Genomic Advancement in Wheat (*Triticum aestivum* L.): Harnessing Technological Breakthroughs for Future Strategies

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Review Article

ABSTRACT

Wheat can be greatly improved by identifying genes that are significant to agronomy. Although progress in wheat genetics and genomics has been limited by the genome's vast size and complexity. Wheat has a high-quality genome sequence in light of recent developments in genome sequencing and sequence assembly. Here, we propose that wheat biology can benefit from the same approaches that have been used to define biological systems in model species, such as the generation and characterization of mutants, methods for gene cloning, and enhanced transgenic technology. These tactics will encourage the establishment of wheat breeding programs and hasten the field of wheat biology. We also summarize current developments in functional genomics of wheat. In order to contribute to global food security, we conclude by talking about the future of wheat functional genomics and the sensible design-based molecular breeding of new wheat

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varieties. We suggest that researchers studying wheat should embrace the methods utilized for functional genomic analysis in other model species. One method for doing this is using gene cloning to find the gene causing an intriguing phenotype, deciphering the biological role of genes through the analysis of their corresponding mutants, and creating a related wheat mutant from the proposed wild-type gene to confirm the target gene's functionality.

1. INTRODUCTION

Wheat [*Triticum aestivum* L.], is a staple crop that is grown in many different types of environments and provides food for 30% of the world's population [1]. As the world's number one cereal, wheat (2n=6x=42) is a self-pollinated, hexaploid species, belonging to the Poaceae family, is known as the "King of Cereals" because of its large acreage under cultivation, abundant annual output, and commanding position in the global market for food grains [79]. With 9 billion people anticipated to live on the planet by 2050, future global food security depends on a large rise in wheat productivity [2]. Additionally, wheat grain quality needs to be improved; New wheat breeding techniques are therefore required because old breeding methods have passed their limit in terms of improving present wheat varieties to raise grain quality and yield. It is anticipated that rational design-based molecular breeding will improve selection accuracy and reduce breeding times by utilizing the genetic foundation in terms of agriculture significant features [3]. In fact, scientists using this technology in less than five years have successfully created super rice varieties featuring excellent yields and premium kernels a significant time savings over conventional breeding methods [4]. Therefore, molecular breeding techniques based on rational design have a lot of potential for crop breeding.

Our growing knowledge of the genetic basis of key agronomic features has made it possible for scientists to combine multiple traits and generate a multitude of elite lines, which has contributed to the success of molecular design breeding for rice [5,6]. The first vital step in applying this method to create novel, desirable wheat cultivars is identifying the genes encoding important agronomic traits. The genetic foundation for significant allohexaploid wheat features has been extensively studied; but, owing to its incredibly huge and complicated genome, we are not as able to perform functional genomic analysis on wheat as we are on rice or maize. Nonetheless, wheat genome assemblies, both near-complete and draft, have been created [7] owing to recent developments in genome assembly and sequencing technology. The resulting assemblies will help with wheat genome functional classification.

In this review, we suggest that researchers studying wheat should embrace the methods utilized for functional genomic analysis in other model species. One method for doing this is using gene cloning to find the gene causing an intriguing phenotype, deciphering the biological role of genes through the analysis of their corresponding mutants, and creating a related wheat mutant from the proposed wild-type gene to confirm the target gene's functionality. Furthermore, the characterisation of wheats molecular functions will be made possible by gene cloning, and the cloned genes will be employed in design-based molecular breeding initiatives to produce wheat varieties with improved traits. In addition, we provide an overview of recent developments in wheat functional genetic research and wheat genome sequencing.

2. STRATEGIES USED IN WHEAT

The technique of creating and characterizing mutants, cloning the gene causing a mutant phenotype, and using transformation to validate the found candidate gene has allowed modern biology to advance quickly. However, several techniques employed in other model organisms are not commonly used to wheat research because of the wheat genome's size and complexity as well as challenges with genetic transformation of wheat. As a result, we don't know much about wheat biology. Nevertheless, its imperative for wheat biologists to use the same research techniques as their counterparts in other fields, considering the most recent developments in genome sequencing and integration, as well as enhanced techniques for cloning and editing wheat genes.

2.1 Genetic Transformation of Wheat

To confirm that a gene is responsible for the observed phenotype, it is important to produce

mutant plants expressing the corresponding wild-type gene once a candidate gene was identified from a mutant. Biosynthetic transformation and agrobacterium mediated genetic transformation are the two most widely used methods for gradually introducing foreign genes of interest into plant cells [8]. Successful plant transformation is a significant benefit for gene function research. However, because wheat is hard to change and regenerate, advances in basic and applied genetics, as well as genetic engineering, have not kept up with the advancements made in other plant species. However, it took a lot of work to create the first transgenic wheat line to be productive, which was achieved by bombarding an embryogenic callus with micro projectiles [9,86]. Later, it was claimed that wheat had undergone its first Agrobacterium-mediated transformation [10]. Nevertheless, the process's efficiency was quite poor. Since then, a lot of work has been done to increase the effectiveness of wheat transformation [e.g., more selectable marker genes, enhanced culture media components, a wider range of target tissues, and better DNA delivery techniques] [11,12]. Compared to *Agrobacterium*-based techniques, transformation by biolistic irradiation is preferable because of its better efficiency (about 10%) and reduced genotype dependence on transformation outcomes. Because the vector framework is occasionally integrated into the host genome and the host genome contains several transgene copies, the process is complex. *Agrobacterium* drives the transformation process, transgenic expression is stable, and the incorporation of foreign genes inside the host genome remains intact. It is also easy to carry out and inexpensive. Agrobacterium-mediated transformation of immature Fielder cultivar embryos can produce transgenic wheat plants with excellent efficiency according to a recent technique established by the Japan Tobacco Company [13]. Later, more cultivars were successfully modified and this approach was implemented [14,15]. Genomic approaches for improving drought tolerance in wheat studied by Paudel *et al.* [80]; [86] and in chickpea by Tutlani *et al.* [81].

