Review article

Advancements of Breeding and Genomics in Wheat (*Triticum aestivum* L.): Enhancing Yield and Nutritional Value for Sustainable Agriculture

Abstract

Advancements in wheat breeding and genomics presently explores the genomic interventions driving focusing on quantitative trait loci (QTL) mapping, marker-assisted selection (MAS) and genomic selection (GS). QTL mapping emerges as a pivotal method for pinpointing markers linked with desirable traits, facilitating MAS. Furthermore, genomic selection (GS) holds immense potential for crop improvement. It also delves into the current landscape of MAS and explores various prospects of GS for wheat biofortification. Looking ahead, accelerated mapping studies combined with MAS and GS schemes are poised to further enhance wheat breeding efficiency. Dense molecular maps and a large set of ESTs (Expressed Sequence Tags)have enabled genome-wide identification of gene-rich and gene-poor regions, as well as QTL, including eQTL (Expression quantitative trait loci). Additionally, markers associated with major economic traits have facilitated MAS programs in some countries and enabled map-based cloning of several genes/QTL. Resources for functional genomics, such as TILLING and RNA interference (RNAi), alongside emerging approaches like epigenetics and association mapping, are further enriching wheat genomics research. In this review, we initially present cutting-edge genome-editing technologies in crop plants, with a specific focus on wheat, addressing both functional genomics and genetic enhancement. We subsequently delineate the utilization of additional technologies, including GWAS, high-throughput genotyping and phenotyping, speed breeding, and synthetic biology, within the context of wheat breeding. We assert that integrating genome editing with other molecular breeding strategies will significantly expedite the genetic enhancement of wheat, thus contributing to sustainable global production.

Keywords:*GWAS, Genotyping, Phenotyping, TILLING, MAS, QTL, Speed Breeding*

1. INTRODUCTION

Common wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) stands as one of the world's vital crops, contributing over 30% of the total calories consumed globally [1,59]. It serves as the primary ingredient in numerous cereal-based processed foods like bread, cookies, and noodles. Despite significant production increases since the 'Green Revolution' of 1960 and the adoption of marker-assisted molecular breeding [2,60], wheat faces unprecedented challenges due to global climate change, burgeoning world population, and water scarcity in arid and semi-arid regions [3-4, 62]. Moreover, excessive use of fertilizers and pesticides aggravates environmental

degradation and pollution concerns. Additionally, the hexaploid nature and gene redundancy in wheat complicate genetic selection, often prolonging the process or rendering it impossible due to gene linkage or drag [5]. To ensure food and ecosystem security worldwide, it becomes imperative to bolster the resilience of wheat production while mitigating environmental impact through the integration of advanced technologies.Wheat stands as one of the paramount staple food crops globally, covering approximately 17% of total crop acreage worldwide [6]. It serves as a vital source of sustenance, nourishing nearly half of the world's population and contributing about one-fifth of total food calories and protein in human diets [7, 57-58]. Despite witnessing steady and substantial growth in wheat production over the past four decades, recent years have seen a decline, culminating in the lowest global wheat stocks recorded since 1948/49.

Genome-editing technologies have sparked a revolution in plant research, offering immense potential for crop enhancement. Among these, the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-CRISPR-associated protein (CRISPR-Cas) system stands out as a versatile, straightforward, and cost-effective tool for precise modifications of DNA sequences [8]. This includes targeted mutagenesis for gene knockout, single-base substitution, and gene/allele replacement in vivo. In recent years, CRISPR/Cas has emerged as the dominant force in genome editing, shaping advancements in the field.The applications of CRISPR/Cas hold particular significance in both plant biology and crop improvement [63], especially given the challenges posed by global climate change and the pressing agricultural [9], environmental [10], and ecological issues of our time [11]. Harnessing the power of CRISPR/Cas technology offers a promising avenue for addressing these challenges, ushering in a new era of precision breeding and sustainable agriculture [12].

