bai and Jin, 2024). Histone Ubiquitination (H2Bub1) Promoting gene expression and plant flexibility. In *Arabidopsis*, the HUB1 and HUB2 enzymes influence the seed dormancy and resistance to the disease by modifying the amount of H2Bub1. This demonstrates the significance of H2Bub1 in plant development as well as in their defences. Plants also regulate the chromatin by replacing the normal histones with altered versions such as: H2A.Z, H3.3 and one specifically found in plants called H2AW. Examples: The presence of H2A.Z in the correct locations is required

to make the plant remember heat stresses experienced in the past and respond rapidly to heat events in maize and Arabidopsis. H2A.W variant H2A.W makes dense chromatin that suppresses the activation of mobile genetic elements and maintains the stability of the genome. As examples: H3K27me3 and H2Aub collaborate in the polycomb system to maintain some genes in a non-active state in early development (Bar-Oz and Schmidt, 2025). H3K9me2 is part of a collaboration with DNA methylation to ensure that the transposable elements do not move throughout development in the crops within rice and maize and thereby to keep the genome intact such that those elements that are useful to inherit are retained and inherited in the reliable way (Xie *et al.*, 2025).

Chromatin remodelling complexes: Central players in gene regulation

SWI/SNF complex, or the BRAHMA complex, is an ATP-based chromatin remodeler that assists in activating genes that are situated beneath it. It also participates in the physical positioning and/or deposition of nucleosomes to allow transcription to occur, *e.g.* in tomatoes, the SWI/SNF complex plays a role in controlling genes linked to fruit-fruitletting to regulate the texture and quality of the tomato fruit. The INO80/ISWI complexes are known to be involved in

repairing DNA damage under stress conditions and maintaining the stability of the genome.

Mechanistic workflow: How histone modifications regulate plant genes

Multi-layered histone signaling: From environmental cues to gene expression shown in Fig 1.

Step 1: Single perception the plant receives external or internal signals, such as cold, drought, day length, growth stage, *etc.*

Step 2: Recruitment of regulatory enzymes-Writers (such as methyltransferases and acetyltransferases) do add some marks. -These marks-Erasers (HDACs and demethylases) remove them. The marks are read and deciphered by the readers (as are the bromo-chromodomain proteins).

Step 3: Chromatin Remodeling Groups of proteins including SWI/SNF, CHD and INO80 relocate the nucleosomes around and this is what determines: -Open chromatin, meaning: the genes are now active. Gates closed that is, genes are inactive.

Step 4: Epigenetic memory formation some of these stable marks, *e.g.* H3K27me3 and H3K9me2, maintain the genes in an off state and assist the plant to

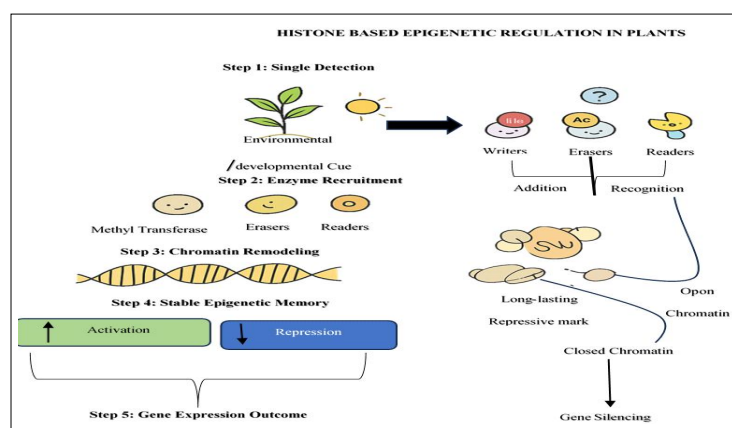


Fig 1: Multi-layered histone signaling: From environmental cues to gene expression.

