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HARNESSING EPIGENETICS FOR STRESS RESILIENCE IN WHEAT AND MAIZE

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ABSTRACT

Abiotic stresses such as drought, heat, and salinity significantly threaten wheat and maize production, two staple crops critical for global food security. As traditional breeding approaches face limitations in rapidly addressing these challenges, epigenetics has emerged as a promising avenue for enhancing stress tolerance in crops. Epigenetic mechanisms, including DNA methylation, histone modifications and RNA-mediated regulation, play pivotal roles in how plants respond to environmental stresses. This review explores the current understanding of these epigenetic processes in wheat and maize, highlighting their contributions to stress tolerance. DNA methylation has been shown to modulate the expression of stress-responsive genes, while histone modifications and RNA-mediated regulation fine-tune gene activity, enabling plants to adapt to changing environments. Recent advancements in molecular biology have provided tools such as bisulfite sequencing, ChIP-seq, and RNA-seq, which have greatly enhanced our ability to study these epigenetic modifications. Furthermore, CRISPR/Cas9-based epigenome editing offers new possibilities for creating stable, heritable changes that could improve crop resilience. However, challenges remain, particularly in understanding the long-term stability and heritability of these modifications. The integration of epigenetics into conventional breeding programs, alongside continued research into the mechanisms underlying stress tolerance, holds great promise for developing climate-resilient wheat and maize varieties. As the field progresses, epigenetic approaches may become essential tools in ensuring global food security in the face of climate change.

Key words : Wheat and maize, Epigenetics, Resilience, Bisulfite sequencing, ChIP-seq, RNA-seq.

Introduction

Wheat and maize are among the most important staple crops worldwide, providing a significant portion of the caloric intake for billions of people. Wheat is a primary food source in many regions, particularly in Europe, North America and Asia, where it is consumed as bread, pasta, and other products. Wheat is majorly growing as *rabi* crop and globally has a produced. The trade estimate is lower than the Ministry of Agriculture's MY 2022-23 estimate at 110.55 million mt for MY 2023-24, the ministry targeted wheat output at 114 million mt (Food and Agriculture Organization of the United Nations, 2022) Additionally, Maize, is a versatile crop grown across diverse climates and is used not only for direct human consumption but also as animal feed, in industrial products, and for biofuel production (Shiferaw *et al.*, 2011). It is mainly growing as a *kharif* crop and the global production 1,163 million tonnes of maize in 2022, which is an increase from 318 million tonnes in 1973. Production of Maize in the country during 2022-23 is estimated at (record) 346.13 lakh tonnes, which is higher by 8.83 lakh tonnes than the previous year production of 337.30 lakh tonnes (Food and Agriculture Organization of the United Nations, 2022).

Despite their global importance, the productivity of wheat and maize is increasingly under threat due to various abiotic stresses. These stresses include, drought is considered as most important one and water scarcity is one of the most significant challenges faced by agriculture worldwide, particularly in arid and semi-arid regions. Drought stress can severely limit crop growth and yield by affecting various physiological and biochemical processes, including photosynthesis, nutrient uptake, and plant-water relations (Farooq *et al*., 2012). Wheat and maize are particularly susceptible to drought during critical growth stages such as flowering and grain filling. Additionally, heat stress is the most devastating stress in the wheat production. The rising temperatures due to climate change are exacerbating heat stress in crop production. Wheat and maize are sensitive to high temperatures, especially during reproductive stages, which can lead to reduced grain size, lower yields and compromised grain quality (Cossani and Reynolds, 2012). Heat stress also affects pollen viability and kernel development, leading to poor seed set and reduced grain yield. Terminal heat stress due to delayed sowing practices in case of wheat it affects approximately 13.5 million hectares of wheat cultivation (Pandey *et al*., 2021). These abiotic stresses are often interrelated, and plants may experience them simultaneously or sequentially. The impact of these stresses on crop productivity is profound, leading to significant yield losses and posing a major threat to food security, especially in the context of global climate change.

