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Harnessing Epigenetic Modifications for Targeted Trait Manipulation in Pakistani Wheat

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ABSTRACT

This study explores the potential of harnessing epigenetic modifications for targeted trait manipulation in Pakistani wheat varieties. Epigenetic regulation is pivotal in modulating gene expression patterns and can be leveraged to enhance agronomic traits. A diverse panel of Pakistani wheat varieties was subjected to comprehensive epigenetic profiling, encompassing DNA methylation and histone modification analyses. CRISPR-based epigenetic editing targeted specific genes associated with disease resistance, yield, and nutritional content. Field trials were conducted to evaluate the agronomic performance of edited lines. Epigenetic profiling revealed distinct DNA methylation and histone modification patterns across the selected wheat varieties. Targeted epigenetic editing amplified disease resistance, enhanced yield potential, and enriched nutritional content. Disease incidence and severity were significantly reduced in edited lines. Additionally, increased tiller number, larger grain size, and elevated levels of essential nutrients were observed. This research demonstrates the feasibility and efficacy of employing epigenetic modifications for precise trait manipulation in Pakistani wheat. The study provides valuable insights into the regulatory mechanisms underlying disease resistance, yield, and nutritional traits. Collaborative efforts among geneticists, molecular biologists, and plant breeders are essential in further refining and implementing these innovative breeding techniques. The findings pave the way for developing resilient and nutritionally enriched wheat varieties, contributing to global food security and agricultural sustainability.

INTRODUCTION

Wheat (*Triticum aestivum* L.) holds an unparalleled significance in global agriculture, serving as a fundamental staple for over 30% of the world's population (Li *et al.*, 2021). In Pakistan, the importance of wheat transcends its role as a mere dietary staple; it is the cornerstone of food security, accounting for nearly 60% of the total caloric intake in the country (Pakistan Economic Survey, 2021). The agroecological diversity of Pakistan, spanning from the fertile plains of Punjab to the arid regions of Balochistan, necessitates a tailored approach to wheat cultivation (Zulfikar & Thapa, 2017). However, this vital crop faces a litany of challenges, including evolving pest pressures, unpredictable climatic shifts, and the ever-present threat of crop diseases (Penaar, 2020). The interplay of these factors underscores the urgency of developing innovative strategies to fortify the resilience and productivity of Pakistani wheat varieties.

Epigenetic Mechanisms: A New Frontier in Crop Improvement

In recent decades, the field of crop improvement has witnessed a paradigm shift with the recognition of epigenetic mechanisms as crucial regulators of gene expression. Epigenetic modifications, encompassing DNA methylation and histone modifications, represent dynamic and heritable alterations in the chromatin

landscape that do not involve changes in the underlying DNA sequence (Feng *et al.*, 2010). These epigenetic marks serve as a regulatory interface between the genome and the environment, allowing plants to adapt and respond to diverse stimuli, including biotic and abiotic stresses (Lämke and Bäurle, 2017).

The Promise of Epigenetic Engineering in Crop Agriculture

Epigenetic engineering offers a powerful toolset for precise trait manipulation in crops. Through targeted modifications of epigenetic marks, researchers can modulate the expression of specific genes, thereby conferring desired traits such as disease resistance, drought tolerance, and enhanced yield (Zhang *et al.*, 2018; Xu *et al.*, 2019). This approach holds immense potential for tailoring crops to meet the evolving demands of modern agriculture, particularly in the face of climate change-induced disruptions.

Epigenetics in Wheat: A Frontier Yet to be Fully Explored

While the application of epigenetics in crop improvement has made significant strides, its implementation in wheat, especially within the context of Pakistani varieties, remains an area ripe for exploration. The hexaploid nature of the wheat genome, characterised by its

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complexity and sheer size, poses unique challenges for epigenetic studies. However, advancements in sequencing technologies and the emergence of precise epigenetic editing tools have propelled the field forward, enabling targeted investigations into the epigenetic regulation of critical traits in this vital cereal crop (Ramirez-Gonzalez *et al.*, 2018; Zhang *et al.*, 2021).

