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Current strategies and advances in wheat biology☆

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ABSTRACT

The characterization of agronomically important genes has great potential for the improvement of wheat. However, progress in wheat genetics and functional genomics has been impeded by the high complexity and enormous size of the wheat genome. Recent advances in genome sequencing and sequence assembly have produced a high-quality genome sequence for wheat. Here, we suggest that the strategies used to characterize biological mechanisms in model species, including mutant preparation and characterization, gene cloning methods, and improved transgenic technology, can be applied to wheat biology. These strategies will accelerate progress in wheat biology and promote wheat breeding program development. We also outline recent advances in wheat functional genomics. Finally, we discuss the future of wheat functional genomics and the rational design-based molecular breeding of new wheat varieties to contribute to world food security.

Contents

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1. Introduction

Wheat (*Triticum aestivum* L.) is an important staple food crop worldwide; it is cultivated in very different environments and serves as a food source for 30% of the human population [1]. It is estimated that the world's population will reach 9 billion by 2050; thus, a substantial increase in wheat yield is needed to ensure future global food security [2]. The grain quality of wheat also requires improvement; however, conventional breeding approaches have reached their limit in terms of increasing grain quality and yield through the enhancement of current wheat varieties. Therefore, a new breeding strategy for wheat is needed.

Rational design-based molecular breeding takes advantage of the genetic basis of agronomically important traits; thus, it is predicted to increase selection accuracy and shorten breeding times [3]. Indeed, scientists have successfully used this strategy to develop super-rice lines with high yields and superior-quality grains in less than five years, which is significantly shorter than the time that would be required using traditional breeding methods [4]. Thus, rational design-based molecular breeding strategies hold great promise for crop breeding.

The success of rice breeding using molecular design is mainly due to advances in our understanding of the genetic basis of important agronomic traits; this has enabled scientists to create different trait combinations and produce numerous elite lines [5,6]. To utilize this strategy to create desirable new wheat cultivars, the most important step is to identify genes that control important agronomic traits. Much effort has been made to uncover the genetic basis for important traits in allohexaploid wheat; however, due to its complex and monstrously large genome, our ability to conduct functional genomic analyses in wheat is limited compared to that in rice or maize. Nevertheless, given recent progress in genome sequencing and assembly technologies, draft and near-complete genome assemblies of wheat have been generated [7] that will be useful for the functional annotation of the wheat genome.

In this review, we propose that the strategies used for functional genomic analysis in other model organisms should be adopted by wheat researchers. These strategies include revealing the biological function of genes by characterizing their corresponding mutants, identifying the gene responsible for an interesting phenotype by gene cloning, and verifying the function of a target gene by transforming the candidate wild-type gene into the corresponding wheat mutant. Additionally, gene cloning will enable the characterization of the molecular mechanisms in wheat, and the cloned genes will be used for rational design-based molecular breeding of wheat varieties. We also summarize recent progress in determining the sequence of the wheat genome and in functional genetic studies of wheat.

2. Strategies used in wheat biology

Modern biology has developed rapidly by following a strategy of mutant preparation and characterization, cloning of the gene responsible for a mutant phenotype, and transformation to confirm the identified candidate gene. However, due to the enormous size and complexity of the wheat genome and difficulties with the genetic transformation of wheat, certain strategies used in other model organisms have not been widely applied to wheat research. Consequently, our understanding of wheat biology is limited. Still, given recent advances in genome sequencing and assembly, as well as improved methods for gene cloning and transformation in wheat, it is time for wheat biologists to utilize the same research tools as their colleagues in other fields.

2.1. *Mutagenesis, mutant preparation, and characterization*

Mutants are the major driving force in genetics; they are critical for the analysis of gene function and the genetic improvement of crops. As species evolve, natural mutants are generated; such mutants have played an important role in functional genomics and molecular breeding [8,9]. For example, the global wheat yield has been greatly improved by the adoption of a natural mutant of the 'green revolution' gene [10]. However, the probability of natural variation is extremely low at 10^{-5} to 10^{-8} events per base pair per generation in higher plants [11]. Therefore, various strategies for mutagenesis, including chemical mutagenesis (e.g., using ethyl methanesulfonate [EMS] or sodium azide) and physical mutagenesis (e.g., using fast neutron or gamma radiation), have been utilized to generate random mutations on a genome-wide scale in a variety of plant species. The resulting populations are then screened for mutants with an interesting phenotype, and the gene thought to be responsible for this phenotype is revealed by mutant characterization. Notably, these mutants are also valuable resources for crop improvement.

EMS is a commonly used chemical agent for seed mutagenesis [12]. The mutations it creates are high in density and randomly distributed in the genome, usually as single nucleotide changes (G to A or C to T) [13]. Several groups have generated EMS-mutagenized populations of different wheat varieties and screened and characterized the resulting mutants of interest [14,15]. EMS-based mutagenesis offers several advantages to researchers, including high-efficiency, large-scale, irreversible, and non-transgenic mutagenesis.

When seeds are bombarded by ionizing particles such as fast neutrons or gamma rays, their DNA is damaged by strand breaks. Seeds sent to space can also be mutated by cosmic radiation. The patterns observed in such mutants include large DNA sequence deletions and chromosomal rearrangements of different sizes, ranging from one base pair to millions of base pairs [16]. This approach has produced new plant varieties with improved properties, but it is difficult to locate the mutations in the genome.

These methods of mutagenesis have proven to be effective at generating mutants that can be used to characterize the relationship between genotype and phenotype. However, the mutagenesis is random; therefore, large-scale screens for the intended mutants are necessary. In addition, common wheat, as an allohexaploid plant, has three copies of most genes in its genome. This results in functional redundancy among the homologous genes from the three subgenomes such that no phenotype may be observed in response to the mutation of a single homolog. However, the recent development of genome editing technologies, especially clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems, has enabled the production of targeted mutations in wheat [17–20]. Such approaches will play an important role in uncovering the functions of wheat genes. However, the application of CRISPR/Cas systems relies on sequence information about the targeted gene. Therefore, random mutagenesis and CRISPR/Cas-induced targeted mutations should be considered complementary approaches for wheat mutant production.

2.2. Gene cloning methods

After characterizing the phenotype of an interesting mutant, the next step is to isolate the affected gene responsible for the observed phenotype. As genomic sequence information is the basis for gene cloning, we will first provide a summary of recent advances in wheat genome sequencing (Table 1) before introducing the methods used for gene cloning.

2.2.1. Progress in wheat genome sequencing

High-quality genomic sequences and assemblies are beneficial to basic research, including gene cloning, and genetic improvement in wheat. Therefore, enormous effort has been made to study the wheat genome, and great progress has been achieved in the last few years. Due to the large genome size of bread wheat, sequencing and mapping the expressed portion of the genome is a priority for gene discovery.

In 2005, the International Wheat Genome Sequencing Consortium (IWGSC), with 2400 members from 68 countries, was established to obtain a high-quality genome sequence of hexaploid bread wheat. Because of the complexity and high similarity among the three subgenomes (A, B, and D; $2n = 6x = 42$), the 21 chromosomes of wheat (Chinese Spring) were separated by flow cytometry; bacterial artificial chromosome (BAC) libraries were then constructed for each chromosome or chromosome arm, and the construction of physical maps and sequencing of the BACs were undertaken by IWGSC members [21]. A physical map of 3B, the largest chromosome in wheat, was generated using a chromosome-based BAC-by-BAC sequencing strategy [22]. Later, the sequences of the chromosomes (or parts of the chromosomes) were released.

Table 1 – Recent advances in wheat genome sequencing.

Sequencing species	Description	Sequencing strategy	Reference
Chinese Spring	Physical map of chromosome 3B	Chromosome-based BAC-by-BAC sequencing	Paux et al. [22]
Chinese Spring	First snapshot of the bread wheat genome	Whole-genome shotgun sequencing and multiple assemblies of the whole genome	Brenchley et al. [23]
Triticum urartu	Draft genome sequence of the A genome	Whole-genome shotgun strategy	Ling et al. [24]
Aegilops tauschii	Draft genome sequence of the D genome	Whole-genome shotgun strategy	Jia et al. [25]
Chinese Spring	An ordered draft sequence of the bread wheat genome	Chromosome-based sequencing and shotgun assembly	IWGSC [1]
Chinese Spring	A high-quality genome sequence of chromosome 3B	Sequencing of 8452 BACs in pools and construction of a pseudomolecule	Choulet et al. [28]
Chinese Spring	9.1 gigabases of the highly repetitive genome of hexaploid wheat	Short-read sequencing technology and whole-genome shotgun assembly	Chapman et al. [26]
Triticum turgidum	10.1-gigabase assembly of the 14 chromosomes of wild tetraploid wheat	Sequencing of several whole-genome shotgun libraries	Avni et al. [29]
Chinese Spring	An improved assembly and annotation of bread wheat	Combination of optimized data types and improved assembly algorithms	Clavijo et al. [30]
Aegilops tauschii	High-quality sequence of the short arm of chromosome 3 (At3DS)	Combined approach of BAC pooling and next-generation sequencing	Xie et al. [31]
Chinese Spring	An annotated assembly (RefSeq v1.0) of the 21 chromosomes of bread wheat	Whole-genome sequencing by short-read sequencing-by-synthesis	IWGSC [32]

Thanks to these technological advances in sequencing and sequence assembly, complete genomic sequences and assemblies of hexaploid bread wheat (Chinese Spring) and its diploid ancestors (*Triticum urartu* and *Aegilops tauschii*) have been reported [23–25]. They have provided a snapshot of the wheat genome and its gene loci; however, they are highly fragmented and contain a large number of unordered scaffolds [27]. Using chromosome-based sequencing and shotgun assembly, an ordered draft sequence of bread wheat was prepared; most genes were evenly distributed along the homeologous chromosomes, and highly similar and conserved gene copies were retained in each subgenome [1]. Meanwhile, a high-quality reference sequence of chromosome 3B [28] was published that features much more contiguity than the sequence reported by the IWGSC [1].