Breeding approaches for stress tolerance

Over the years, plant breeders have made significant strides in developing stress-tolerant varieties of wheat and maize. Different Breeding approaches used for stress tolerance in maize and wheat illustrated in Fig. 1. Traditional breeding approaches primarily involve:

- **Selection and hybridization:** Breeders have selected for desirable traits, such as drought or heat tolerance, from within the existing genetic diversity of wheat and maize populations. Hybridization, or crossing different varieties, has been used to combine these traits into new cultivars that perform better under stress conditions (Tester and Langridge, 2010).
- **Marker assisted selection (MAS):** The advent of molecular markers has revolutionized traditional breeding by enabling the identification of specific genes associated with stress tolerance. MAS allows breeders to select plants with the desired traits more efficiently, speeding up the development of new varieties (Collard and Mackill, 2008). It includes marker assisted back cross and marker assisted recurrent selection.
- **Biotechnological interventions:** Advances in genetic engineering and biotechnology offer new avenues for introducing stress tolerance traits into crops. This includes the use of transgenic approaches to insert specific genes from other organisms that confer drought, heat, or salinity tolerance (Zhang *et al*., 2016).
- **Genomic selection:** is a modern breeding method that uses genome-wide markers to predict the performance of breeding lines. This approach can significantly accelerate the breeding process by allowing the selection of superior genotypes at an early stage (Meuwissen *et al*., 2001).
- **Epigenetics and epigenome editing:** Epigenetic modifications, which do not involve changes to the DNA sequence but can influence gene expression, are emerging as important players in stress tolerance. Understanding the role of epigenetics in stress responses can lead to the development of epigenetic markers for breeding and the use of tools like CRISPR/Cas9 for targeted epigenome editing to enhance stress tolerance (Springer and Schmitz, 2017).

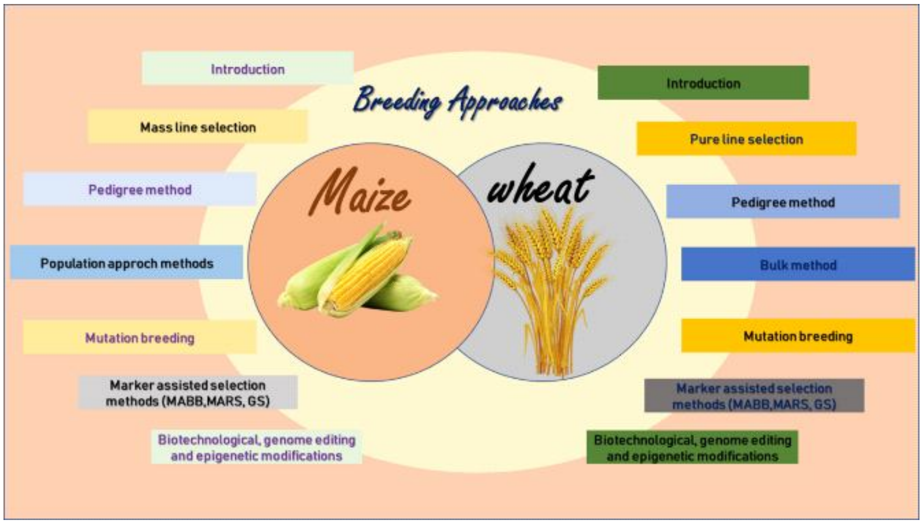

Fig. 1 : Different Breeding approaches used for stress tolerance in maize and wheat.

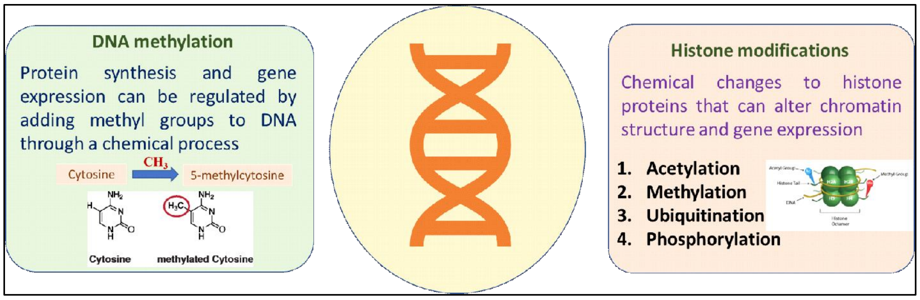

Fig. 2 : Overview of epigenetics and modifications.

Epigenetics, mechanisms in stress tolerance induced by abiotic stress

Epigenetics refers to heritable changes in gene expression that do not involve alterations to the underlying DNA sequence. This field has gained significant attention due to its role in regulating gene activity in response to environmental stimuli. Key mechanisms of epigenetic regulation include DNA methylation, histone modifications, and RNA interference, which collectively influence how genes are expressed without changing the genetic code itself (Allis and Jenuwein, 2016). These mechanisms are not static; they are dynamic and responsive to various external factors, making them crucial for plants to adapt to stressful environments (Zhang *et al*., 2018). Overview of epigenetics and modifications illustrated in Fig. 2.