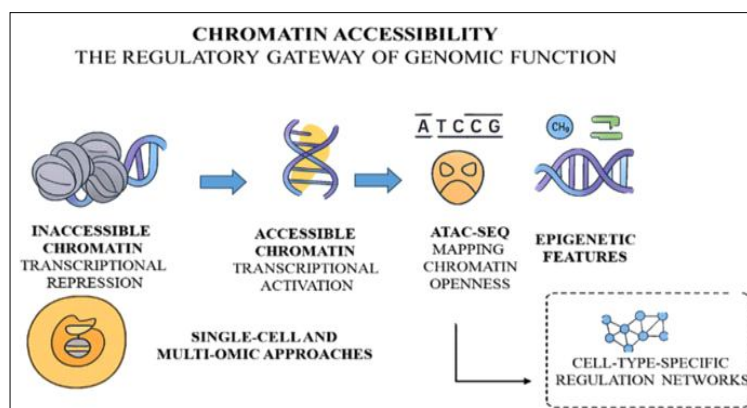


Fig 2: Chromatin accessibility.

maintain its constant response to development and to remember its response to stress.

Step 5: Gene Expression Output-Active marks result in the production of proteins, which manifest as traits visible to us. Repressive marks maintain the genes in the inactive state and safeguard the plant against the stress, unwanted processes and also regulate transposable elements.

Small RNA pathways and RdDM: Directing epigenetic marks with sequence precision

Small RNAs, especially siRNAs and miRNAs, are another important part of how plants control their genes through epigenetic means. These small RNAs help guide enzymes that add methyl groups to DNA, a process called RNA-directed DNA methylation. Small interfering RNAs produced by transposable elements or repetitive elements of the genome, in this process, target proteins called ARGONAUTE proteins and certain plant RNA polymerases, Pol IV and Pol V, which subsequently catalyse the addition of methyl groups to DNA at specific loci and operate to protect the genome by making transposable elements transcriptionally silent and fortifying the structure of repetitive sequences. As such, this system is a protective mechanism of undesirable genetic mutations. However, these small RNAs have more functions beyond the stability of the genome. They also regulate the growth of plants, mediate the responses of nutrients and the way the plants deal with environmental and biotic stresses.

An obvious example of this system can be observed in maize, in which one gene silences another gene, however, the DNA sequence of the latter does not change. This effect is indicative of the fact that phenotypic characters are capable of being passed on in the manner in which they constitute one of the forms of epigenetic inheritance, thus preserving the original genetic code. These findings dispute the traditional rules of genetics and have potential to develop new methods of improving crops through epigenetic interaction among genes. Moreover, plants produce particular groups of small RNAs when they are exposed to stressors (high temperatures, salinity or disease). These RNAs stay in the plant and make them to be more responsive to the further events of the same stress. These stress-memory small RNAs are now being studied by researchers to be used as an addition to crop resilience through epigenetics (Xue *et al.*, 2025).

Chromatin accessibility: Functional interface of regulatory potential

Chromatin accessibility (Fig 2) refers to the general receptiveness of a slice of DNA, *i.e.*, whether the DNA is receptive to such processes as transcription factors and polymerases to attach to the otherwise the DNA is conceived with barriers. When open, the chromatin is typical at the promoter region of a gene, enhancers and other control spots and assists the gene in being turned on. When the chromatin is negative it is typically referred to as heterochromatin and it keeps the genes closed.

Table 1: Major epigenetic layers, their functions and crop examples.

Epigenetic layer	Primary role	Mechanisms	Examples in crops	Outcome/trait influenced	References
DNA methylation (CG, CHG, CHH)	Gene silencing, transposon control, imprinting	RdDM pathway, de/methyltransferases	Rice: Drought-induced methylation changes; Maize: TE methylation maintaining genome stability	Stress memory, improved drought tolerance, stable trait inheritance	Li <i>et al.</i> (2022)
Histone modifications	Activation or repression of transcription	H3K4me3, H3K9ac (active); H3K27me3 (repressive)	Wheat: H3K27me3 regulates vernalization; Tomato: Histone acetylation enhances fruit ripening	Flowering regulation, ripening control, developmental transitions	Khaskheli <i>et al.</i> (2021)
Chromatin remodeling	Structural reorganization of nucleosomes	SWI/SNF, INO80, CHD complexes	Maize: Remodeling at enhancer regions during seed development	Activation of developmental genes, improved seed traits	Farahat and Belopukhov, (2021)
Small RNA pathways (siRNA, miRNA)	Post-transcriptional regulation, DNA methylation targeting	miRNA-guided cleavage, siRNA-mediated RdDM	Arabidopsis and Rice: miR156/miR172 regulate transitions; Maize: siRNAs silence transposons	Flowering time control, stress adaptation, genome integrity	Shi <i>et al.</i> (2023)
Chromatin accessibility	Openness for TF binding	ATAC-seq accessible peaks at promoters/enhancers	Rice: Accessibility shifts under drought; Wheat: Root growth transitions	Activation of stress-responsive genes, developmental plasticity	Erol <i>et al.</i> (2025)