Several technological and conceptual achievements in genome sequencing and assembly have vastly improved our understanding of the wheat genome. For example, a high-quality genome sequence of wild emmer (*Triticum turgidum*) was obtained [29] and validated using genetic and Hi-C data. Also, an ordered and annotated genome sequence of bread wheat (IWGSC RefSeq v1.0) was released. The quality and contiguity of IWGSC RefSeq v1.0, assessed through alignments with radiation hybrid maps of the subgenomes and independent data from coding and noncoding sequences, exceed those of previous sequences [32]. Very recently, IWGSC RefSeq v2.0 (i.e., version 2 of the reference sequence of wheat) has become available (https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies). Apart from these draft and near-complete genome sequences of wheat, major advances in the isolation and characterization of genes endowing important agronomic traits for the molecular breeding of wheat are in the pipeline.

2.2.2. Traditional gene cloning methods

In the pre-reference genome era, only genetic information was available for a great number of genes responsible for agronomically important traits in crops. Map-based cloning, or positional cloning, is a useful method to clone genes of interest. The creation of high-resolution mapping populations, genotyped with molecular markers and phenotyped by the trait of interest, is a prerequisite. Using this strategy, one can gradually narrow down the location of a mutated gene by utilizing mapping populations consisting of a few hundred or thousand plants generated through genetic recombination (Fig. 1) [27,33]. By combining the phenotypic and molecular marker data, a genetic map covering the target locus is produced. Then, the corresponding physical map is pieced together by chromosome walking, in which newly developed closest flanking markers are used to screen a BAC library and subclones of the identified BACs [16]. A contig spanning the target locus is established, and the candidate gene is identified and verified by sequencing [34].

Map-based cloning even works when no information about the gene product is available. It has been successfully used to clone a great number of genes, including *Xa21*, *HM1*, and *Lr10* from rice, maize, and wheat, respectively [8,35,36]. Notably, nearly half of these genes are disease resistance genes (Table 2). However, map-based cloning is laborious and time-consuming. Moreover, when the genes are located in centromeric regions, it is impossible to clone them using a map-based strategy [27].

2.2.3. Genetic mapping based on sequencing

With the rapid advances in sequencing and bioinformatic technologies, innovative and accelerated gene cloning strategies, including homology-based cloning, mutational mapping (MutMap), mutant chromosome sequencing (MutChromSeq), targeted chromosome-based cloning via long-range assembly (TACCA), and mutagenesis resistance gene enrichment and sequencing (MutRenSeq), have been created to overcome the limitations of traditional gene cloning methods.

Given that genes are evolutionarily conserved, orthologous genes in different species may have similar sequences and conserved functions. Homology-based cloning, which relies on sequence similarities between known and target genes, has been used successfully to isolate wheat genes. For example, the wheat *rht-1* mutant exhibits a short stature, impaired responsiveness to gibberellin, and increased gibberellin levels *in planta*, similar to maize *d8* and *Arabidopsis gai*; based on these similarities, the DNA sequence of *Rht-D1* in wheat was obtained [10]. However, when no relevant sequence information is available in other species, it is impossible to clone a target gene using this method.

MutMap is a rapid gene isolation method based on mutagenesis, gene mapping, and whole-genome resequencing. In MutMap, a plant with an interesting phenotype is selected, crossed to its wild-type parent, and then selfed. The F_2 progeny will exhibit mutant and wild-type phenotypes. Bulked DNAs from F_2 progeny displaying the mutant phenotype are subjected to whole-genome sequencing, and the sequences are aligned to the reference genome. Single nucleotide polymorphisms (SNPs) showing an SNP index of 1 are thought to harbor the gene responsible for the mutant phenotype [85]. This approach has been used to identify the genomic positions of genes controlling agronomically important traits in rice. For species with a relatively small genome and a high-quality reference genome, MutMap is a good choice for isolating mutated genes.

In comparison, MutChromSeq is a fast method for gene cloning from large genomes. It combines mutagenesis, the reduction of genome complexity by chromosome sorting, and high-throughput sequencing. A candidate gene with a clear phenotype is identified through sequencing and a comparison of wild-type and mutant chromosomes. This method, which does not require positional fine mapping of genes, was used to clone the barley *Eceriferum-q* gene and wheat *Pm2* gene [64]. When the requirements of the method (i.e., mutagenesis, the identification of target genes conferring strong phenotypes, and the isolation of individual chromosomes) are met, it is possible to clone previously intractable genes.

TACCA enables rapid gene cloning from complex polyploid genomes through the use of flow-sorted chromosomes to reduce genome complexity, next-generation DNA sequencing, and cultivar-specific de novo assembly. Using this method, the wheat leaf rust resistance gene *Lr22a* was isolated from bread wheat [67]. When chromosome isolation is feasible and a preliminary gene map is available, TACCA is a good option for gene isolation.