DNA Methylation

DNA methylation is a well-studied epigenetic mechanism in which methyl groups are added to cytosine bases in DNA. This process can lead to the repression or activation of genes, depending on the context. In plants, stress conditions often trigger changes in DNA methylation patterns, leading to the regulation of stress-responsive genes (Chinnusamy and Zhu, 2009). In maize, drought stress has been shown to cause significant changes in DNA methylation patterns. Eichten and Springer (2015) conducted a study where they observed that drought conditions led to alterations in methylation at specific genomic loci, which in turn affected the expression of genes involved in stress adaptation. For instance, some genes that are typically suppressed under normal conditions were activated under drought stress due to demethylation, allowing the plant to better cope with water scarcity. This study highlights how DNA methylation can play a critical role in enabling maize to adapt to drought stress by modulating gene expression. In maize, drought-induced changes in DNA methylation have been extensively studied. For example, Luo *et al*. (2017) found that drought stress led to significant alterations in DNA methylation patterns at loci associated with root development and water uptake. This modification affected the expression of key genes involved in these processes, such as *ZmNAC111*, a transcription factor crucial for root growth under drought conditions. The study demonstrated that demethylation of the promoter region of *ZmNAC111* under drought stress led to its upregulation, thereby enhancing the plant's ability to extract water from deeper soil layers.

Histone Modifications

Histone modifications involve chemical changes to the histone proteins around which DNA is wound. These modifications, such as acetylation, methylation, phosphorylation, and ubiquitination, play a critical role in chromatin remodelling and gene expression (Bannister and Kouzarides, 2011). These alterations can either loosen or tighten the chromatin structure, thereby facilitating or hindering the transcription of specific genes. In wheat, histone modifications have been linked to the regulation of genes involved in responses to abiotic stress, such as drought and heat. For example, Kim *et al*. (2015) reported that histone acetylation and methylation are associated with the activation of the dehydration-responsive element-binding protein (DREB) family of transcription factors. These factors are crucial for the plant's response to dehydration and other stress conditions. The study found that specific histone marks on the promoters of *DREB* genes were altered in response to drought, leading to their increased expression and, consequently, enhanced stress tolerance in wheat. Histone acetylation also plays a pivotal role in regulating drought-responsive genes in wheat. Kumar *et al*. (2020) highlighted that drought stress resulted in increased acetylation of histones at the promoters of *dehydration-responsive element-binding (DREB)* genes, which are key regulators of drought tolerance. In wheat, heat stress has been shown to induce specific histone modifications associated with the activation of heat shock transcription factors (HSFs). Qin *et al*. (2014) conducted a study showing that heat stress leads to increased histone acetylation at the promoters of *HSFA2* and *HSFA3* genes. These HSFs are responsible for the transcriptional activation of HSPs, which function as molecular chaperones, stabilizing proteins and preventing their aggregation under high temperatures. Similarly, in maize, heat stress has been associated with changes in histone modifications that regulate the expression of stress-responsive genes. Lämke and Bäurle (2017) discussed how heat stress induces histone methylation and acetylation at the loci of HSPs, enhancing their expression.

RNA-Mediated Epigenetic regulation

RNA-mediated epigenetic regulation involves small RNAs, such as microRNAs (miRNAs) and small interfering RNAs (siRNAs), which regulate gene expression through mechanisms like RNA-directed DNA methylation (RdDM) and post-transcriptional gene silencing (PTGS) (Zhai *et al*., 2015). These small RNAs can target specific mRNAs for degradation or inhibit their translation, thereby fine-tuning the plant's response to stress. In maize, miRNAs have been implicated in the regulation of genes involved in drought and heat stress tolerance. Kakrana *et al*. (2014) conducted a study that tracked the expression of miRNAs across different developmental stages of maize under stress conditions. They discovered that certain miRNAs were upregulated in response to drought and heat, leading to the downregulation of target genes that would otherwise be detrimental under stress. This regulation helped maize plants conserve energy and resources, enhancing their survival during adverse conditions. This case study underscores the role of RNA-mediated regulation in modulating the plant's stress responses at a post-transcriptional level.

Epigenetic Tools and Techniques for studying Stress Tolerance

Advancements in molecular biology and bioinformatics have equipped researchers with sophisticated tools to study and manipulate epigenetic modifications. These tools have enhanced our understanding of how plants respond to environmental stresses at the epigenetic level. The technique for detecting epigenetic changes is to be understanding the epigenetic basis of stress tolerance in plants requires precise detection and mapping of epigenetic modifications such as DNA methylation, histone modifications, and non-coding RNA expression. Advances in high-throughput sequencing technologies have revolutionized the ability to study these changes in a comprehensive and detailed manner.