This comprehensive introduction lays the groundwork for a focused investigation into harnessing epigenetic modifications for targeted trait manipulation in Pakistani wheat varieties. The subsequent sections of this research article will elucidate the methodology employed in this study, present the results of our investigations, and engage in a discussion of the implications and future directions of this research.

LITERATURE REVIEW

Epigenetic Mechanisms in Plant Development and Stress Response

Epigenetic modifications play a pivotal role in regulating gene expression patterns in response to developmental cues and environmental stresses. Among these modifications, DNA methylation and histone modifications are prominent regulators of chromatin structure and gene accessibility (Feng *et al.*, 2010). DNA methylation involves the addition of a methyl group to the cytosine base, predominantly occurring at CpG dinucleotides; this modification can lead to transcriptional repression by hindering the binding of transcriptional machinery, thereby influencing various aspects of plant growth and development. (Zhang *et al.*, 2018).

Histone modifications, on the other hand, entail the covalent modification of histone proteins, including acetylation, methylation, phosphorylation, and ubiquitination. These modifications alter the chromatin landscape, influencing the accessibility of DNA to the transcriptional machinery (Kouzarides, 2007). For instance, histone acetylation is generally associated with gene activation, while histone methylation can either activate or repress gene expression, depending on the specific residue and methylation state (Bannister & Kouzarides, 2011).

In plants, epigenetic modifications have been implicated in a myriad of processes, including flowering time regulation, stress responses, and pathogen defence; these findings underscore the dynamic interplay between epigenetic mechanisms and the intricate regulatory networks that govern plant development and adaptation to changing environmental conditions (Baulcombe & Dean, 2014; Robert H Downen *et al.*, 2012; Lämke & Bäurle, 2017).

Epigenetic Engineering: A Revolutionary Approach in Crop Improvement

The recognition of epigenetic modifications as powerful regulators of gene expression has spurred the emergence of epigenetic engineering as a transformative tool in crop improvement; by precisely modulating specific epigenetic marks, researchers can fine-tune the expression of target

genes, thereby conferring desirable traits, this approach offers a level of precision that complements traditional breeding methods, allowing for the rapid development of crop varieties with tailored characteristics (Xu *et al.*, 2015). A study by Zhang *et al.* (2018) on various crops has demonstrated the efficacy of epigenetic engineering in trait manipulation. For example, in rice, targeted demethylation of a flowering-time regulator resulted in altered flowering behaviour, showcasing the potential for precise control over developmental processes (Zhang *et al.*, 2018). Similarly, in maize, modulation of histone acetylation patterns led to enhanced drought tolerance, highlighting the versatility of epigenetic engineering in addressing abiotic stress responses (Sun *et al.*, 2018).

Epigenetic Applications in Wheat: Progress and Potential

While the application of epigenetics in crop improvement has made significant strides, its implementation in wheat, particularly within the context of Pakistani varieties, is an area ripe for exploration, the intricacies of the wheat genome, characterised by its hexaploid nature and large genome size, present unique challenges for epigenetic studies, recent advancements in sequencing technologies and the emergence of precise epigenetic editing tools have propelled the field forward, enabling targeted investigations into the epigenetic regulation of critical traits in this vital cereal crop (Karanthamalai *et al.*, 2020; Ramírez-González *et al.*, 2018).

A study by Demetriou *et al.* (2009) on wheat has begun to unveil the potential of epigenetic modifications in trait enhancement. For instance, research has shown that altering DNA methylation patterns can influence traits related to disease resistance and abiotic stress tolerance (Demetriou *et al.*, 2009). Additionally, investigation into histone modifications has revealed their involvement in key developmental processes, including grain filling and tiller initiation (Liu *et al.*, 2007). These studies provide a promising foundation for further exploration of epigenetic approaches in wheat improvement.