2.2 Mutation Breeding

Genetics is mostly driven by mutations, which are essential for understanding gene function and developing crops with improved genetics [84]. Natural mutants that arise from the evolution of species have been significant in the

fields of molecular breeding and functional genomics [16,17]. Research [18] has shown that the acceptance of naturally occurring mutation of the "green revolution" gene has greatly increased the global wheat output. Higher plants experience infrequent natural changes that happen at a rate of 10 to 15 – 10 to 8 for each base pair every production [19]. Different mutagenesis techniques have been used to cause random changes throughout the genomes of different plant species. These techniques include chemical mutagenesis (using sodium azide or ethyl methane sulfonate {EMS}) and physical mutagenesis (using gamma or fast neutron radiation). A variety of mutagenesis techniques have been used to introduce random mutations into the genomes of several plant species. These strategies include chemical mutagenesis (e.g., sodium azide or EMS) and physical mutagenesis caused by gamma or rapid neutron radiation. After that, the populations are sorted to identify any mutants displaying phenotypes of interest. Mutant characterisation reveals the gene that is considered to be responsible for its trait. Interestingly, these mutations can be useful instruments for improving crops (Fig. 1). EMS is a chemical agent that is commonly used in seed mutagenesis [20].

High-density, randomly dispersed mutations, typically resulting in only single nucleotide alterations like guanine to adenine or cytosine to thymine, are produced by it [21]. Numerous organizations have created EMS-mutagenized populations of various wheat kinds, after which they characterized and screened the mutants of interest that surfaced [22, 23]. Greater effectiveness, extensive, permanent, and non-transgenic mutagenesis are just a few benefits that researchers can gain from EMS-based mutagenesis. Strand breaks occur in the DNA of seeds exposed to ionizing particles like gamma rays or fast neutrons. Cosmological radiation can also alter seeds that are sent to space. Various sized chromosomal rearrangements from a single bp to thousands of bp, and large deletions of DNA sequences, are among the patterns seen in such mutants [24]. Although new plant types with better qualities have been created using this method, it is challenging to identify the changes in the genome.

These mutagenesis techniques have demonstrated their efficacy in producing mutants that allow for the characterization of the genotype-phenotype link. Large-scale screens
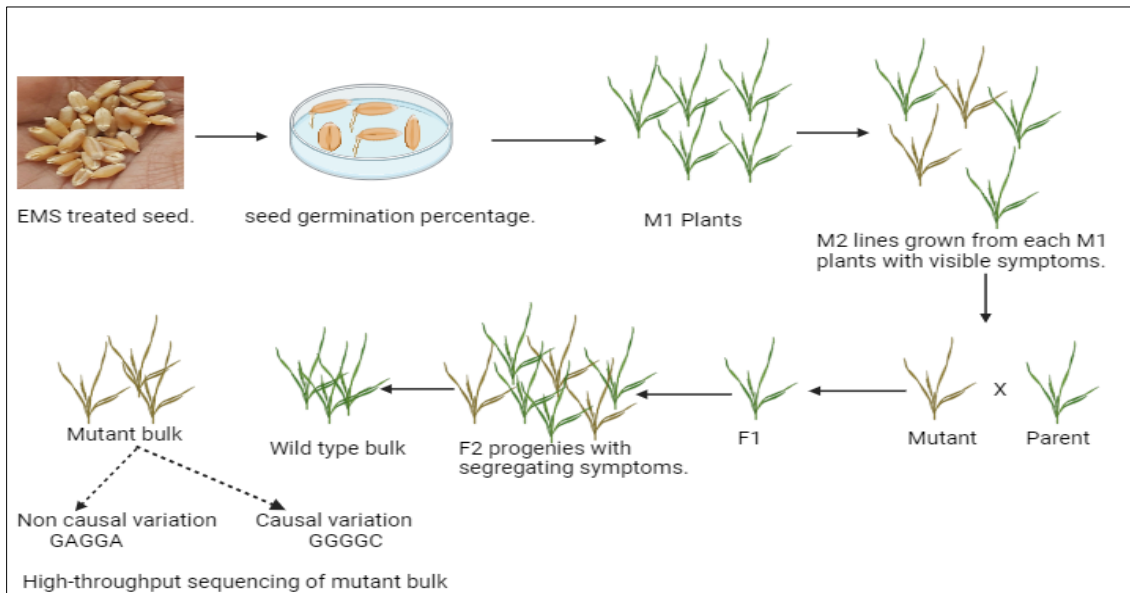
Fig. 1. The MutMap (Mutation Mapping) approach, a genetic method that uses next-generation sequencing

are required to find the intended mutations since mutagenesis is unpredictable. Furthermore, bread wheat is an allohexaploid, whose genome are duplicated three times. A mutation in a single homolog cannot alter any phenotypic trait due to the functional duplication between the homologous genes of the three sub-genomes. However, the ability to produce specific mutations in wheat has been made possible by the new advancement of genome editing technologies, particularly those involving in clustered regularly interspaced short palindromic repeats (CRISPR)/ CRISPR associated (Cas) system [25–28]. These methods will be crucial in learning more about the roles of wheat genes. Nevertheless, the sequence of the targeted gene must be known in order to employ CRISPR/Cas system. Consequently, it is best to think of CRISPR/Cas-induced targeted mutations and random mutagenesis as complimentary methods for producing wheat mutants.

2.3 Gene Cloning Methods

The next step after phenotyping an intriguing mutant is to identify the mutated gene that causes the trait that has been observed. Since the knowledge of genomic sequences is the foundation for gene cloning.

