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


## 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 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.



***Key words**: cloning, mutation, Genetic transformation, Whole genome sequencing*




## 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 ((IWGSC) et al., 2014). With 9 billion people anticipated to live on the planet by 2050, future global food security depends on a large rise in wheat productivity (Foley et al., 2011). 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 (Qian et al., 2016). 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 (Zeng et al., 2017). 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 (Z.-K. Li & Zhang, 2013; Q. Zhang, 2007). 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 (M. Jia et al., 2018) 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.



**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.

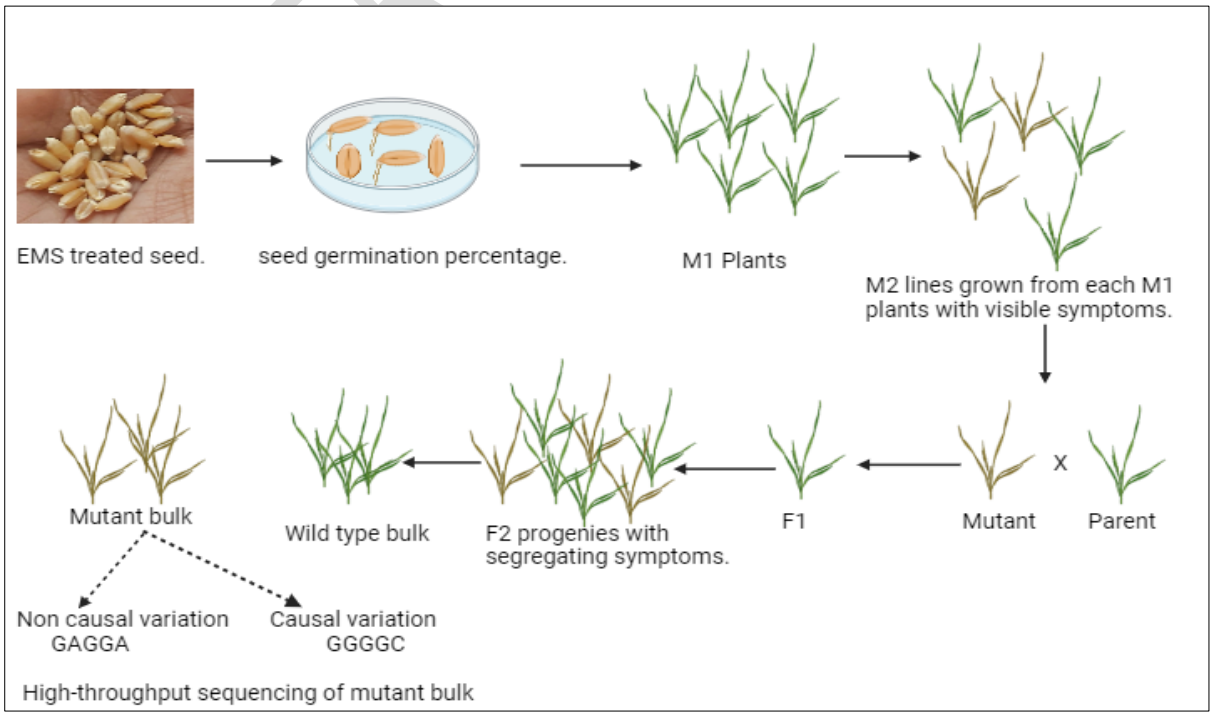
**Genetic transformation of wheat:**

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 (Xia et al., 2012). 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 microprojectiles(Vasil et al., 1992). Later, it was claimed that wheat had undergone its first Agrobacterium-mediated transformation (Cheng et al., 1997). 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) (Harwood, 2012; Shrawat & Armstrong, 2018). 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(Ishida et al., 2015). Later, more cultivars were successfully modified and this approach was implemented (Richardson et al., 2014; K. Wang et al., 2018a).

**Mutation breeding:**

Genetics is mostly driven by mutations, which are essential for understanding gene function and developing crops with improved genetics. Natural mutants that arise from the evolution of species have been significant in the fields of molecular breeding and functional genomics(Gu et al., 2005; Song et al., 1995).Research (Peng et al., 1999a) 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 (Jiang & Ramachandran, 2010).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.