Various genomic approaches, including quantitative trait loci (QTL) mapping, marker-assisted selection (MAS), and genomic selection, have been widely utilized for enhancing the biofortification of wheat [13]. Numerous techniques exist for mapping QTL in experimental crosses. However, the molecular basis of QTLs presents a challenge, even in model plants like Arabidopsis and rice, due to difficulties in precisely narrowing intervals to single genes [14]. Factors such as experimental design, the type of plant population analysed, and the level of polymorphisms between parental genomes also influence QTL predictions.Statistical methods for identifying quantitative trait loci (QTL) necessitate a plethora of molecular markers and high-resolution genetic maps [15]. This approach stands as a cornerstone of genomics, aimed at dissecting complex phenotypes. Several QTL mapping studies have successfully identified a range of stable and consistent QTLs, offering valuable insights into the genetic underpinnings of biofortification traits. These findings contribute to our understanding of wheat genetics and hold promise for future breeding efforts aimed at enhancing nutritional quality.

A variety of molecular markers, derived from both ESTs and genomic DNA, have played a crucial role in uncovering relationships between genomes and comparing marker-trait associations across different crops [16]. Comparative genomics, particularly among major crop grasses like wheat, has not only shed light on evolutionary relationships but has also guided the design of crop improvement initiatives. While functional genomics research in wheat has

historically lagged behind that of other major food crops such as maize and rice, recent advancements have been notable. Technologies like RNA interference, TILLING, and "expression genetics" have significantly contributed to mapping eQTLs and elucidating the functions of individual genes [17]. These developments have facilitated the identification of candidate genes for specific traits, aiding in both understanding trait biology and developing diagnostic markers for map-based gene cloning and marker-assisted selection.To expedite the sequencing of the Greater Region of the wheat genome, the International Genome Research on Wheat (IGROW) initiative was initially launched, evolving into the International Wheat Genome Sequencing Consortium (IWGSC) [18]. Timeline of advancements in genotyping of whole-genomeillustrated in **Figure 1**. This multinational collaboration is poised to accelerate genome sequencing progress and enable comprehensive analysis of the structure and function of the wheat genome.In light of these advancements, Somers identified five primary thrust areas for wheat improvement research: genetic mapping, QTL analysis, molecular breeding, association mapping, and software development [19]. These areas serve as key focal points for advancing wheat breeding and genomics, paving the way for enhanced crop productivity and sustainability.

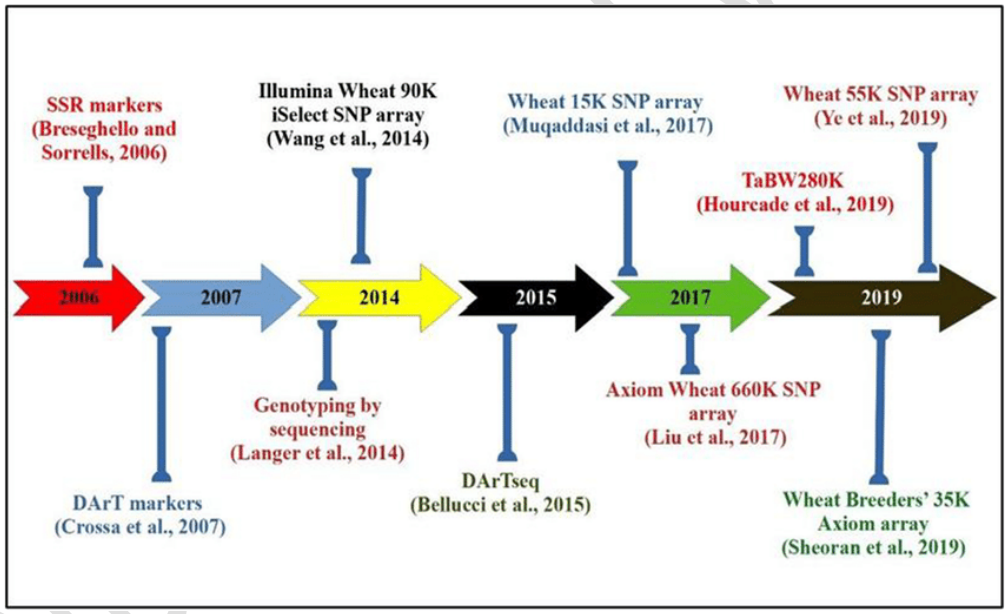

Figure 1: Timeline of advancements in genotyping of whole-genome

2. APPLICATION OF GENOMICS TOMOLECULAR BREEDING OFWHEAT

2.1 Association mapping in wheat

The application of genomics in molecular breeding of wheat represents a pivotal advancement in modern agriculture. By leveraging genomic tools and techniques, researchers and breeders can enhance the efficiency and precision of wheat breeding programs. Genomics offers a wide array of methodologies, including but not limited to quantitative trait loci (QTL) mapping [20], marker-assisted selection (MAS) [21], genomic selection (GS) [22], association mapping, functional genomics, genome sequencing and assembly [23], and gene editing technologies such as CRISPR-Cas9 [8, 24]. These approaches empower breeders to develop wheat varieties with improved yield, quality, resilience to biotic and abiotic stresses, and

