Review Article

Genomic Innovations in Wheat (*Triticum aestivum* L.): A Comprehensive Review of Recent Developments and Future Directions

ABSTRACT

Genomic innovations have revolutionized the field of wheat breeding by providing advanced tools to enhance its biofortification and overall productivity. This comprehensive review synthesizes recent developments in wheat genomics, focusing on the integration of various approaches such as genome-wide association studies (GWAS), marker-assisted selection (MAS), and quantitative trait locus (QTL) mapping. These genomic techniques have significantly advanced our understanding of the genetic basis underlying key traits, facilitating the development of wheat varieties with improved nutritional profiles and agronomic characteristics. Association mapping, leveraging natural variation within wheat populations, has identified numerous genetic loci associated with important traits, including grain quality and resistance to abiotic and biotic stresses. Marker-assisted selection (MAS) has been instrumental in accelerating the breeding process by enabling the selection of desirable traits based on molecular markers linked to these traits. Additionally, QTL mapping has provided valuable insights into the genetic architecture of complex traits, guiding breeders in the development of high-yielding and nutritionally enhanced wheat varieties. The convergence of these genomic approaches, including GWAS, MAS, and QTL mapping, has ushered in a new era of precision breeding. This review highlights the synergy between these methodologies, illustrating their combined impact on improving wheat biofortification. Future directions in wheat genomics will likely focus on integrating these approaches with emerging technologies, such as CRISPR-based genome editing and advanced phenotyping techniques, to address the challenges of global food security and climate change. The continuous advancement of these genomic tools promises to further enhance the resilience and nutritional quality of wheat, ensuring its pivotal role in global agriculture.

Keywords: *Biofortification; Productivity; GWAS; MAS; QTL; Association mapping; CRISPR*

1. INTRODUCTION

Common wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) is a crucial global crop, providing more than 30% of the world's total calorie intake [1-2]. It is a key component in a variety of cereal-based processed foods, including bread, cookies, and noodles. Wheat is cultivated, consumed, and traded worldwide, ranking third in cereal production. It contains significant amounts of carbohydrates (78.1%), protein (14.7%), fat (2.1%), and minerals (2.1%) [3]. Micronutrient deficiencies are a primary cause of global malnutrition and profoundly affect diets, especially in developing regions. Hidden hunger, prevalent in less developed countries, significantly impacts health. The importance of addressing mineral micronutrient deficiencies, particularly iron and zinc, is critical. Iron deficiency anemia, affecting many women and children of reproductive age, is a notable issue [4]. In populations where cereal-based foods like wheat are dietary staples, the severity of iron and zinc deficiencies is heightened due to the loss of these micronutrients during grain processing, leading to "hidden hunger" [5].

Despite considerable increases in production since the 1960s 'Green Revolution' and the use of marker-assisted molecular breeding [6], wheat faces unprecedented challenges from global climate change, rapid population growth, and water shortages in arid and semi-arid regions [7-8]. Additionally, the excessive use of fertilizers and pesticides exacerbates environmental degradation and pollution. The hexaploid nature and gene redundancy of wheat further complicate genetic selection, often extending the process or making it difficult due to gene linkage or drag [9]. To ensure global food and ecosystem security, it is crucial to enhance the resilience of wheat production while minimizing environmental impacts through the adoption of advanced technologies. Wheat is a major staple crop worldwide, occupying about 17% of global crop acreage [10]. It is essential for feeding nearly half of the world's population and provides roughly one-fifth of the total food calories and protein in human diets [11].

Although wheat production has seen steady growth over the past forty years, recent trends indicate a decline, with current global wheat stocks reaching their lowest levels since 1948/49.

Genome-editing technologies have revolutionized plant research, offering substantial potential for crop improvement. Among these technologies, the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-CRISPR-associated protein (CRISPR-Cas) system is particularly notable for its versatility, simplicity, and cost-effectiveness in making precise DNA modifications [12]. This system enables targeted mutagenesis, including gene knockout, single-base substitutions, and gene or allele replacements in vivo. Recently, CRISPR/Cas has become a leading tool in genome editing, driving progress in the field. Its applications are especially relevant for advancing plant biology and crop improvement in the face of global climate change and pressing agricultural [13], environmental [14], and ecological challenges [15]. Leveraging CRISPR/Cas technology presents a promising path toward addressing these issues, paving the way for precision breeding and sustainable agriculture [16].

