

## REVIEW

# Accelerating wheat improvement through trait characterization: advances and perspectives

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## Abstract

Wheat (*Triticum* spp.) is a primary dietary staple food for humanity. Many wheat genetic resources with variable genomes have a record of domestication history and are widespread throughout the world. To develop elite wheat varieties, agronomical and stress-responsive trait characterization is foremost for evaluating existing germplasm to promote breeding. However, genomic complexity is one of the primary impediments to trait mining and characterization. Multiple reference genomes and cutting-edge technologies like haplotype mapping, genomic selection, precise gene editing tools, high-throughput phenotyping platforms, high-efficiency genetic transformation systems, and speed-breeding facilities are transforming wheat functional genomics research to understand the genomic diversity of polyploidy. This review focuses on the research achievements in wheat genomics, the available omics approaches, and bioinformatic resources developed in the past decades. Advances in genomics and system biology approaches are highlighted to circumvent bottlenecks in genomic and phenotypic selection, as well as gene transfer. In addition, we propose conducting precise functional genomic studies and developing sustainable breeding strategies for wheat. These developments in understanding wheat traits have speed up the creation of high-yielding, stress-resistant, and nutritionally enhanced wheat varieties, which will help in addressing global food security and agricultural sustainability in the era of climate change.

## 1 | INTRODUCTION

Wheat (*Triticum* spp.) stands as a prominent source of carbohydrates and is one of the staple foods of humanity. Genetically diverse wheat genetic resources show variable ploidy levels (diploid, tetraploid and hexaploid) due to prolonged evolution and domestication (Shewry 2009; Jordan et al., 2015). Wheat production has increased significantly over the past few decades, but the rate of increase in annual wheat production has declined due to climatic variabilities (Frankin et al., 2021; Fadida-Myers et al., 2022; Roychowdhury et al., 2023a).

By 2050, wheat production will likely be insufficient to meet the food demands of the global population (Ray et al., 2012, 2013). As an allopolyploid crop, wheat breeding and genetic investigation are more challenging with stabilized trait characterization. Generally, wheat traits are broadly classified into four major groups to make the future crop – abiotic stress tolerance, disease resistance, agronomic breeding traits and grain quality traits. Abiotic stresses include drought, heat, cold, salinity, excess water, heavy metals, high soil minerals, etc., and disease resistance is for various fungal, bacterial, and viral pathogens, which highly affect wheat yield and grain quality. Agronomic traits are

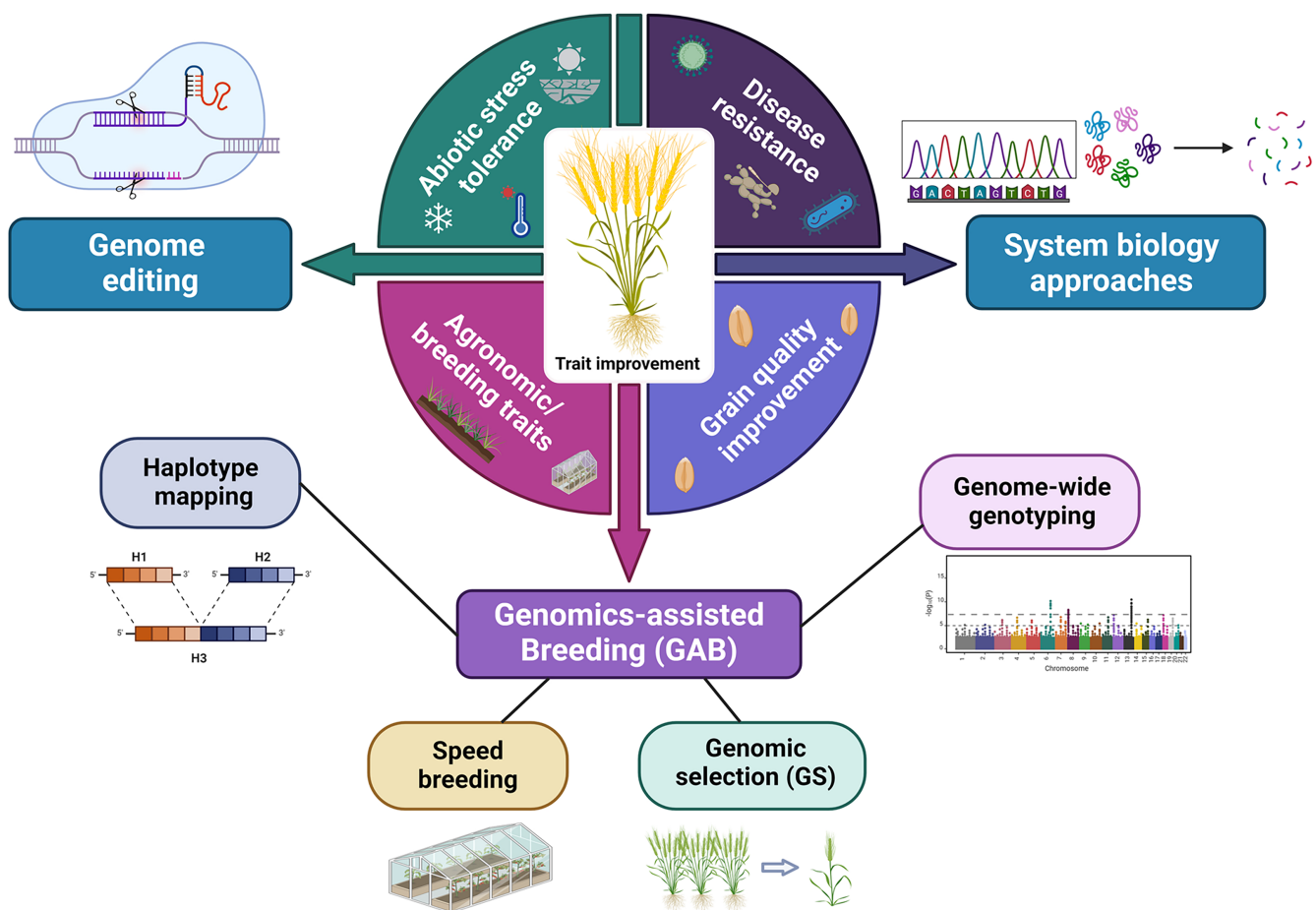
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important parameters for breeding, including crop phenology like heading and flowering time, biomass in the form of vegetative and reproductive dry matter, and yield components, e.g. grain weight, grain number, thousand kernel weight, harvest index, etc. Grain quality traits include grain protein content, amylase, gluten and mineral content (e.g. iron, zinc, etc.). Due to technological advances, such wheat traits can be improved through precise molecular characterization, manipulation and validation by applying genomics-assisted breeding (GAB), gene editing (GE) and system biology approaches, respectively (Figure 1) that can maintain or increase the wheat productivity in changing climate scenarios.

Understanding the molecular basis of different traits in wheat can help to develop improved varieties through breeding programs (Brinton et al., 2020). The large genome size (~17 Gb), extensive stretches of repetitive DNA (>80%), and the allohexaploid nature of the bread wheat have challenged the genome sequencing and hampered its application for crop improvement. However, the current advancements in sequencing technologies have successfully addressed these issues. Therefore, the genetic makeup and detailed

profiles of gene expression for bread wheat and its ancestors have been effectively unravelled (Muthamilarasan and Prasad 2014; Borrill et al., 2015). This has given the unique opportunity to advance the molecular characterization of many traits by identifying and selecting desirable genes, enabling breeders to develop wheat varieties with enhanced traits that can contribute to increased productivity, resilience to stresses, and nutritional value. Besides conventional breeding approaches, GAB utilizes the genomics toolbox with the available reference genomes to decipher genotype-phenotype associations (Weckwerth et al., 2020; Varshney et al., 2021b). Molecular marker-assisted breeding (MAB) and speed breeding (SB) are the recent trends to enhance the germplasm to achieve elite lines and also breed new lines to combat adverse environmental challenges and food security (Wolter et al., 2019; Weckwerth et al., 2020). More specifically, the upgrading of sequencing technologies has led to opening the avenue for re-sequencing of low-copy genomic regions (Nyine et al., 2019) in a geographically and genetically diverse wheat haplotype mapping population with reduced crop-cycle by incorporating SB or fast-forward breeding towards wheat improvement (Ghatak



**FIGURE 1** Characterization of major categories of traits for wheat improvement through genomics-assisted breeding, gene editing and system biology approaches. The diagram highlights key agronomic, biotic, and abiotic traits targeted for enhancement of yield potential, disease resistance, drought tolerance, and nutritional quality in wheat. Each trait is linked to specific genomic interventions, such as marker-assisted selection through haplotype mapping, speed breeding, genomic selection and CRISPR-based gene editing, genome-wide genotyping and multi-omics integration (through system biology), illustrating how advanced biotechnological tools are being employed to accelerate wheat breeding and address global food security challenges.

et al., 2017; Varshney et al., 2021a; Jordan et al., 2022). In conjugation with haplotype mapping and SB, genomic selection (GS) can precisely predict genotype-phenotype or genotype-trait associations, which aided GS for future breeding programs. GS can utilize genomic information to predict the performance of wheat genotypes for various traits by involving the use of high-density molecular markers, such as single nucleotide polymorphisms (SNPs), to capture the genetic variation present in the wheat genome (Verges et al., 2021; Ficht et al., 2023; Kumar et al., 2023; Li et al. 2024).