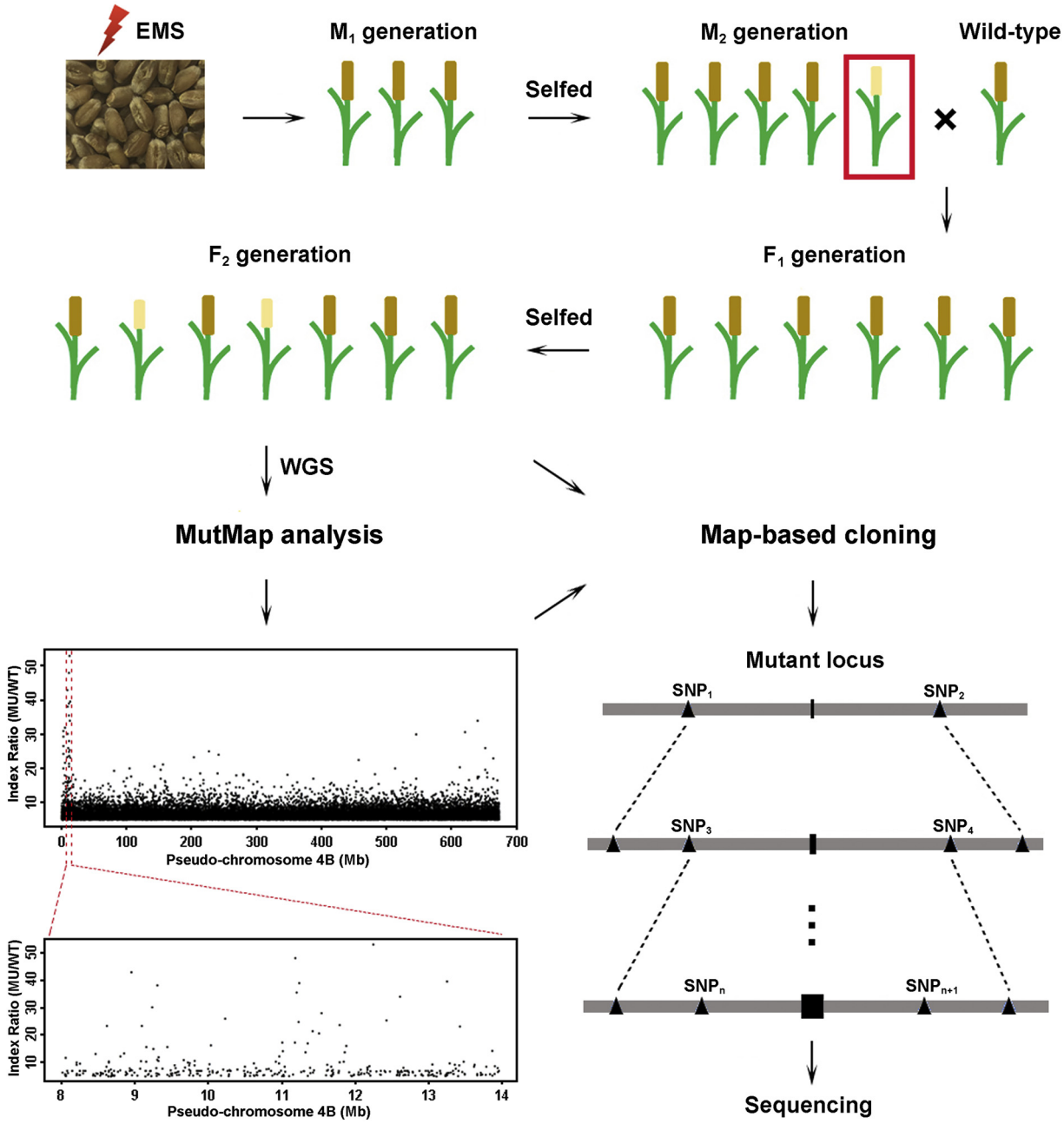

Fig. 1 – Overview of the isolation of mutated genes from a complex genome by MutMap-based cloning. Seeds of a cultivar are mutagenized by EMS, resulting in M_1 plants, which are selfed to generate a large number of M_2 seeds. An M_2 plant with an interesting phenotype is selected and crossed to its wild-type parent. In the F_2 generation, plants exhibiting mutant and wild-type phenotypes are segregated. Bulked DNA from F_2 plants displaying interesting phenotypes and wild-type plants is then subjected to whole-genome sequencing (WGS). SNPs between the wild-type and mutant plants are identified through MutMap analysis; these SNPs are used as markers for traditional map-based cloning to narrow down the region of interest. After one to several rounds of mapping, the target gene is pinned down to a small interval.

MutRenSeq is a rapid gene cloning method for the isolation of nucleotide binding and leucine-rich repeats (NLR) genes. It involves chemical mutagenesis, exome capture, and sequencing. Since most resistance genes encode proteins with NLRs, exome capture is used to enrich the NLR-specific bait library and sequencing of the resistant wild-type parent and susceptible loss-of-function mutants is performed. The mutant reads are aligned with those from the parent, allowing the identification of genes responsible for the resistance. This method, which was used to clone two fungal stem rust resistance genes (*Sr22* and *Sr45*) from bread wheat [61], does not require positional fine mapping and can be applied to isolate NLR-type resistance genes from most crops and their wild relatives.

Table 2 – Some of the genes cloned and their functions in wheat.

Genes	Identification method	Function(s)	Reference
Rht-1	Homology-based cloning	Short and increased grain yield	Peng et al. [10]
Q	Map-based cloning	Influencing threshing and spike phenotypes	Faris et al. [37]
Lr21	Map-based cloning	Leaf rust resistance	Huang et al. [38]
Lr10	Map-based cloning	Leaf rust resistance	Feuillet et al. [36]
VRN1	Map-based cloning	Vernalization, controlling flowering	Yan et al. [39]
Pm3b	Map-based cloning	Powdery mildew resistance	Yahiaoui et al. [40]
VRN2	Map-based cloning	A flowering repressor down-regulated by vernalization	Yan et al. [41]
VRN3	Map-based cloning	Vernalization, an ortholog of *FT*	Yan et al. [42]
Gpc-B1	Map-based cloning	Regulating senescence and increasing grain protein, zinc, and iron	Uauy et al. [43]
Ph1	Map-based cloning	Controlling homologous chromosome pairing	Griffiths et al. [44]
Lr1	Map-based cloning	Leaf rust resistance	Cloutier et al. [45], Qiu et al. [46]
Lr34	Map-based cloning	Leaf rust and powdery mildew resistance	Krattinger et al. [47]
Yr36/WKS1	Map-based cloning	Stripe rust resistance	Fu et al. [48]
Tsn1	Map-based cloning	Stagonospora nodorum blotch, tan spot resistance	Faris et al. [49]
TmMla1	Homology-based cloning	Powdery mildew resistance	Jordan et al. [50]
Pm8	Homology-based cloning	Powdery mildew resistance	Hurni et al. [51]
Sr33	Map-based cloning	Stem rust resistance	Periyannan et al. [52]
Sr35	Map-based cloning	Stem rust resistance	Saintenac et al. [53]
PHS1	Map-based cloning	Preharvest sprouting resistance	Liu et al. [54]
VRN-D4	Map-based cloning	Vernalization	Kippes et al. [55]
Bo1, Bo4	Map-based cloning	Boron transporter	Pallotta et al. [56]
Yr10	Map-based cloning	Stripe rust resistance	Liu et al. [57]
Sr50	Map-based cloning	Stem rust resistance	Mago et al. [58]
Lr67	Map-based cloning	Rust and powdery mildew resistance	Moore et al. [59]
Snn1	Map-based cloning	Septoria nodorum blotch resistance	Shi et al. [60]
Sr22, Sr45	MutRenSeq	Stem rust resistance	Steuernagel et al. [61]
Fhb1	Map-based cloning	Fusarium head blight resistance	Su et al. [62]; Li et al. [63]
Pm2	MutChromSeq	Powdery mildew resistance	Sanchez-Martin et al. [64]
Phs-A1	Map-based cloning	Seed dormancy	Torada et al. [65]
TaEDR1	Homology-based cloning	Powdery mildew resistance	Zhang et al. [66]
Lr22a	TACCA	Leaf rust resistance	Thind et al. [67]
Sr13	Map-based cloning	Stem rust resistance	Zhang et al. [68]
TaVIT2	Homology-based cloning	Iron transport	Connorton et al. [69]
TtBtr1	Sequencing	Controlling shattering, a key domestication trait	Avni et al. [29]
TaTAR2.1-3A	Homology-based cloning	Improve yield and N use efficiency	Shao et al. [70]
AVR-Rmg8	Map-based cloning	Blast resistance	Anh et al. [71]
Ms2	Map-based cloning	Male fertility	Ni et al. [72]; Xia et al. [73]
Ms1	Map-based cloning; MutMap-based cloning	Male fertility	Tucker et al. [74]; Wang et al. [15]
Stb6	Map-based cloning	Septoria tritici blotch resistance	Saintenac et al. [75]
Pm60	Map-based cloning	Powdery mildew resistance	Zou et al. [76]
Pm21	Map-based cloning	Powdery mildew resistance	Xing et al. [77]; He et al. [78]
Sr21	Map-based cloning	Stem rust resistance	Chen et al. [79]
Yr7, *Yr5*, *YrSP*	MutRenSeq	Stripe rust resistance	Marchal et al. [80]
Yr15	Map-based cloning	Stripe rust resistance	Klymiuk et al. [81]
Ms5	Map-based cloning	Male fertility	Pallotta et al. [82]
Yr28	Map-based cloning	Stripe rust resistance	Zhang et al. [83]
Sr60	Map-based cloning	Stem rust resistance	Chen et al. [84]

2.2.4. *MutMap-based cloning*

Map-based cloning can be used to clone any gene; it works even when no information about the gene product is available. However, it is laborious, time-consuming, and requires a large number of markers to narrow down the chromosomal location of the target gene. Thus, occasionally it is impossible to isolate a gene using map-based cloning due to a lack of molecular markers. Considering that map-based cloning is sometimes unusable and MutMap is only suitable for organisms with small genomes, we developed MutMap-based cloning, a rapid gene cloning method for use with complex genomes (Fig. 1). It combines MutMap with traditional map-based cloning [15].

MutMap-based cloning consists of EMS mutagenesis, genome sequencing, and mapping. Initially, SNPs are identified between wild-type plants and homozygous mutants (segregated from the progeny of heterozygous mutants) and the chromosomal region in which the target gene is located is

determined by MutMap. Second, the SNPs identified by MutMap are used as markers to narrow down the region of interest. Eventually, the target gene is pinned down to a small interval by traditional map-based cloning using these SNP markers. Using MutMap-based cloning, we successfully mapped *Ms1* to a 198-kb interval and cloned *Ms1* from allohexaploid bread wheat using 676 F_2 plants derived from the progeny of heterozygous *ms1e* mutants [15].

It is estimated that the SNPs generated by EMS mutagenesis are evenly distributed throughout the genome and that the markers generated by MutMap analysis are sufficient for mapping (usually 3–5 SNPs per 1 Mb). Therefore, the required mapping population is smaller than that in traditional map-based cloning. Furthermore, the mapping population is built from the progeny of heterozygous mutants. This saves one to several generations of wheat breeding time, and it simplifies the genetic background (compared to crossing with different varieties); therefore, it shortens the experimental time and overcomes potential problems due to genetic complexity arising from crosses between varieties. MutMap-based cloning can be used to clone previously intractable genes from any organism (especially organisms with complex genomes), including wheat, which lacks a sufficient marker density to allow the region containing a gene of interest to be narrowed down.

The above-mentioned gene cloning methods exhibit distinct advantages and disadvantages, and each method works in at least some cases (Table 2). However, in general, those methods that take greater advantage of recent advances in genome sequencing have greater potential for high-efficiency gene cloning.