**Fig I: MutMap Approach**



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. EMS is a chemical agent that is commonly used in seed mutagenesis. (Sega, 1984). High-density, randomly dispersed mutations, typically resulting in only single nucleotide alterations like guanine to adenine or cytosine to thymine, are produced by it(Uauy et al., 2017). Numerous organizations have created EMS-mutagenized populations of various wheat kinds, after which they characterized and screened the mutants of interest that surfaced (Krasileva et al., 2017; Z. Wang et al., 2017). 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 (Periyannan, 2018). 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 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 (J. Li et al., 2019; K. Wang et al., 2018b; Y. Wang et al., 2014a; Y. Zhang et al., 2016).

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.

**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.

***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 pheno-typed 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(Johal & Briggs, 1992; Keller et al., 2018). 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 (Feuillet et al., 2003a; Johal & Briggs, 1992; Song et al., 1995). 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 (Keller et al., 2018).

**Table 1: Function of the genes cloned in wheat**

| Genes | Function | Identification method | Reference |
|---|---|---|---|
| Lr21 | Leaf rust resistance | Map-based cloning | (Huang et al., 2003) |
| Lr10 | Leaf rust resistance | Map-based cloning | (Feuillet et al., 2003b) |
| Lr34 | Leaf rust resistance | Map-based cloning | (Krattinger et al., 2009a) |
| Yr36 | Stripe rust resistance | Map-based cloning | (Fu et al., 2009a) |
| Yr10 | Stripe rust resistance | Map-based cloning | (Liu et al., 2014) |
| Sr33 | Stem rust resistance | Map-based cloning | (Periyannan et al., 2013a) |
| Sr35 | Stem rust resistance | Map-based cloning | (Saintenac et al., 2013a) |
| Pm2 | Powdery mildew resistance | MutChromSeq | (Sánchez-Martín et al., 2016a) |
| Fhb1 | Fusarium head blight resistance | Map-based cloning | (G. Li et al., 2019; Su et al., 2019) |

| | | | |
|---|---|---|---|
| TaTAR2.1-3A | Boost production and nitrogen usage effectiveness | Homology-based cloning | (Shao et al., 2017a) |
| Bo1, Bo4 | Boron transporter | Map-based cloning | (Pallotta et al., 2014) |
| Gpc-B1 | Boosting iron, zinc, and kernel protein by controlling senescence. | Map-based cloning | (Uauy et al., 2006) |

***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 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 (Paux et al., 2008a).

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 (Brenchley et al., 2012a; J. Jia et al., 2013a; Z. Ling et al., 2013) 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 (Keller et al., 2018). 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 ((IWGSC) et al., 2014). In the meantime, substantially more contiguity is present in the chromosome 3B

high-quality reference sequence (Choulet et al., 2014a) than in the sequence provided by the IWGSC ((IWGSC) et al., 2014).

The A and D draft genome sequences for the whole genome shotgun approach are derived from the *T. Urartu* and *Ae. tauschii* (H.-Q. Ling et al., 2013) (J. Jia et al., 2013b). First snapshot of the bread wheat genome sequenced from *Chinese spring* for whole genome shotgun sequencing (Brenchley et al., 2012b). 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 (Xie et al., 2017). 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 (Avni et al., 2017a) (Chapman et al., 2015). 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 (Consortium, 2018a). Physical map and great quality of 3B chromosome genome sequencing of *Chinese spring* species for BAC-by-BAC and 8452 BACs in pools sequencing (Paux et al., 2008b) (Choulet et al., 2014b).

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(Avni et al., 2017b) 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 (Consortium, 2018b). 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.

***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 (Peng et al., 1999b). 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 (Abe et al., 2012). 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 (Sánchez-Martín et al., 2016b). 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 (Thind et al., 2017). 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) (Steuernagel et al., 2016). 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.

**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.

***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 (Shao et al., 2017b). 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(Ouyang et al., 2016). 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.

***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 (Marchal et al., 2018)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 durablely resistant to disease. In wheat rusts are the most common and dangerous disease, which include stripe rust, stem rust, and leaf rust. Resistance to certain rusts and mildews is provided by Lr34. 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 (Krattinger et al., 2009b). 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(Fu et al., 2009b). Recently, Yr28, an ancestral NB-LRR that gives wheat resistance to stripe rust, was successfully cloned(C. Zhang et al., 2019). 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 (Periyannan et al., 2013b; Saintenac et al., 2013b).