To develop climate-resilient wheat, we must integrate various advanced genomics and allied omics tools for future breeding programs. Pre-breeding and genomics/molecular-assisted breeding are important for efficiently developing climate-resilient wheat (Weckwerth et al., 2020; Kitashova et al., 2023; Chaturvedi et al., 2024). Additionally, crop modelling can aid in developing climate-smart wheat varieties. However, relying solely on conventional breeding tools and elite germplasm leads to genetic gains of only around 1% per year. This lack of significant yield gains in modern wheat varieties emphasizes the need to complement conventional approaches with modern pre-breeding methods. Trait manipulation in wheat can be achieved through gene editing techniques that enable precise modifications in the wheat genome, allowing targeted changes to specific genes or regulatory elements (Roychowdhury et al., 2020; Kottakota et al., 2021). Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) are the versatile and widely used genome editing system in wheat with ongoing increasing successful instances of trait manipulation (Singh et al., 2020; Kottakota et al., 2021). Trait validation in wheat using systems biology approaches involves integrating high-throughput molecular or multi-omics data with computational modelling analysis to understand the underlying biological processes and their relationship to specific traits (Weckwerth et al., 2020). The system biology approach includes the integration of transcriptomics, proteomics, and metabolomics (Roychowdhury et al., 2023b), which provides a holistic view of the underlying biological processes of the given trait under biotic or abiotic stress conditions. The systems biology approach, GAB, and genome editing technique jointly can contribute to a deeper understanding of trait characterization, manipulation, and validation. These can guide targeted breeding or genome engineering strategies for trait improvement in wheat.

## 2 | GENOMICS-ASSISTED BREEDING (GAB) FOR TRAIT CHARACTERIZATION AND IMPROVEMENT IN WHEAT

GAB is a modern crop breeding approach that combines sequence-based genomics strategies with molecular breeding pipelines. GAB in a polyploid species like wheat poses significant challenges. It contributes to germplasm enhancement and cultivar development to characterize allelic variations for important agronomic traits related to yield, grain quality and stress resilience (Varshney et al., 2005).

### 2.1 | Haplotype-based breeding (HBB) and involvement of speed breeding in haplotype mapping for wheat genetic resources

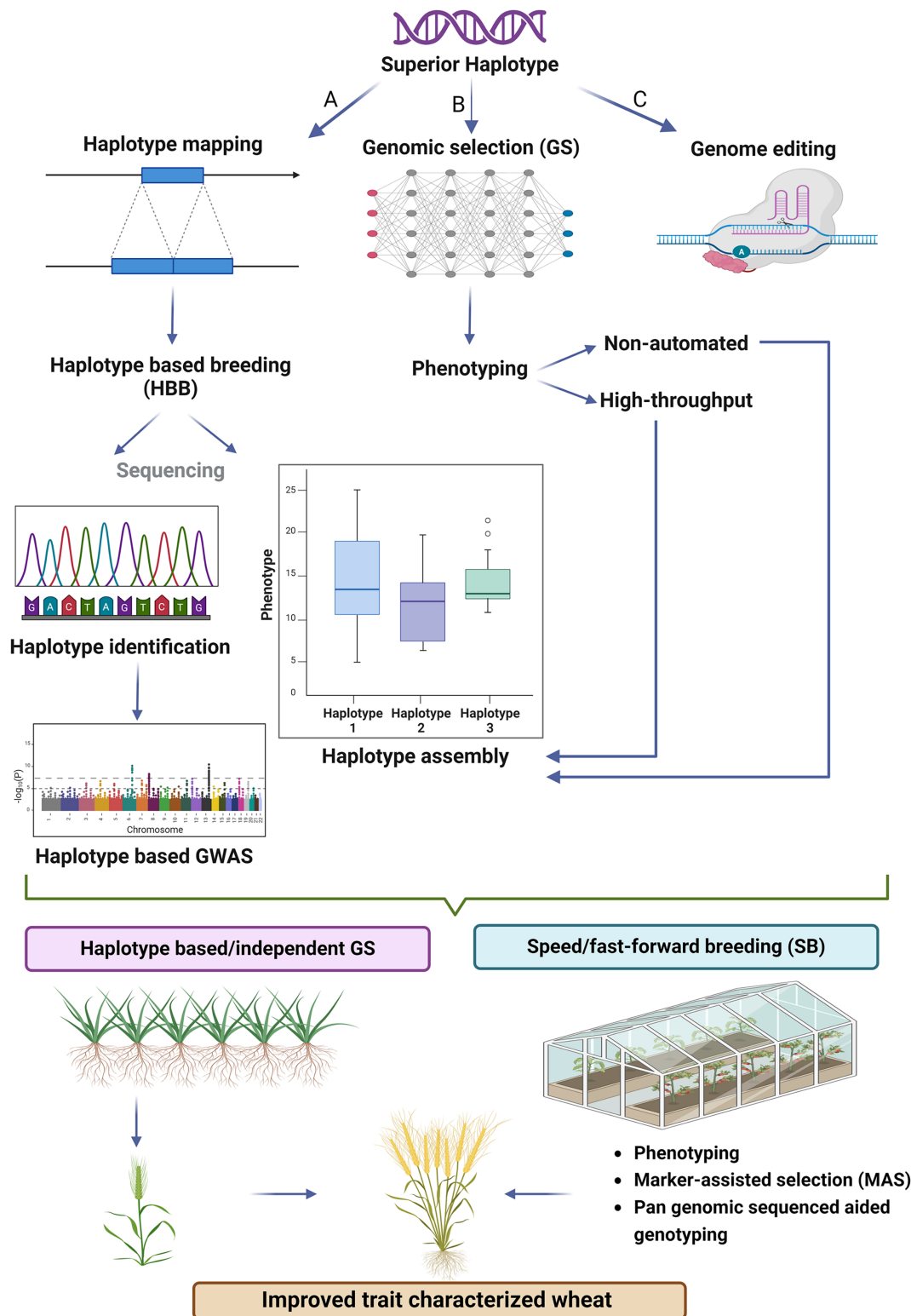
Recent advancements in crop genetics have triggered the development of innovative technologies that attempt to combine desirable phenotypes. The method of haplotype construction developed by Bevan et al., (2017) based on sequencing genotypes begins with discovering and confirming the morphological characteristics of the haplo group (Varshney et al., 2021b). Aiming for haplotype mapping, many crop species, including wheat, have also been constructed utilizing the huge SNP data gathered from numerous genotypes using genome sequence-based technologies that can be used in various agricultural research and breeding applications (Varshney et al., 2021b). The crop breeding cycle plays a crucial role in stabilizing homozygote lines and increasing genetic gain via hybridization and traditional breeding schemes in plant breeding. Some advanced approaches such as double haploid (DH), shuttle breeding, and embryo tissue culture may effectively reduce the generation time (Bhatta et al., 2021). However, DH approaches are quite intractable for wheat.

The use of a novel and advanced breeding technique, referred to as SB, has enabled the acceleration of agricultural innovation by reducing the duration of the wheat growth cycle and expediting the process of generation advancement (Hickey et al., 2017, 2019; Ghosh et al., 2018; Watson et al., 2018). SB initiative implementation includes extending the duration of light exposure for the specific crop species. Immediately after that, grain harvesting occurs to expedite reproduction, hence decreasing the duration required for some day-neutral and long-day plants to generate subsequent generations (Wolter et al., 2019). In wheat SB, the fundamental principle is to exploit early flowering by altering the photoperiod (day length) and temperature in form of vernalization or cold requirement, in a controlled condition (Ghosh et al., 2018). By synchronising the flowering time i.e. anthesis, it is possible to produce haplotypes and enhanced new varieties of the same species, which can then be included in the marker-assisted molecular breeding program (Morton et al., 2019). The development of haplotype-linked biomarkers has enabled the selection of new combinations of haplotypes. However, constructing haplotypes from short-read sequences obtained through a second-generation sequencing platform is much more challenging due to the low likelihood of finding allelic variations in the form of SNPs or insertion-deletions (InDels). However, there is a further opportunity to create the haplotype utilizing long-read sequences (Maestri et al., 2020). Identifying the haplotype is much easier due to the widespread availability of sequencing information from diverse genotypes. The process of creating haplotypes utilizing breeding line sequencing data involves identifying and assessing the alterations in the physical characteristics of the haplotype “component” using whole genome re-sequencing (WGRS) data (Bevan et al., 2017). Utilizing the process of constructing haplotypes between neighbouring SNPs on a chromosome is an alternative approach that may enhance the potential of genome-wide association studies (GWAS). Haplotypes refer to specific combinations of alleles that are identified on a single

chromosome and passed down collectively with limited possibility for future recombination (Pal et al., 2022). A recent study on *Triticum* spp. and other field crops has shown that conducting GWAS investigations using haplotypes may be more advantageous than using a single marker when evaluating the effects of allelic variations (Li et al., 2019; Shokat et al., 2020). Haplotype-based breeding (HBB) aims to create a tailored crop variety by pyramiding superior haplotypes in a single plant. The development of DNA markers based on haplotypes allows for the detection of new combinations of haplogroups. However, because of the larger selection of genetic markers that are related to haplotypes, wheat breeders have an increased probability of creating high-performing hybrids that are free from the negative effects of linkage drag (Varshney et al., 2021a). Under short-day (SD) conditions, it may be feasible to synchronize the flowering time of both wheat parents used in the crossing experiments and the subsequent propagation of future generations compactly and efficiently. Additionally, the shortened time it takes for this polyploid crop to reproduce would allow for the efficient evaluation of the transformed plants for additional selection and the use of markers to investigate and enhance grain yield, nutritional quality, agronomic traits, flowering time, and adaptations to environmental fluctuations and disease resistance (Scheben and Edwards 2018). In addition to examining the lines for both abiotic and biotic stress responses, the SB procedure and tools may be modified to combine beneficial/resistance genes to develop climate-resilient wheat (Bekele et al., 2019). SB facilitates the use of superior haplotypes including exotic and adaptable alleles for HBB, GS, and genome editing. Utilizing the SB methods, the process of faster generation will be beneficial in producing a wider range of enhanced traits. This will be achieved by subjecting the plants to high-throughput phenotyping, marker-assisted selection (MAS), genotyping and sequencing (Figure 2).