nutritional content [61]. By integrating genomics into wheat breeding programs, researchers aim to address the challenges posed by changing environmental conditions, increasing food demand, and evolving pest and disease pressures, ultimately contributing to global food security and sustainability.

The application of genomics in molecular breeding of wheat encompasses various methodologies, including association mapping. This high-resolution technique for mapping quantitative trait loci (QTL) leverages linkage disequilibrium (LD) and shows significant potential for dissecting complex traits [25]. Association mapping offers several advantages, extensively deliberated in literature. In wheat, certain genomic regions exhibit higher amenability to LD/association mapping for QTL detection and fine mapping compared to others. This variability in the level of LD across the chromosome length underscores the utility of association mapping in unraveling the genetic architecture of wheat traits [26].

2.2 Marker-assisted selection in wheat

In recent decades, the identification of numerous marker-trait associations has facilitated the adoption of molecular markers for marker-assisted selection (MAS) in bread wheat, a practice gaining traction in various countries. Major MAS programs in wheat are currently underway in the USA, Australia, and at CIMMYT in Mexico. In the USA, a wheat MAS consortium, comprising over 20 wheat-breeding programs, was established with the aim of integrating MAS into public wheat breeding initiatives [27]. Through these programs, MAS has been instrumental in transferring up to 27 insect and pest resistance genes and 20 alleles associated with improved bread making and pasta quality into approximately 180 lines adapted to primary US production regions [28]. This effort has resulted in the release of 45 MAS-derived germplasm lines. Similarly, the Australian program has focused on enhancing 20 different traits, including resistance to various abiotic stresses, leading to the release of improved cultivars. MAS has become the preferred method for selecting agronomical important traits, particularly where conventional bioassays were costly or inconclusive, as observed in the selection for cereal cyst nematode resistance by Agriculture Victoria [29].

Additionally, MAS has been integrated into backcross breeding to introgress QTL for improving transpiration efficiency and for negative selection against undesirable traits such as yellow flour color [30]. Australian scientists have also employed computer simulations to design economically efficient marker-assisted wheat breeding strategies, combining restricted backcrossing and doubled haploid (DH) technology to reduce breeding costs by up to 40% [31]. This MAS strategy has been practically validated in wheat breeding programs aimed at improving quality and resistance against rust disease. At CIMMYT, markers associated with 25 different genes governing insect pest resistance, protein quality, homoeologous pairing, and other agronomic traits are utilized in wheat breeding programs to develop improved cultivars [32]. Some of these markers are perfect markers derived from available nucleotide sequences of these genes. Future large-scale sequencing efforts of gene-rich regions (GRRs) by IWGSC(International Wheat Genome Sequencing Consortium) are expected to facilitate the

isolation of important genes for producing improved transgenic crops and developing perfect markers for agronomically significant traits to be used in MAS [33].

Table 1: CRISPR/Cas-mediated genome editing in wheat. (Source: Bapela *et al.* [32])

Target gene	Nucleases	Transformation	Improvedagronomic 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

3. EPIGENETICS IN WHEAT

Epigenetics encompasses heritable changes not attributable to alterations in DNA sequence but rather to chemical modifications of nucleotides in DNA or its associated histone proteins within chromatin [12]. Recent studies have initiated investigations into epigenetic modifications within the wheat genome. For example, methylationsensitive amplified polymorphism (MSAP) analysis has been employed to examine DNA methylation levels at four distinct stages (2d, 4d, 8d, and 30d after pollination) during seed development in bread wheat. The findings revealed that 36–38% of CCGG sites exhibited either full methylation at internal Cs or hemimethylation at external Cs across the corresponding stages [28,32].