2.3. Genetic transformation of wheat

Following the isolation of a candidate gene from a mutant, the gene should be confirmed to be responsible for the observed phenotype by transforming the corresponding wild-type gene into mutant plants. Biolistic transformation and *Agrobacterium*-mediated genetic transformation are the two most commonly used methods to stably introduce foreign genes of interest into plant cells [86]. The ability to transform plants successfully is a powerful advantage in studies of gene function.

Wheat, however, is difficult to transform and regenerate, so progress in genetic engineering, basic research, and applied genetics has lagged behind that in other plants. After significant effort, however, the first fertile transgenic wheat line was obtained by the microprojectile bombardment of an embryogenic callus [87]. Later, the first *Agrobacterium*-mediated transformation of wheat was reported [88]. However, the efficiency of the process was very low. Since then, significant progress has been made to improve the efficiency of wheat transformation (e.g., better DNA delivery methods, a broader choice of target tissues, improved culture media components, and new selectable marker genes) [89,90].

Transformation by biolistic bombardment is preferred over *Agrobacterium*-based methods, mainly because success is less genotype-dependent and the efficiency is higher (approximately 10%). However, the procedure is complex, with multiple copies of the transgene in the host genome and occasional integration of the vector backbone into the genome. In contrast, *Agrobacterium*-mediated transformation is simple to perform and is cost-effective; moreover, it produces a low copy number, intact integration of foreign genes into the host genome, and stable transgene expression. Recently, the Japan Tobacco Company developed an efficient protocol in which transgenic wheat plants could be obtained from immature embryos of the cultivar Fielder by *Agrobacterium*-mediated transformation with high efficiency [91]. This protocol was later successfully applied with modifications to additional cultivars [20,92].

The strategies mentioned above have been used to characterize several model species, and they are being applied to wheat functional genomics and wheat biology. Given the many experimental methodologies available, it is time for the scientists who study wheat to use these strategies in their research. This will accelerate progress in wheat functional genomics and wheat biology and promote the development of new alleles for wheat breeding.

3. Advances in wheat functional genomics

Elucidating the functions of genes responsible for important agronomic traits is the most pressing issue in the postgenomic era. However, due to the complex, large genome of bread wheat, progress in wheat biology has fallen behind that in other crops. Still, significant progress in wheat functional genomics has been made recently. Indeed, a growing number of genes controlling, but not limited to, vernalization and flowering, disease resistance, yield and quality improvements, male sterility, and nutrient uptake efficiency have been cloned and elucidated, making rational design-based molecular breeding in wheat possible. Here, we provide a brief introduction to recent achievements in wheat functional genomics (Table 2).

3.1. Vernalization and flowering

Winter wheat requires extended exposure to cold temperatures to induce flowering. This biological process, called vernalization, ensures that wheat plants will not flower before winter. The winter growth habit of winter wheat is determined by both VRN2, which represses flowering, and VRN1, which promotes flowering after vernalization [39,41].

VRN2 is a dominant repressor of flowering. After vernalization, the expression of *VRN2* is down-regulated, releasing the expression of *VRN3*, an ortholog of *Arabidopsis FLOWERING LOCUS T* (FT), which encodes a protein that is transferred from leaves to the shoot apical meristem [42]. FT participates in the floral activation complex, which induces the expression of *VRN1* and promotes the transition of the apical meristem from the vegetative stage to the reproductive stage [42]. Recently, VRN-D4 was identified in the ancient wheat from South Asia, and mutations in *VRN-D4* were shown to cause delayed flowering [55].

For proper flowering induction, *VRN1* mRNA must accumulate in response to prolonged cold exposure. A novel mechanism for VRN1 induction during vernalization was recently revealed. Under non-vernalization conditions, GRP2 binds to *VRN1* pre-mRNA to repress its accumulation.

Vernalization induces *VER2* expression and elevates the level of O-GlcNAcylated GRP2 (GRP2-G). Next, phosphorylated VER2 interacts with GRP2-G, releasing the repressive effect of GRP2 on *VRN1* and resulting in *VRN1* mRNA accumulation [93]. Moreover, tae-miR408-mediated expression of *TIMING OF CAB EXPRESSION* is needed for the regulation of heading time [94].

3.2. Disease resistance

Fungus-induced diseases, including rusts, powdery mildew, head blight/scab, and septoria tritici blotch (STB), reduce wheat yields around 25% per annum [80] and could threaten world food security. Enormous effort has been made to identify genes responsible for disease resistance in order to breed durable and broadly disease-resistant wheat varieties.

Rusts, including leaf rust, stripe rust, and stem rust, are the most widespread and devastating diseases in wheat. *Lr34* confers resistance to multiple rusts and a mildew. Although it has been used for wheat improvement since the 20th century, it wasn't until 2009 that the *Lr34* gene, encoding an ATP-binding cassette transporter, was cloned. The LR34 transporter functions in adult plants; similar to the pleiotropic drug resistance protein PEN3 in *Arabidopsis*, it confers resistance to pathogens by exporting metabolites to affect fungal growth [47]. *Yr36*, which provides broad resistance to stripe rusts, was also cloned. It contains a functional enzymatic (kinase) domain and a putative START domain, both of which are necessary for disease resistance [48]. Very recently, *Yr28*, which is an ancestral NB-LRR and confers stripe rust resistance in wheat, was cloned [83]. Further, two genes (*Sr33* and *Sr35*) conferring resistance to wheat stem rust race Ug99 have been cloned from *A. tauschii* and *Triticum monococcum*, respectively [52,53].

Powdery mildew, a destructive disease caused by *Blumeria graminis*, can reduce wheat yields dramatically. To date, 78 powdery mildew resistance alleles and 50 loci have been identified and assigned designations, respectively. However, only a few genes have been molecularly and functionally characterized. For instance, *Pm21* encodes a CC-NBS-LRR protein that confers broad-spectrum resistance to powdery mildew [77,78], while *Pm60*, cloned from *T. urartu*, was recently found to encode an NB-LRR protein that plays a major role in Bgt E09 resistance [76].

STB caused by *Zymoseptoria tritici* could result in economic losses worldwide. There are 21 *Stb* resistance genes in wheat. However, only one gene, *Stb6*, has been cloned and characterized. It encodes a conserved wall-associated receptor-like kinase that confers resistance to the pathogen without a hypersensitive response [75].

3.3. Wheat yield- and quality-related genes

As the global population continues to increase and living standards improve, it will be essential to identify and characterize genes regulating grain yield and quality to promote the rational design-based molecular breeding of new wheat varieties.

The stems of tall wheat are not strong enough to support heavy grains (they fall over). The introduction of dwarfing or semi-dwarfing genes into wheat is thus crucial to increase world wheat yields. The *Rht* gene of wheat has been isolated and characterized. It encodes a transcription factor containing an SH2-like domain that participates in gibberellin signaling [10]. New varieties carrying mutated versions of *Rht* (and which respond abnormally to gibberellin) are short and exhibit increased grain yields; such plants have enabled the Green Revolution. Recently, the mutant allele *gw2-A1* was characterized; it increases the thousand grain weight of wheat by producing wider and longer grains [95].

Wheat grain quality is affected by many traits, including starch composition, nutritional value, and end-use, which are determined by numerous genes. However, only a few relevant genes have been cloned. For example, the NAC gene *Gpc-B1* was mapped to chromosome arm 6BS and cloned. It encodes a transcription factor that regulates senescence and is responsible for increasing nutrient remobilization, which in turn improves the protein, zinc, and iron contents of wheat grains [43]. Wheat lines carrying mutations in *starch branching enzyme II* genes have elevated levels of amylose and resistant starch, which are beneficial for human health [96].

3.4. Male sterility

Male sterility is a valuable agricultural trait for hybrid seed production. Although five genic male sterility (*Ms*) loci have been identified in wheat, only *Ms1*, *Ms2*, and *Ms5* have been cloned and characterized.

Ms1 is a recessive gene cloned by two independent groups. Only *Ms1* from the B subgenome is expressed; its homologs from the A and D subgenomes are epigenetically silenced. *Ms1*, which is specifically expressed in microsporocytes, encodes a phospholipid-binding protein that is necessary for pollen exine development and male fertility [15,74].

The *ms2* mutant is male sterile but lacks other unfavorable agronomic phenotypes; thus, it has been extensively used to breed numerous wheat cultivars in China. In 2017, the molecular characterization of *Ms2* was reported. A TRIM insertion gives rise to the activation of *Ms2*, which encodes an orphan protein. It is specifically expressed in the anthers of *ms2* plants, where it confers dominant male sterility [72,73]. *Ms5* has also been cloned. It is a recessive gene expressed from subgenomes A and D that encodes a glycosylphosphatidylinositol-anchored lipid transfer protein necessary for pollen exine development and male fertility [82].

3.5. Nutrient uptake in wheat

Fertilizers containing nitrogen (N) and phosphorus (P) contribute to improved crop yields, including wheat yields. However, the widespread overuse of fertilizers has reduced the nutrient use efficiency of wheat plants and created adverse environmental effects. Therefore, it is important to identify genes regulating N and P use in wheat in order to understand nutrient uptake and further improve use efficiency.