It is necessary to monitor the transmission of haplotypes within genetic groups. This may aid in identifying the most suitable parents for breeding in order to create offspring with the necessary adaptive features, which is essential for creating new genetic combinations. By combining vast genome sequencing and haplo-phenotyping databases, researchers have successfully found valuable haplotypes (Abbai et al., 2019; Sinha et al., 2020). The creation of selection signatures is mostly attributable to choosing genetic alleles influencing desirable trait of concern through crop evolution and domestication (Qian et al., 2017). Success in the restoration of polyploid species' haplotypes will have a significant influence on their future breeding. Polyploid crops, such as wheat, may generate haplotypes via the use of single-cell methods and advanced sequencing technologies like Pacific Biosciences (PacBio) and Oxford Nanopore Technology (ONT). These technologies provide high-quality long-read sequencing and have the potential to reveal far higher levels of genetic variation (Torkamaneh and Belzile 2022). Haplotype diversity is much more typical for a particular crop species (Sun et al., 2022; Torkamaneh and Belzile 2022). Brinton et al., (2020) identified haplotype blocks in wheat in which SNP markers were unable to identify the different haplotypes (seven haplotypes, namely H1, H2,....., H7) that anticipated for the strongly preserved genomic area on the chromosome 6A for the corresponding gene *TaGW2-A*, regulating numerous increased yield characteristics

(Varshney et al., 2021b). Luján Basile et al., (2019) characterized a haplotype block and GWAS in Argentinian bread wheat using genetic, molecular markers and SNP profiling. The analysis uncovered many haplotype blocks that span the whole genome and include conserved genomic areas, such as the 1BL/1RS wheat/rye translocation site located on chromosome 1BS. Furthermore, the majority of these acquired haplotypes have a very promising and considerable impact on the yield characteristics in multi-locational breeding experiments. The study focused on using a haplotype-based GWAS to analyse the epistatic interactions in multi-locational breeding experiments of spring wheat genetic resources at CIMMYT, Mexico (Sehgal et al., 2020). The objective of this research is to investigate the genomic areas that are consistently stable in haplotypes, to enhance yield components and to understand haplotype relationships. By using linkage disequilibrium (LD) techniques, a multitude of haplotype blocks were created, covering a range of over 14 megabases in the wheat genome. Through haplotype-based GWAS, it was shown that these blocks consistently showed a correlation with drought stress conditions, particularly in certain chromosomal regions of high activity. This study provides evidence supporting the need to develop genetic markers and use them in agricultural crop production. These markers should be based on haplotypes rather than relying only on SNPs, since SNPs are not always causative. Selection signatures, sometimes called persistent haplotype blocks and selective sweeps, are a collection of genes collectively controlled by a significant regulatory gene (Qian et al., 2016; Bhat et al., 2021). These signatures may be found in every population. The selection signatures' evidence of a correlation between different traits may be attributable to genuine linkage among the genes or else to the pleiotropic action of the same genes (Qian et al., 2016; Dixon et al., 2020; Bhat et al., 2021). Because of this, grain breeders must focus their attention on those genomic areas to better understand the influence these regions have on the desirable characteristics. Therefore, it is necessary to monitor the transfer of haplotypes via breeding lineages. This is a crucial step in constructing novel genomic variants since it helps to choose the most suitable progenitors for breeding in order to produce offspring with the necessary characteristics in combination. In addition, the incorporation of genomic information into the process of determining the recombinants created by the mating of different sets of parents may be of tremendous assistance in easing the perplexity around metrical characteristics. It would increase the performance with which certain features in contemporary varieties may be improved to better adapt such varieties to harsh situations (Jensen et al., 2020). For crops with genomes that include large blocks of LD, an HBB technique becomes increasingly relevant. LD blocks are areas of genetic variation that tend to remain unchanged. The creation of haplotype blocks commonly employs the following three procedures in sequence: the (1) length specified by the user, (2) sliding-window, and (3) LD. The user-specified range of haplotype block lengths (2–15 bp) is a straightforward approach. However, the resulting haplotypes do not accurately reflect genomic phenomena such as crossover or linkage disequilibrium (Gabriel et al., 2002; Sehgal et al., 2020), nor do they capture a shared evolutionary process (Templeton et al., 2005). The second option is often



**FIGURE 2** Schematic workflow representation using genotyping (by sequencing) and phenotyping in haplotype-based breeding (HBB) and involvement of speed breeding in haplotype mapping to characterize wheat genetic resources. The diagram illustrates key steps to identify superior haplotype-contained wheat lines, including haplotype mapping (A), genomic selection (B) and genome editing (C). Haplotype mapping can be further used in sequence-based mapping to identify haplotypes for a gene that enables the grouping of the population based on the phenotypic values and used in genome-wide association studies (GWAS) and haplotype-based breeding (HBB). On the other hand, GS applies non-automated and high-throughput phenotyping, which indirectly involves wheat HBB. As a whole, all these genomics tools, in addition to the haplotype-based GS and speed breeding of the crop, help identify the potential wheat lines with improved traits through precise phenotyping, marker-assisted selection and pan-genomic sequence-aided genotyping. These approaches enable rapid characterization and utilization of wheat genetic resources, facilitating the selection of superior haplotypes for breeding programs to improve agronomic performance and stress tolerance.

used by GWAS researchers for haplotype creation (Braz et al., 2019). This approach is easily used, however, it becomes redundant when adjacent SNPs are highly correlated, rendering it less advantageous compared to utilising individual SNPs alone. It is difficult to determine the best window size for a comprehensive analysis of the whole genome when the LD rates vary across substantial genetic variations (Sehgal et al., 2020). In wheat, the use of haplotype phasing and scaffolding has become more beneficial because of the advancements in chromosomal configuration monitoring using Hi-C sequencing (Hi-C; Zhang et al., 2019b) and Bionano Genomics (BNG) optical mapping-based genomic assemblies (Zhu et al., 2019). The combination of SB with single seed descent (SSD) may be advantageous for advancing haplotypes and other breeding populations with many parents. This approach has potential applications in MAB and precise genome editing to achieve specific traits.

## 2.2 | Advantages of using HBB and its integration with speed breeding for wheat improvement

HBB in wheat offers several key advantages over traditional breeding approaches, particularly when integrated with SB techniques, to significantly accelerate and enhance the improvement of wheat varieties. Such a modern breeding approach focuses on using haplotypes, which are combinations of alleles at adjacent loci that are inherited together, rather than individual markers (Roychowdhury et al., 2023c). This approach provides a more comprehensive and accurate representation of the genetic variation associated with complex traits such as wheat grain yield, disease resistance, and abiotic stress tolerance. By capturing LD and epistatic interactions more effectively, HBB allows for more precise identification of genomic regions influencing these traits (Sivabharathi et al., 2024). This enhanced genetic resolution improves the accuracy of marker-trait associations (MTA) and enables breeders to make more informative selection decisions. In addition, HBB can utilize historical recombination events, offering insights into the evolutionary and domestication history and functional significance of specific haplotypes, which can be leveraged to predict and select advantageous genetic combinations more effectively. Integrating HBB with SB amplifies these benefits by dramatically reducing the breeding cycle duration, allowing for multiple generations to be grown and evaluated annually (Tyagi et al., 2024). SB uses controlled environments with extended photoperiods and optimized growth conditions to accelerate plant development and flowering times. This rapid cycling not only hastens the introgression and fixation of beneficial haplotypes identified through HBB, but also increases the selection intensity as more generations and selection rounds can be conducted within the same time frame (Roychowdhury et al., 2023d). Consequently, genetic gains are achieved faster and more efficiently compared to traditional breeding methods, which typically rely on one generation per year. Moreover, the combination of HBB and SB facilitates the rapid validation of predicted haplotypes, enabling breeders to quickly confirm the phenotypic effects of selected genetic regions and make adjustments in real-time scenarios (Saini et al., 2024). The

integration of these advanced techniques will enhance the ability to respond to emerging challenges such as climate change, new pests, and diseases by enabling the rapid development and deployment of resilient wheat varieties (Roychowdhury, 2014). The high-throughput nature of SB technology, when combined with the precise genetic insights from HBB, ensures that breeding programs can keep pace with the increasing demands for wheat production and the need for sustainable agricultural practices. Furthermore, this integrated approach supports the maintenance of genetic diversity within breeding populations by allowing the exploration and utilization of a broader range of genetic resources in a shorter time frame. As a whole, the synergy between HBB and SB represents a transformative advancement in wheat breeding, providing a robust, efficient, and responsive strategy to develop superior wheat varieties that meet the global challenges of food security and environmental sustainability more effectively than traditional breeding approaches.

## 2.3 | Genomic selection (GS) in wheat

In general, GS is a way for GAB to shortlist the plants, populations, breeding lines, varieties, etc., based on different genomic signatures, models, prediction, and validation pipelines for the targeted trait of interest (Meuwissen et al., 2001). Genomic signatures could be different molecular markers depending on the genome sequence. In addition, the phenomics platform also accelerates the process of GS through higher predictive values with available genotyping tools. The usage of genome-wide markers reveals genomic estimated breeding values (GEBV), and its incorporation through GS in the MAB helps the selection process faster than the classical genotyping-phenotyping approaches (Meuwissen et al., 2001; Isidro et al., 2015). Genome-wide markers can identify even the short-effect QTLs or trait-linked genes to compute all sorts of genetic variations of the populations, and each trait-linked QTL is in LD to at least a single marker. The higher LD between the marker's alleles and QTL signify accurate GS, facilitating rapid genotypic selection or a subset of populations with improved trait performance. However, the GS makes it much more challenging for the polyploid wheat genome to characterize major traits, as stated before.