ATAC-seq is the favorite method of finding these open spots and it is highly accurate. In plants, ATAC-seq has also revealed such large regulation variants as those in the maize seed development, the drought response in rice and the integration of chromatin openness, DNA modification and histone tweaks, where they interact in connecting genes expression.

Methods of interrogating epigenomic history and introduction and mechanism in table

Systematic study of plant epigenomics started in the early 1990s when scientists first found patterns of cytosine methylation and used enzymes that are sensitive to methylation to look at epigenetic differences (Zhu *et al.*, 2025). However, these early methods weren't good enough for studying the whole genome's epigenetic features. A big improvement came with the use of bisulfite sequencing from 1999 to 2005, which allowed researchers to look at DNA methylation at the level of individual bases (Wang and Bart, 2025). Soon after, in 2007 and 2008, the techniques ChIP-Seq and RNA-Seq became available, helping scientists link chromatin states with gene expression on a large scale. Starting in 2013, new methods like ATAC-seq, CUT and RUN and CUT and Tag provided more efficient and low-input ways to study chromatin accessibility and how histones interact with DNA. Epigenomic history from 1999 to 2025 illustrated in Fig 3.

Mechanism and methods for interrogating epigenomic

Epigenomic profiling tools provide complementary insights into DNA methylation, chromatin state and genome organization that underpin trait regulation and stress adaptation in plants (Table 2). Whole-genome bisulfite sequencing (WGBS) enables single-base resolution mapping of 5-mC methylation across the genome, albeit at high cost and analytical complexity, while reduced-representation bisulfite sequencing (RRBS) offers a cost-effective alternative focused on CpG-rich regions such as gene promoters (de Abreu *et al.*, 2025; Hsieh *et al.*, 2024). Chromatin-level regulation is elucidated through ChIP-seq

and CUT and Tag, which profile histone modifications and transcription factor binding, with CUT and Tag being particularly suited for low-input tissues (Agius *et al.*, 2023; Liao *et al.*, 2024). ATAC-seq rapidly identifies accessible chromatin and regulatory elements responsive to stress, whereas small RNA-seq captures miRNA and siRNA-mediated pathways involved in transposon silencing and epigenetic inheritance (Ueda *et al.*, 2023; Qi *et al.*, 2023). Higher-order genome organization and epigenetic variation are resolved through Hi-C and Oxford Nanopore long-read sequencing, enabling analysis of 3D chromatin interactions, epialleles and structural variation without bisulfite conversion (Abdulraheem *et al.*, 2024; Qi *et al.*, 2023).

Challenges and future prospects in plant epigenomics

Plant epigenomics is becoming a critical field of understanding and controlling of complex traits; nonetheless, a succession of obstacles is hindering extensive implementation of the technology to crops enhancement. The heterogeneity of epigenetic marks depending on cell type and tissue is a key issue, as well as their quite high temporal variance. Fig 4 highlights that major advances in plant epigenomics will depend on resolving tissue-specific and dynamic epigenetic variation, integrating green epigenomics, epiallele-based breeding and developing robust advanced analytical pipelines to translate epigenetic knowledge into crop improvement outcomes. The differences in chromatin accessibility, histone modifications and the extent of DNA methylation amongst different cells and different developmental stages blur the definition of precise regulatory landscape. This requires the use of high resolutions and single-cell approaches (Bai and Jin, 2024). Even with the advances in technology, the existing methods are not sufficient to study the rare cell populations or volatile regulation states in large plant tissues. This situation is worsened by plants with large, polyploid and highly repetitive genomes which include wheat, sugarcane and canola. Such genomic complications complicate the matching of DNA reads, categorization of transposable elements and accurate