Future Prospects

The integration of epigenetic engineering into crop improvement strategies represents a revolutionary advancement in agriculture. In the context of Pakistani wheat, a crop of paramount importance to food security, harnessing epigenetic modifications holds immense potential for developing varieties that are not only resilient to evolving environmental challenges but also tailored to meet the specific needs of local agricultural systems.

However, it is essential to approach this frontier with a nuanced understanding of the complex interplay between epigenetic regulation and genetic background. Furthermore, comprehensive field trials and rigorous characterisation of edited lines will be imperative to ensure the stability and efficacy of the introduced epigenetic modifications.

This study aims to investigate and apply epigenetic

modifications for targeted trait manipulation in Pakistani wheat varieties, with a focus on enhancing disease resistance, yield, and nutritional content. The objectives encompass the comprehensive characterisation of the epigenetic landscape through DNA methylation and histone modification profiling, identification of key epigenetic marks associated with desirable traits, precise manipulation of epigenetic marks using CRISPR-based techniques, rigorous evaluation of agronomic performance under controlled and field conditions, assessment of stability and heritability across subsequent generations, and in-depth analysis of epigenetic and phenotypic data to uncover underlying regulatory mechanisms and potential biomarkers. This research endeavours to contribute valuable knowledge and methodologies for the application of epigenetic engineering in crop improvement, holding significant implications for sustainable agriculture and food security in Pakistan.

METHODOLOGY

Study Design

This study was conducted over one year, from 2022 to 2023, at the SZABIST University, Karachi.

Germplasm Selection

A diverse panel of Pakistani wheat varieties was selected based on their genetic diversity, adaptability to local environmental conditions, and representation of major agroecological zones within Pakistan; this selection process ensured a comprehensive representation of the genetic pool, allowing for robust epigenetic analyses and trait manipulation.

Epigenetic Profiling

DNA Methylation Analysis

Genomic DNA was extracted from young leaf tissues of selected wheat varieties using a modified cetyltrimethylammonium bromide (CTAB) method. Bisulfite conversion was performed to detect cytosine methylation patterns. High-throughput bisulfite sequencing was carried out using Illumina technology, generating single-base resolution methylation data, which enabled the comprehensive assessment of DNA methylation patterns across the genome.

Histone Modification Analysis

Histone proteins were extracted from leaf tissues, and chromatin immunoprecipitation (ChIP) was performed using antibodies specific to histone modifications associated with gene regulation. ChIP-seq was conducted to obtain genome-wide maps of histone modification patterns; this approach provided insights into the dynamic epigenetic regulation of gene expression through histone modifications.

Epigenetic Editing

CRISPR-based epigenetic editing was employed to target specific genes associated with the selected traits.

Guide RNAs (gRNAs) were designed to direct epigenetic modifying enzymes to precise genomic loci. The dCas9-SunTag system was used for multiplexed epigenetic editing, allowing for the simultaneous modification of multiple epigenetic marks. This precise editing approach enabled the enhancement of desired traits while minimising unintended effects.

Trait Manipulation

Disease Resistance

Analysis of DNA methylation patterns was used to identify epigenetic marks associated with disease resistance genes. Epigenetic editing was applied to modulate the methylation status of these genes, aiming to enhance their expression and bolster the natural defence mechanisms of wheat against prevalent pathogens. Field trials were conducted to assess disease resistance in the edited lines.

Yield Enhancement

Histone modification profiling was used to identify regulatory regions associated with yield-related traits. Epigenetic editing was employed to modulate histone modification patterns in these regions, with the goal of increasing tiller number and grain size. Field evaluations were conducted to confirm the improvement in overall yield potential in the edited lines.

Nutritional Enrichment

Epigenetic editing of genes involved in nutrient metabolism pathways was performed to enhance the nutritional content of wheat grains. This involved targeted modifications of epigenetic marks associated with nutrient-related genes. Analysis of grain samples was conducted to quantify the increase in essential nutrients, addressing critical dietary deficiencies.

Field Evaluation

Epigenetically modified wheat lines, along with control lines, were subjected to comprehensive field trials under varying environmental conditions to assess their agronomic performance. This included evaluations of disease resistance, yield potential, and nutritional quality. Statistical analyses were employed to compare the performance of edited lines with control lines.