Various genomic methods, such as quantitative trait loci (QTL) mapping, marker-assisted selection (MAS), and genomic selection, have been extensively used to improve wheat biofortification [17]. While numerous techniques are available for QTL mapping in experimental crosses, identifying the molecular basis of QTLs remains challenging. This is true even in well-studied model plants like Arabidopsis and rice, due to the difficulty of precisely pinpointing QTLs to single genes [18]. Factors such as experimental design, the type of plant population studied, and the level of polymorphism between parental genomes also affect QTL predictions. Statistical approaches for identifying QTLs require a large number of molecular markers and high-resolution genetic maps [19]. This methodology is fundamental to genomics, aimed at unraveling complex phenotypes. Several QTL mapping studies have successfully pinpointed a variety of stable and reliable QTLs, providing valuable insights into the genetic basis of biofortification traits. These discoveries enhance our understanding of wheat genetics and offer promising avenues for future breeding programs focused on improving nutritional quality.

A range of molecular markers, derived from expressed sequence tags (ESTs) and genomic DNA, has been instrumental in revealing genome relationships and comparing marker-trait associations across various crops [20]. Comparative genomics, especially among major cereal crops like wheat, has illuminated evolutionary relationships and informed crop improvement strategies. Although functional genomics research in wheat has historically lagged behind that of crops such as maize and rice, recent advancements have been significant. Techniques such as RNA interference, TILLING, and "expression genetics" have greatly contributed to mapping expression quantitative trait loci (eQTLs) and understanding the functions of specific genes [21]. These advancements have helped identify candidate genes for targeted traits, aiding both in trait biology comprehension and the development of diagnostic markers for gene cloning and marker-assisted selection. To accelerate wheat genome sequencing, the International Genome Research on Wheat (IGROW) initiative was established and has since evolved into the International Wheat Genome Sequencing Consortium (IWGSC) [22]. This international collaboration is set to enhance genome sequencing efforts and provide a thorough analysis of the wheat genome's structure and function. In light of these developments, Somers identified five key areas for wheat improvement research: genetic mapping, QTL analysis, molecular breeding, association mapping, and software development [23]. These areas are crucial for advancing wheat breeding and genomics, leading to improved crop productivity and sustainability.

2. APPLICATION OF GENOMICS TO MOLECULAR BREEDING OF WHEAT

2.1 Association mapping in wheat

The integration of genomics into wheat molecular breeding represents a significant leap forward in modern agriculture. By utilizing advanced genomic tools and techniques, researchers and breeders can significantly improve the efficiency and accuracy of wheat breeding programs. Genomics provides a broad range of methodologies, including quantitative trait loci (QTL) mapping [24], marker-assisted selection (MAS) [25], genomic selection (GS) [26], association mapping, functional genomics, genome sequencing and assembly, and gene editing technologies such as CRISPR-Cas9 [27]. These techniques enable the development of wheat varieties with enhanced yield, quality, resistance to biotic and abiotic stresses, and improved nutritional content [28]. Incorporating genomics into wheat breeding aims to tackle challenges arising from environmental changes, rising food demand, and evolving pest and disease pressures, thereby contributing to global food security and sustainability.

The use of genomics in wheat molecular breeding includes various methodologies, notably association mapping. This high-resolution technique for identifying quantitative trait loci (QTL) capitalizes on linkage disequilibrium (LD) and has significant potential for dissecting complex traits [29]. Association mapping offers numerous advantages, as extensively discussed in the literature. In wheat, certain genomic regions are more amenable to LD/association mapping for QTL detection and fine mapping than others. This variability in LD across different chromosome regions highlights the effectiveness of association mapping in uncovering the genetic architecture of wheat traits [30].

2.2 Marker-assisted selection in wheat

In recent decades, the discovery of numerous marker-trait associations has advanced the use of molecular markers for marker-assisted selection (MAS) in bread wheat, a technique increasingly adopted worldwide. Major MAS programs are currently active in the USA, Australia, and at CIMMYT in Mexico. In the USA, a wheat MAS consortium, which includes over 20 wheat-breeding programs, was formed to incorporate MAS into public wheat breeding efforts [31]. These programs have enabled the transfer of up to 27 insect and pest resistance genes and 20 alleles linked to improved bread-making and pasta quality into approximately 180 lines tailored to key US production areas [32]. This initiative has led to the release of 45 MAS-derived germplasm lines. Similarly, Australia's program has targeted 20 different traits, including resistance to various abiotic stresses, resulting in the development of enhanced cultivars. MAS has become the preferred approach for selecting crucial agronomic traits, especially where traditional bioassays were costly or inconclusive, as demonstrated by Agriculture Victoria's selection for cereal cyst nematode resistance [33].