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(Zou et

al., 2018). Similarly, Pm21 encodes a CC-NBS-LRR protein that provides broad-spectrum resistance to powdery mildew(He et al., 2018; Xing et al., 2018). 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.

***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(Peng et al., 1999c). 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(Simmonds et al., 2016).

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 (Uauy et al., 2006). 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 (Slade et al., 2012).

**Conclusion and 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(Consortium, 2018c). 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 (Y. Wang et al., 2014b). 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.



**REFERENCE**

Abe, A., Kosugi, S., Yoshida, K., Natsume, S., Takagi, H., Kanzaki, H., Matsumura, H., Yoshida, K., Mitsuoka, C., & Tamiru, M. (2012). Genome sequencing reveals

agronomically important loci in rice using MutMap. *Nature Biotechnology*, *30*(2), 174–178.

Avni, R., Nave, M., Barad, O., Baruch, K., Twardziok, S. O., Gundlach, H., Hale, I., Mascher, M., Spannagl, M., & Wiebe, K. (2017a). Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. *Science*, *357*(6346), 93–97.

Avni, R., Nave, M., Barad, O., Baruch, K., Twardziok, S. O., Gundlach, H., Hale, I., Mascher, M., Spannagl, M., & Wiebe, K. (2017b). Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. *Science*, *357*(6346), 93–97.

Brenchley, R., Spannagl, M., Pfeifer, M., Barker, G. L. A., D'Amore, R., Allen, A. M., McKenzie, N., Kramer, M., Kerhornou, A., & Bolser, D. (2012a). Analysis of the bread wheat genome using whole-genome shotgun sequencing. *Nature*, *491*(7426), 705–710.

Brenchley, R., Spannagl, M., Pfeifer, M., Barker, G. L. A., D'Amore, R., Allen, A. M., McKenzie, N., Kramer, M., Kerhornou, A., & Bolser, D. (2012b). Analysis of the bread wheat genome using whole-genome shotgun sequencing. *Nature*, *491*(7426), 705–710.

Chapman, J. A., Mascher, M., Buluç, A., Barry, K., Georganas, E., Session, A., Strnadova, V., Jenkins, J., Sehgal, S., & Oliker, L. (2015). A whole-genome shotgun approach for assembling and anchoring the hexaploid bread wheat genome. *Genome Biology*, *16*, 1–17.

Cheng, M., Fry, J. E., Pang, S., Zhou, H., Hironaka, C. M., Duncan, D. R., Conner, T. W., & Wan, Y. (1997). Genetic transformation of wheat mediated by Agrobacterium tumefaciens. *Plant Physiology*, *115*(3), 971–980.

Choulet, F., Alberti, A., Theil, S., Glover, N., Barbe, V., Daron, J., Pingault, L., Sourdille, P., Couloux, A., & Paux, E. (2014a). Structural and functional partitioning of bread wheat chromosome 3B. *Science*, *345*(6194), 1249721.

Choulet, F., Alberti, A., Theil, S., Glover, N., Barbe, V., Daron, J., Pingault, L., Sourdille, P., Couloux, A., & Paux, E. (2014b). Structural and functional partitioning of bread wheat chromosome 3B. *Science*, *345*(6194), 1249721.

Consortium, I. W. G. S. (2018a). Shifting the limits in wheat research and breeding through a fully annotated and anchored reference genome sequence. *Science*, *361*.

Consortium, I. W. G. S. (2018b). Shifting the limits in wheat research and breeding through a fully annotated and anchored reference genome sequence. *Science*, *361*.

Consortium, I. W. G. S. (2018c). Shifting the limits in wheat research and breeding through a fully annotated and anchored reference genome sequence. *Science*, *361*.

Feuillet, C., Travella, S., Stein, N., Albar, L., Nublat, A., & Keller, B. (2003a). 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*, *100*(25), 15253–15258.

Feuillet, C., Travella, S., Stein, N., Albar, L., Nublat, A., & Keller, B. (2003b). 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*, *100*(25), 15253–15258.

Foley, J. A., Ramankutty, N., Brauman, K. A., Cassidy, E. S., Gerber, J. S., Johnston, M., Mueller, N. D., O'Connell, C., Ray, D. K., & West, P. C. (2011). Solutions for a cultivated planet. *Nature*, *478*(7369), 337–342.