Several models have been used in genomic prediction-based selections like genomic best linear unbiased prediction (G-BLUP), ridge-regression genomic best linear unbiased prediction (RR-BLUP), Bayes, Bayes LASSO, reproducing kernel Hilbert space regression (RKHSR), random forest (RF), least-squares (LS), etc. (Burgueño et al., 2012; Jiang et al., 2017; Juliana et al., 2017a; Sun et al., 2017b; Rapp et al., 2018; Kumar et al., 2023). G-BLUP and RR-BLUP are widely used for wheat GS. G-BLUP uses a genomic association matrix and perhaps parental information to assess gene efficiency and information. How much genetic information a crop shares with others determines the genetic link matrix, which is more accurate than lineage-based ones. RR-BLUP estimates marker effects in the validation group and determines selection candidates' GEBVs by combining their marker values (Isidro et al., 2015). GS can capture small effect

resistance QTLs, allows the use of whole existing additive genetic variance, and enables a large number of breeding line evaluations for both abiotic and biotic resistance to enhance the selection efficiency. The reduced breeding cycles and further selection accuracy and intensity through GS accelerate the advancement of stress-resilient varieties. Numerous researches have been attempted to identify the resistant wheat lines for diseases to incorporate into the resistance breeding programs (Isidro et al., 2015; Jiang et al., 2017; Juliana et al., 2017a; Muleta et al., 2017; Crain et al., 2018; Herter et al., 2019; Sarinelli et al., 2019; Alemu et al., 2023). Genetic gain in yield is positively correlated with multiple agronomic traits, viz., spike length, thousand kernel weight, grain dimension, fertile spikelet, etc., and they collectively impact grain yield. The grain yield, a highly complex trait, exhibits lower heritability than the other key agronomic traits and is significantly impacted by environmental factors. Therefore GS breeding provided better solutions by reducing the requirements for extensive phenotyping, minimizing replication, and lowering data collection costs. Several studies have been undertaken on wheat grain yield improvements (Juliana et al., 2017b; Hu et al., 2019; Mérida-García et al., 2019; Ficht et al., 2023), flowering time (Burgueño et al., 2012), thousand kernel weight (Poland et al., 2012) and grain quality parameters like grain protein (Guzman et al., 2016; Würschum et al., 2016; Rapp et al., 2018), flour quality (Heffner et al., 2011a, b; Battenfield et al., 2016; Guzman et al., 2016; Hayes et al., 2017) and nutrient components (Velu et al., 2016; Manickavelu et al., 2017) through GS (please see details in Table 1).

### 2.3.1 | Integration of GS with advanced breeding tools

Comprehensive crop breeding using different methods and technologies achieves targeted genetic gain. Integrating phenotyping, genotyping, advanced biotechnological tools and bioinformatic pipelines can harness the germplasms' potential and use GS for rapid crop development. Here, we have introduced different key approaches that can be implemented within the framework of GS (He and Li 2020). GS-speed breeding integration efforts would boost wheat genetic gain. Employing GS to segregate wheat populations and speed breeding would accelerate breeding line selection for the subsequent generation. As GS shortened breeding cycles and SB reduced the generation cycle, their combination (GS-SB) would increase genetic gains in each cropping cycle. Multi-locational field trials can validate the advanced generation lines and improve prediction models (Krishnappa et al., 2021).

Along with genotyping, precise phenotyping of multiple traits with great efficiency has been a primary obstacle to confirming the potential and scalability of wheat breeding. Nevertheless, high-throughput phenotyping platforms (HTPPs) have solved this issue by enabling precise and non-destructive measurements of variant phenotypes in wheat lines. In GS, HTP platforms are used in two ways. First, they use the directly generated phenotypic data as primary traits while training genomic prediction models. For instance, Hassan et al. (2019) utilized UAV remote sensing in wheat to identify QTL underlying plant

height much faster than conventional measurements. Subsequently, it enhances the prediction precision by integrating genetically correlated secondary traits measured via HTP platforms. To enhance grain yield prediction, Rutkoski et al. (2016) and Sun et al. (2017b) employed canopy temperature (CT) and normalized difference vegetation index (NDVI) and improve GS accuracy. Implementing HTP and GS together provides robustness, reduces measurement errors, and captures time-series data for continual plant growth monitoring, which is cost-effective and labour-intensive (Crain et al., 2018).

Genotype-environment interactions (GxE) may accelerate backcrossing between elite lines and exotic material, strengthening GS-assisted breeding. These exotic lines have valuable biotic and abiotic stress tolerance genes and may be exploited to develop sustainable wheat cultivars. Once the relevant allele is introduced, GE interaction may use GS to enhance recombination with previously adapted alleles. GS then uses genotyping data to predict breeding values, selecting individuals with the most desirable traits to accelerate crop improvement by efficiently introducing and recombining beneficial alleles (Nadeem et al., 2018). Rodríguez-Leal et al. (2017) suggested altering the regulatory sequence to develop unique transcriptional alleles for precise control over gene expression, allowing selection of the most desired allele for breeding programs. The frequent overlook of non-additive gene effects, viz., epistasis and genome imprinting, limits the prediction accuracy in the classical GS approach as well as neglects the genotype interaction, especially in polyploid species where non-additive effects serve a pivotal role (Varona et al., 2018; Hunt et al., 2020). Hence, novel machine learning (ML) and deep learning (DL) techniques can be implemented with GS to address these limitations, as they can capture minor non-additive effects and interactions between phenotypes or genotypes. Similarly, a study on wheat data set compared multi-layer perceptions, support vector machines, and Bayesian threshold genomic BLUP (TGBLUP), where TGBLUP performed superiorly (Montesinos-López et al., 2019). Another study by Wang et al. (2023a) introduced a DL method, deep neural network genomic prediction (DNNGP), for the integration of multi-omics data in crop plants. DNNGP was trained over wheat, tomato and maize datasets and compared with extensively used GS approaches viz., GBLUP, LightGBM, SVR, DL GS (DeepGS), and DL genome-wide association study (DLGWAS), and surpasses them in performance. DNNGP, with this study, proved to be a reliable and practically fit approach to efficiently use multi-omics data for precise trait predictions in large and diverse datasets within the framework of GS.

### 2.3.2 | Bridging GS and wheat complex traits through HTPPs

Implementing GS in wheat for complex traits such as grain yield presents several significant challenges, stemming primarily from the intricate nature of these traits and the practical difficulties in applying advanced genomic technologies in breeding programs. Grain yield is a highly polygenic trait, influenced by numerous genes contributing to a small effect, which complicates the capture of the full genetic

TABLE 1 Genomic selection in wheat for predicting various trait characterization.

Trait categories	Characterizing trait(s)	Remarks/Note	GS tools used	Validation type and/or Prediction Accuracy	References
Abiotic stress tolerance	Lodging	Used in +1K soft winter wheat with 38893 SNPs based GBS	G-BLUP	0.12 to 0.59	Isidro et al., (2015)
	Terminal drought stress	286 bread wheat cultivars were genotyped with 2047 SNP markers using GBS	RR-BLUP, G-BLUP, BRR	-0.32 to 0.52	Shabannejad et al., (2021)
	Salinity stress	A total of 298 Iranian bread wheat accessions were evaluated	G-BLUP, BRR, RR-BLUP	-	Javid et al., (2022)
Disease resistance	Stem rust	Used in historic wheat lines	G-BLUP, BayesR	Forward validation- 0.20 to 0.40 Cross validation- 0.55	Rutkoski et al., (2015)
	Fusarium head blight (FHB)	Compared with marker-assisted selection by using independent and cross-validation	MAS models	Cross-validation 0.01 to 0.62 Independent validation- 0.01 to 0.46	Jiang et al., (2017)
	Yellow/stripe rust	419 winter wheat genotypes were evaluated with haplotype-tagged SNPs to improve genomic prediction accuracy	BRR and RR-BLUP	Cross validation-0.07	Alemu et al., (2023)
Breeding/ agronomic traits	Powdery mildew	Used for adult plant resistance for spring wheat accessions	RR-BLUP with SNP based array	Cross-validation 0.45 to 0.65	Muleta et al., (2017)
	Flowering time	Used in historical winter wheat lines	RR-BLUP	Cross-validation 0.36 to 0.57	Sarinelli et al., (2019)
	GY, DTH, TKW	599 wheat genotypes were evaluated in 4 different environments and genotyped with 1279 DARTs	Pedigree-based, marker-based, pedigree + marker based models	10 fold cross-validation- Model I (-0.27 to 0.50) and Model II (0.22 to 0.607)	Burgueño et al., (2012)
Grain quality trait	Spike width	254 semiarid wheat advanced breeding lines were genotyped with 41,371 SNPs through GBS	BLUP model	7 fold cross validation, 0.28 to 0.45 (for GY), 0.3-0.5 (for overall traits)	Poland et al., (2012)
	GY	419 winter wheat genotypes were evaluated with haplotype-tagged SNPs to improve genomic prediction accuracy	BRR and RR-BLUP	Cross validation-0.092	Alemu et al., (2023)
	P, K, Mn, Fe, Mg, Zn	Used 1870 winter wheat genotypes to evaluate best selection tool for each agronomic trait	RR-BLUP, RKHS and feed-forward neural networks	0.34 (yield by RKHS)	Ficht et al., (2023)
Grain quality trait	Grain protein, flour yield, grain milling and baking properties	Used 3 cycles of recombination (C <sub>1</sub> , C <sub>2</sub> , and C <sub>3</sub> ) of bi-parental F <sub>1</sub> s through rapid-cycle recurrent GS to achieve 12.3% of genetic gain	G-BLUP, G-BLUP+P, RKHS+KA, P+RKHS-KA	0.32 to 0.94	Dreisigacker et al., (2023)
	P, K, Mn, Fe, Mg, Zn	5520 wheat lines were genotyped and 20,833 SNPs were generated using the TASSEL 5 v2 pipeline	RR-BLUP, GAUSS, PLSR, EN, RF	Cross validation- 0.32 to 0.62	Battenfield et al., (2016)
		Used in Afghan wheat landraces			



TABLE 1 (Continued)