MAS has also been incorporated into backcross breeding to introduce QTLs that enhance transpiration efficiency and to perform negative selection against undesirable traits, such as yellow flour color [34]. In Australia, scientists have utilized computer simulations to create cost-effective marker-assisted wheat breeding strategies, combining restricted backcrossing with doubled haploid (DH) technology to reduce breeding costs by up to 40% [35]. This MAS approach has been successfully applied in wheat breeding programs focused on improving quality and rust disease resistance. At CIMMYT, markers linked to 25 different genes controlling traits such as insect pest resistance, protein quality, homoeologous pairing, and other agronomic characteristics are employed in breeding programs to develop enhanced cultivars tabulated in **Table 1** [36]. Some of these markers are "perfect markers" derived from the nucleotide sequences of these genes. Future large-scale sequencing of gene-rich regions (GRRs) by the International Wheat Genome Sequencing Consortium (IWGSC) is anticipated to aid in isolating key genes for generating improved transgenic crops and developing perfect markers for significant agronomic traits to be used in MAS [37].

Table 1: CRISPR/Cas-mediated genome editing in wheat. Source: Bapela *et al.* [36]

Target gene	Nucleases	Transformation	Improved agronomic traits	Edit
TaMLO	Cas9	bombardment	powdery mildew resistance	knockout
TaLOX2	Cas9	bombardment	varied grain size, weight and increased storability	knockout
TaPHO2-A1	Cas9	Agrobacterium	increased phosphorus uptake	knockout
TaGASR7	Cas9	bombardment	increased yield	knockout
TaEDR1	Cas9	Agrobacterium	powdery mildew resistance	knockout
TaGW2	Cas9	bombardment	increased yield	knockout
TaZIP4	Cas9	Agrobacterium	increased homoeologous CO frequency	knockout

TaHRC	Cas9	bombardment	Fhb resistance	knockout
TaMs1	Cas9	bombardment	male sterility	knockout
TaSBEIIa	Cas9	bombardment	high amylose	knockout
TaDA1/TaPDS/ TaNCED1	Cas9	Agrobacterium	–	knockout
TaCENH3a	Cas9	Agrobacterium	high haploid induction rate	knockout
TaQsd1,TaARE1, TaNPT1, TaSBEIIa, TaSPDT	Cas9	bombardment	–	knockout and multiplexing
TaLOX2	nCas9-D10A/ dCas9	Agrobacterium /bombardment	improved wheat quality	base editing
TaALS, TaACC	nCas9-D10A	bombardment	herbicide resistance	base editing
Ubi10, TaGW2, TaGASR7, TaDME1 TaLOX2,TaMLO,	nCas9-H840A	protoplast transformation	–	primer editing
Ubiquitin	Cas9	bombardment	–	HDR replicon

2.3 CRISPR/Cas9-mediated knockout and its applications in wheat

To date, the predominant approach in genome-editing studies has been the use of non-homologous end joining (NHEJ) to create loss-of-function mutations at specific gene loci in various crops, including wheat. CRISPR/Cas9 has become a widely utilized tool for improving wheat yields and quality (Fig. 1). For example, targeting the lipoxygenase (LOX) gene, which plays multiple roles in plant growth, development, and defense, led to changes in grain size, weight, and improved storability in wheat [38]. Additionally, simultaneous targeting of all three gibberellin-regulated TaGASR7 genes, which affect grain size, resulted in a significant increase in thousand-kernel weight. The deletion of the phosphate 2 gene TaPHO2-A1 enhanced phosphorus uptake and grain yield under low-phosphorus conditions, while knocking out the RING-type E3 ligase gene TaGW2 increased grain size and, consequently, grain yield [39].

Enhancing wheat quality to satisfy varied consumer needs is a central objective of wheat breeding programs. One group of gluten proteins, α-gliadin, plays a significant role in the end-use properties of wheat in food processing but also contains major immunogenic epitopes that can lead to health issues such as celiac disease [40]. Mutations in genes associated with gliadin, Waxy, and VIT2 have been shown to reduce gliadin content and increase branched starch content. Recently, high-amylose wheat has been developed through targeted mutagenesis of the TaSBEIIa gene using CRISPR/Cas9, offering potential for creating new wheat varieties with improved nutritional value [41]. Base editing, a precise technique for inducing single-nucleotide point mutations, is also emerging as a valuable method for modifying critical agronomic traits in wheat and other crop plants.