Similarly, explored genetic and epigenetic alterations among three homoeologs in two class E-type wheat genes associated with flower development, namely, wheat SEPALLATA (WSEP) and wheat LEAFY HULL STERILE1 (WLHS1) [34]. Analysis of gene structure, expression

patterns, and protein functions revealed no alterations in the WSEP homoeologs. In contrast, the three WLHS1 homoeologs displayed genetic and epigenetic modifications, with WLHS1-B predominantly silenced by cytosine methylation [35]. This suggests that the expression of the three homoeologous genes is differentially regulated by genetic or epigenetic mechanisms. Similar investigations have been conducted for several other genes, such as TaHd1 involved in the photoperiodic flowering pathway, Ha for grain hardness, and TaBx for benzoxazinone biosynthesis [36].

4. CRISPR/Cas9-mediated knockout and its applications in wheat

Until now, the predominant method in genome-editing studies involves NHEJ to induce loss-of-function mutations at specific gene loci in various crop plants, wheat included. CRISPR/Cas9 has emerged as a widely used tool for enhancing wheat yields and quality**(Figure 2)**. For instance, targeting lipoxygenase (LOX), a gene with multiple functions in plant growth, development, and defense mechanisms, resulted in altered grain size, weight, and enhanced storability of wheat [37]. Simultaneously targeting all three gibberellin-regulated TaGASR7 genes, known to influence grain size, significantly increased thousand-kernel weight. Additionally, deleting the phosphate 2 gene TaPHO2-A1 improved phosphorus uptake and grain yield under low-phosphorus conditions, while knockout of the RING-type E3 ligase-encoding gene TaGW2 increased grain size, thereby enhancing grain yield [18].

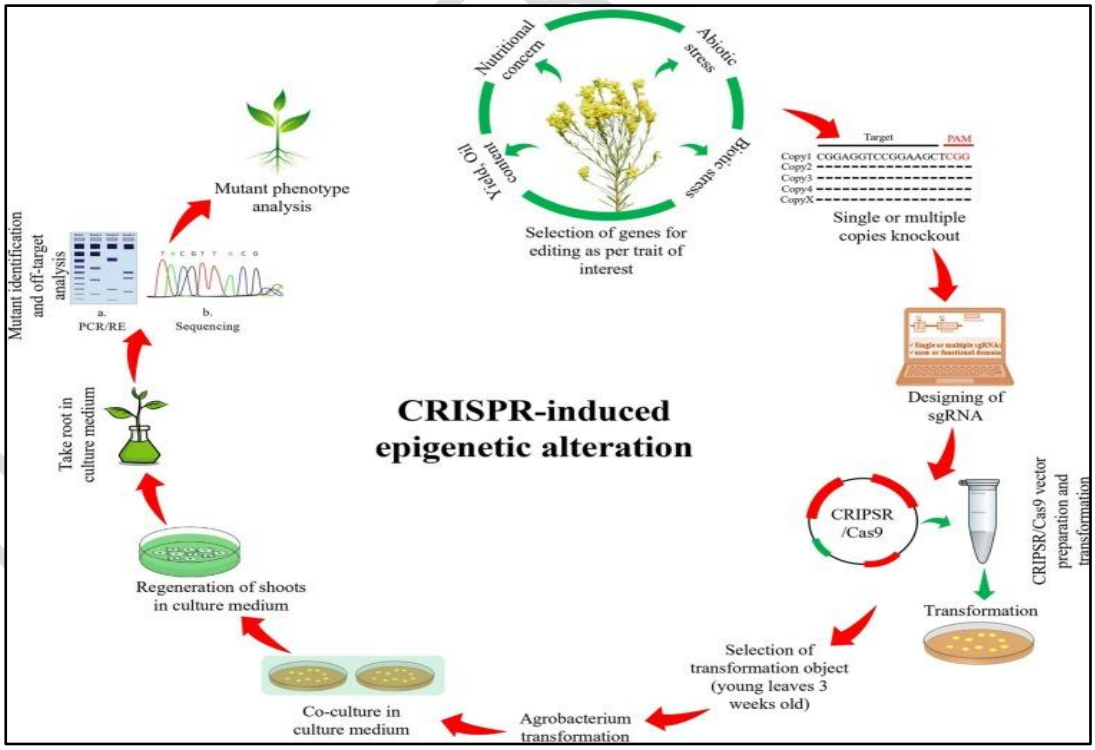

Figure 2: Epigenetics for Crop Improvement using CRISPR [18]

Improving wheat quality to meet diverse consumer demands is a key goal in wheat breeding programs. One group of gluten proteins, a-gliadin, contributes to end-use properties in food processing but contains major immunogenic epitopes that can cause health issues such as celiac

disease [38]. Mutations in genes related to gliadin, Waxy, and VIT2 resulted in decreased gliadin content and increased branched starch content. Recently, high-amylose wheat was developed through targeted mutagenesis of TaSBEIIa using CRISPR/Cas9, offering potential for breeding novel wheat varieties with enhanced nutritional value [39].Base editing offers a precise method to induce single-nucleotide point mutations, which underlie variations in crucial agronomic traits in crop plants like wheat.