N and P use efficiency is complex and is influenced by diverse physiological traits. Only a few genes, including *NAC* and *NFYB*, regulating N and P use in wheat have been identified. For example, in a genome-wide analysis performed to identify *TAA1/TAR* genes in wheat, 15 *TaTAR* genes were identified, among which *TaTAR2.1* is mainly expressed in

roots [70]. *TaTAR2.1* expression is up-regulated under low-N conditions, promoting root growth. Therefore, TaTAR2.1 may play an important role in improving wheat yields and N use efficiency. P is also a macronutrient essential for plant growth and crop yields. The knockout of *TaPHO2-A1* in wheat could improve P uptake and wheat yields under low-P conditions [97]. As the genes regulating N and P use in wheat are identified, our understanding of nutrient uptake and the N and P use efficiencies of wheat will increase. This in turn may lead to increased wheat yields with less fertilizer input.

4. Conclusions and future perspectives

Rational design-based molecular breeding, which involves the characterization and utilization of agronomically important genes, has great potential for wheat improvement. Considerable effort has been made to elucidate the functions of genes conferring important agronomic traits to wheat. However, progress in functional wheat genomics and wheat biology has been hindered by the complex and monstrously large genome, which is 135 times larger than that of *Arabidopsis* and 37 times larger than that of rice. As the technologies used for genome sequencing and sequence assembly have advanced and the cost of sequencing has fallen, a near-complete assembly of the bread wheat genome has been generated [32]. In addition, significant progress in genetic mapping techniques and wheat transformation methods has been made over the last two decades. As a result, a growing number of wheat genes have been cloned and dissected (Table 2).

Wheat is an allohexaploid species containing three distinct subgenomes (AABBDD), and most genes show functional redundancy. Recent developments in genome editing have been especially useful in studies of wheat because they have enabled targeted mutations to be produced in all copies of a gene (i.e., not only in a single gene). For instance, the simultaneous knockout of all three copies of *Mlo* confers strong resistance in wheat to powdery mildew [17]. Therefore, this approach offers a shortcut to relate phenotypes to sequence variants or to inactivate genes conferring undesirable traits and thus enable the genetic improvement of wheat.

Several powerful tools are now available to analyze the functions of wheat genes, including mutagenesis, high-quality genome sequences, extended genetic maps, improved transgenic technology, and advanced genome editing techniques. The combined use of random mutagenesis and genome editing should enable the mutation of any gene in the wheat genome; other approaches can then be applied to reveal the biological function of the mutated gene.

Strategies and techniques are now in place that can greatly advance wheat biology. The entry of scientists who study other model plants or bioinformatics into the wheat biology field will greatly promote progress in wheat functional genomics. We predict that many more agronomically important genes, which are valuable resources for molecular breeding, will be isolated and characterized by applying the strategies used for functional genomic analysis in model organisms. We also believe that rational design-based molecular breeding is a promising avenue for creating elite wheat varieties that will promote future global food security.

Declaration of competing interest

The authors declare no conflicts of interest.

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Author contributions

Ligeng Ma conceived the idea; Jun Li and Ligeng Ma wrote the paper; Jing Yang gathered data; Yan Li prepared the figure. All authors contributed to the revisions.

REFERENCES

[1] Wheat Genome Sequencing Consortium (IWGSC), A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome, Science 345 (2014), 1251788. .

[2] J.A. Foley, N. Ramankutty, K.A. Brauman, E.S. Cassidy, J.S. Gerber, M. Johnston, N.D. Mueller, C. O'Connell, D.K. Ray, P.C. West, C. Balzer, E.M. Bennett, S.R. Carpenter, J. Hill, C. Monfreda, S. Polasky, J. Rockström, J. Sheehan, S. Siebert, D. Tilman, D.P.M. Zaks, Solutions for a cultivated planet, Nature 478 (2011) 337–342.

[3] Q. Qian, L.B. Guo, S.M. Smith, J.Y. Li, Breeding high-yield superior quality hybrid super rice by rational design, Natl. Sci. Rev. 3 (2016) 283–294.

[4] D. Zeng, Z. Tian, Y. Rao, G. Dong, Y. Yang, L. Huang, Y. Leng, J. Xu, C. Sun, G. Zhang, J. Hu, L. Zhu, Z. Gao, X. Hu, L. Guo, G. Xiong, Y. Wang, J. Li, Q. Qian, Rational design of high-yield and superior-quality rice, Nat. Plants 3 (2017) 17031.

[5] Q. Zhang, Strategies for developing Green super rice, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 16402–16409.

[6] Z. Li, F. Zhang, Rice breeding in the post-genomics era: from concept to practice, Curr. Opin. Plant Biol. 16 (2013) 261–269.

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

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

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

[10] J. Peng, D.E. Richards, N.M. Hartley, G.P. Murphy, K.M. Devos, J.E. Flintham, J. Beales, L.J. Fish, A.J. Worland, F. Pelica, D. Sudhakar, P. Christou, J.W. Snape, M.D. Gale, N.P. Harberd, "Green revolution" genes encode mutant gibberellin response modulators, Nature 400 (1999) 256–260.