2.3.1 Traditional gene cloning

Prior to the discovery of reference genomes, many genes causing crucial agricultural features

in crops were solely known by their genetic makeup. Cloning genes of interest via positional cloning/ map-based cloning, is a helpful technique. A necessary step is to create high-resolution mapping populations that are phenotyped by the desired trait and genotyped using molecular markers. Through the use of mapping populations composed of several hundred or thousand plants created via genetic recombination, one can gradually identify the site of a mutant gene using this method Mutmap based cloning(29,30). By integrating information from phenotypic and molecular markers, a genetic map covering the target locus is created. The gene product need not be known in order for map-based cloning to function. Several genes have been successfully cloned using it, including Lr10, HM1, and Xa21 from rice, wheat, and maize accordingly [16, 30-31]. Notably, Table 1 indicates that about half of these genes are resistant to illness. Map-based cloning, however, requires a lot of work and time. Furthermore, cloning genes via a map-based approach is not feasible when the genes are situated in centromeric areas [29].

2.3.2 Advances in wheat genome sequencing

Gene cloning and wheat genetic improvement are two applications of fundamental research that benefit from great quality genomic assemblies and sequencing. Consequently, a considerable deal of work has gone into studying the wheat genome, and in the last several years, significant

Table 1. Function of the genes cloned in wheat

Genes	Function	Identification method	Reference
Lr21	Leaf rust resistance	Map-based cloning	[32]
Lr10	Leaf rust resistance	Map-based cloning	[33]
Lr34	Leaf rust resistance	Map-based cloning	[34]
Yr36	Stripe rust resistance	Map-based cloning	[35]
Yr10	Stripe rust resistance	Map-based cloning	[36]
Sr33	Stem rust resistance	Map-based cloning	[37]
Sr35	Stem rust resistance	Map-based cloning	[38]
Pm2	Powdery mildew resistance	MutChromSeq	[39]
Fhb1	Fusarium head blight resistance	Map-based cloning	[40-41]
TaTAR2.1-3A	Boost production and nitrogen usage effectiveness	Homology-based cloning	[42]
Bo1, Bo4	Boron transporter	Map-based cloning	[43]
Gpc-B1	Boosting iron, zinc, and kernel protein by controlling senescence.	Map-based cloning	[44]

advancements have been made. Bread wheat has a large genome, in order to discover new genes, it is crucial to sequence and map the region of the genome that is expressed. Reaching a high standard of sequencing the hexaploid bread wheat genome in 2005 was the aim of the international wheat genome sequencing consortium's (IWGSC) objective, which is shared by its 2400 members from 68 nations. After bacterial artificial chromosome (BAC) libraries were created for each chromosome or chromosomal arm, IWGSC members worked on creating physical maps and sequencing the BACs due to high degree of similarity and complexity of the three subgenomes (A, B, and D; 2n=6x=42). Following separation by flow cytometry, the 21 chromosome of Chinese Spring wheat were sequenced. BAC-by-BAC sequencing was created using a chromosomal map of wheat's biggest chromosome, 3B [45].

Eventually, the chromosomal sequences (or portions of them) were made public. Whole genome sequences and assemblies for the diploid *Triticum Urartu* and *Aegilops tauschii*, as well as hexaploidy common wheat known as Chinese spring, have been reported [46–48] due to these technical developments in sequencing and assembling sequences. Despite their considerable fragmentation and abundance of unordered scaffolds, they have provided us with an insight into the wheat genome and its locations for genes [29]. By utilizing shotgun assembly and chromosome-based sequencing, an ordered draft sequence of common wheat was produced; the majority of the genes were uniformly distributed among homologous chromosomes and each subgenome comprised

duplicates of highly conserved and comparable genes [1]. In the meantime, substantially more contiguity is present in the chromosome 3B high-quality reference sequence [49] than in the sequence provided by the IWGSC [1].

The A and D draft genome sequences for the whole genome shotgun approach are derived from the *T. Urartu* and *Ae. tauschii*. First snapshot of the bread wheat genome sequenced from *Chinese spring* for whole genome shotgun sequencing [51]. Grate quality AT3DS sequencing for the short arm of chromosome 3, taken from the *Aegilops tauschii* species for next generation sequencing and combined approach of BAC pooling [52]. 10.1gigabase of tetraploid wheat and 9.1 gigabases of hexaploidy wheat sequenced from *T. turgidum* and *Chinese spring* for whole genome shotgun libraries and short-read sequencing technology. A reference dataset (RefSeqv1.0) including all 21 chromosomes of common wheat, derived from the *chinese spring* sequencing species, intended for short read sequencing whole genome sequencing [54]. Physical map and great quality of 3B chromosome genome sequencing of *Chinese spring* species for BAC-by-BAC and 8452 BACs in pools sequencing.

Our understanding of wheat genome assembly and sequencing has greatly increased due to a number of conceptual and technological advances. For instance, the genomic sequence of the wild emmer (*Triticum turgidum*) was recovered in excellent quality [56] and confirmed by Hi-C and genetic data. Additionally, the IWGSC (RefSeq v1.0), genome sequence of common wheat, which was organized and annotated, was released. Alignments with

separate information from exons and introns sequences and subgenome hybrid maps induced by radiation, shows the consistency and quality of the IWGSC RefSeq v1.0 are better than those of previous sequences [57]. Apart from these preliminary and nearly whole wheat genome sequences, notable progress is being made in identifying and isolating the genes that encode crucial agronomic traits for wheat molecular breeding.