Data Analysis and Interpretation

Epigenetic data, phenotypic data, and field trial results were analysed to gain insights into the regulatory mechanisms underlying the enhanced traits. Statistical methods and bioinformatics tools were utilised to identify significant correlations and patterns. Potential epigenetic biomarkers for trait improvement were identified.

Ethical Considerations

All experiments were conducted in compliance with relevant ethical guidelines and regulations. Proper protocols for handling genetically modified organisms

(GMOs) were followed, and necessary approvals were obtained from relevant authorities.

Reproducibility and Quality Control

Experiments were conducted in replicates to ensure the reproducibility of results. Quality control measures were implemented at each step of the methodology, including DNA extraction, epigenetic profiling, and editing procedures.

RESULTS AND DISCUSSIONS

DNA Methylation and Histone Modification Profiling

The epigenetic profiling of the selected Pakistani wheat varieties provided a comprehensive understanding of the DNA methylation and histone modification patterns. The analysis revealed distinct epigenetic signatures across the varieties, indicating potential regulatory mechanisms underlying specific traits. DNA methylation levels ranged from 76.4% in Variety C to 82.1% in Variety E, with an average of 79.6%. Notably, regions associated with disease resistance genes exhibited higher methylation levels, suggesting an epigenetic regulatory role in defence mechanisms, as shown in Table 1.

Table 1 illustrates the DNA methylation levels and

Table 1: Epigenetic Profiling Summary

Wheat Variety	DNA Methylation (%)	H3K4me3 Enrichment (RPKM)
Variety A	78.2	45.6
Variety B	81.8	55.3
Variety C	76.4	48.9
Variety D	79.5	50.2
Variety E	82.1	53.7

H3K4me3 enrichment in selected wheat varieties. Histone modification analysis revealed enrichment patterns of H3K4me3, a histone mark associated with active gene expression. The enrichment ranged from 45.6 RPKM (Reads Per Kilobase Million) in Variety A to 55.3 RPKM in Variety B, indicating variability in the activation of specific genomic regions. This dynamic histone modification landscape correlated with traits such as grain size and nutritional content.

Disease Resistance Enhancement

Field trials conducted under natural pathogen pressure demonstrated a substantial reduction in disease incidence and severity in the edited lines compared to control plants, as shown in Table 2.

Table 2 outlines the disease resistance field trial results, showcasing the reduced incidence and severity in the edited lines. Epigenetic editing targeted disease resistance genes identified through DNA methylation analysis. The edited lines exhibited a significant increase in the expression levels of these genes, indicating successful epigenetic manipulation.

Table 2: Disease Resistance Field Trials

Wheat Line	Disease Incidence (%)	Disease Severity (Scale 1-10)
Edited Line 1	12.4	3.2
Edited Line 2	10.8	2.9
Control Line	28.7	6.8

Yield Enhancement and Nutritional Enrichment

Epigenetic modification of regulatory regions associated with yield-related traits led to a significant increase in tiller number and grain size. Additionally, edited lines exhibited elevated levels of essential nutrients, including iron and zinc, in the wheat grains, which is present in Table 3. Table 3 presents the nutritional content analysis, demonstrating elevated levels of iron and zinc in the edited wheat lines.

Table 3: Nutritional Content Analysis

Wheat Line	Iron Content (mg/kg)	Zinc Content (mg/kg)
Edited Line 1	48.6	32.1
Edited Line 2	50.2	34.5
Control Line	42.3	28.7

The results collectively highlight the success of employing epigenetic modifications for targeted trait manipulation in Pakistani wheat varieties. The combination of DNA methylation and histone modification analyses elucidated the regulatory mechanisms underlying disease resistance, yield, and nutritional traits. The application of epigenetic editing techniques demonstrated precise control over these traits, leading to substantial improvements in agronomic performance. These findings hold significant implications for the development of more resilient and productive wheat varieties tailored to the unique environmental conditions of Pakistan. The methodology and results presented in this study provide a solid foundation for further research in epigenetic-based crop improvement strategies, with potential applications extending to other staple crops worldwide. Collaborative efforts among geneticists, molecular biologists, and plant breeders are encouraged to advance and implement these innovative breeding techniques.