[11] S. Jiang, S. Ramachandran, Natural and artificial mutants as valuable resources for functional genomics and molecular breeding, Int. J. Biol. Sci. 6 (2010) 228–251.
[12] G.A. Sega, A review of the genetic effects of ethyl methanesulfonate, Mutat. Res. Rev. Genet. Toxicol. 134 (2002) 113–142.
[13] C. Uauy, B. Wulff, J. Dubcovsky, Combining traditional mutagenesis with new high-throughput sequencing and genome editing to reveal hidden variation in polyploid wheat, Annu. Rev. Genet. 51 (2017) 435–454.
[14] K.V. Krasileva, H.A. Vasquez-Gross, T. Howell, P. Bailey, F. Paraiso, L. Clissold, J. Simmonds, R.H. Ramirez-Gonzalez, X. Wang, P. Borrill, C. Fosker, S. Ayling, A.L. Phillips, C. Uauy, J. Dubcovsky, Uncovering hidden variation in polyploid wheat, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) E913–E921.
[15] Z. Wang, J. Li, S. Chen, Y. Heng, Z. Chen, J. Yang, K. Zhou, J. Pei, H. He, X.W. Deng, L. Ma, Poaceae-specific *MS1* encodes a phospholipid-binding protein for male fertility in bread wheat, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 12614–12619.
[16] S. Periyannan, Sustaining global agriculture through rapid detection and deployment of genetic resistance to deadly crop diseases, New Phytol. 219 (2018) 45–51.
[17] Y. Wang, X. Cheng, Q. Shan, Y. Zhang, J. Liu, C. Gao, J. Qiu, Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew, Nat. Biotechnol. 32 (2014) 947–951.
[18] Y. Zhang, Z. Liang, Y. Zong, Y. Wang, J. Liu, K. Chen, J. Qiu, C. Gao, Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA, Nat. Commun. 7 (2016) 12617.
[19] J. Li, Y. Li, L. Ma, CRISPR/Cas9-based genome editing and its applications for functional genomic analyses in plants, Small Methods 3 (2019) 1800473.
[20] K. Wang, B. Riaz, X. Ye, Wheat genome editing expedited by efficient transformation techniques: progress and perspectives, Crop J. 6 (2018) 22–31.
[21] X. Shi, H. Ling, Current advances in genome sequencing of common wheat and its ancestral species, Crop J. 6 (2018) 15–21.
[22] E. Paux, P. Sourdille, J. Salse, C. Saintenac, F. Choulet, P. Leroy, A. Korol, M. Michalak, S. Kianian, W. Spielmeyer, E. Lagudah, D. Somers, A. Kilian, M. Alaux, S. Vautrin, H. Bergès, K. Eversole, R. Appels, J. Safar, H. Simkova, J. Dolezel, M. Bernard, C. Feuillet, A physical map of the 1-gigabase bread wheat chromosome 3B, Science 322 (2008) 101–104.
[23] R. Brenchley, M. Spannagl, M. Pfeifer, G.L. Barker, R. D'Amore, A.M. Allen, N. McKenzie, M. Kramer, A. Kerhornou, D. Bolser, S. Kay, D. Waite, M. Trick, I. Bancroft, Y. Gu, N. Huo, M.C. Luo, S. Sehgal, B. Gill, S. Kianian, O. Anderson, P. Kersey, J. Dvorak, W.R. McCombie, A. Hall, K.F. Mayer, K.J. Edwards, M.W. Bevan, N. Hall, Analysis of the bread wheat genome using whole-genome shotgun sequencing, Nature 491 (2012) 705–710.
[24] H.Q. Ling, S. Zhao, D. Liu, J. Wang, H. Sun, C. Zhang, H. Fan, D. Li, L. Dong, Y. Tao, C. Gao, H. Wu, Y. Li, Y. Cui, X. Guo, S. Zheng, B. Wang, K. Yu, Q. Liang, W. Yang, X. Lou, J. Chen, M. Feng, J. Jian, X. Zhang, G. Luo, Y. Jiang, J. Liu, Z. Wang, Y. Sha, B. Zhang, H. Wu, D. Tang, Q. Shen, P. Xue, S. Zou, X. Wang, X. Liu, F. Wang, Y. Yang, X. An, Z. Dong, K. Zhang, X. Zhang, M.C. Luo, J. Dvorak, Y. Tong, J. Wang, H. Yang, Z. Li, D. Wang, A. Zhang, J. Wang, The draft genome of *Triticum urartu*, Nature 496 (2013) 487–490.
[25] J. Jia, S. Zhao, X. Kong, Y. Li, G. Zhao, W. He, R. Appels, M. Pfeifer, Y. Tao, X. Zhang, R. Jing, C. Zhang, Y. Ma, L. Gao, C. Gao, M. Spannagl, K.F. Mayer, D. Li, S. Pan, F. Zheng, Q. Hu, X. Xia, J. Li, Q. Liang, J. Chen, T. Wicker, C. Gou, H. Kuang, G. He, Y. Luo, B. Keller, Q. Xia, P. Lu, J. Wang, H. Zou, R. Zhang, J. Xu, J. Gao, C. Middleton, Z. Quan, G. Liu, J. Wang, International Wheat Genome Sequencing Consortium, H. Yang, X. Liu, Z. He, L. Mao, J. Wang, *Aegilops tauschii* draft genome sequence reveals a gene repertoire for wheat adaptation, Nature 496 (2013) 91–95.
[26] J.A. Chapman, M. Mascher, A. Buluc, K. Barry, E. Georganas, A. Session, V. Strnadova, J. Jenkins, S. Sehgal, L. Oliker, J. Schmutz, K.A. Yelick, U. Scholz, R. Waugh, J.A. Poland, G.J. Muehlbauer, N. Stein, D.S. Rokhsar, A whole-genome shotgun approach for assembling and anchoring the hexaploid bread wheat genome, Genome Biol. 16 (2015) 26.
[27] B. Keller, T. Wicker, S.G. Krattinger, Advances in wheat and pathogen genomics: implications for disease control, Annu. Rev. Phytopathol. 56 (2018) 67–87.
[28] F. Choulet, A. Alberti, S. Theil, N. Glover, V. Barbe, J. Daron, L. Pingault, P. Sourdille, A. Couloux, E. Paux, P. Leroy, S. Mangenot, N. Guilhot, J. Le Gouis, F. Balfourier, M. Alaux, V. Jamilloux, J. Poulain, C. Durand, A. Bellec, C. Gaspin, J. Safar, J. Dolezel, J. Rogers, K. Vandepoele, J.M. Aury, K. Mayer, H. Berges, H. Quesneville, P. Wincker, C. Feuillet, Structural and functional partitioning of bread wheat chromosome 3B, Science 345 (2014) 1249721.
[29] R. Avni, M. Nave, O. Barad, K. Baruch, S.O. Twardziok, H. Gundlach, I. Hale, M. Mascher, M. Spannagl, K. Wiebe, K.W. Jordan, G. Golan, J. Deek, B. Ben-Zvi, G. Ben-Zvi, A. Himmelbach, R.P. MacLachlan, A.G. Sharpe, A. Fritz, R. BenDavid, H. Budak, T. Fahima, A. Korol, J.D. Faris, A. Hernandez, M.A. Mikel, A.A. Levy, B. Steffenson, M. Maccaferri, R. Tuberosa, L. Cattivelli, P. Faccioli, A. Ceriotti, K. Kashkush, M. Pourkheirandish, T. Komatsuda, T. Eilam, H. Sela, A. Sharon, N. Ohad, D.A. Chamovitz, K.F.X. Mayer, N. Stein, G. Ronen, Z. Peleg, C.J. Pozniak, E.D. Akhunov, A. Distelfeld, Wild emmer genome architecture and diversity elucidate wheat evolution and domestication, Science 357 (2017) 93–97.
[30] B.J. Clavijo, L. Venturini, C. Schudoma, G.G. Accinelli, G. Kaithakottil, J. Wright, P. Borrill, G. Kettleborough, D. Heavens, H. Chapman, J. Lipscombe, T. Barker, F. Lu, N. McKenzie, D. Raats, R.H. Ramirez-Gonzalez, A. Coince, N. Peel, L. Percival-Alwyn, O. Duncan, J. Trösch, G. Yu, D. Bolser, G. Namaati, A. Kerhornou, M. Spannagl, H. Gundlach, G. Haberer, R.P. Davey, C. Fosker, F.D. Palma, A. Phillips, A.H. Millar, P.J. Kersey, C. Uauy, K.V. Krasileva, D. Swarbreck, M.W. Bevan, M.D. Clark, An improved assembly and annotation of the allohexaploid wheat genome identifies complete families of agronomic genes and provides genomic evidence for chromosomal translocations, Genome Res. 27 (2017) 885–896.
[31] J. Xie, N. Huo, S. Zhou, Y. Wang, G. Guo, K.R. Deal, S. Ouyang, Y. Liang, Z. Wang, L. Xiao, T. Zhu, T. Hu, V. Tiwari, J. Zhang, H. Li, Z. Ni, Y. Yao, H. Peng, Q. Sun, Sequencing and comparative analyses of *Aegilops tauschii* chromosome arm 3DS reveal rapid evolution of *Triticeae* genomes, J. Genet. Genom. 44 (2017) 51–61.
[32] International Wheat Genome Sequencing Consortium (IWGSC), Shifting the limits in wheat research and breeding through a fully annotated and anchored reference genome sequence, Science 361 (2018) 661.
[33] J. Bettgenhaeuser, S.G. Krattinger, Rapid gene cloning in cereals, Theor. Appl. Genet. 132 (2018) 699–711.
[34] S. Krattinger, T. Wicker, B. Keller, Map-based cloning of genes in *Triticeae* (wheat and barley), in: G. Muehlbauer, C. Feuillet (Eds.), Genetics and Genomics of the Triticeae. Plant Genetics and Genomics: Crops and Models, 7, Springer, New York, NY, USA 2009, pp. 337–357.
[35] G.S. Johal, S.P. Briggs, Reductase activity encoded by the *HM1* disease resistance gene in maize, Science 258 (1992) 985–987.
[36] C. Feuillet, S. Travella, N. Stein, L. Albar, A. Nublat, B. Keller, Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 15253–15258.