Fu, D., Uauy, C., Distelfeld, A., Blechl, A., Epstein, L., Chen, X., Sela, H., Fahima, T., & Dubcovsky, J. (2009a). A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. *Science*, *323*(5919), 1357–1360.

Fu, D., Uauy, C., Distelfeld, A., Blechl, A., Epstein, L., Chen, X., Sela, H., Fahima, T., & Dubcovsky, J. (2009b). A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. *Science*, *323*(5919), 1357–1360.

Gu, K., Yang, B., Tian, D., Wu, L., Wang, D., Sreekala, C., Yang, F., Chu, Z., Wang, G.-L., & White, F. F. (2005). R gene expression induced by a type-III effector triggers disease resistance in rice. *Nature*, *435*(7045), 1122–1125.

Harwood, W. A. (2012). Advances and remaining challenges in the transformation of barley and wheat. *Journal of Experimental Botany*, *63*(5), 1791–1798.

He, H., Zhu, S., Zhao, R., Jiang, Z., Ji, Y., Ji, J., Qiu, D., Li, H., & Bie, T. (2018). Pm21, encoding a typical CC-NBS-LRR protein, confers broad-spectrum resistance to wheat powdery mildew disease. *Molecular Plant*, *11*(6), 879–882.

Huang, L., Brooks, S. A., Li, W., Fellers, J. P., Trick, H. N., & Gill, B. S. (2003). Map-based cloning of leaf rust resistance gene Lr21 from the large and polyploid genome of bread wheat. *Genetics*, *164*(2), 655–664.

Ishida, Y., Tsunashima, M., Hiei, Y., & Komari, T. (2015). Wheat (Triticum aestivum L.) transformation using immature embryos. *Agrobacterium Protocols: Volume 1*, 189–198.

(IWGSC), I. W. G. S. C., Mayer, K. F. X., Rogers, J., Doležel, J., Pozniak, C., Eversole, K., Feuillet, C., Gill, B., Friebe, B., & Lukaszewski, A. J. (2014). A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. *Science*, *345*(6194), 1251788.

Jia, J., Zhao, S., Kong, X., Li, Y., Zhao, G., He, W., Appels, R., Pfeifer, M., Tao, Y., & Zhang, X. (2013a). Aegilops tauschii draft genome sequence reveals a gene repertoire for wheat adaptation. *Nature*, *496*(7443), 91–95.

Jia, J., Zhao, S., Kong, X., Li, Y., Zhao, G., He, W., Appels, R., Pfeifer, M., Tao, Y., & Zhang, X. (2013b). Aegilops tauschii draft genome sequence reveals a gene repertoire for wheat adaptation. *Nature*, *496*(7443), 91–95.

Jia, M., Guan, J., Zhai, Z., Geng, S., Zhang, X., Mao, L., & Li, A. (2018). Wheat functional genomics in the era of next generation sequencing: an update. *The Crop Journal*, *6*(1), 7–14.

Jiang, S.-Y., & Ramachandran, S. (2010). Natural and artificial mutants as valuable resources for functional genomics and molecular breeding. *International Journal of Biological Sciences*, *6*(3), 228.

Johal, G. S., & Briggs, S. P. (1992). Reductase activity encoded by the HM1 disease resistance gene in maize. *Science*, *258*(5084), 985–987.

Keller, B., Wicker, T., & Krattinger, S. G. (2018). Advances in wheat and pathogen genomics: implications for disease control. *Annual Review of Phytopathology*, *56*, 67–87.

Krasileva, K. V, Vasquez-Gross, H. A., Howell, T., Bailey, P., Paraiso, F., Clissold, L., Simmonds, J., Ramirez-Gonzalez, R. H., Wang, X., & Borrill, P. (2017). Uncovering hidden variation in polyploid wheat. *Proceedings of the National Academy of Sciences*, *114*(6), E913–E921.

Krattinger, S. G., Lagudah, E. S., Spielmeyer, W., Singh, R. P., Huerta-Espino, J., McFadden, H., Bossolini, E., Selter, L. L., & Keller, B. (2009a). A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science*, *323*(5919), 1360–1363.