One of the methods is **Bisulfite Sequencing for DNA methylation** analysis. It is a powerful technique used to analyse DNA methylation at a single-nucleotide resolution. In this method, DNA is treated with sodium bisulfite, which converts unmethylated cytosines to uracil while leaving methylated cytosines unchanged. The treated DNA is then sequenced, and the methylation status of each cytosine can be determined by comparing the treated and untreated sequences. This technique has been widely used to study stress-induced DNA methylation changes in plants. In maize, Luo *et al*. (2017) used bisulfite sequencing to map DNA methylation changes in response to drought stress.

Additionally, **ChIP-Seq for histone modification mapping** that is chromatin immunoprecipitation followed by sequencing (ChIP-seq) is a technique used to identify the locations of specific histone modifications across the genome. In this method, DNA-protein complexes are crosslinked, and the chromatin is fragmented. Antibodies specific to the histone modification of interest are used to immunoprecipitated the associated DNA, which is then sequenced to identify the genomic regions where the modification occurs. Qin *et al*. (2014) used ChIP-seq to map histone acetylation marks in wheat under heat stress. The study revealed that heat stress-induced acetylation at the promoters of heat shock transcription factors (HSFs) was crucial for the rapid activation of heat shock proteins (HSPs), highlighting the role of histone modifications in heat stress tolerance.

The RNA-Seq for non-coding RNA analysis that (RNA-seq) is a high-throughput technique used to analyse the expression of non-coding RNAs, including microRNAs (miRNAs) and small interfering RNAs (siRNAs), which play critical roles in epigenetic regulation. RNA-seq provides a comprehensive view of the transcriptome, allowing researchers to identify differentially expressed non-coding RNAs in response to stress. In maize, RNA-seq has been used to identify miRNAs involved in drought and heat stress responses. Kakrana *et al*. (2014) used RNA-seq to profile miRNAs in maize under drought conditions, revealing the role of specific miRNAs in regulating genes involved in water uptake and stress adaptation.

CRISPR/Cas9 and epigenome editing, initially developed for genome editing, has been adapted for epigenome editing, allowing targeted modifications of epigenetic marks. This technology offers a precise and powerful tool for studying the functional roles of specific epigenetic modifications in stress tolerance and holds great promise for developing stress-tolerant crop varieties. Epigenome editing using CRISPR/Cas9 involves the fusion of the deactivated Cas9 (dCas9) protein with effector domains that can add or remove specific epigenetic marks. By guiding dCas9 to specific genomic loci using a guide RNA (gRNA), researchers can selectively modify DNA methylation, histone acetylation, or other epigenetic marks, thereby altering gene expression without changing the underlying DNA sequence. Vojta *et al.* (2016) demonstrated the use of CRISPR/dCas9 fused with the DNA methyltransferase DNMT3A to target and methylate specific gene promoters in mammalian cells. This approach has since been adapted for use in plants, providing a tool to study the causal relationship between epigenetic marks and gene expression in stress responses. In maize, CRISPR/Cas9-based tools have been developed to target epigenetic modifications associated with stress-responsive genes. Wang *et al.* (2017) used a CRISPR/dCas9 system fused with histone acetyltransferase (HAT) to specifically acetylate histones at the promoter region of the *ZmDREB2A* gene, a key regulator of drought tolerance. The targeted acetylation enhanced the expression of ZmDREB2A, leading to improved drought tolerance in maize plants. This study demonstrates the potential of CRISPR/Cas9-based epigenome editing for developing crops with enhanced stress tolerance.

Epigenetics in Crop Improvement: Challenges and opportunities

The application of epigenetics in crop improvement is a burgeoning field that offers exciting prospects for enhancing stress tolerance in plants. However, this area also faces significant challenges that must be addressed to realize its full potential. Despite the promising potential of epigenetic modifications in crop improvement, several challenges impede their effective utilization in breeding programs.

Transient Nature of Epigenetic Changes, this is the one of the primary challenges in harnessing epigenetic variation is the transient nature of some epigenetic modifications. Unlike genetic mutations, which are stable and heritable, epigenetic changes can be reversible and may not be consistently transmitted across generations. For example, environmental factors such as temperature, water availability, or nutrient levels can induce temporary epigenetic modifications, which may revert once the stress is removed. This variability makes it difficult to ensure that beneficial epigenetic traits will be retained in the progeny, complicating their use in breeding programs (Springer and Schmitz, 2017). Another challenge lies in distinguishing between adaptive and non-adaptive epigenetic modifications. Not all epigenetic changes induced by stress are beneficial; some may be neutral or even detrimental to plant fitness. Identifying which modifications contribute to enhanced stress tolerance is crucial for their effective use in crop improvement. This requires a deep understanding of the specific genes and pathways regulated by epigenetic modifications under different stress conditions, which is still an area of ongoing research. Technical challenges also exist in accurately detecting and manipulating epigenetic modifications. High-throughput sequencing technologies like bisulfite sequencing and ChIP-seq are powerful tools, but they require significant resources, expertise, and computational power. Moreover, off-target effects and unintended consequences remain concerns in epigenome editing using tools like CRISPR/Cas9. Addressing these challenges requires continued technological advancements and methodological refinements.