[37] J.D. Faris, Z. Zhang, H. Lu, S. Lu, L. Reddy, S. Cloutier, J.P. Fellers, S.W. Meinhardt, J.B. Rasmussen, S.S. Xu, R.P. Oliver, K. J. Simons, T.L. Friesen, A unique wheat disease resistancelike gene governs effector-triggered susceptibility to necrotrophic pathogens, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 13544–13549.
[38] L. Huang, S. Brooks, W. Li, J. Fellers, H. Trick, B. Gill, Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat, Genetics 164 (2003) 655–664.
[39] L. Yan, A. Loukoianov, G. Tranquilli, M. Helguera, T. Fahima, J. Dubcovsky, Positional cloning of the wheat vernalization gene *Vrn1*, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 6263–6268.
[40] N. Yahiaoui, P. Srichumpa, R. Dudler, B. Keller, Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat, Plant J. 37 (2004) 528–538.
[41] L. Yan, A. Loukoianov, A. Blechl, G. Tranquilli, W. Ramakrishna, P. SanMiguel, J.L. Bennetzen, V. Echenique, J. Dubcovsky, The wheat *Vrn2* gene is a flowering repressor down-regulated by vernalization, Science 30 (2004) 1640–1644.
[42] L. Yan, D. Fu, C. Li, A. Blechl, G. Tranquilli, M. Bonafede, A. Sanchez, M. Valarik, S. Yasuda, J. Dubcovsky, The wheat and barley vernalization gene *Vrn3* is an orthologue of FT, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 19581–19586.
[43] C. Uauy, A. Distelfeld, T. Fahima, A. Blechl, J. Dubcovsky, A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat, Science 314 (2006) 1298–1301.
[44] S. Griffiths, R. Sharp, T.N. Foote, I. Bertin, M. Wanous, S. Reader, I. Colas, G. Moore, Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat, Nature 439 (2006) 749–752.
[45] S. Cloutier, B. McCallum, C. Loutre, T. Banks, T. Wicker, C. Feuillet, B. Keller, M.C. Jordan, Leaf rust resistance gene *Lr1*, isolated from bread wheat (*Triticum aestivum* L.) is a member of the large psr567 gene family, Plant Mol. Biol. 65 (2007) 93–106.
[46] J. Qiu, A. Christina, S. Yahiaoui, L. Dong, H. Fan, Z. Zhang, B. Keller, H. Ling, Physical mapping and identification of a candidate for the leaf rust resistance gene *Lr1* of wheat, Theor. Appl. Genet. 115 (2007) 159–168.
[47] S.G. Krattinger, E.S. Lagudah, W. Spielmeyer, R.P. Singh, J. Huerta-Espino, H. McFadden, E. Bossolini, L.L. Selter, B. Keller, A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat, Science 323 (2009) 1360–1363.
[48] D.L. Fu, C. Uauy, A. Distelfeld, A. Blechl, L. Epstein, X.M. Chen, H. Sela, T. Fahima, J. Dubcovsky, A kinase-START gene confers temperature-dependent resistance to wheat stripe rust, Science 323 (2009) 1357–1360.
[49] J.D. Faris, J.P. Fellers, S.A. Brooks, B.S. Gill, A bacterial artificial chromosome contig spanning the major domestication locus Q in wheat and identification of a candidate gene, Genetics 164 (2003) 311–321.
[50] T. Jordan, S. Seeholzer, S. Schwizer, A. Toller, I. Somssich, B. Keller, The wheat *Mla* homologue *TmMla1* exhibits an evolutionarily conserved function against powdery mildew in both wheat and barley, Plant J. 65 (2011) 610–621.
[51] S. Hurni, S. Brunner, G. Buchmann, G. Herren, T. Jordan, P. Krukowski, T. Wicker, N. Yahiaoui, R. Mago, B. Keller, Rye *Pm8* and wheat *Pm3* are orthologous genes and show evolutionary conservation of resistance function against powdery mildew, Plant J. 76 (2013) 957–969.
[52] S. Periyannan, J. Moore, M. Ayliffe, U. Bansal, X. Wang, L. Huang, K. Deal, M. Luo, X. Kong, H. Bariana, R. Mago, R. McIntosh, P. Dodds, J. Dvorak, E. Lagudah, The gene *Sr33*, an ortholog of barley *Mla* genes, encodes resistance to wheat stem rust race Ug99, Science 341 (2013) 786–788.
[53] C. Saintenac, W. Zhang, A. Salcedo, M.N. Rouse, H.N. Trick, E. Akhunov, J. Dubcovsky, Identification of wheat gene *Sr35* that confers resistance to Ug99 stem rust race group, Science 341 (2013) 783–786.
[54] S. Liu, S. Sehgal, M. Lin, J. Li, H. Trick, B. Gill, G. Bai, Independent mis-splicing mutations in *TaPHS1* causing loss of preharvest sprouting (PHS) resistance during wheat domestication, New Phytol. 208 (2015) 928–935.
[55] N. Kippes, J.M. Debernardi, H.A. Vasquez-Gross, B.A. Akpinar, H. Budak, K. Kato, S. Chao, E. Akhunov, J. Dubcovsky, Identification of the *VERNALIZATION 4* gene reveals the origin of spring growth habit in ancient wheats from South Asia, Proc. Natl. Acad. Sci. U. S. A. 31 (2015) 5401–5410.
[56] M. Pallotta, T. Schnurbusch, J. Hayes, A. Hay, U. Baumann, J. Paull, P. Langridge, T. Sutton, Molecular basis of adaptation to high soil boron in wheat landraces and elite cultivars, Nature 514 (2014) 88–91.
[57] W. Liu, M. Frick, R. Huel, C.L. Nykiforuk, X.M. Wang, D.A. Gaudet, F. Eudes, R.L. Conner, A. Kuzyk, Q. Chen, Z.S. Kang, A. Laroche, The stripe rust resistance gene *Yr10* encodes an evolutionary-conserved and unique CC-NBS-LRR sequence in wheat, Mol. Plant 7 (2014) 1740–1755.
[58] R. Mago, P. Zhang, S. Vautrin, H. Šimková, U. Bansal, M.C. Luo, M. Rouse, H. Karaoglu, S. Periyannan, J. Kolmer, Y. Jin, M.A. Ayliffe, H. Bariana, R.F. Park, R. McIntosh, J. Doležel, H. Bergès, W. Spielmeyer, E.S. Lagudah, J.G. Ellis, P.N. Dodds, The wheat *Sr50* gene reveals rich diversity at a cereal disease resistance locus, Nat. Plants 1 (2015) 15186.
[59] J.W. Moore, S. Herrera-Foessel, C. Lan, W. Schnippenkoetter, M. Ayliffe, J. Huerta-Espino, M. Lillemo, L. Viccars, R. Milne, S. Periyannan, X. Kong, W. Spielmeyer, M. Talbot, H. Bariana, J.W. Patrick, P. Dodds, R. Singh, E. Lagudah, A recently evolved hexose transporter variant confers resistance to multiple pathogens in wheat, Nat. Genet. 47 (2015) 1494–1498.
[60] G. Shi, Z. Zhang, T.L. Friesen, D. Raats, T. Fahima, R.S. Brueggeman, S. Lu, H.N. Trick, Z. Liu, W. Chao, The hijacking of a receptor kinase-driven pathway by a wheat fungal pathogen leads to disease, Sci. Adv. 2 (2016), e1600822.
[61] B. Steuernagel, S. Periyannan, I. Hernandez-Pinzon, K. Witek, M.N. Rouse, G.T. Yu, A. Hatta, M. Ayliffe, H. Bariana, J.D.G. Jones, E.S. Ladudah, B.H. Wulff, Rapid cloning of disease resistance genes in plants using mutagenesis and sequence capture, Nat. Biotechnol. 34 (2016) 652–655.
[62] Z. Su, A. Bernardo, B. Tian, H. Chen, S. Wang, H. Ma, S. Cai, D. Liu, D. Zhang, T. Li, H. Trick, P.S. Amand, J. Yu, Z. Zhang, G. Bai, A deletion mutation in *TaHRC* confers *Fhb1* resistance to Fusarium head blight in wheat, Nat. Genet. 51 (2019) 1099–1105.
[63] G. Li, J. Zhou, H. Jia, Z. Gao, M. Fan, Y. Luo, P. Zhao, S. Xue, N. Li, Y. Yuan, S. Ma, Z. Kong, L. Jia, X. An, G. Jiang, W. Liu, W. Cao, R. Zhang, J. Fan, X. Xu, Y. Liu, Q. Kong, S. Zheng, Y. Wang, B. Qin, S. Cao, Y. Ding, J. Shi, H. Yan, X. Wang, C. Ran, Z. Ma, Mutation of a histidine-rich calcium-binding protein gene in wheat confers resistance to Fusarium head blight, Nat. Genet. 51 (2019) 1106–1112.
[64] J. Sanchez-Martin, B. Steuernagel, S. Ghosh, G. Herren, S. Hurni, N. Adamski, J. Vrana, M. Kubalakova, S.G. Krattinger, T. Wicker, J. Doležel, B. Keller, B.B. Wulff, Rapid gene isolation in barley and wheat by mutant chromosome sequencing, Genome Biol. 17 (2016) 221.
[65] A. Torada, M. Koike, T. Ogawa, Y. Takenouchi, K. Tadamura, J. Wu, T. Matsumoto, K. Kawaura, Y. Ogihara, A causal gene for seed dormancy on wheat chromosome 4A encodes a MAP kinase kinase, Curr. Biol. 26 (2016) 782–787.
[66] Y. Zhang, Y. Bai, G. Wu, S. Zou, Y. Chen, C. Gao, D. Tang, Simultaneous modification of three homoeologs of TaEDR1