Krattinger, S. G., Lagudah, E. S., Spielmeyer, W., Singh, R. P., Huerta-Espino, J., McFadden, H., Bossolini, E., Selter, L. L., & Keller, B. (2009b). A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science*, *323*(5919), 1360–1363.

Li, G., Zhou, J., Jia, H., Gao, Z., Fan, M., Luo, Y., Zhao, P., Xue, S., Li, N., & Yuan, Y. (2019). Mutation of a histidine-rich calcium-binding-protein gene in wheat confers resistance to Fusarium head blight. *Nature Genetics*, *51*(7), 1106–1112.

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

Li, Z.-K., & Zhang, F. (2013). Rice breeding in the post-genomics era: from concept to practice. *Current Opinion in Plant Biology*, *16*(2), 261–269.

Ling, H.-Q., Zhao, S., Liu, D., Wang, J., Sun, H., Zhang, C., Fan, H., Li, D., Dong, L., & Tao, Y. (2013). Draft genome of the wheat A-genome progenitor Triticum urartu. *Nature*, *496*(7443), 87–90.

Ling, Z., Liu, X., Wang, Y., Li, L., & Xiang, C. (2013). Pyrosequencing analysis of the salivary microbiota of healthy Chinese children and adults. *Microbial Ecology*, *65*, 487–495.

Liu, W., Frick, M., Huel, R., Nykiforuk, C. L., Wang, X., Gaudet, D. A., Eudes, F., Conner, R. L., Kuzyk, A., & Chen, Q. (2014). The stripe rust resistance gene Yr10 encodes an evolutionary-conserved and unique CC–NBS–LRR sequence in wheat. *Molecular Plant*, *7*(12), 1740–1755.

Marchal, C., Zhang, J., Zhang, P., Fenwick, P., Steuernagel, B., Adamski, N. M., Boyd, L., McIntosh, R., Wulff, B. B. H., & Berry, S. (2018). BED-domain-containing immune receptors confer diverse resistance spectra to yellow rust. *Nature Plants*, *4*(9), 662–668.

Ouyang, X., Hong, X., Zhao, X., Zhang, W., He, X., Ma, W., Teng, W., & Tong, Y. (2016). Knock out of the PHOSPHATE 2 gene TaPHO2-A1 improves phosphorus uptake and grain yield under low phosphorus conditions in common wheat. *Scientific Reports*, *6*(1), 29850.

Pallotta, M., Schnurbusch, T., Hayes, J., Hay, A., Baumann, U., Paull, J., Langridge, P., & Sutton, T. (2014). Molecular basis of adaptation to high soil boron in wheat landraces and elite cultivars. *Nature*, *514*(7520), 88–91.

Paux, E., Sourdille, P., Salse, J., Saintenac, C., Choulet, F., Leroy, P., Korol, A., Michalak, M., Kianian, S., & Spielmeyer, W. (2008a). A physical map of the 1-gigabase bread wheat chromosome 3B. *Science*, *322*(5898), 101–104.

Paux, E., Sourdille, P., Salse, J., Saintenac, C., Choulet, F., Leroy, P., Korol, A., Michalak, M., Kianian, S., & Spielmeyer, W. (2008b). A physical map of the 1-gigabase bread wheat chromosome 3B. *Science*, *322*(5898), 101–104.

Peng, J., Richards, D. E., Hartley, N. M., Murphy, G. P., Devos, K. M., Flintham, J. E., Beales, J., Fish, L. J., Worland, A. J., & Pelica, F. (1999a). 'Green revolution' genes encode mutant gibberellin response modulators. *Nature*, *400*(6741), 256–261.

Peng, J., Richards, D. E., Hartley, N. M., Murphy, G. P., Devos, K. M., Flintham, J. E., Beales, J., Fish, L. J., Worland, A. J., & Pelica, F. (1999b). 'Green revolution' genes encode mutant gibberellin response modulators. *Nature*, *400*(6741), 256–261.

Peng, J., Richards, D. E., Hartley, N. M., Murphy, G. P., Devos, K. M., Flintham, J. E., Beales, J., Fish, L. J., Worland, A. J., & Pelica, F. (1999c). 'Green revolution' genes encode mutant gibberellin response modulators. *Nature*, *400*(6741), 256–261.