2.3.2 Genetic mapping based on sequencing

Innovative and accelerated strategies for gene cloning have been developed in response to the rapid advancements in sequencing and bioinformatic technologies. The previously mentioned techniques comprise homology-based cloning, mutant chromosome sequencing (MutChromSeq), mutagenesis resistance gene improvement and sequencing (MutRenSeq), mutational mapping (MutMap), and targeted chromosomal based cloning through extended data gathering (TACCA). Due to evolutionary conservation, genes that are similar across species it may share same unique functions and sequences. Through the use of homology cloning, which is predicated regarding sequence similarity between target and known genes, wheat genes were effectively isolated. For example, the maize d8 and Arabidopsis gai share traits with the wheat rht-1 mutant, encompassing small size, reduced gibberellin responsiveness, and increased GA3 levels in planta. Rht-D1's DNA sequence in wheat was found based on these similarities [58]. Productivity of fodder maize (*Zea mays* L.) SFM-1 under varied sowing dates and nitrogen levels studied by [83]. However, this approach cannot be used to clone a target gene if pertinent sequence information from other species is not available. MutMap is a fast approach of isolating genes based on whole-genome resequencing, gene mapping, and mutagenesis. An intriguing phenotype-displaying plant is chosen for MutMap, after crossing with its parent of wild kind, it selfed. The offspring of the F2 will have both wild-type and mutant characteristics. Whole genome sequencing is used to large scale DNA samples of F2 offspring exhibiting the mutant phenotype, and the sequences obtained are compared with the reference genome. One hypothesis is that single nucleotide polymorphism (SNPs) with an SNP index of 1 carry the gene causing the mutant phenotype [59]. Using this method, the genomic locations of the genes governing rice features that are significant to agronomy have been found. MutMap is a great alternative for obtaining altered genes from species with tiny genomes and a high-quality reference genome.

MutChromSeq, in contrast, offers a quick way to clone genes from big genomes. This technique combines high throughput sequencing, mutagenesis, and chromosomal separating to minimize genome heterogeneity. By sequencing and comparing chromosomes from the wild-type and mutant strains, a potential gene with a distinct phenotype is found. Using this technique, the wheat Pm2 gene and the barley Eceriferum-q gene were cloned without the necessity for positionally accurate gene mapping (60). When the conditions of the method namely, mutagenesis, finding target genes that give strong phenotypes, and isolating single chromosomes are satisfied, previously unclonable genes can now be cloned. Flow-sorted chromosomes are used by TACCA to streamline genome assembly, enable next-generation DNA sequencing, and reduce genome complexity, hence enabling the rapid cloning of genes from complex polyploid genomes. The wheat leaf rust resistance gene Lr22a was isolated from bread wheat using this method [61].

For gene isolation, TACCA is an excellent choice if chromosomal isolation is possible and a draft gene map is available. The fast gene cloning method known as MutRenSeq can be used to isolate genes containing nucleotide binding and leucine rich repeats (NLRs). Chemical mutagenesis, sequencing, and exome capture are all involved. Since most resistance genes encode proteins that have non coding RNAs, exome capture is employed to enhance the NLR specific bait library and sequencing is carried out on both the vulnerable loss of function mutants and the resistant wild type parent. The genes in charge of the resistance can be identified since the mutant readings align with the parent's. NLR-type resistance genes can be isolated using this method from the majority of crops and their wild cousins. It was used to clone two genes from bread wheat that are resistant to fungal stem rust (Sr22 and Sr45) [62]. It does not require positional precise mapping. Several model species have been characterized using the previously outlined methodologies, which are currently being used in the fields of wheat biology and functional genomics. It's time for wheat scientists to employ these tactics in their research, as there are numerous experimental procedures at their disposal. This will encourage

the emergence of new alleles for wheat breeding and hasten the advancement of wheat functional genomics and biology.

3. ADVANCEMENTS IN WHEAT FUNCTIONAL GENOMICS

In the postgenomic age, the most essential problem is figuring out how the genes that cause significant agronomic features work. However, wheat biology has not advanced as quickly as other crops because of the bread wheat's huge and complicated genome. Interestingly, significant improvements have been made recently in wheat functional genomics. In fact, an increasing number of genes governing several characteristics including resistance to disease, improved yield and quality, and efficiency in absorbing nutrients have been cloned and elucidated, paving the way for sensible, design based molecular breeding in wheat. A concise overview of the latest developments in wheat functional genomics is presented here.

3.1 Nutrient Uptake in Wheat

Fertilizers that are rich in phosphorus (P) and nitrogen (N) can increase crop yields, especially those of wheat. Unfortunately, the extensive misuse of fertilizers has had a negative impact on the ecosystem and decreased wheat plants' ability to utilise nutrients efficiently. To improve nutrient absorption and use efficiency, it is essential to discover the genes controlling N and P uptake in wheat. The efficiency of using N and P is complicated and depends on a variety of physiological characteristics. There are very few genes known to control N and P uptake in wheat, notably NFYB and NAC. To find TAA1/TAR genes in wheat, for instance, fifteen TaTAR genes were found through a genome wide search, with TaTAR2.1 being mostly expressed in roots [63]. Low-N environments cause an upregulation of TaTAR2.1 expression, which encourages root development. Consequently, TaTAR2.1 might be crucial for raising wheat yields and optimizing N usage. P is another macronutrient that crops and plant growth depend on. In low-P environments, wheat yields and P absorption may be enhanced by TaPHO2-A1 deletion [64]. Our knowledge of nitrogen uptake and wheat's N and P utilization efficiency will grow when the genes controlling these processes are discovered. Thus, wheat yields could rise while using less fertilizer. With changing climate condition in the country put an alarming situation towards production and productivity of wheat crop [82].

3.2 Disease Resistant

Diseases caused by fungi, such as *Septoria tritici* blotch (STB), rusts, powdery mildew, and head blight/scab can reduce wheat yields by approximately 25% every year [65] and pose a risk to global food security. A great deal of work has gone into figuring out which genes cause disease resistance in order to create wheat types that are both broadly and durably resistant to disease. In wheat rusts are the most common and dangerous disease, which include stripe rust, stem rust, and leaf rust. Lr34 provides resistance to certain rusts and mildews. It has been utilized to improve wheat since the 20th century, despite the fact that the ATP binding cassette transporter-coding gene, Lr34 was not cloned until 2009. Similar to the pleiotropic drug resistance protein PEN3 in Arabidopsis, in adult plants the Lr34 transporter contributes to fungal development modification through exporting compounds that confer resistance to infections (66). Additionally, Yr36 which offers widespread defense against stripe rusts was cloned. Both the putative START domain and the functional enzymatic (kinase) domain are essential for disease resistance [67]. Recently, Yr28, an ancestral NB-LRR that gives wheat resistance to stripe rust, was successfully cloned [68]. Furthermore, two genes that provide protection against the wheat stem rust race *Ae. tauschii* and *T. monococcum* are the sources of Ug99 clones respectively, and named Sr33 and Sr35 [69-70].