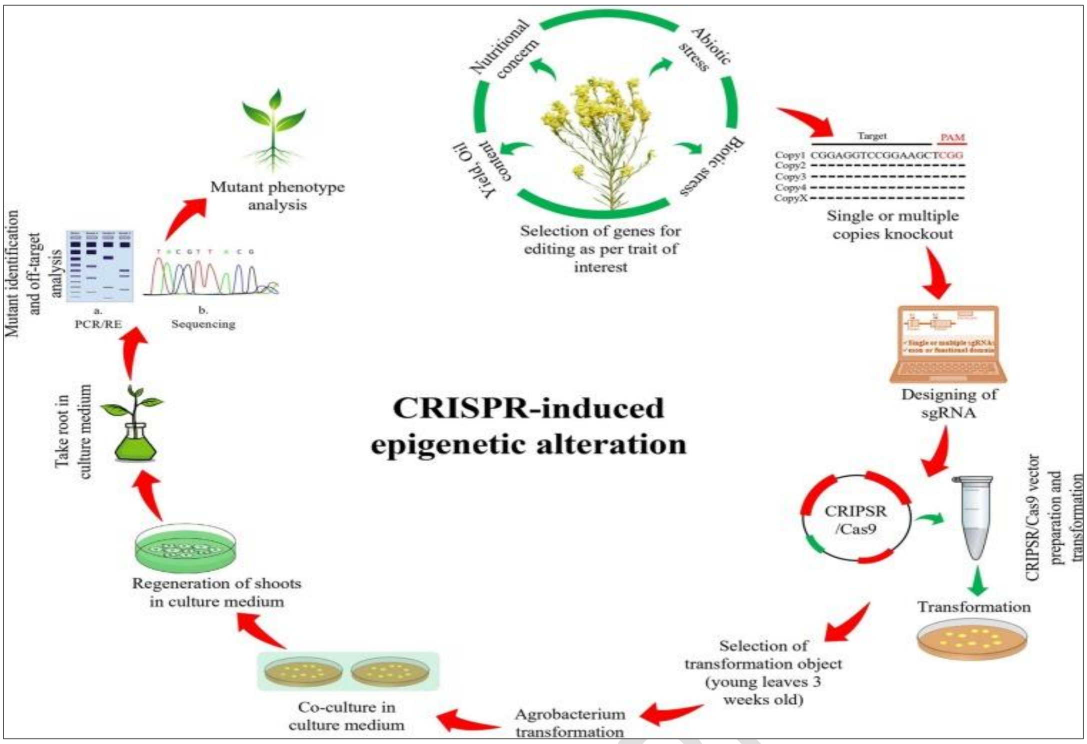

Fig. 1: Epigenetics for Crop Improvement using CRISPR [39]

3. QUANTITATIVE TRAIT LOCUS (QTL) MAPPING

Several studies have focused on mapping quantitative trait loci (QTLs) for grain iron (Fe) and zinc (Zn) concentrations in wheat. In 1997, the first QTL associated with iron and zinc concentration, known as Gpc-B1, was identified on chromosome 6BS in a recombinant inbred line (RIL) population derived from a cross between durum wheat and wild emmer (Triticum turgidum) [42]. This QTL was found to increase iron concentration by 18% and zinc concentration by 12%. Numerous QTL studies have been conducted to explore the genomic regions linked to iron and zinc content in wheat grains. Various biparental mapping populations have been employed, primarily using bread wheat cultivars as parents, though other hexaploid species such as Triticum spelta, stabilized synthetic hexaploid wheat (SHW) lines, and tetraploid parents have also been utilized. Additionally, many studies leverage multi-environment trial data to identify stable QTLs for grain Fe and Zn content [43].