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

Periyannan, S., Moore, J., Ayliffe, M., Bansal, U., Wang, X., Huang, L., Deal, K., Luo, M., Kong, X., & Bariana, H. (2013a). The gene Sr33, an ortholog of barley Mla genes, encodes resistance to wheat stem rust race Ug99. *Science*, *341*(6147), 786–788.

Periyannan, S., Moore, J., Ayliffe, M., Bansal, U., Wang, X., Huang, L., Deal, K., Luo, M., Kong, X., & Bariana, H. (2013b). The gene Sr33, an ortholog of barley Mla genes, encodes resistance to wheat stem rust race Ug99. *Science*, *341*(6147), 786–788.

Qian, Q., Guo, L., Smith, S. M., & Li, J. (2016). Breeding high-yield superior quality hybrid super rice by rational design. *National Science Review*, *3*(3), 283–294.

Richardson, T., Thistleton, J., Higgins, T. J., Howitt, C., & Ayliffe, M. (2014). Efficient Agrobacterium transformation of elite wheat germplasm without selection. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *119*, 647–659.

Saintenac, C., Zhang, W., Salcedo, A., Rouse, M. N., Trick, H. N., Akhunov, E., & Dubcovsky, J. (2013a). Identification of wheat gene Sr35 that confers resistance to Ug99 stem rust race group. *Science*, *341*(6147), 783–786.

Saintenac, C., Zhang, W., Salcedo, A., Rouse, M. N., Trick, H. N., Akhunov, E., & Dubcovsky, J. (2013b). Identification of wheat gene Sr35 that confers resistance to Ug99 stem rust race group. *Science*, *341*(6147), 783–786.

Sánchez-Martín, J., Steuernagel, B., Ghosh, S., Herren, G., Hurni, S., Adamski, N., Vrána, J., Kubaláková, M., Krattinger, S. G., & Wicker, T. (2016a). Rapid gene isolation in barley and wheat by mutant chromosome sequencing. *Genome Biology*, *17*, 1–7.

Sánchez-Martín, J., Steuernagel, B., Ghosh, S., Herren, G., Hurni, S., Adamski, N., Vrána, J., Kubaláková, M., Krattinger, S. G., & Wicker, T. (2016b). Rapid gene isolation in barley and wheat by mutant chromosome sequencing. *Genome Biology*, *17*, 1–7.

Sega, G. A. (1984). A review of the genetic effects of ethyl methanesulfonate. *Mutation Research/Reviews in Genetic Toxicology*, *134*(2–3), 113–142.

Shao, A., Ma, W., Zhao, X., Hu, M., He, X., Teng, W., Li, H., & Tong, Y. (2017a). The auxin biosynthetic TRYPTOPHAN AMINOTRANSFERASE RELATED TaTAR2. 1-3A increases grain yield of wheat. *Plant Physiology*, *174*(4), 2274–2288.

Shao, A., Ma, W., Zhao, X., Hu, M., He, X., Teng, W., Li, H., & Tong, Y. (2017b). The auxin biosynthetic TRYPTOPHAN AMINOTRANSFERASE RELATED TaTAR2. 1-3A increases grain yield of wheat. *Plant Physiology*, *174*(4), 2274–2288.

Shrawat, A. K., & Armstrong, C. L. (2018). Development and application of genetic engineering for wheat improvement. *Critical Reviews in Plant Sciences*, *37*(5), 335–421.

Simmonds, J., Scott, P., Brinton, J., Mestre, T. C., Bush, M., Del Blanco, A., Dubcovsky, J., & Uauy, C. (2016). 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*, *129*, 1099–1112.

Slade, A. J., McGuire, C., Loeffler, D., Mullenberg, J., Skinner, W., Fazio, G., Holm, A., Brandt, K. M., Steine, M. N., & Goodstal, J. F. (2012). Development of high amylose wheat through TILLING. *BMC Plant Biology*, *12*, 1–17.

Song, W.-Y., Wang, G.-L., Chen, L.-L., Kim, H.-S., Pi, L.-Y., Holsten, T., Gardner, J., Wang, B., Zhai, W.-X., & Zhu, L.-H. (1995). A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. *Science*, *270*(5243), 1804–1806.

Steuernagel, B., Periyannan, S. K., Hernández-Pinzón, I., Witek, K., Rouse, M. N., Yu, G., Hatta, A., Ayliffe, M., Bariana, H., & Jones, J. D. G. (2016). Rapid cloning of disease-resistance genes in plants using mutagenesis and sequence capture. *Nature Biotechnology*, *34*(6), 652–655.