The dangerous illness powdery mildew, caused by *Blumeria graminis*, could drastically decrease wheat harvests. Fifty loci and seventy-eight powdery mildew resistance alleles have been identified and named thus far. However, the molecular and functional characteristics of just a small number of genes are known. For example, Pm60, which was cloned from *T.urartu* was recently shown to encode NB-LRR protein that is important for Bgt E09 resistance [71]. Similarly, Pm21 encodes a CC-NBS-LRR protein that provides broad-spectrum resistance to powdery mildew [72-73]. Global economic damages may arise from STB caused by *Zymoseptoria tritici*. Wheat contains 21 genes related to Stb resistance. Nevertheless, only one gene Stb6 has undergone characterization and cloning. Resistance to the pathogen is conferred without causing hypersensitivity via its wall associated receptor-kinase.

4. WHEAT YIELD AND QUALITY

To promote the rational design based molecular breeding of novel wheat varieties, it will become more vital to identify and define the genes influencing grain production and quality as the world's population and standard of living rise. Heavy grains collapse down on tall wheat stems because they are too weak to support them. Thus, in order to increase wheat yields worldwide, semi-dwarfing and dwarfing genes should be inserted into wheat. We have identified and isolated the wheat Rht gene. It produces a transcription factor involved in gibberellin signalling that has an SH2-like domain [74]. The Green Revolution was made possible by new Rht-mutant plant kinds that are shorter and produce more grain; these plants also react strangely to gibberellin. The wheat mutant allele gw2-A1 has been characterized recently; it produces broader and longer grains, which raise the wheat's thousand grain weight [75].

Numerous characteristics, which are influenced by multiple genes, control the end-use, nutritional value, and starch composition of wheat grains. Still, very few of the necessary genes have been successfully cloned. As an illustration, the NAC gene Gpc-B1 was cloned and localized to chromosomal arm6BS. It contains a transcription factor that increases nutrient remobilization and regulates senescence, raising the levels of iron, zinc, and protein in wheat grains (44). When the starch branching enzyme II gene is mutated in wheat lines, the resulting increased quantities of resistant starch and amylose are beneficial to human health.

5. FUTURE PERSPECTIVES

Wheat research has a great deal of potential when agronomically important genes are identified and used through sensible molecular breeding designs. The functions of the genes that give wheat its significant agronomic features have been well studied. The intricate and enormously huge genome of wheat, which is 135 times larger than Arabidopsis and 37 times larger than rice, has, however, hampered advances in functional wheat genomics and metabolism. The genome of bread wheat has been almost fully assembled as a result of advancements in sequencing technologies and sequencing cost reduction [77]. Not to mention, throughout the past 20 years, considerable advancements have been made in wheat transformation and genetic mapping techniques. The majority of the genes in

wheat, an allohexaploid species with three unique sub-genomes (AABBDD), exhibit functional redundancy. Because specific mutations may now be created in all copies of a gene, rather than just one, most recently, genome editing has been made possible help particularly in wheat research. For example, wheat exhibits significant resistance to powdery mildew when all three copies of Mlo are simultaneously knocked off [78]. As a result, this method provides a quick way to link phenotypes to sequence variations or to deactivate genes causing unwanted features, allowing wheat to be genetically improved. The activities of wheat genes can now be examined using a number of potent tools, such as high-quality genome sequencing, expanded genetic maps, mutagenesis, enhanced transgenic technologies, and sophisticated genome editing methods. It should be possible to alter any gene in the wheat genome by combining random mutagenesis with genome editing; further methods can then be used to determine the changed gene's biological function. There are current strategies and techniques available that can significantly progress wheat biology. The subject of wheat biology will benefit enormously from the involvement of scientists studying other model plants or bioinformatics, as it will push wheat functional genomics forward. We anticipate that many agronomically significant genes will be identified and isolated through the application of functional genomic analysis techniques in model organisms. These genes will be important resources for molecular breeding. Furthermore, we believe that creating elite wheat varieties through molecular breeding based on rational design holds enormous potential for guaranteeing future food security worldwide.

6. CONCLUSION

Wheat research stands at the cusp of a significant transformation, driven by the identification and utilization of agronomical important genes through innovative molecular breeding techniques. The complex and extensive genome of wheat has historically posed challenges to progress, with its size being 135 times larger than Arabidopsis and 37 times larger than rice. However, recent advancements in sequencing technologies and genetic mapping have substantially reduced these barriers, leading to a near-complete assembly of the bread wheat genome. This breakthrough, combined with the ongoing development of wheat transformation and sophisticated genome