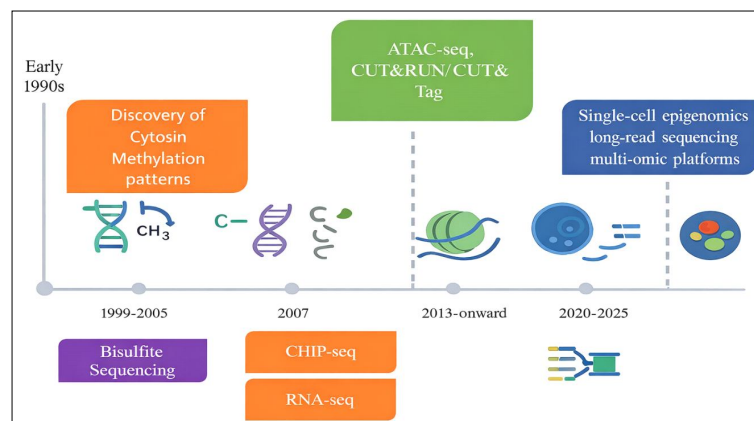


Fig 3: Epigenomic history from 1999 to 2025.

Table 2: High-throughput epigenomic and chromatin profiling techniques used in plant research.

Method	What it measures	Principle	Advantages	Limitations	Applications	References
WGBS	5 mC DNA methylation (genome wide)	Bisulfite conversion + NGS mapping	Single base resolution; Genome-wide	Costly; DNA degradation; Complex analysis	Trait epigenetics; Stress response; Methylome diversity	(de Abreu <i>et al.</i> , 2025)
RRBS	High CpG methylation	Restriction enzyme enrichment + bisulfite sequencing	Cheaper than WGBS; High resolution in CpG regions	Bias toward CpG rich regions	Gene promoter methylation; Targeted epigenetics	(Hsieh <i>et al.</i> , 2024)
ChIP seq	Histone modifications (e.g., H3K27me3, H3K9me2)	Antibody pull down + sequencing	Genome wide histone landscape	Requires high quality antibodies; High input	Regulation of flowering, stress memory	(Agius <i>et al.</i> , 2023)
ATAC seq	Chromatin accessibility	Tn5 transposase insertion in open chromatin	Low input; Fast; High resolution	Sensitive to sample quality	Identifying regulatory elements; Stress-responsive enhancers	(Ueda <i>et al.</i> , 2023)
Small RNA seq	miRNAs, siRNAs, RdDM-associated RNAs	Sequencing of 18-30 nt RNAs	Highly sensitive; Detects RdDM pathway signals	Cannot directly detect methylation	Transposon silencing; Hybrid vigor studies	(Qi <i>et al.</i> , 2023)
CUT and Tag	Histone marks, TF binding	Antibody-tethered Tn5 tagging	Low input; High signal to noise	New method; Requires optimization	Low input/seed tissue epigenomics	(Liau <i>et al.</i> , 2024)
HiC	3D genome structure	Chromatin cross linking + proximity ligation	Maps chromatin loops and compartments	Complex library prep; High cost	3D genome dynamics under stress; Enhancer-gene loops	(Abdulraheem <i>et al.</i> , 2024)
ONT long read	Direct DNA methylation + structural variants	Nanopore current disruption reveals modifications	No bisulfite needed; Long reads	Higher error rate vs. short reads	Epialleles; Transposons; Pan epigenomics	(Qi <i>et al.</i> , 2023)