5. Base editing and its applications in wheat

In the editing process, deamination of substrate nucleotides within the editing window leads to the formation of uracil and hypoxanthine. Cas nickases, such as nCas9 (D10A), cut the gRNA target DNA strand, promoting DNA repair using the edited DNA strands as templates, resulting in precise base pair transitions at target sites [40]. However, the conversion from cytosine to uracil may be hindered by endogenous uracil glycosylase, which recognizes unnatural U-G pairing. The efficiency and accuracy of the CBE system can be enhanced by using uracil DNA glycosylase inhibitors (UGIs).Further optimization of ABEs has been conducted to enhance base editing efficiency, resulting in the development of the TadA8e system, which significantly improves base editing efficiency [41]. Moreover, surrogate systems and optimization of fusion protein joint sequences, along with an increase in UGI functional domains and nuclear localization sequences (NLSs), can further enhance the efficiency of CBEs or ABEs [42].Base editing has been successfully applied to various crop plants, including rice, maize, soybean, cotton, and oilseeds. Integration of existing base editor types into a single module has enabled the generation of herbicide-resistant rice lines through artificial evolution of the OsACC or OsALS gene [43]. Directed artificial evolution of agriculturally important genes holds promise for generating novel gene resources to improve crops.

6. Prime editing and its applications in wheat

A search-and-replace genome-editing technique, known as prime editing (PE), has been developed to enable precise modifications such as small indels, single or multiple substitutions (including transitions and transversions), and their combinations at specific gene loci in human cells, without the need for Double-Strand Breaks (DSBs) or a DNA Repair Template (DRT) [44]. The PE system utilizes a catalytically impaired Cas9 (H840A) (nCas9) fused with a reverse transcriptase, M-MLV-RT (Moloney murine leukemia virus reverse transcriptase), and employs a prime editing guide RNA (pegRNA) to target the desired site and encode the intended genetic alterations.The pegRNA consists of three components: a single-guide RNA (sgRNA) for target specificity, a reverse transcription (RT) template encoding the desired edit, and a primer-binding site (PBS) for initiating reverse transcription [45]. In the PE system, the protein complex binds to the target DNA, nicks the non-target strand, and the resulting 3' DNA terminus hybridizes with the PBS in the pegRNA, initiating reverse transcription of new DNA containing the desired edit. Subsequent DNA repair incorporates the edited DNA flap into the non-target strand and copies the edit into the complementary target strand, resulting in stable DNA editing [46].

Advancements in protein engineering and pegRNA design have led to the development of three PE systems: PE1, PE2, PE3, and PE3b, each with improvements in editing efficiency and/or product purity [47]. For instance, PE2 replaced the original M-MLV-RT with an engineered version with six mutations to enhance efficacy, while PE3 further increased editing efficiency by introducing another nicking sgRNA to induce a second cut in the non-edited strand. However, the PE3 system exhibited higher indel frequencies due to induced NHEJ near opposite DNA strands. To mitigate this, PE3b uses a specific sgRNA to guide the edited DNA sequence, ensuring the second nicking occurs after resolving the edited strand flap [48].Prime editing offers enhanced precision in genome editing efficiency and product purity compared to conventional HDR strategies, addressing limitations of base editors.

7. BEYOND GENOME EDITING: Genome sequencing

Genome sequencing is pivotal for unlocking genetic insights essential for functional genomics, genome editing, and marker-assisted breeding in wheat [49-50]. Extensive research efforts have been dedicated to expediting wheat enhancement through the genome sequencing of various wheat accessions or their relatives. These endeavors, spanning over years, have significantly advanced our understanding of wheat genetics.In 2004, genome sequencing shed light on the origin of the D genome in the allopolyploid species *Triticum aestivum* and Aegilops cylindrical, catalyzing investigations into the polyploid formation mode in common wheat. The International Wheat Genome Sequencing Consortium (IWGSC) has embarked on an ambitious journey to flow-sort and sequence individual wheat chromosomes, achieving notable progress with chromosomes like 3B and 4A [51]. Whole-genome shotgun sequence analysis of bread wheat and its diploid relatives has attributed over 60% of genes to the A, B, and D genomes with a high level of confidence [52].