Su, Z., Bernardo, A., Tian, B., Chen, H., Wang, S., Ma, H., Cai, S., Liu, D., Zhang, D., & Li, T. (2019). A deletion mutation in TaHRC confers Fhb1 resistance to Fusarium head blight in wheat. *Nature Genetics*, *51*(7), 1099–1105.

Thind, A. K., Wicker, T., Šimková, H., Fossati, D., Moullet, O., Brabant, C., Vrána, J., Doležel, J., & Krattinger, S. G. (2017). Rapid cloning of genes in hexaploid wheat using cultivar-specific long-range chromosome assembly. *Nature Biotechnology*, *35*(8), 793–796.

Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., & Dubcovsky, J. (2006). A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science*, *314*(5803), 1298–1301.

Uauy, C., Wulff, B. B. H., & Dubcovsky, J. (2017). Combining traditional mutagenesis with new high-throughput sequencing and genome editing to reveal hidden variation in polyploid wheat. *Annual Review of Genetics*, *51*, 435–454.

Vasil, V., Castillo, A. M., Fromm, M. E., & Vasil, I. K. (1992). Herbicide resistant fertile transgenic wheat plants obtained by microprojectile bombardment of regenerable embryogenic callus. *Bio/Technology*, *10*(6), 667–674.

Wang, K., Riaz, B., & Ye, X. (2018a). Wheat genome editing expedited by efficient transformation techniques: progress and perspectives. *The Crop Journal*, *6*(1), 22–31.

Wang, K., Riaz, B., & Ye, X. (2018b). Wheat genome editing expedited by efficient transformation techniques: progress and perspectives. *The Crop Journal*, *6*(1), 22–31.

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., & Qiu, J.-L. (2014a). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnology*, *32*(9), 947–951.

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., & Qiu, J.-L. (2014b). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnology*, *32*(9), 947–951.

Wang, Z., Li, J., Chen, S., Heng, Y., Chen, Z., Yang, J., Zhou, K., Pei, J., He, H., & Deng, X. W. (2017). Poaceae-specific MS1 encodes a phospholipid-binding protein for male fertility in bread wheat. *Proceedings of the National Academy of Sciences*, *114*(47), 12614–12619.

Xia, L., Ma, Y., He, Y., & Jones, H. D. (2012). GM wheat development in China: current status and challenges to commercialization. *Journal of Experimental Botany*, *63*(5), 1785–1790.

Xie, J., Huo, N., Zhou, S., Wang, Y., Guo, G., Deal, K. R., Ouyang, S., Liang, Y., Wang, Z., & Xiao, L. (2017). Sequencing and comparative analyses of Aegilops tauschii chromosome arm 3DS reveal rapid evolution of Triticeae genomes. *Journal of Genetics and Genomics*, *44*(1), 51–61.

Xing, L., Hu, P., Liu, J., Witek, K., Zhou, S., Xu, J., Zhou, W., Gao, L., Huang, Z., & Zhang, R. (2018). Pm21 from Haynaldia villosa encodes a CC-NBS-LRR protein conferring powdery mildew resistance in wheat. *Molecular Plant*, *11*(6), 874–878.

Zeng, D., Tian, Z., Rao, Y., Dong, G., Yang, Y., Huang, L., Leng, Y., Xu, J., Sun, C., & Zhang, G. (2017). Rational design of high-yield and superior-quality rice. *Nature Plants*, *3*(4), 1–5.

Zhang, C., Huang, L., Zhang, H., Hao, Q., Lyu, B., Wang, M., Epstein, L., Liu, M., Kou, C., & Qi, J. (2019). An ancestral NB-LRR with duplicated 3′ UTRs confers stripe rust resistance in wheat and barley. *Nature Communications*, *10*(1), 4023.

Zhang, Q. (2007). Strategies for developing green super rice. *Proceedings of the National Academy of Sciences*, *104*(42), 16402–16409.

Zhang, Y., Liang, Z., Zong, Y., Wang, Y., Liu, J., Chen, K., Qiu, J.-L., & Gao, C. (2016). Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. *Nature Communications*, *7*(1), 12617.

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