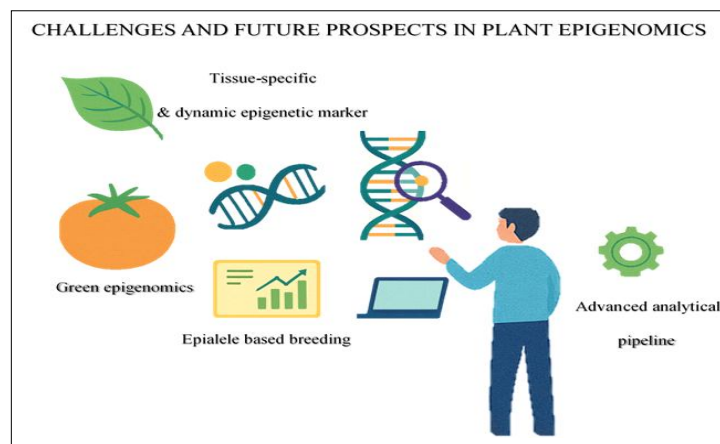


Fig 4: Future direction in plant epigenomics.

identifying of patterns of methylation, which makes it difficult to distinguish between real and artificial regulatory variances and technical artefacts, which remains a major challenge and as a result, its drawback. Another urgent problem is the inability to separate stable and heritable epigenetic alterations and the ones caused by the environment and subject to reversal. This distinction is vital towards the identification of powerful indicators in durable breeding programs (Bar-Oz and Schmidt, 2025).

Reasoning and coordinating disparate layers of biology, such as genetic difference, chromatin states, genetic activity, protein interactions and metabolic profiles, requires complex multi-omics planning along with high-performance computing and complex statistical methodologies and powerful machine-learning algorithms. This kind of integration is essential when trying to explain the nexus of the genotype, epigenotype and phenotype, but is still both computationally and technically challenging (Azeem *et al.*, 2026). Despite these retardations, the field is moving forward at quicker rates towards more useful and practical applications. New methods of genomic editing, such as those provided by CRISPR/dCas9-based systems that can insert or remove methylation tags, provide a means of selective regulation of particular regulatory elements (Fal *et al.*, 2025).

CONCLUSION

Epigenomics has emerged as a transformative paradigm in crop science, effectively bridging the long-standing gap between genotype and phenotype through heritable yet reversible regulatory mechanisms. By integrating DNA methylation, histone modifications and small RNA pathways, it provides a deeper understanding of gene expression dynamics without altering DNA sequences. Advances in high-resolution technologies have enabled precise mapping of regulatory landscapes, uncovering novel loci associated with complex agronomic traits. The development of epialleles and epiRILs further

highlights the significant contribution of epigenetic variation to yield, adaptability, and stress tolerance.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Department of Genetics and Plant Breeding, SR University, Warangal and co-authors for completion of the manuscript.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

REFERENCES

- Abdulraheem, M.I., Xiong, Y., Moshood, A.Y., Cadenas-Pliego, G., Zhang, H. and Hu, J. (2024). Mechanisms of plant epigenetic regulation in response to plant stress: Recent discoveries and implications. *Plants*. **13**(2): 163.
- Agius, D.R., Kapazoglou, A., Avramidou, E., Baranek, M., Carneros, E., Caro, E. and Vassileva, V. (2023). Exploring the crop epigenome: A comparison of DNA methylation profiling techniques. *Frontiers in Plant Science*. **14**: 1181039.
- Ahmar, S., Gill, R.A., Jung, K.H., Faheem, A., Qasim, M.U., Mubeen, M. and Zhou, W. (2020). Conventional and molecular techniques from simple breeding to speed breeding in crop plants: Recent advances and future outlook. *International Journal of Molecular Sciences*. **21**(7): 2590.
- Azeem, A., Zia, M.A.B., Khan, S., Yasmin, T., Abbas, N. and Azeem, S. (2026). Epigenetic-Based Breeding Strategies for Stress-Resilient Crops. In *Epigenetic Mechanisms in Plant Stress Adaptation*. IGI Global Scientific Publishing. (pp. 1-28).