Opportunities for Breeding Stress-Tolerant varieties

Despite the challenges, there are significant opportunities to integrate epigenetics into crop breeding programs, particularly in developing stress-tolerant varieties. As our understanding of plant epigenetics deepens, new avenues for crop improvement are emerging. The integration of epigenetic information into conventional and molecular breeding approaches offers a promising path forward. By identifying epigenetic markers associated with stress tolerance, breeders can use marker-assisted selection (MAS) to incorporate these traits into new crop varieties. This approach could accelerate the development of plants better equipped to withstand abiotic stresses such as drought, heat, and salinity. Gupta *et al.* (2021) highlighted the potential of using epigenetic markers in MAS to improve stress tolerance in crops. The authors suggested that epigenetic markers, once identified and validated, could complement existing genetic markers, providing a more comprehensive strategy for breeding stress-tolerant varieties. Epigenome editing technologies like CRISPR/Cas9 offer new opportunities to create stable epigenetic variants with enhanced stress tolerance. By precisely targeting and modifying specific epigenetic marks, researchers can potentially develop crops that retain beneficial traits across generations. This approach could be particularly valuable in regions facing frequent and severe environmental stresses due to climate change. Kumar *et al.* (2020) discussed the potential of CRISPR/Cas9-based epigenome editing in crop improvement. They highlighted its ability to create stable, heritable epigenetic changes that could be used to enhance stress tolerance. The development of stable epigenetic variants through targeted epigenome editing could open new frontiers in crop breeding, particularly for traits that are difficult to achieve through traditional genetic approaches.

Conclusion and Future Directions

Epigenetic mechanisms, including DNA methylation, histone modifications, and RNA-mediated regulation, play pivotal roles in how wheat and maize manage and adapt to environmental stresses such as drought, heat, and salinity. These mechanisms allow plants to modulate gene expression in response to environmental changes, enhancing their resilience without altering the underlying DNA sequence. For example, DNA methylation changes in maize have been associated with the regulation of genes involved in root development and water uptake under drought conditions (Luo *et al*., 2017), while histone modifications in wheat have been linked to the activation of heat shock transcription factors that help the plant cope with heat stress (Qin *et al*., 2014). Despite these advances, the field of epigenetics in plant stress tolerance is still emerging, and significant challenges remain. One major challenge is understanding the long-term stability and heritability of stress-induced epigenetic changes, which is crucial for their effective utilization in breeding programs (Springer and Schmitz, 2017). Furthermore, distinguishing between adaptive and non-adaptive epigenetic modifications is essential for identifying which changes can be beneficially harnessed for crop improvement.

Future research should focus on understanding the long-term stability and inheritance of epigenetic modifications under field conditions. Additionally, there is a need to explore the interaction between genetic and epigenetic factors in shaping plant responses to environmental stresses. Integrating epigenomics with other omics approaches, such as genomics, transcriptomics, and proteomics, could provide a more holistic understanding of plant stress tolerance and inform the development of more resilient crop varieties. The future is deepening our understanding of the complex interactions between different epigenetic modifications and environmental stresses. This includes exploring how DNA methylation, histone modifications, and non-coding RNAs interact to regulate stress-responsive gene networks, as well as how these modifications are influenced by external stress factors. Advances in epigenetic tools and techniques, such as CRISPR/Cas9-based epigenome editing, offer promising avenues for precisely manipulating these epigenetic marks to enhance stress tolerance in crops (Wang *et al*., 2017). For instance, targeted epigenome editing could be used to induce or suppress specific epigenetic modifications associated with stress tolerance, creating crops that are more resilient to adverse environmental conditions.

Moreover, integrating epigenetic insights into conventional breeding programs could significantly accelerate the development of stress-tolerant wheat and maize varieties. Marker-assisted selection (MAS) utilizing epigenetic markers associated with stress tolerance could enhance the efficiency of breeding programs, allowing for the rapid selection of varieties that are better suited to withstand the impacts of climate change (Gupta *et al*., 2021). As research in this field progresses, the potential for developing climate-resilient crops through epigenetic approaches will become increasingly important in ensuring global food security.

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