editing techniques, offers new avenues for the study and enhancement of wheat's genetic makeup. The potential to simultaneously target all copies of a gene in wheat's allohexaploid genome (AABBDD) allows researchers to explore functional redundancy and achieve significant results, such as increased resistance to powdery mildew through the knockout of all three copies of the Mlo gene. The convergence of high-quality genome sequencing, advanced genetic mapping, mutagenesis, and refined transgenic technologies provides a robust toolkit for exploring and manipulating the wheat genome. This approach enables the linking of specific phenotypes with sequence variations and the targeted deactivation of undesirable traits, leading to genetically improved wheat strains. The future of wheat research is promising, with functional genomic analysis techniques poised to identify and isolate a multitude of agronomically significant genes. These discoveries will serve as vital resources for molecular breeding, paving the way for the rational design of elite wheat varieties. The participation of scientists from other model plant studies and bioinformatics is crucial to accelerating wheat functional genomics, potentially ensuring global food security through the development of high-yield, resilient wheat varieties. The path forward holds great potential, not only for enhancing wheat production but also for fostering a sustainable and secure agricultural future.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. (IWGSC) IWGSC, Mayer KFX, Rogers J, Doležel J, Pozniak C, Eversole K, et al. A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. Science (1979). 2014;345(6194):1251788.
2. Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS, Johnston M, et al. Solutions for a cultivated planet. Nature. 2011;478(7369):337–42.
3. Qian Q, Guo L, Smith SM, Li J. Breeding high-yield superior quality hybrid super rice by rational design. Natl Sci Rev. 2016;3(3):283–94.
4. Zeng D, Tian Z, Rao Y, Dong G, Yang Y, Huang L, et al. Rational design of high-yield and superior-quality rice. Nat Plants. 2017;3(4):1–5.
5. Zhang Q. Strategies for developing green super rice. Proceedings of the national Academy of Sciences. 2007;104(42):16402–9.
6. Li ZK, Zhang F. Rice breeding in the post-genomics era: from concept to practice. Curr Opin Plant Biol. 2013;16(2):261–9.
7. Jia M, Guan J, Zhai Z, Geng S, Zhang X, Mao L, et al. Wheat functional genomics in the era of next generation sequencing: an update. Crop J. 2018;6(1):7–14.
8. Xia L, Ma Y, He Y, Jones HD. GM wheat development in China: current status and challenges to commercialization. J Exp Bot. 2012;63(5):1785–90.
9. Vasil V, Castillo AM, Fromm ME, Vasil IK. Herbicide resistant fertile transgenic wheat plants obtained by microprojectile bombardment of regenerable embryogenic callus. Bio/technology. 1992;10(6):667–74.
10. Cheng M, Fry JE, Pang S, Zhou H, Hironaka CM, Duncan DR, et al. Genetic transformation of wheat mediated by Agrobacterium tumefaciens. Plant Physiol. 1997;115(3):971–80.
11. Harwood WA. Advances and remaining challenges in the transformation of barley and wheat. J Exp Bot. 2012;63(5):1791–8.
12. Shrawat AK, Armstrong CL. Development and application of genetic engineering for wheat improvement. CRC Crit Rev Plant Sci. 2018;37(5):335–421.
13. Ishida Y, Tsunashima M, Hiei Y, Komari T. Wheat (*Triticum aestivum* L.) transformation using immature embryos. Agrobacterium Protocols. 2015;1:189–98.
14. Wang K, Riaz B, Ye X. Wheat genome editing expedited by efficient transformation techniques: progress and perspectives. Crop J. 2018;6(1):22–31.
15. Richardson T, Thistleton J, Higgins TJ, Howitt C, Ayliffe M. Efficient Agrobacterium transformation of elite wheat germplasm without selection. Plant Cell, Tissue and Organ Culture (PCTOC). 2014;119:647–59.

16. Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, et al. A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. Science (1979). 1995;270(5243):1804–6.

17. Gu K, Yang B, Tian D, Wu L, Wang D, Sreekala C, et al. R gene expression induced by a type-III effector triggers disease resistance in rice. Nature. 2005;435(7045):1122–5.

18. Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, et al. 'Green revolution'genes encode mutant gibberellin response modulators. Nature. 1999;400(6741):256–61.

19. Jiang SY, Ramachandran S. Natural and artificial mutants as valuable resources for functional genomics and molecular breeding. Int J Biol Sci. 2010;6(3):228.

20. Sega GA. A review of the genetic effects of ethyl methanesulfonate. Mutation Research/Reviews in Genetic Toxicology. 1984;134(2–3):113–42.

21. Uauy C, Wulff BBH, Dubcovsky J. Combining traditional mutagenesis with new high-throughput sequencing and genome editing to reveal hidden variation in polyploid wheat. Annu Rev Genet. 2017;51:435–54.

22. Krasileva K V, Vasquez-Gross HA, Howell T, Bailey P, Paraiso F, Clissold L, et al. Uncovering hidden variation in polyploid wheat. Proceedings of the National Academy of Sciences. 2017;114(6):E913–21.

23. Wang Z, Li J, Chen S, Heng Y, Chen Z, Yang J, et al. Poaceae-specific MS1 encodes a phospholipid-binding protein for male fertility in bread wheat. Proceedings of the National Academy of Sciences. 2017;114(47):12614–9.

24. Periyannan S. Sustaining global agriculture through rapid detection and deployment of genetic resistance to deadly crop diseases. New Phytologist. 2018; 219(1):45–51.

25. Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, et al. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol. 2014;32(9):947–51.

26. Zhang Y, Liang Z, Zong Y, Wang Y, Liu J, Chen K, et al. Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. Nat Commun. 2016;7(1):12617.

27. Li J, Li Y, Ma L. CRISPR/Cas9-based genome editing and its applications for functional genomic analyses in plants. Small Methods. 2019;3(3):1800473.

28. Wang K, Riaz B, Ye X. Wheat genome editing expedited by efficient transformation techniques: progress and perspectives. Crop J. 2018;6(1):22–31.

29. Keller B, Wicker T, Krattinger SG. Advances in wheat and pathogen genomics: implications for disease control. Annu Rev Phytopathol. 2018;56:67–87.

30. Johal GS, Briggs SP. Reductase activity encoded by the HM1 disease resistance gene in maize. Science (1979). 1992;258(5084):985–7.

31. Feuillet C, Travella S, Stein N, Albar L, Nublat A, Keller B. Map-based isolation of the leaf rust disease resistance gene Lr10 from the hexaploid wheat (*Triticum aestivum* L.) genome. Proceedings of the National Academy of Sciences. 2003;100(25):15253–8.