Trait categories	Characterizing trait(s)	Remarks/Note	GS tools used	Validation type and/or Prediction Accuracy	References
Grain protein		Used in bread and durum wheat lines	G-BLUP, RKHS, RF, RR, EN, LASSO RR-BLUP in GBS based genotyping.	10 fold cross validation- 0.3 to 0.5	Manickavelu et al., (2017) Würschum et al., (2016); Rapp et al., (2018)
Gluten index, alveograph measures and protein content		Used 2 durum wheat BP, one with 170 varieties and advanced breeding lines, and the other with DH population of 154 lines were evaluated	RR-BLUP, G-BLUP, BayesA, BayesB, Bayesian LASSO, and RKHS for ST and MT- BayesA, MT-Matrix, and MT-SI for MT	5 fold cross validation- 0.5 to 0.8 (for ST)	Haile et al., (2018)
Baking quality		A 495 advanced wheat breeding lines were genotyped and phenotyped	BRR as a single trait model and Bayesian multivariate Gaussian model for a multi trait model	Cross validation (0.43)	Lado et al., (2018)
Semolina quality		Used in 1184 durum wheat lines	RR-BLUP, Bayesian Lasso, BRR, RKHS, G-BLUP	5-fold cross validation (0.27-0.66)	Fiedler et al., (2017)

Abbreviations: Bayesian LASSO- Bayesian Least Absolute Shrinkage and Selection Operator; BayesR- Bayesian Regression; BP- Breeding population; BRR- Bayesian Ridge Regression; DART- Diversity Arrays Technology; DTH- Days to Heading; EN- Elastic net; GAUSS- Gaussian kernel; GBS- Genome based sequencing; GS- Genomic selection; GY- Grain yield; KA- Kernel averaging; MT- Multi trait; P- Pedigree information; PLSR- Partial least squares regression; RF- Random forest; RR-BLUP- Ridge-regression Genomic Best Linear Unbiased Prediction; SNP- Single Nucleotide Polymorphism; TKW- Thousand kernel weight; TP- Training population; ST- Single trait.

architecture necessary for accurate trait predictions and outputs. Additionally, genetic interactions (e.g. epistasis) and GxE further complicate the genetic models, as these interactions are complex to quantify and incorporate into prediction models. Phenotyping for grain yield is also particularly challenging because it requires precise, high-throughput methods that can reliably measure yield across different environments and growth stages (Roychowdhury et al., 2023a). Environmental variability significantly influences grain yield, necessitating extensive multi-environment trials to capture the range of possible conditions, which can be both logistically challenging and expensive. On the genomic side, sufficient marker density and high-quality data are critical to capturing the relevant genetic variation, but genotyping errors, missing data, and population structure can introduce biases and reduce model reliability (Roychowdhury et al., 2023b). The computational complexity of GS models also poses challenges: selecting appropriate statistical models that balance complexity and computational feasibility while accurately capturing the genetic architecture of yield is difficult, and large datasets required for these models demand substantial computational resources. Designing an effective training population is another hurdle in wheat breeding. It must be large and representative enough to ensure robust model training, and differences between the training and breeding populations can reduce model applicability. Integrating GS into traditional breeding programs requires careful alignment with existing breeding objectives and thorough training of personnel, which can be resource-intensive (Tiware et al., 2023). Furthermore, the economic considerations are significant, as the initial investment in genotyping infrastructure, computational tools, and training must be justified by long-term genetic gains and economic benefits. Ethical and regulatory issues, such as data privacy and compliance with international frameworks, add additional layers of complexity. Finally, maintaining genetic diversity is crucial to prevent the narrowing of the genetic base due to intensive selection, ensuring the long-term sustainability and adaptive potential of breeding programs (Özbek et al., 2024). Addressing these multifaceted challenges requires a coordinated effort across disciplines, integrating advances in genomics, phenomics, computational biology, and traditional breeding practices to fully realize the potential of genomic selection in wheat improvement.

The integration of HTPPs into GS for complex traits in wheat holds significant potential for advancing breeding programs. HTPPs provide a means to collect large-scale, precise phenotypic data rapidly and consistently across various environments, which is crucial for the accurate modelling and prediction of complex traits (Roychowdhury et al., 2023c). These platforms leverage advanced technologies like remote sensing, imaging, and automated data collection to measure a wide array of plant traits, including growth rates, biomass, canopy structure, and physiological parameters such as chlorophyll content and water use efficiency (Roychowdhury et al., 2023b). By generating comprehensive and high-resolution phenotypic datasets, HTPPs enable breeders to capture the full spectrum of phenotypic variation and the interaction of plants with the environment. This detailed phenotypic information is essential for training robust GS models that can accurately predict the performance of genotypes under diverse conditions. Furthermore, HTPPs facilitate the identification of subtle

phenotypic traits and dynamic changes over time, which are often missed in traditional phenotyping methods but are critical for understanding the genetic basis of complex traits (Mansoor et al., 2024). Integrating HTPP data into GS pipelines enhances the power to detect genotype-phenotype associations and improves the accuracy of genomic predictions by reducing phenotyping errors and environmental noise. Additionally, the high-throughput nature of these platforms allows for the assessment of large breeding populations, thereby increasing the selection intensity and accelerating the breeding cycle. This integration also supports the development of more precise selection indices that combine multiple trait data, optimizing the simultaneous improvement of yield and other important agronomic traits. By providing real-time, non-destructive phenotyping, HTPPs enable continuous monitoring and rapid decision-making, further streamlining the breeding process (Roth et al., 2024). Moreover, the use of HTPPs aligns with the increasing demand for precision agriculture, offering data-driven insights that can be used to optimize field management practices and enhance crop performance. In short, the integration of high-throughput phenotyping platforms into genomic selection frameworks represents a transformative approach in wheat breeding, enhancing the efficiency, accuracy, and scope of breeding programs aimed at improving complex traits like grain yield. This integration not only accelerates genetic gain but also supports the development of wheat varieties that are better adapted to changing environmental conditions and capable of meeting future food security challenges.

## 2.4 | Genome-wide genotyping

A variety of DNA-based molecular markers, including simple sequence repeats (SSRs), amplified fragment length polymorphisms (AFLP), restriction fragment length polymorphisms (RFLP), competitive allele-specific PCR (KASP) based markers are indispensable means for the genetic analysis of plants (Röder et al., 1995) and have been successfully used previously (Roychowdhury, 2014). The advent of Next-Generation-Sequencing (NGS) such as Genotyping-by-sequencing (GBS) is gradually replacing older marker systems due to their high throughput and low cost per data point. GBS, a reduced representation sequencing method, is particularly effective for genotyping and discovering new SNP in complex genome crops such as wheat. It is especially useful in crops without prior sequencing information or a reference genome (Getachew et al., 2019). GBS reduces genome complexity using two restriction enzymes (PstI/MspI) and simultaneously discovers and genotypes genome-wide variations. This technology has been successfully used in crops such as wheat, barley, rice, maize, cassava, potato, and soybean (Elshire et al., 2011). Additionally, GWAS, along with genotypic and phenotypic data, has been validated as a robust approach for detecting quantitative trait loci (QTLs) associated with desired and targeted attributes (Sukumaran et al., 2015). This approach offers a wider scope of genomic regions and allele frequencies at maximum resolution without relying on any bi-parental mapping population (Tadesse et al., 2015). GWAS also delves into the genetic mechanisms underlying the responsible genes. This technique proved valuable, yielding more precise results due to increased

genetic diversity and historical recombination of alleles within the associated panels (Ain et al., 2015; Bhatta et al., 2018). Association mapping (AM) detects the genomic regions associated with drought tolerance and yield traits. It involves different statistical analyses to minimize false positives and minor allele effects. Studies have successfully determined the accurate genomic locations of key genes viz., reduced height (Rht), vernalization (Vrn), and photoperiod responsiveness (Ppd) that serve as references for incorporating phenotypic diversity and markers in Argentinean bread wheat research (Gomez et al., 2014). Also, genetic references influence adaptive stress abilities and various physiological processes like heading date, plant height and maturity (Reynolds et al., 2012). Likewise, 105 bread wheat genotypes were investigated under control, drought, and heat-stress conditions, revealing 541 significant marker-trait associations (MTAs) by crossing with FDR < 0.05 threshold. Among these, 195, 179, and 167 MTAs were linked to control, drought, and heat-stressed conditions, respectively (Ahmed et al., 2022). GWAS characterized novel allelic variations for 13 yield-related traits in 163 bread wheat cultivars across 14 environments at three locations over three years (Sun et al., 2017a).

### 3 | GENE EDITING IN WHEAT FOR TRAIT IMPROVEMENT

Wheat genome decoding opens new avenues for genome modifications and gene editing, demonstrating the polyploid genome's potential in breeding pipelines and agricultural advancements (Kottakota et al., 2021; Hussain et al., 2022). Due to the hexaploid genome, bread wheat transformation is recalcitrant and limits gene editing in wheat genetic resources (Shan et al., 2014; Bhowmik et al., 2018). Gene editing technologies provide tools to create targeted mutations, insertions/deletions (indels), and precise sequence modifications through nucleases to alter a crop's DNA. In past years, transcription activator-like nucleases (TALENs), meganucleases, zinc-finger nucleases (ZFNs), and CRISPR-Cas have been developed and applied for gene editing in crops (Singh et al., 2020; Kottakota et al., 2021). CRISPR-Cas9-mediated gene editing is the most advanced and adaptable genome editing tool for improving single wheat traits like abiotic stress tolerance, disease resistance, agronomic breeding traits, grain quality, etc. or multi-traits through multiplexing over conventional plant breeding and transgenic technologies (Roychowdhury et al., 2020; Singh et al., 2020). This section summarizes CRISPR-mediated wheat genome editing for abiotic stress tolerance, disease and pest resistance, gluten-free grain quality and nutritional improvement in grain and flour.