DISCUSSION

The comprehensive epigenetic profiling conducted in this study has yielded invaluable insights into the intricate regulatory mechanisms governing key agronomic traits in Pakistani wheat varieties. The discerned DNA methylation patterns, particularly within regions intricately associated with disease resistance genes, strongly imply a pivotal epigenetic role in orchestrating the plant's defence responses, a discovery congruent with earlier research findings (R. H. Downen *et al.*, 2012). The successful enhancement of disease resistance through

the judicious application of targeted epigenetic editing stands as a significant breakthrough in contemporary crop improvement strategies. Moreover, the precision with which DNA methylation patterns in pivotal defence genes were modulated manifested concretely in the field trials, where a marked reduction in disease incidence and severity was observed. This dynamic exemplification underscores the potential of epigenetic engineering not merely in theoretical realms but in pragmatic, field-ready applications.

Equally significant were the outcomes of the epigenetic modification targeted towards regulatory regions associated with yield-related traits. This intervention yielded substantial improvements, with discernible increases in both tiller number and grain size. Such findings resonate with and affirm prior research endeavours that delved into the epigenetic regulation of yield-associated genes in cereals (Liu *et al.*, 2007). Furthermore, the edited lines exhibited enriched levels of essential nutrients—particularly noteworthy were heightened concentrations of iron and zinc—addressing critical dietary deficiencies. This holds profound implications in the broader context of global nutrition, aligning seamlessly with conscientious initiatives to combat malnutrition through strategic biofortification strategies (Bouis & Saltzman, 2017).

The collective outcomes of this study dovetail harmoniously with and build upon the foundation established by antecedent research endeavours that explored epigenetic interventions in the sphere of crop enhancement. For example, studies in rice have adeptly demonstrated the modulatory potential of DNA methylation patterns in augmenting disease resistance and fortifying stress tolerance (Zhang *et al.*, 2018). Similarly, research in maize has shed light on the prospect of histone modification-based strategies in bolstering drought tolerance (Sun *et al.*, 2018). These cohesive insights underline the adaptability and efficacy of epigenetic engineering across diverse crop species, underscoring the robustness of this approach in the pursuit of agricultural sustainability and food security.

Looking ahead, these findings have profound implications for agricultural practices in Pakistan and beyond. The success of this research in wielding epigenetic modifications as precision tools for trait manipulation presents a transformative stride towards the realisation of more resilient, high-yielding, and nutrient-dense wheat varieties. The potential applications extend far beyond the confines of wheat, embracing a diverse array of staple crops, thereby promising a tangible impact on global food security. Collaborative synergies between geneticists, molecular biologists, and plant breeders will undoubtedly be instrumental in further refining and deploying these innovative techniques. In summation, this study not only illuminates the potential of epigenetic engineering as a catalyst for transformative change in crop improvement but also establishes a robust platform for future research endeavours, transcending the boundaries of wheat-centric inquiry and charting a course towards a more food-secure world (Li *et al.*, 2021; Sun *et al.*, 2018; Zhang *et al.*, 2018)

CONCLUSION

In conclusion, this research demonstrates the potential of epigenetic engineering in enhancing key traits of Pakistani wheat. Targeted editing led to improved disease resistance, higher yield, and enriched nutritional content, offering tailored solutions for global agriculture. These findings advance our understanding and lay a foundation for future crop improvement strategies. Collaboration among geneticists, molecular biologists, and plant breeders will be pivotal in refining and deploying these innovative techniques, contributing to a more resilient and nourished world.

LIMITATIONS

The limitations of this study are that the potential for off-target effects in epigenetic editing, requiring precise targeting strategies. Additionally, the long-term stability of epigenetic modifications and their heritability across generations may warrant further investigation for practical implementation.

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