Figure 3 illustrates the breeding process for developing a green super wheat variety through CRISPR/Cas-mediated gene editing and other breeding technologies. Here's a breakdown of each component:

(A) Genome sequencing: Wheat genome and pan-genome sequencing provide fundamental information for designing sgRNA targets and evaluating off-target effects in wheat genome editing.

(B) GWAS analysis: Genome-wide association studies (GWAS) enable the identification of specific genes, SNPs, or markers associated with particular traits on a chromosome.

(C) CRISPR/Cas-mediated multiplex system: A multiplex system allows for the simultaneous knockout (KO) of multiple genes using CRISPR/Cas technology.

(D) CRISPR/Cas-mediated HDR investigation: Research into CRISPR/Cas-mediated homology-directed repair (HDR) aims to enhance HDR efficiency in wheat.

(E) Development of a module for simultaneous HDR and/or base editing (BE): Creating a module for simultaneous HDR and/or base editing (BE) and knockout would expedite the translational breeding process by pyramiding favorable alleles in elite wheat varieties more efficiently.

(F) Development of diverse genotype-independent strategies: Genotype-independent strategies facilitate the transformation of recalcitrant wheat varieties, making genome editing accessible in diverse elite wheat germplasm.

(G) Gene stacking by synthetic biology: Synthetic biology enables the accumulation of multiple transgenes in the same plant genome to stack beneficial traits or create novel traits.

(H) Speed breeding: Speed breeding shortens the generation time for seed harvesting in wheat, accelerating breeding efforts.