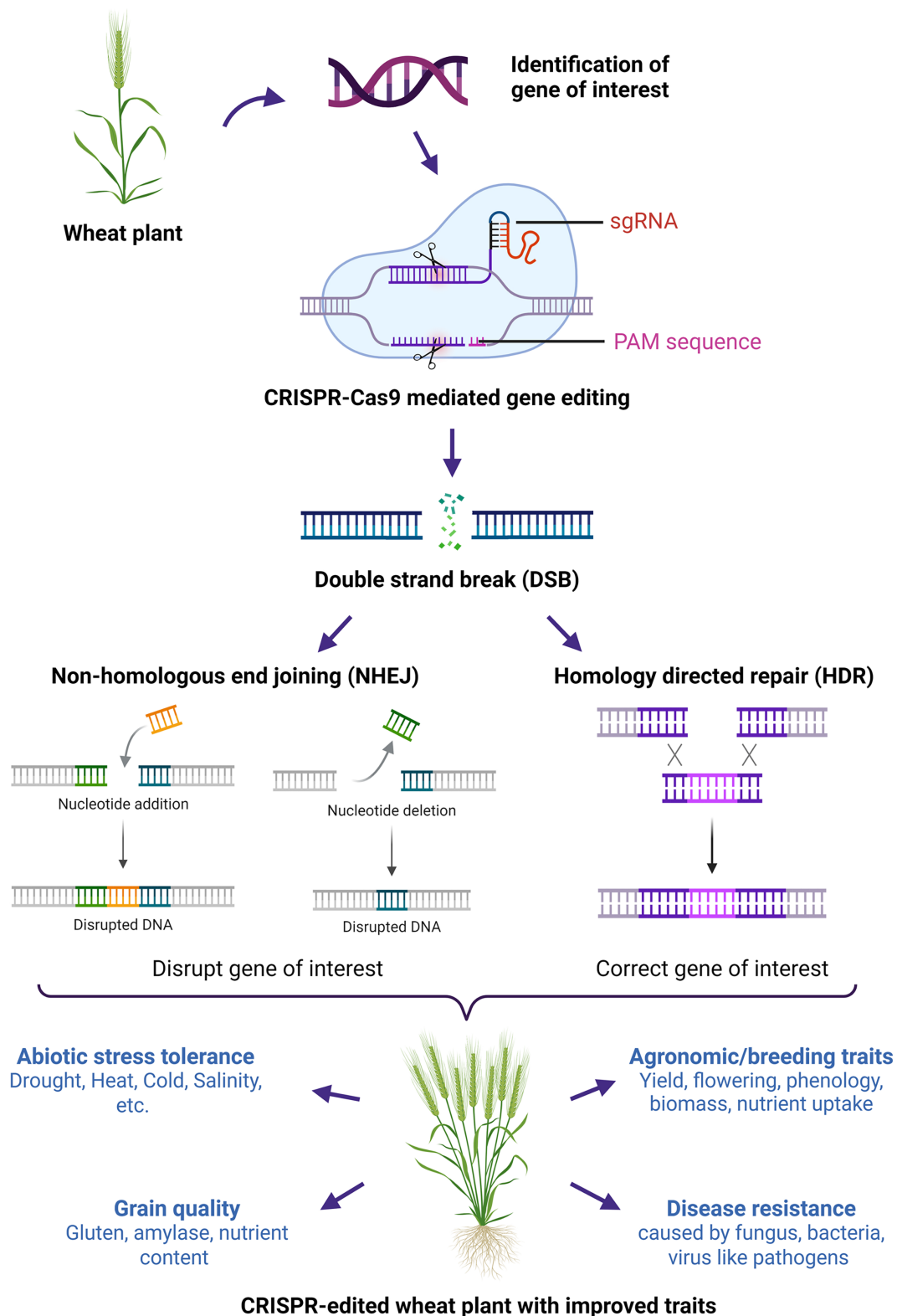
#### 3.1 | CRISPR-based gene editing: a mechanistic overview

CRISPR-Cas uses an adaptive immune system deciphered in bacteria and archaea to acquire resistance from viral DNA and foreign plasmids

(Marraffini and Sontheimer 2010). This system contains Cas9 and a single guide RNA molecule (sgRNA), which may be synthesized from a DNA template (Cong et al., 2013). CRISPR-Cas12a, the type V (Class-II), is also showing substantial improvement for genome editing that uses a sgRNA trans-activating CRISPR RNA to target T-rich regions of the genome where Cas9 is inappropriate for use (Bandyopadhyay et al., 2020; Weckwerth et al., 2020). In CRISPR, the 20 nucleotides at the 5' end of a sgRNA act as a site where the sgRNA-Cas9 complex binds with the target genomic region. This region is situated directly to the upstream of the protospacer adjacent motif or PAM (NGG, 2-5 base pair in length). *Streptococcus pyogenes* Cas9 protein cleaves target DNA and yields a blunt-ended double-strand break (DSB), which is repaired by the host cellular mechanism i.e. homology-directed repair (HDR) and non-homologous end joining (NHEJ) (Mei et al., 2016). Programmable sequence-specific nucleases (SSNs) in the CRISPR-Cas system create site-specific DSBs in the target genomic sequence and use the endogenous DSB repair machinery to cause mutations. HDR and NHEJ processes may rejoin both ends without additional processing (Liu et al., 2018). NHEJ is vulnerable to random indels at the cleavage site generating frameshift mutations or premature stop codons (Yang et al., 2020). On the other hand, HDR is highly accurate and utilizes a homologous DNA template to achieve precise gene insertion or replacement by incorporating a donor DNA with sequence homology at the anticipated DSB site (Yang et al., 2020). The schematic illustration of CRISPR-Cas9-based genome editing in wheat for improving traits is illustrated in Figure 3.

#### 3.2 | CRISPR-mediated gene editing in wheat

To explore and delineate the functions of wheat genes under several developmental phases and environmental conditions, it is crucial to access whole genome assemblies of wheat global accessions (Hussain et al., 2022). The CRISPR-Cas9-mediated gene editing approach is intricate in bread and durum wheat due to large genomes, orthologous gene copies, and repetitive regions. Recent editing advances that allow concurrent several-gene targeting or genome multiplexing have opened up new CRISPR applications in polyploid wheat, which has several homologous and paralogous copies of the same gene (Xie et al., 2015). No off-target mutations or transgenic gene inclusions existed throughout the development of new ribonucleoprotein-based (RNP) genome editing, which altered wheat genomes in just 7-9 weeks (Liang et al., 2017). The goal is to change harmful negative alleles rapidly and effectively in elite wheat lines by direct induction or modification. RNP-based biolistic delivery regulates off-target mutations by transiently expressing and rapidly degrading Cas9 (Liang et al., 2017). It is effectively used to gene-edit bread wheat (Liang et al., 2018). Permanent genomic integration using CRISPR-Cas9 leads to stable targeted gene expressions. Recently, a GRF-GIF (Growth-Regulating Factor-GRF-Interacting Factor) recombinant protein construct has revolutionized the regeneration in recombinant wheat lines by 100% (Debernardi et al., 2020). The main obstacles of implementing CRISPR in polyploidy wheat, are



**FIGURE 3** Schematic model depicting the mechanism of CRISPR-Cas9-mediated genome editing in wheat for various traits improvement. The diagram illustrates the CRISPR-Cas9 system, where the Cas9 protein, guided by a specific RNA sequence, introduces targeted double-strand breaks at desired genomic locations. Subsequent repair via non-homologous end joining (NHEJ) or homology-directed repair (HDR) enables precise modification of genes related to abiotic stress tolerance, disease resistance, and important agronomic traits, such as yield and improved grain quality. This genome-editing approach accelerates the development of improved wheat varieties with enhanced performance under challenging environmental conditions.

homologous genes and their genetic influence during transformation cell/callus culture. Rey et al., (2018) found that *Tazip4-B2 ph1b* mutant-rye hybrids grown on the culture media of Hoagland solution had more chromosomal recombination through chiasma formation. They extended the study to *Tazip4-B2 TILLING* and the CRISPR mutant-*Ae variabilis* hybrids. When watered with 1 mM  $Mg^{2+}$ , all hybrids showed remarkable homologous co-occurrence. These results showed that magnesium improves wheat genomic introgression from wild relatives (Rey et al., 2018). The complexity of three sub-genomes in wheat makes mutation detection difficult, especially for traits regulated by recessive genes. Agrobacterium-mediated CRISPR-Cas9 was used to create hexaploid wheat mutant lines with inactivated *Qsd1* homoalleles to control barley seed dormancy (Abe et al., 2019). Compared to the wild-type, these wheat mutants have longer seed dormancy periods, which may reduce pre-harvest sprouting. By screening genome-edited *TaCENH3* $\alpha$ -heteroallelic combinations, a commercially viable paternal haploid induction line was found in wheat, with a ~7% haploid induction rate. In contrast to Arabidopsis, the altered alleles inhibited transmission in female gametophytes, and heterozygous genotypes induced more haploid than homozygous combinations. These advances may make the CENTROMERIC HISTONE (CENH3) haploid induction technique easier to adopt in diverse crop species (Lv et al., 2020). Recently, researchers identified optimized CRISPR-Cas9 and CRISPR-Cas12a systems with specific codon optimization, intron inclusion, promoter selection, and protein engineering that enabled highly efficient simultaneous multi-gene editing in wheat, with mutation rates over 90% in some cases (Lawrenson et al., 2024). An upgraded engineered plant prime editor, ePPEplus, significantly enhanced prime editing efficiency in hexaploid wheat, enabling robust multiplex editing of up to ten genes simultaneously in protoplasts and eight genes in regenerated plants for stacking multiple agronomic traits (Ni et al., 2023). A detailed recent example of CRISPR-Cas9 mediated gene editing in wheat for major trait improvement is presented in Table 2.