- Farahat, E.M.M. and Belopukhov, S.L. (2021). Effect of the Different concentrations of protective stimulating complex (HFC) on the growth and development of grape seedlings in the tissue culture at the different nutrient levels. *Indian Journal of Agricultural Research*. **55(4)**: 493-496. doi: 10.18805/IJARE.A-613.
- Bai, Y. and Jin, X. (2024). Hyperspectral approaches for rapid and spatial plant disease monitoring. *Trends in Plant Science*. **29(6)**: 711-712.
- Bar-Oz, G. and Schmidt, J. (2025). Why study the archeo-histories of dryland landraces now? *Trends in Plant Science*. **30(7)**: 724-735.
- Behrens, P., Champagne, C.M., Halford, J.C., Moodie, M., Proietto, J., Rutter, G.A. and Holly, J.M. (2025). Obesity and climate change: Co-crises with common solutions. *Frontiers in Science*. **3**: 1613595.
- Tripathi, G., Gupta, S., Rinka, K., Gupta, T., Selokar, N.L. and Singh, M.K. (2024). Exogenous miR-29b reduces DNA methylation and apoptosis in transgenic cells. *Indian Journal of Animal Research*. doi: 10.18805/IJAR.B-5284.
- de Abreu, A.R., Ibrahim, J., Lemonidis, V., Mateiu, L., Van Camp, G. and Op de Beeck, K. (2025). Comparison of current methods for genome-wide DNA methylation profiling. *Epigenetics and Chromatin*. **18(1)**: 57.
- Erol, T., Çiğ, F., Sabancılar, I., Kara, K., Özek, R. and Ceritoglu, M. (2025). Enhancing salinity tolerance in triticale via synergistic effect of plant growth promoting bacteria and arbuscular mycorrhizal fungi. *Legume Research*. **49(5)**: 814-821. doi: 10.18805/LRF-911.
- Fal, K., El Khoury, S., Le Masson, M., Berr, A. and Carles, C.C. (2025). CRISPR/dCas9-targeted H3K27me3 demethylation at the CUC3 boundary gene triggers ectopic transcription and impacts plant development. *iScience*. **28(5)**: 112475.
- Furci, L., Berthelot, J., Juez, O., Miryeganeh, M. and Saze, H. (2023). Plant Epigenomics. In: *Handbook of Epigenetics*. Academic Press. (pp. 263-286).
- Gogolev, Y.V., Ahmar, S., Akpınar, B.A., Budak, H., Kiryushkin, A.S., Gorshkov, V.Y. and Korzun, V. (2021). OMICs, epigenetics and genome editing techniques for food and nutritional security. *Plants*. **10(7)**: 1423.
- Han, J., Chang, C.Y.Y., Gu, L., Zhang, Y., Meeker, E.W., Magney, T.S. and Sun, Y. (2022). The physiological basis for estimating photosynthesis from Chl a fluorescence. *New Phytologist*. **234(4)**: 1206-1219.
- Hsieh, J.W.A., Lin, P.Y., Wang, C.T., Lee, Y.J., Chang, P., Lu, R.J.H. and Wang, C.J.R. (2024). Establishing an optimized ATAC-seq protocol for the maize. *Frontiers in Plant Science*. **15**: 1370618.
- Shi, P., Fan, W., Yang, Y. and Shi, Y. (2023). Integrative analysis of miRNA and mRNA in dormant alfalfa under different growth states. *Legume Research*. **46(4)**: 432-439. doi: 10.18805/LRF-719.
- Khaskheli, A.J., Zhang, L., Khaskheli, M.I., Khaskheli, A.A. and Qing, L.H. (2021). Genetic relationship of transcriptional factor genes in the regulation of cells death in arabidopsis: A review. *Agricultural Reviews*. **42(4)**: 406-412. doi: 10.18805/ag.R-188.
- Jabeen, A., Kaleem, M., Minhas, L.A. and Mumtaz, A.S. (2026). Epigenomics: A High Throughput Approach to Study Plant Stress Adaptations. In *Epigenetic Mechanisms in Plant Stress Adaptation*. IGI Global Scientific Publishing. (pp. 321-358).