In the studies mentioned, QTLs often varied due to differences in populations, environments, and marker sets, which affected the accuracy and positioning of QTL identification. With the advent of new genome sequences, it is now possible to better compare the actual locations of QTLs identified in different studies using these updated reference sequences. This comparison may help in pinpointing QTLs that consistently enhance iron and zinc concentrations. However, practical constraints limit the number of markers that can be used simultaneously for multiple QTLs in large breeding populations, making marker-assisted selection challenging. An alternative approach is to use marker-assisted selection to stack small-effect QTLs within the same genomic region, creating a haplotype with multiple beneficial QTLs. A haplotype refers to a set of DNA variations that are inherited together on a single chromosome. By selecting this haplotype as a single unit, the number of markers needed for further selection can be reduced. This method also allows for the integration of QTLs for higher iron and zinc content with those for other desirable traits, such as yield or disease resistance, which are likely already being targeted through marker-assisted selection [44].

Several QTL studies have been conducted to identify genomic regions associated with iron (Fe) and zinc (Zn) concentrations in wheat. For example, a double haploid population derived from a cross between Berkut and Krichauff was analyzed, with grain Zn and Fe concentrations measured using X-ray fluorescence. Composite interval mapping revealed three QTLs: one associated with protein (1A) and two linked to Zn, with one QTL also associated with Fe [45]. These QTLs, identified across multiple seasons, appear to be reliable and useful for marker-assisted selection (MAS). In a current study at

CIMMYT in Mexico, a line with high grain zinc content from China was crossed with the Mexican bread wheat cultivar Roelfs F2007 to develop a recombinant inbred line (RIL) mapping population for QTL identification. Using the annotated wheat genome sequence, researchers identified 55 potential genes related to Zn and Fe content in the grain [48]. Additionally, four QTLs associated with grain Zn and Fe concentrations were found in a RIL population generated from a cross between the Indian variety 'WH 542' and a Synthetic Hexaploid Wheat (SHW) line [46]. Large-scale implementation of breeding programs to develop nutrient-rich elite wheat lines should be a priority. Developing countries are particularly affected by micronutrient deficiencies, making bio-fortification of wheat with QTLs that enhance grain iron (Fe) and zinc (Zn) content a cost-effective and sustainable solution. To boost Fe and Zn levels, it is essential to screen diverse sources such as unknown landraces, related species, wild relatives, promising synthetic hexaploid wheat (SHW) lines, and alleles from high-nutrient genetic resources. Mapping populations across various environments is critical for understanding genotype-environment interactions and identifying stable QTLs for these traits. Incorporating reported QTLs into elite genetic backgrounds can lead to the development of nutrient-rich breeding lines, offering a long-term approach to addressing malnutrition [47].

4. GENE CLONING METHODS

After phenotyping a promising mutant, the next critical step is to identify the specific gene responsible for the observed trait. This process relies on genomic sequences as the foundation for gene cloning. Before the advent of reference genomes, many genes associated with important agricultural traits in crops were identified based on their genetic characteristics alone. Positional or map-based cloning is a valuable technique for gene identification. This approach involves creating high-resolution mapping populations that are phenotyped for the desired trait and genotyped using molecular markers. By employing mapping populations consisting of several hundred to thousands of plants generated through genetic recombination, researchers can progressively locate the site of a mutant gene using methods like Mutmap-based cloning [48]. The integration of phenotypic and molecular marker data allows for the construction of a genetic map that covers the target locus. Map-based cloning does not require prior knowledge of the gene product to be effective. This technique has successfully cloned several genes, such as Lr10, HM1, and Xa21 from wheat, maize, and rice, respectively [49]. Notably, approximately half of these cloned genes confer disease resistance, as shown in Table 2. However, map-based cloning is labor-intensive and time-consuming, and it is not feasible for genes located in centromeric regions [50].

Table 2: Function of the genes cloned in wheat

Genes	Function	Identification method	Reference
Lr21	Leaf rust resistance	Map-based cloning	[51]
Lr10	Leaf rust resistance	Map-based cloning	[52]
Lr34	Leaf rust resistance	Map-based cloning	[53]
Yr36	Stripe rust resistance	Map-based cloning	[54]
Yr10	Stripe rust resistance	Map-based cloning	[55]
Sr33	Stem rust resistance	Map-based cloning	[56]
Sr35	Stem rust resistance	Map-based cloning	[57]
Pm2	Powdery mildew resistance	MutChromSeq	[58]
Fhb1	Fusarium head blight resistance	Map-based cloning	[59]
TaTAR2.1-3A	Boost production and nitrogen usage effectiveness	Homology-based cloning	[60]

Bo1, Bo4	Boron transporter	Map-based cloning	[61-62]
Gpc-B1	Boosting iron, zinc, and kernel protein by controlling senescence.	Map-based cloning	[63]

5. FUTURE PERSPECTIVES OF GENOME EDITING

The future trajectory of wheat breeding and genome editing holds immense promise, driven by the ongoing advancements in cutting-edge technologies and innovative strategies outlined in our review. Looking forward, several key developments are expected to shape the field:

1. Advanced Genome Editing Technologies: The refinement and integration of genome editing tools, such as CRISPR/Cas-mediated base editing and prime editing, will offer unprecedented precision and efficiency in modifying the wheat genome. These technologies will enable the targeted alteration of specific genes, improving traits such as yield, disease resistance, and nutritional quality.