by genome editing enhances powdery mildew resistance in wheat, Plant J. 91 (2017) 714–724.
[67] A. Thind, T. Wicker, H. Simkova, D. Fossati, O. Moullet, C. Brabant, J. Vrana, J. Doležel, S.G. Krattinger, Rapid cloning of genes in hexaploid wheat using cultivar-specific long-range chromosome assembly, Nat. Biotechnol. 35 (2017) 793–796.
[68] W. Zhang, S. Chen, Z. Abate, J. Nirmala, M.N. Rouse, J. Dubcovsky, Identification and characterization of *Sr13*, a tetraploid wheat gene that confers resistance to the Ug99 stem rust race group, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 9483–9492.
[69] J. Connorton, E. Jones, I. Rodríguez-Ramiro, S. Fairweather-Tait, C. Uauy, J. Balk, Wheat vacuolar iron transporter TaVIT2 transports Fe and Mn and is effective for biofortification, Plant Physiol. 174 (2017) 2434–2444.
[70] A. Shao, W. Ma, X. Zhao, M. Hu, X. He, W. Teng, H. Li, Y. Tong, The auxin biosynthetic *TRYPTOPHAN AMINOTRANSFERASE RELATED TaTAR2.1-3A* increases grain yield of wheat, Plant Physiol. 174 (2017) 2274–2288.
[71] V.L. Anh, Y. Inoue, S. Asuke, T. Vy, N.T. Anh, S. Wang, I. Chuma, Y. Tosa, *Rmg8* and *Rmg7*, wheat genes for resistance to the wheat blast fungus, recognize the same avirulence gene *AVR-Rmg8*, Mol. Plant Pathol. 19 (2018) 1252–1256.
[72] F. Ni, J. Qi, Q.Q. Hao, B. Lyu, M.C. Luo, Y. Wang, F.J. Chen, S.Y. Wang, C.Z. Zhang, L. Epstein, X.Y. Zhao, H.G. Wang, X.S. Zhang, C.X. Chen, L.Z. Sun, D.L. Fu, Wheat *Ms2* encodes for an orphan protein that confers male sterility in grass species, Nat. Commun. 8 (2017) 15121.
[73] C. Xia, L.C. Zhang, C. Zou, Y.Q. Gu, J.L. Duan, G.Y. Zhao, J.J. Wu, Y. Liu, X.H. Fang, L.F. Gao, Y.N. Jiao, J.Q. Sun, Y.H. Pan, X. Liu, J.Z. Jia, X.Y. Kong, A TRIM insertion in the promoter of Ms2 causes male sterility in wheat, Nat. Commun. 8 (2017) 15407.
[74] E.J. Tucker, U. Baumann, A. Kouidri, R. Suchecki, M. Baes, M. Garcia, T. Okada, C. Dong, Y. Wu, A. Sandhu, M. Singh, P. Langridge, P. Wolters, M.C. Albertsen, A.M. Cigan, R. Whitford, Molecular identification of the wheat male fertility gene *Ms1* and its prospects for hybrid breeding, Nat. Commun. 8 (2017) 869.
[75] C. Saintenac, W.S. Lee, F. Cambon, J.J. Rudd, R.C. King, W. Marande, S.J. Powers, H. Bergès, A.L. Phillips, C. Uauy, K.E. Hammond-Kosack, T. Langin, K. Kanyuka, Wheat receptorkinase-like protein Stb6 controls gene-for-gene resistance to fungal pathogen *Zymoseptoria tritici*, Nat. Genet. 50 (2018) 368–374.
[76] S. Zou, H. Wang, Y. Li, Z. Kong, D. Tang, The NB-LRR gene *Pm60* confers powdery mildew resistance in wheat, New Phytol. 218 (2018) 298–309.
[77] L. Xing, P. Hu, J. Liu, K. Witek, S. Zhou, J. Xu, W. Zhou, L. Gao, Z. Huang, R. Zhang, X. Wang, P. Chen, H. Wang, J.D.G. Jones, M. Karafiátová, J. Vrána, J. Bartoš, J. Doležel, Y. Tian, Y. Wu, A. Cao, *Pm21* from *Haynaldia villosa* encodes a CC-NBS-LRR protein conferring powdery mildew resistance in wheat, Mol. Plant 11 (2018) 874–878.
[78] H. He, S. Zhu, R. Zhao, Z. Jiang, Y. Ji, J. Ji, D. Qiu, H. Li, T. Bie, *Pm21*, encoding a typical CC-NBS-LRR protein, confers broadspectrum resistance to wheat powdery mildew disease, Mol. Plant 11 (2018) 879–882.
[79] S. Chen, W. Zhang, S. Bolus, M.N. Rouse, J. Dubcovsky, Identification and characterization of wheat stem rust resistance gene *Sr21* effective against the Ug99 race group at high temperature, PLoS Genet. 14 (2018), e1007287. .
[80] C. Marchal, J. Zhang, P. Zhang, P. Fenwick, B. Steuernagel, N. M. Adamski, L. Boyd, R. McIntosh, B.B.H. Wulff, S. Berry, E. Lagudah, C. Uauy, BED-domain-containing immune receptors confer diverse resistance spectra to yellow rust, Nat. Plants 4 (2018) 662–668.
[81] V. Klymiuk, E. Yaniv, L. Huang, D. Raats, A. Fatiukha, S. Chen, L. Feng, Z. Frenkel, T. Krugman, G. Lidzbarsky, W. Chang, M.J. Jääskeläinen, C. Schudoma, L. Paulin, P. Laine, H. Bariana, H. Sela, K. Saleem, C.K. Sørensen, M.S. Hovmøller, A. Distelfeld, B. Chalhoub, J. Dubcovsky, A.B. Korol, A.H. Schulman, T. Fahima, Cloning of the wheat *Yr15* resistance gene sheds light on the plant tandem kinase-pseudokinase family, Nat. Commun. 9 (2018) 3735.
[82] M.A. Pallotta, P. Warner, A. Kouidri, E.J. Tucker, M. Baes, R. Suchecki, N. Watson-Haigh, T. Okada, M. Garcia, A. Sandhu, M. Singh, P. Wolters, M.C. Albertsen, A.M. Cigan, U. Baumann, R. Whitford, Wheat *ms5* male-sterility is induced by recessive homoeologous a and D genome non-specific Lipid Transfer Proteins, Plant J. 99 (2019) 673–685.
[83] C. Zhang, L. Huang, H. Zhang, Q. Hao, B. Lyu, M. Wang, L. Epstein, M. Liu, C. Kou, J. Qi, F. Chen, M. Li, G. Gao, F. Ni, L. Zhang, M. Hao, J. Wang, X. Chen, M. Luo, Y. Zheng, J. Wu, D. Liu, D. Fu, An ancestral NB-LRR with duplicated 3′ UTRs confers stripe rust resistance in wheat and barley, Nat. Commun. 10 (2019) 4023.
[84] S. Chen, M.N. Rouse, W. Zhang, X. Zhang, Y. Guo, J. Briggs, J. Dubcovsky, Wheat gene *Sr60* encodes a protein with two putative kinase domains that confers resistance to stem rust, New Phytol. 225 (2020) 948–959.
[85] A. Abe, S. Kosugi, K. Yoshida, S. Natsume, H. Takagi, H. Kanzaki, H. Matsumura, K. Yoshida, C. Mitsuoka, M. Tamiru, H. Innan, L. Cano, S. Kamoun, R. Terauchi, Genome sequencing reveals agronomically important loci in rice using MutMap, Nat. Biotechnol. 30 (2012) 174–178.
[86] L. Xia, Y. Ma, Y. He, H.D. Jones, GM wheat development in China: current status and challenges to commercialization, J. Exp. Bot. 63 (2012) 1785–1790.
[87] V. Vasil, A.M. Castillo, M.E. Fromm, I.K. Vasil, Herbicide resistant fertile transgenic wheat plants obtained by microprojectile bombardment of regenerable embryogenic callus, Nat. Biotechnol. 10 (1992) 667–674.
[88] M. Cheng, J.E. Fry, S. Pang, H. Zhou, C.M. Hironaka, D.R. Duncan, T.W. Conner, Y. Wan, Genetic transformation of wheat mediated by Agrobacterium tumefaciens, Plant Physiol. 115 (1997) 971–980.
[89] W.A. Harwood, Advances and remaining challenges in the transformation of barley and wheat, J. Exp. Bot. 63 (2012) 1791–1798.
[90] A. Shrawat, C. Armstrong, Development and application of genetic engineering for wheat improvement, Crit. Rev. Plant Sci. 37 (2018) 335–421.
[91] Y. Ishida, M. Tsunashima, Y. Hiei, T. Komari, Wheat (*Triticum aestivum* L.) transformation using immature embryos, in: K. Wang (Ed.), Agrobacterium Protocols. Methods in Molecular Biology, 1223, Springer, New York, NY, USA 2014, pp. 189–198.
[92] T. Richardson, J. Thistleton, T.J. Higgins, C. Howitt, M. Ayliffe, Efficient *Agrobacterium* transformation of elite wheat germplasm without selection, Plant Cell Tissue Organ Cult. 119 (2014) 647–659.
[93] J. Xiao, S. Xu, C. Li, Y. Xu, L. Xing, Y. Niu, Q. Huan, Y. Tang, C. Zhao, D. Wagner, C. Gao, K. Chong, OGlcNAc-mediated interaction between VER2 and TaGRP2 elicits *TaVRN1* mRNA accumulation during vernalization in winter wheat, Nat. Commun. 5 (2014) 4572.
[94] X. Zhao, P. Hong, J.Y. Wu, X.B. Chen, X.G. Ye, Y.Y. Pan, J. Wang, X.S. Zhang, The tae-miR408-mediated control of *TaTOC1* genes transcription is required for the regulation of heading time in wheat, Plant Physiol. 170 (2016) 1578–1594.
[95] J. Simmonds, P. Scott, J. Brinton, T.C. Mestre, M. Bush, A. Del Blanco, J. Dubcovsky, C. Uauy, A splice acceptor site mutation in *TaGW2-A1* increases thousand grain weight in tetraploid and hexaploid wheat through wider and longer grains, Theor. Appl. Genet. 129 (2016) 1099–1112.
[96] A.J. Slade, C. McGuire, D. Loeffler, J. Mullenberg, W. Skinner, G. Fazio, A. Holm, K.M. Brandt, M.N. Steine, J.F. Goodstal, V.C.

Knauf, Development of high amylose wheat through TILLING, BMC Plant Biol. 12 (2012) 69.
[97] X. Ouyang, X. Hong, X. Zhao, W. Zhang, X. He, W. Ma, W. Teng, Y. Tong, Knock out of the *PHOSPHATE 2* gene *TaPHO2-A1* improves phosphorus uptake and grain yield under low phosphorus conditions in common wheat, Sci. Rep. 6 (2016) 29850.