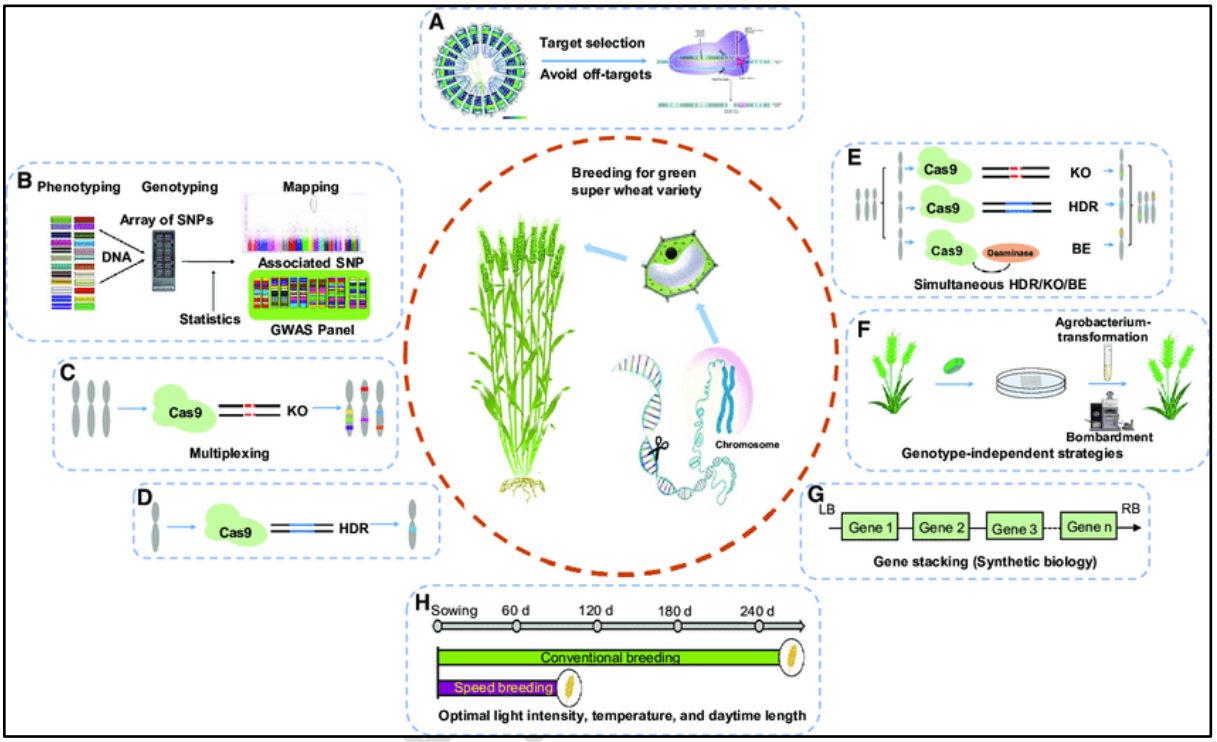

Figure 3: Breeding of a green super wheat variety through various approaches [53]

Moreover, analysis of the wheat D genome donor, *Aegilops tauschii*, revealed the expansion of agronomical significant genes associated with disease resistance, stress tolerance, and grain quality [53]. Similarly, sequencing the diploid progenitor genome of the A genome, *Triticum urartu*, has provided a valuable reference for analyzing polyploid wheat genomes and improving wheat genetics.A haplotype map of allohexaploid wheat showcased distinct selection patterns on homoeologous genomes, potentially broadening the spectrum of selection targets [54]. Subsequently, obtaining a high-quality reference genome sequence of the wheat D genome donor, *Ae. tauschii*, facilitated the characterization of gene expression profiles, methylation, microRNAs, and transposable elements [55]. Despite the challenges posed by wheat's hexaploid nature and gene redundancy, ongoing efforts by the IWGSC and other researchers hold promise for generating comprehensive reference sequences essential for further enhancing wheat breeding and crop improvement [56].

8. Future perspectives of genome editing

The future trajectory of wheat breeding and genome editing holds immense promise, propelled by advancements in cutting-edge technologies and innovative strategies discussed in our review. As we look ahead, continued refinement and integration of genome editing technologies, such as CRISPR/Cas-mediated base editing and prime editing, will enable precise and efficient modification of the wheat genome. The convergence of various genomic approaches, including genome-wide association studies (GWAS), marker-assisted selection (MAS), and quantitative trait locus (QTL) mapping, will facilitate the identification of key genes associated with desirable traits, accelerating the development of improved wheat varieties. Multiplex genome editing systems will enable the simultaneous modification of multiple genes, facilitating the pyramiding of beneficial traits in elite wheat cultivars. Furthermore, genotype-independent strategies for wheat transformation will broaden the application of genome editing across diverse germplasms, fostering inclusivity in breeding programs. Synthetic biology approaches will unlock opportunities for gene stacking, allowing for the accumulation of multiple transgenes to confer complex traits or novel functionalities in wheat. The integration of speed breeding techniques with rapid advancements in genome editing will expedite breeding cycles, enabling faster variety development to meet the evolving needs of agriculture. Collaborative initiatives and knowledge-sharing platforms will play a pivotal role in driving scientific discoveries, promoting technology adoption, and facilitating the development of regionally adapted wheat varieties tailored to diverse agroecological contexts. In essence, the future of wheat breeding and genome editing holds immense potential to address global food security challenges, enhance nutritional quality, and promote sustainable agricultural practices.

9. Conclusion

The integration of advanced genome editing technologies, including CRISPR/Cas-mediated base editing and prime editing, holds promise for precise and efficient modification of the wheat genome, ushering in an era of tailored genetic improvements. Leveraging multiplex genome editing systems and genotype-independent transformation strategies will expedite the development of elite wheat varieties with stacked beneficial traits, enhancing resilience to biotic and abiotic stresses while maximizing productivity and nutritional quality. Furthermore, the convergence of diverse genomic approaches, such as GWAS, MAS, and QTL mapping, will unlock insights into the genetic basis of complex traits, facilitating targeted breeding efforts. Synthetic biology methodologies offer avenues for gene stacking and trait diversification, while speed-breeding techniques expedite breeding cycles, enabling rapid variety development to meet evolving agricultural demands. Collaboration and knowledge-sharing platforms will be instrumental in fostering innovation, promoting technology adoption, and democratizing access to cutting-edge breeding tools. As we navigate the complexities of a changing climate, burgeoning population, and evolving 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 science and technology, we can create resilient, nutritious, and

environmentally sustainable wheat varieties tailored to diverse agroecological contexts, ensuring a brighter future for generations to come.

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