32. Huang L, Brooks SA, Li W, Fellers JP, Trick HN, Gill BS. Map-based cloning of leaf rust resistance gene Lr21 from the large and polyploid genome of bread wheat. Genetics. 2003;164(2):655–64.

33. Feuillet C, Travella S, Stein N, Albar L, Nublat A, Keller B. Map-based isolation of the leaf rust disease resistance gene Lr10 from the hexaploid wheat (*Triticum aestivum* L.) genome. Proceedings of the National Academy of Sciences. 2003;100(25):15253–8.

34. Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, et al. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science (1979). 2009;323(5919):1360–3.

35. Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen X, et al. A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science (1979). 2009;323(5919):1357–60.

36. Liu W, Frick M, Huel R, Nykiforuk CL, Wang X, Gaudet DA, et al. The stripe rust resistance gene Yr10 encodes an evolutionary-conserved and unique CC–NBS–LRR sequence in wheat. Mol Plant. 2014;7(12):1740–55.

37. Periyannan S, Moore J, Ayliffe M, Bansal U, Wang X, Huang L, et al. The gene Sr33, an ortholog of barley Mla genes, encodes resistance to wheat stem rust race Ug99. Science (1979). 2013;341(6147):786–8.

38. Saintenac C, Zhang W, Salcedo A, Rouse MN, Trick HN, Akhunov E, et al. Identification of wheat gene Sr35 that confers resistance to Ug99 stem rust race group. Science (1979). 2013;341(6147): 783–6.

39. Sánchez-Martín J, Steuernagel B, Ghosh S, Herren G, Hurni S, Adamski N, et al. Rapid gene isolation in barley and wheat by mutant chromosome sequencing. Genome Biol. 2016;17:1–7.

40. Su Z, Bernardo A, Tian B, Chen H, Wang S, Ma H, et al. A deletion mutation in TaHRC confers Fhb1 resistance to Fusarium head blight in wheat. Nat Genet. 2019;51(7):1099–105.

41. Li G, Zhou J, Jia H, Gao Z, Fan M, Luo Y, et al. Mutation of a histidine-rich calcium-binding-protein gene in wheat confers resistance to Fusarium head blight. Nat Genet. 2019;51(7):1106–12.

42. Shao A, Ma W, Zhao X, Hu M, He X, Teng W, et al. The auxin biosynthetic tryptophan Aminotransferase related TaTAR2. 1-3A increases grain yield of wheat. Plant Physiol. 2017;174(4):2274–88.

43. Pallotta M, Schnurbusch T, Hayes J, Hay A, Baumann U, Paull J, et al. Molecular basis of adaptation to high soil boron in wheat landraces and elite cultivars. Nature. 2014;514(7520):88–91.

44. Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J. A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science (1979). 2006;314(5803):1298–301.

45. Paux E, Sourdille P, Salse J, Saintenac C, Choulet F, Leroy P, et al. A physical map of the 1-gigabase bread wheat chromosome 3B. Science (1979). 2008;322(5898):101–4.

46. Brenchley R, Spannagl M, Pfeifer M, Barker GLA, D'Amore R, Allen AM, et al. Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature. 2012;491(7426):705–10.

47. Ling Z, Liu X, Wang Y, Li L, Xiang C. Pyrosequencing analysis of the salivary microbiota of healthy Chinese children and adults. Microb Ecol. 2013;65:487–95.

48. Jia J, Zhao S, Kong X, Li Y, Zhao G, He W, et al. Aegilops tauschii draft genome sequence reveals a gene repertoire for wheat adaptation. Nature. 2013;496 (7443):91–5.

49. Choulet F, Alberti A, Theil S, Glover N, Barbe V, Daron J, et al. Structural and functional partitioning of bread wheat chromosome 3B. Science (1979). 2014;345(6194):1249721.

50. Ling HQ, Zhao S, Liu D, Wang J, Sun H, Zhang C, et al. Draft genome of the wheat A-genome progenitor Triticum urartu. Nature. 2013;496(7443):87–90.

51. Brenchley R, Spannagl M, Pfeifer M, Barker GLA, D'Amore R, Allen AM, et al. Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature. 2012;491(7426):705–10.

52. Xie J, Huo N, Zhou S, Wang Y, Guo G, Deal KR, et al. Sequencing and comparative analyses of Aegilops tauschii chromosome arm 3DS reveal rapid evolution of Triticeae genomes. Journal of genetics and genomics. 2017;44(1):51–61.

53. Avni R, Nave M, Barad O, Baruch K, Twardziok SO, Gundlach H, et al. Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. Science (1979). 2017; 357(6346):93–7.

54. Consortium IWGS. Shifting the limits in wheat research and breeding through a fully annotated and anchored reference genome sequence. Science (1979). 2018;361.

55. Paux E, Sourdille P, Salse J, Saintenac C, Choulet F, Leroy P, et al. A physical map of the 1-gigabase bread wheat chromosome 3B. Science (1979). 2008;322(5898):101–4.

56. Avni R, Nave M, Barad O, Baruch K, Twardziok SO, Gundlach H, et al. Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. Science (1979). 2017;357 (6346):93–7.

57. Consortium IWGS. Shifting the limits in wheat research and breeding through a fully annotated and anchored reference genome sequence. Science (1979). 2018;361.

58. Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, et al. 'Green revolution'genes encode mutant gibberellin response modulators. Nature. 1999;400 (6741):256–61.

59. Abe A, Kosugi S, Yoshida K, Natsume S, Takagi H, Kanzaki H, et al. Genome sequencing reveals agronomically important loci in rice using MutMap. Nat Biotechnol. 2012;30(2):174–8.

60. Sánchez-Martín J, Steuernagel B, Ghosh S, Herren G, Hurni S, Adamski N, et al.

Rapid gene isolation in barley and wheat by mutant chromosome sequencing. Genome Biol. 2016;17:1–7.