### 3.2.1 | Abiotic stress tolerance in wheat

Kim et al., (2018) used the CRISPR-Cas9 on wheat protoplast to modify drought-responsive transcription factor genes- dehydration-responsive element binding protein 2 (*TaDREB2*) and ethylene response factor 3 (*TaERF3*) with temporary production of sgRNA and Cas9 protein. The significance of the wheat *TaHAG1* gene for salinity tolerance was confirmed by a CRISPR-mediated knock-out. Under salt stress, its overexpression caused enhanced H3 acetylation and transcriptional activation of a particular group of genes important for reactive oxygen species (ROS) accumulation (Zheng et al., 2021). Base-editing of two potent wheat genes- acetolactate synthase (*TaALS*) and acetyl-coenzyme A carboxylase (*TaACC*) create transgene-free mutants resistant to a varied range of commercial herbicide and by stacking such traits into the wheat lines may be useful for weed control during cropping season (Zhang et al., 2019a). CRISPR-based gene editing has been done to knock-out wheat gene

*DIW1/TaPP2C158* which resulted in drought tolerant plants (Wang et al., 2023b).

### 3.2.2 | Disease resistance in wheat

The CRISPR-Cas9 system has been utilized to knock out pathogen-sensitive genes to generate disease resistant wheat lines. By mutating the gene responsible for enhanced disease resistance1 (*TaEDR1*)- a negative regulator of powdery mildew (Pm), using CRISPR-Cas9, researchers successfully developed Pm-resistant wheat plants (Zhang et al., 2016b). Mutation in all six *MLO* alleles resulted in the *mlo* triple mutant which conferred enhanced resistance to Pm obtained through transient protoplast expression (Shan et al., 2014; Wang et al., 2014). Additionally, Wang et al., (2014) mutated a single *TaMLO-A1* allele in the A genome resulting in increased resistance to Pm. Also, the CRISPR-based knockout of histidine-rich calcium-binding protein (*TaHRC*) in hexaploid bread wheat confers resistance to Fusarium head blight (FHB) disease. *TaHRC* is directly associated with QTL *Fhb1*, responsible for FHB susceptibility, and the deletion in the start codon of the gene *TaHRC* makes it non-functional and governs resistance to FHB (Su et al., 2019). Another example is the knockdown of wheat calcineurin B-like protein (CBL)-interacting protein kinases (*TaCIPKs*), which are the susceptibility factors for wheat stripe (yellow) rust fungal pathogen (*Pst*) infections. Such knockdown significantly enhance disease resistance in wheat through the higher accumulation of ROS and overexpression of pathogenesis-related (PR) genes (He et al., 2023; Li et al., 2023). It has been also reported that the CRISPR mediated manipulation of salicylic (SA) and jasmonic acid (JA) responsive genes, especially their biosynthesis genes are modulated to boost wheat immunity against a varied range of pathogens (Mishra et al., 2024; Roychowdhury et al., 2024a, 2024b).

### 3.2.3 | Agronomic breeding trait improvement of wheat

Thousand kernel weight (TKW) and seed size of wheat were improved through the knockout of three homoalleles of the *TaGW2* gene using the CRISPR-Cas9 technology (Wang et al., 2018). Disruption of three genes has been done using CRISPR-based editing of *TaNP1* homoalleles in wheat, resulting in *TaNP1* triple mutants displaying complete male sterility (Li et al., 2020). A mutation in the centromeric histone *TaCENH3a* was induced through CRISPR-Cas9, which produced paternal haploid inducer wheat lines (Lv et al., 2020) resulting in promoting fertilization and crop growth. The knockout of *TaPHO2-A1* in bread wheat improved phosphorus (Pi) acquisition and grain yield under reduced P environments without any negative impact under high P conditions (Ouyang et al., 2016). These CRISPR-lines have the potential to be released as a variety or used as germplasm for further advancements (Hussain et al., 2022). A study by Zhang et al., (2021a) investigated a novel approach to enhance wheat yield potential using CRISPR-Cas9. They generated mutant lines of

TABLE 2 CRISPR-Cas mediated gene editing in wheat for trait improvement.

Trait categories	Target gene	Transformation method	Editing type	Nucleases	Improved traits	References
Abiotic stress tolerance	<i>TaDREB2</i> , <i>TaDREB3</i> , <i>TaERF3</i>	<sup>1</sup> AM	Knockout	Cas9	Drought tolerance	Kim et al., (2018)
	<i>TaDREB2</i> , <i>TaDREB3</i> , <i>TaERF3</i>	<sup>2</sup> PM	Knockout	Cas9	Drought tolerance	Kim et al., (2018)
	<i>TaSal1</i>	PM	Knockout	Cas9	Drought tolerance	Abdallah et al., (2022)
	<i>TaHAG1</i>	AM	Knockout	Cas9	Salinity tolerance	Zheng et al., (2021)
	<i>TaALS</i> , <i>TaACC</i>	<sup>3</sup> BM	Base editing	nCas9-D10A	Herbicide resistance	Zhang et al., (2019a)
	<i>TaDIW1</i> / <i>TaPP2C158</i>	AM	Knockout	Cas9	Drought tolerance	Wang et al., (2023b)
	<i>TaMLO</i>	BM	Knockout	Cas9	Powdery mildew resistance	Wang et al., (2014)
Disease resistance	<i>TaEDR1</i>	AM	Knockout	Cas9	Powdery mildew resistance	Zhang et al., (2016b)
	<i>TaHRC</i>	BM	Knockout	Cas9	Fusarium head blight (FHB) resistance	Su et al., (2019)
	<i>TaCIPK14</i>	AM	Knockout	Cas9	Resistance to stripe(yellow) rust	He et al., (2023)
	<i>TaGW2</i>	BM	Knockout	Cas9	Increased grain weight	Wang et al., (2018)
	<i>TaNIP1</i>	BM	Knockout	Cas9	Male sterility	Ni et al., (2023)
	<i>TaMs1</i>	BM	Knockout	Cas9	Male sterility	Li et al., (2020)
	<i>TaCENH3<math>\alpha</math></i>	AM	Knockout	Cas9	High haploid induction rate	Okada et al., (2019)
Agronomic (breeding) traits	<i>TaLOX2</i>	BM	Knockout	Cas9	High haploid induction rate	Lv et al., (2020)
	<i>TaGASR7</i>	BM	Knockout	Cas9	Varied grain size, weight, and increased storage	Zhang et al., (2016b)
	<i>TaPHO2-A1</i>	AM	Knockout	Cas9	Increased grain yield	Zhang et al., (2016b)
	<i><math>\alpha</math>-gladin</i>	BM	Knockout	Cas9	Increased phosphorus uptake and yield	Ouyang et al., (2016)
	<i>TaSBEII<math>\alpha</math></i>	BM	Knockout	Cas9	Gluten content	Sánchez-Leon et al., (2018)
	<i>TaLOX2</i>	AM, BM	Base editing	nCas9-D10A/ dCas9	High amylose	Li et al., (2021)
	<i>pinb-47</i> , <i>waxy-2</i> , <i>ppo-7</i> , <i>psy-13</i>	AM	Base editing	Cas9	Improved wheat quality	Zong et al., (2017)
Grain quality	<i>Inositol pentakisphosphate 2-kinase 1 (TaIPK1)</i>	AM	Knockout	Cas9	Grain hardness, starch quality and dough colour	Zhang et al., (2021b)
					Fe and Zn biofortification	Ibrahim et al., (2022)

<sup>1</sup>AM – Agrobacterium mediated, <sup>2</sup>PM – PEG mediated, <sup>3</sup>BM – Biolistic mediated.

the wheat variety ‘ZhengMai 7698’ by targeting the *Abnormal Cytokinin Response1 Repressor1 (ARE1)* gene. *ARE1* represses the enzyme Ferredoxin-dependent Glutamate 2-Oxoglutarate Aminotransferase (Fd-GOGAT), which is crucial for nitrogen assimilation. The mutant lines exhibited improved tolerance to nitrogen starvation, delayed senescence, and higher yields. This suggests that CRISPR-Cas9 editing of *ARE1* is a promising strategy for breeding wheat cultivars with better nitrogen use efficiency and increased yield.

### 3.2.4 | Improving grain quality in wheat

The CRISPR-Cas system in wheat has been used for targeted mutagenesis of  $\alpha$ -gladin genes to minimize gluten content in wheat grains (Sánchez-León et al., 2018). Furthermore, a CRISPR-Cas9-based mutation in the *TaSBE1a* gene has effectively produced high amylase wheat with a considerably improved starch content (Li et al., 2021). Zhang et al., (2021b) studied four grain-quality related genes viz. *pinb*, *waxy*, *ppo* and *psy*, which were implicated to be involved in wheat grain hardness, starch quality and dough colour, respectively, and targeted them for gene editing to compare their functionality and expression pattern in wild type (non-gene edited). This study revealed new allelic variations of the target genes (*pinb*, *waxy*, *ppo* and *psy*) to be introduced in the modern wheat line using Agrobacterium-mediated CRISPR delivery. CRISPR-Cas9 has been reported to modulate the *Inositol pentakisphosphate 2-kinase 1 (TaIPK1)* that lowers phytic acid in the grains to make iron and zinc biofortification (Ibrahim et al., 2022).

## 4 | SYSTEM BIOLOGY APPROACH FOR WHEAT TRAIT IMPROVEMENT

In recent years, omics technologies and the omics-integration generated large data which have offered numerous research openings in plant systems. The omics methodologies have been employed to target the central dogma: first for DNA analysis (genomics), then extended to the RNA (transcriptomics), protein (proteomics), and small metabolites i.e. metabolomics (Roychowdhury et al., 2023b), which are significant in identifying potential genes for cereal improvement (Ghatak et al., 2017).