- Kaya, C., Uğurlar, F. and Adamakis, I.D.S. (2024). Epigenetic modifications of hormonal signaling pathways in plant drought response and tolerance for sustainable food security. *International Journal of Molecular Sciences*. **25(15)**: 8229.
- Khan, Z. and Shahwar, D. (2023). Genome Editing in Plants via CRISPR/Cas9: A Genomic Scissor Borrowed from Bacterial Immune System. In: *Genome Editing and Global Food Security*. Routledge. (pp. 1-16).
- Li, Y., La, Z.A.B., Cao, L. and Zhao, S. (2022). Establishment of a DNA barcoding database for legume and grass species identification. *Legume Research*. **45(6)**: 659-668. doi: 10.18805/AF-727.
- Liau, Y., Whibley, A., Hill, A.M., Vanga, B.R., Pither-Joyce, M., Hilario, E. and Lizamore, D. (2024). Low-pass nanopore sequencing for measurement of global methylation levels in plants. *BMC Genomics*. **25(1)**: 1235.
- Liu, J. and He, Z. (2020). Small DNA methylation, big player in plant abiotic stress responses and memory. *Frontiers in Plant Science*. **11**: 595603.
- Qi, Q., Hu, B., Jiang, W., Wang, Y., Yan, J., Ma, F. and Xu, J. (2023). Advances in plant epigenome editing research and its application in plants. *International Journal of Molecular Sciences*. **24(4)**: 3442.
- Seem, K., Kaur, S., Kumar, S. and Mohapatra, T. (2024). Epigenome editing for targeted DNA (de) methylation: A new perspective in modulating gene expression. *Critical Reviews in Biochemistry and Molecular Biology*. **59(1-2)**: 69-98.
- Talarico, E., Zambelli, A., Araniti, F., Greco, E., Chiappetta, A. and Bruno, L. (2024). Unravelling the epigenetic code: DNA methylation in plants and its role in stress response. *Epigenomes*. **8(3)**: 30.
- Ueda, J., Yamazaki, T. and Funakoshi, H. (2023). Toward the development of epigenome editing-based therapeutics: Potentials and challenges. *International Journal of Molecular Sciences*. **24(5)**: 4778.
- Varotto, S., Tani, E., Abraham, E., Krugman, T., Kapazoglou, A., Melzer, R. and Miladinović, D. (2020). Epigenetics: Possible applications in climate-smart crop breeding. *Journal of Experimental Botany*. **71(17)**: 5223-5236.
- Wang, Z. and Bart, R.S. (2025). Using targeted genome methylation for crop improvement. *Journal of Experimental Botany*. **76(9)**: 2394-2404.
- Kumar, I., Nirwan, A., Khan, A., Lalhmingmawii, S. and Vyas, J. (2024). Epigenetics: Unraveling hidden phenotypic diversity in livestock. *Agricultural Science Digest*. **46(4)**: 564-572. doi: 10.18805/ag.D-5964.
- Xie, G., Du, X., Hu, H. and Du, J. (2025). Molecular mechanisms underlying the establishment, maintenance and removal of DNA methylation in plants. *Annual Review of Plant Biology*. **76**: 143-170.

- Xue, Y., Cao, X., Chen, X., Deng, X., Deng, X.W., Ding, Y. and Liu, Q. (2025). Epigenetics in the modern era of crop improvements. *Science China Life Sciences*. pp 1-40.
- Yang, X., Sanchez, R., Kundariya, H., Maher, T., Dopp, I., Schwegel, R. and Mackenzie, S.A. (2020). Segregation of an MSH1 RNAi transgene produces heritable non-genetic memory in association with methylome reprogramming. *Nature Communications*. **11(1)**: 2214.
- Yang, X., Sohail, H., Noor, I., Costa, F.C.L., Zhong, S., Zhang, L. and Chen, X. (2025). Epigenetic crop improvement: Integrating ENCODE strategies into horticultural breeding. *Horticulture Research*. **12(11)**: uhaf213.
- Zhu, R., Xue, Y. and Qian, W. (2025). Molecular mechanisms and biological functions of active DNA demethylation in plants. *Epigenetics and Chromatin*. **18(1)**: 41.