61. Thind AK, Wicker T, Šimková H, Fossati D, Moullet O, Brabant C, et al. Rapid cloning of genes in hexaploid wheat using cultivar-specific long-range chromosome assembly. Nat Biotechnol. 2017;35(8):793–6.

62. Steuernagel B, Periyannan SK, Hernández-Pinzón I, Witek K, Rouse MN, Yu G, et al. Rapid cloning of disease-resistance genes in plants using mutagenesis and sequence capture. Nat Biotechnol. 2016;34(6):652–5.

63. Shao A, Ma W, Zhao X, Hu M, He X, Teng W, et al. The auxin biosynthetic tryptophan aminotransferase related TaTAR2. 1-3A increases grain yield of wheat. Plant Physiol. 2017;174(4):2274–88.

64. Ouyang X, Hong X, Zhao X, Zhang W, He X, Ma W, et al. Knock out of the Phosphate 2 gene TaPHO2-A1 improves phosphorus uptake and grain yield under low phosphorus conditions in common wheat. Sci Rep. 2016;6(1):29850.

65. Marchal C, Zhang J, Zhang P, Fenwick P, Steuernagel B, Adamski NM, et al. BED-domain-containing immune receptors confer diverse resistance spectra to yellow rust. Nat Plants. 2018;4(9):662–8.

66. Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, et al. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science (1979). 2009;323(5919):1360–3.

67. Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen X, et al. A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science (1979). 2009;323(5919):1357–60.

68. Zhang C, Huang L, Zhang H, Hao Q, Lyu B, Wang M, et al. An ancestral NB-LRR with duplicated 3′ UTRs confers stripe rust resistance in wheat and barley. Nat Commun. 2019;10(1):4023.

69. Periyannan S, Moore J, Ayliffe M, Bansal U, Wang X, Huang L, et al. The gene Sr33, an ortholog of barley Mla genes, encodes resistance to wheat stem rust race Ug99. Science (1979). 2013;341(6147):786–8.

70. Saintenac C, Zhang W, Salcedo A, Rouse MN, Trick HN, Akhunov E, et al. Identification of wheat gene Sr35 that confers resistance to Ug99 stem rust race group. Science (1979). 2013;341 (6147):783–6.

71. Zou S, Wang H, Li Y, Kong Z, Tang D. The NB-LRR gene Pm60 confers powdery mildew resistance in wheat. New Phytologist. 2018;218(1):298–309.

72. Xing L, Hu P, Liu J, Witek K, Zhou S, Xu J, et al. Pm21 from Haynaldia villosa encodes a CC-NBS-LRR protein conferring powdery mildew resistance in wheat. Mol Plant. 2018;11(6):874–8.

73. He H, Zhu S, Zhao R, Jiang Z, Ji Y, Ji J, et al. Pm21, encoding a typical CC-NBS-LRR protein, confers broad-spectrum resistance to wheat powdery mildew disease. Mol Plant. 2018;11(6):879–82.

74. Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, et al. 'Green revolution'genes encode mutant gibberellin response modulators. Nature. 1999;400 (6741):256–61.

75. Simmonds J, Scott P, Brinton J, Mestre TC, Bush M, Del Blanco A, et al. A splice acceptor site mutation in TaGW2-A1 increases thousand grain weight in tetraploid and hexaploid wheat through wider and longer grains. Theoretical and Applied Genetics. 2016;129:1099–112.

76. Slade AJ, McGuire C, Loeffler D, Mullenberg J, Skinner W, Fazio G, et al. Development of high amylose wheat through TILLING. BMC Plant Biol. 2012;12:1–17.

77. Consortium IWGS. Shifting the limits in wheat research and breeding through a fully annotated and anchored reference genome sequence. Science (1979). 2018;361.

78. Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, et al. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol. 2014;32(9):947–51.

79. Santhoshini A, Dubey N, Avinashe HA, Thonta R, Kumar R. Inheritance Studies in Segregating Population of Bread Wheat (*Triticum aestivum* L.). International Journal of Environment and Climate Change. 2023;13(9):277-87.

80. Paudel P, Pandey MK, Subedi M, Paudel P, Kumar R. Genomic approaches for improving drought tolerance in wheat (*Triticum aestivum* L.): A Comprehensive Review. Plant Archives. 2024;24(1):1289-300.

81. Tutlani A, Kumar R, Kumari S, Chouhan S. Correlation and path analysis for yield and its phenological, physiological, morphological and biochemical traits under salinity stress in chickpea (*Cicer arietinum* L.). International Journal of Bio-resource and Stress Management. 2023;14(Jun, 6):878-90.

82. Rathore M, Yellanki P, Kumar, R, Tutlani A, Aggarwal N. Enhancing seed quality and insect management in wheat (*Triticum aestivum* L.) through optimization of storage treatments with natural and chemical compounds. Plant Archives. 2024;24(1):26-36.

83. Nisar S, Rashid Z, Touseef A, Kumar R, Nissa SU, Faheem J, Angrez A, Sabina N, Shabeena M, Tanveer A, Amal S. Productivity of fodder maize (Zea mays L.) SFM-1 under varied sowing dates and nitrogen levels. International Journal of Bio-resource and Stress Management. 2024;15(Jan, 1):01-12.

84. Aggarwal N, Rathore M, Kumar, R, Tutlani A, Kumari S and Yellanki P. Identification of Induced Chemical Mutants for Improving Yield and Its Attributing Traits in Wheat (*Triticum aestivum* L.) Against Changing Climate. Agricultural Science Digest. 2024; 1-12.

85. Vasil IK. Molecular genetic improvement of cereals: transgenic wheat (*Triticum aestivum* L.). Plant cell reports. 2007;26:1133-54.

86. Eid MH. Estimation of heritability and genetic advance of yield traits in wheat (*Triticum aestivum* L.) under drought condition. International Journal of Genetics and Molecular Biology. 2009;1(7): 115-20.

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