### 4.1 | Transcriptomics: the era of high-throughput RNA sequencing

The use of high-throughput RNA-Seq and *de novo* transcriptome assembly in gene expression studies has facilitated the discovery of potential genes for forecasting gene functions and the examination of transcriptional regulatory networks in different crop plants, including wheat (Yang et al., 2016; Yu et al., 2016; Zhang et al., 2016a; Powell et al., 2017). The study of expressed sequence tags (ESTs) revealed that some wheat genes may be present in one genome not in other

genomes during gene expression (Xiao et al., 2013). The microarray is an effective tool for genome-wide transcriptome profiling under abiotic stress conditions. However, these studies are limited to wheat due to the polyploidy genome (Fernie and Schauer 2009). RNA-Seq, a powerful deep genome sequencing tool, allows for efficient gene expression analysis and this approach has been shown to be very effective in discovering specific genomic alterations. Next-generation sequencing (NGS) technologies have been developed to enhance sequencing accuracy. The absence of a completely sequenced and intricate genome (hexaploid) in wheat poses several challenges to “OMICS” investigations.

Poersch-Bortolon et al., (2016) evaluated the expression profiles of roots and leaves of wheat plants under extreme drought stress, which was beneficial for the marker development and the selection of significant, essential genes. Similarly, RNA-seq analysis has been implemented to ascertain the impact of drought on the wheat genome during reproductive stages (Ma et al., 2017). Further differential root transcriptome analyses identified salt stress-responsive gene networks and functional annotation was done by Goyal et al., (2016) leading to root mediated salt stress response in wheat and can be useful in making salinity resistant wheat lines.

### 4.2 | Proteomics: functional interplay of the wheat genes

Proteins play a role in plant stress responses, therefore, it is important to characterize and isolate stress-responsive proteins to understand the stress-response mechanisms in plants. Wheat is frequently cultivated under water deficit conditions as a dry winter cereal. Consequently, it is imperative to comprehend the mechanisms by which wheat plants respond to adversity. Numerous drought-responsive candidate proteins have been identified through proteomic analysis of various wheat cultivars. For instance, ROS proteins under arid stress in various tolerant wheat varieties and their susceptible counterparts has been crucial in the development of stress tolerance (Hajheidari et al., 2007).

Matrix-Assisted Laser Desorption/Ionization (MALDI) coupled to time-of-flight (MS) mass spectrometry (MALDI-TOF MS) was employed to investigate the proteome changes in wheat leaves under arid stress conditions (Caruso et al., 2009). Before harvesting wheat leaf tissue for protein extraction, this investigation subjected 8-day-old plants to a seven-day stress period. Twelve of the 36 proteins identified were up-regulated, while the twenty-four protein-coding genes were down-regulated (Caruso et al., 2009). Under stress conditions, the RuBisCo SSU protein and the ATP synthase CF1 alpha subunit phosphoribulokinase were downregulated. These findings were consistent with the research conducted by Plomion et al., (2006) on poplar, which demonstrated that the mRNA that encodes drought-responsive protein which is down-regulated under drought-stress. Further, it was also found that those stress-responsive proteins involved in ROS scavenging, energy production and stress defence were down-regulated.

The first comparative large-scale label-free quantitative shotgun proteomics study between two important cereals, viz. pearl millet and wheat, under drought stress was performed by Ghatak et al., (2021). In this study, it was found that compared to the pearl millet, harvested wheat leaves, root, and seed tissues contain higher amounts of drought stress specific protein markers. However, wheat is a C<sub>3</sub> plant and pearl millet is a C<sub>4</sub> plant, therefore, another study aimed to reveal the differences corresponding to different photosynthetic capacities. Proteins such as rubisco subunits, late embryogenesis-abundant (LEA) proteins, ATP synthase, and ABA-responsive proteins can be suggested as markers of drought stress conditions for wheat and pearl millet (Ghatak et al., 2021). Proteomic and metabolomic analyses in seed development have also helped to advance valuable and potentially important agricultural strategies for improving wheat plant yield, quality, and stress tolerance (Zhang et al., 2021c). These analyses have revealed novel molecular crosstalk during the grain-developing process in various tissues, including seed coat, endosperm, embryo and cavity fluid (Zhang et al., 2021c). Furthermore, a cell type-specific analysis of the developing endosperm showed interactions between proteins and metabolites, indicating different activity levels in nitrogen assimilation and sugar pathways in the various cell types from 15 to 26 days after anthesis. These findings can be linked to the specific accumulation of proteins and metabolites in the endosperm (Zhang et al., 2023).

### 4.3 | Metabolomics studies in wheat for final phenotypic readout

High-throughput metabolomics identifies and quantifies small molecules or metabolites, for example amino acids, sugars, organic acids, etc., in a given biological sample (Roessner and Bowne 2009). The data from metabolomic analyses demonstrated profound advantages in bridging the gap between the genotype and phenotype relationship (Fiehn 2002; Weckwerth 2003, 2011; Ghatak et al., 2018). For instance, a metabolomic assessment of growing grain in the wheat cultivar Zhongmai 175 revealed 74 metabolites including amino acids, carbohydrates, organic acids, lipids/fatty acids, etc. in various phases of grain development (Zhen et al., 2016a). This metabolic profiling helps to build a metabolic map when the breeding program seek to enhance wheat grain's nutritional value. In the same way, a metabolomic study of wheat's response to high nitrogen fertilizers during grain filling found the high accumulation of metabolites involved in carbon and nitrogen metabolism, especially in TCA cycle and starch synthesis, and proved that high-N fertilizer can promote grain protein content, drought viscosity and grain softening (Zhen et al., 2016b). Similarly, many studies have used metabolome profiling to evaluate wheat stress responses, revealing significant changes in metabolite composition, such as the accumulation of osmoprotectants, antioxidants, and secondary metabolites. These metabolic shifts provide insights into the biochemical pathways activated during stress, such as drought, heat, and salinity, and help identify key biomarkers and potential targets for improving wheat stress tolerance through breeding and

genetic modification (Lowe et al., 2008; Bowne et al., 2012; Gunnaiah and Kushalappa 2014).

### 4.4 | Integration of high throughput data - machine learning and mathematical tools

The complicated interactions and high dimensionality of distinct omics datasets create difficulty in identifying appropriate techniques for integrating multi-omics data into meaningful representations of biological functions. In the context of plant breeding, this entails identifying and isolating the key characteristics of genes, transcripts, proteins and metabolites and their interrelationships (hence, 'multi-omics' or 'panomics') that serve as the most accurate indicators of a certain trait, such as high seed production or the ability to withstand abiotic stresses (Weckwerth et al., 2020). Data integration poses challenges due to the heterogeneous nature and varying scales of data provided by different high-throughput technologies, including machine sensitivity, error rate, and data structure, which complicates their combining. Data integration is often carried out using three methodologies: (i) Various 'omics' datasets are examined separately to discover crucial 'characteristics'. Once the key traits are identified, they are interconnected to get the comprehensive model route of the system. (ii) Specialised tools are accessible for combining multi-omics data sets in an integrated data analysis method prior to any analysis and interpretation (Kuo et al., 2013), e.g. COBRA, Mapman, MetaboAnalyst, mixOmics, COVAIN, SIMCA. (iii) Systems modelling and simulation approaches rely on mathematical equations, which serve as helpful tools for comprehending and perhaps forecasting the causal functional connection of complex biological systems in relation to their surroundings (Weckwerth 2019). These integration approaches heavily depend on the well-specified qualitative or quantitative structure of the system being studied in order to compare fresh experimental results. Accurate comprehension of the wheat system biology is often reliant on possessing large genome sequences, transcriptomic and metabolomic data (Weckwerth 2019). These modelling systems may incorporate dynamic/kinetic models that solve systems using differential, partial differential, inverse stochastic Lyapunov matrix equations (Weckwerth 2019) or flux-balance models (Orth et al., 2010). Recently, a mathematical model was used to predict the winter wheat yield in a semi-arid zone. The developed model produced an average accuracy of 2.48% compared to the other available models.

## 5 | CONCLUSION AND FUTURE OUTLOOK

In the coming years, worldwide wheat production will significantly reduced due to climate change, poor irrigation, and excessive use of chemical fertilizers, leading to poor soil quality. To tackle this problem, we must develop a strong breeding pipeline to identify genes and molecular signatures to characterize, manipulate, and validate the major traits of wheat like abiotic stress tolerance, disease resistance, agronomic/breeding traits and grain quality. Precise trait



characterization in polyploid wheat is very challenging for further sustainable improvement and food security for humanity. We predict that integrating multi-omics techniques with contemporary plant breeding methods, high-throughput experimental techniques, sophisticated bioinformatics, and computational modelling tools in a systems biology approach will expedite the creation of environmentally sustainable, high-yielding, and nutritive wheat varieties that are resilient to climate change. Traditional wheat breeding methods face limitations like high costs, lengthy timelines, and potential loss of desired traits over generations. However, the CRISPR-Cas9 genome editing tool offers a precise and efficient approach for targeted genetic modifications in wheat. Its integration into breeding programs promises acceleration of superior wheat cultivars with improved quality, productivity, and stress tolerance. As this technology continues advancing, it holds immense potential in addressing food security challenges, enhancing crop productivity, and developing climate-resilient wheat varieties, paving the way for a more sustainable food for future. It is our unique attempt to combine all the research material available in the trait improvement of wheat and provide the research community with a significant update on implementing these state-of-the-art tools that can easily surpass the barrier of wheat genomic complexity and provide improved bread and durum wheat lines.

#### AUTHOR CONTRIBUTIONS

R.R. conceptualized the study, wrote the original draft, reviewed and edited the draft, worked on illustration visualization, project administration and supervised the work; A.G. and W.W. wrote the original draft, edited and reviewed the draft; M.K. and K.S. wrote the original draft; P.C. conceptualized the study, wrote the original draft, reviewed and edited the draft, funding acquisition and supervised the work. All authors have read and agreed to submit the final version of the manuscript.

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The authors declare no known competing personal or financial interests that could have appeared to influence the work in this manuscript.

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