2. Convergence of Genomic Approaches: The synergy of genome-wide association studies (GWAS), marker-assisted selection (MAS), and quantitative trait locus (QTL) mapping will enhance the identification of crucial genes linked to desirable traits. This convergence will accelerate the development of improved wheat varieties by providing comprehensive insights into the genetic basis of key agronomic and nutritional traits.

3. Multiplex Genome Editing: The advent of multiplex genome editing systems will facilitate the simultaneous modification of multiple genes. This capability will enable the pyramiding of beneficial traits, such as improved disease resistance and enhanced nutritional content, into elite wheat cultivars, leading to more robust and high-performing varieties.

4. Genotype-Independent Transformation: Advances in genotype-independent wheat transformation methods will broaden the applicability of genome editing across diverse germplasms. This inclusivity will support the development of wheat varieties suited to a wide range of environmental conditions and agricultural contexts.

5. Synthetic Biology and Gene Stacking: Synthetic biology approaches will offer new opportunities for gene stacking, allowing the accumulation of multiple transgenes to confer complex traits or introduce novel functionalities in wheat. This capability will be particularly valuable for addressing multifaceted challenges in wheat production.

6. Speed Breeding Integration: The integration of speed breeding techniques with rapid advancements in genome editing will expedite breeding cycles. This combination will enable the faster development of new wheat varieties, helping to meet the evolving demands of agriculture and address food security challenges.

7. Collaborative Initiatives and Knowledge Sharing: Collaborative research efforts and knowledge-sharing platforms will play a crucial role in advancing scientific discoveries, promoting the adoption of new technologies, and developing regionally adapted wheat varieties. These initiatives will enhance the collective ability to address local and global agricultural challenges.

In summary, the future of wheat breeding and genome editing is poised to revolutionize agricultural practices. By leveraging these emerging technologies and strategies, we can enhance nutritional quality, improve yields, and promote sustainable practices, ultimately contributing to global food security and agricultural resilience.

6. CONCLUSION

The future of wheat breeding and genome editing is poised for transformative advancements, driven by the integration of cutting-edge technologies and innovative strategies. The adoption of advanced genome editing tools, such as CRISPR/Cas-mediated base editing and prime editing, promises precise and efficient modifications to the wheat genome, ushering in an era of tailored genetic improvements. These technologies will enable the development of elite wheat varieties with enhanced traits, improving resilience to both biotic and abiotic stresses while maximizing productivity and nutritional quality. The use of multiplex genome editing systems and genotype-independent transformation strategies will further accelerate the breeding process by allowing the simultaneous modification of multiple genes and expanding the range of germplasms that can be targeted. This will facilitate the creation of wheat varieties with stacked beneficial traits, addressing critical challenges in modern agriculture. The convergence of diverse genomic approaches, including genome-wide association studies (GWAS), marker-assisted selection (MAS), and quantitative trait locus (QTL) mapping, will provide valuable insights into the genetic basis of complex traits. This understanding will enable more targeted and effective breeding efforts, leading to the development of varieties that meet specific agricultural and nutritional needs. Synthetic biology methodologies will offer new opportunities for gene stacking and trait diversification, while speed-breeding techniques will shorten breeding cycles, allowing for rapid variety development in response to evolving demands. Collaborative initiatives and knowledge-sharing platforms will play a crucial role in fostering innovation, promoting the adoption of new technologies, and ensuring equitable access to advanced breeding tools. As we face the challenges of a changing climate, growing population, and shifting agricultural landscapes, the synergy between wheat breeding and genome editing offers a beacon of hope for sustainable food production and global food security. By harnessing the power of scientific and technological advancements, we can develop resilient, nutritious, and environmentally sustainable wheat varieties tailored to diverse agroecological contexts, ensuring a brighter future for generations to come.

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