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Advances in Wheat and Pathogen Genomics: Implications for Disease Control

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Abstract

The gene pool of wheat and its wild and domesticated relatives contains a plethora of resistance genes that can be exploited to make wheat more resilient to pathogens. Only a few of these genes have been isolated and studied at the molecular level. In recent years, we have seen a shift from classical breeding to genomics-assisted breeding, which makes use of the enormous advancements in DNA sequencing and high-throughput molecular marker technologies for wheat improvement. These genomic advancements have the potential to transform wheat breeding in the near future and to significantly increase the speed and precision at which new cultivars can be bred. This review highlights the genomic improvements that have been made in wheat and its pathogens over the past years and discusses their implications for disease-resistance breeding.

INTRODUCTION

The cereal crop plants, including maize, rice, wheat, barley, sorghum, millets, oats, and rye, play a pivotal role in providing food for humankind. Tetraploid pasta wheat (*Triticum durum*) and hexaploid bread wheat (*Triticum aestivum*) are among the most widely cultivated cereal plants. In 2016, the global wheat production reached a new record of 760 million tons (FaoStat; **http://www.fao.org/faostat/en/#home**). Despite this impressive number, the production of wheat and other crops is constantly at risk and threatened by biotic and abiotic stresses that impair crop yield and quality. Since the dawn of agriculture, crop diseases, pests, droughts, and other stresses have affected crop production, with sometimes dramatic impacts on human history. Plant diseases caused by pathogenic viruses, bacteria, oomycetes, and fungi have been a major cause of crop losses. In the past twenty years, the emergence of new and highly virulent races of the wheat stem rust fungus, collectively referred to as Ug99 after their first occurrence in Uganda in the year 1999, caused serious damage to wheat production in Africa and the Middle East (88). In 2016, wheat blast, a disease previously restricted to Brazil and neighboring South American countries, ravaged wheat fields in Bangladesh (47). These examples highlight the impact of plant diseases on food security and human health. Not surprisingly, one of the main objectives in breeding and agriculture is the selection and cultivation of crops that are able to withstand disease. The basis of disease resistance, however, was not comprehensively understood until the description of the laws of inheritance by Gregor Mendel in the mid-nineteenth century, which laid the foundation for modern genetics and plant breeding as we know it today.

Since the Green Revolution in the 1960s (79), the genetics of disease resistance in crops has been systematically studied and dissected into its single genetic components, which are the disease-resistance genes encoded in a plant's genome. In wheat, this systematic effort has led to the cataloging of several hundred different disease-resistance genes (65). However, only a small fraction of these genes have been cloned and had their molecular function studied in detail.

The wheat genome is among the largest and most complex of all cultivated plants. With 17 billion (17 Gb) nucleotides, the bread-wheat genome is five times larger than the human genome. Bread wheat is a hexaploid species that arose through natural hybridization of three closely related wild grass species (83). In addition, the proportion of repetitive sequences within the wheat genome is higher than 80%. The huge and complex genome of wheat has greatly complicated the isolation of individual genes in the past. The first disease-resistance genes cloned in wheat were *Lr10* and *Lr21*, which both confer race-specific resistance against the fungal leaf rust disease (*Puccinia triticina*). Both genes encode intracellular immune receptors of the nucleotide binding-site–leucine-rich repeat (NLR) family (28, 37). The cloning of *Lr10* and *Lr21* occurred in 2003, almost eight years after the first disease-resistance gene was isolated from rice (89). In comparison to the wheat genome, the rice genome with its 380 million nucleotides is considerably smaller (43), which makes gene cloning in rice more feasible. The large size and high complexity of the wheat genome, however, has inspired wheat researchers to think of novel genomic approaches that facilitate genomics-assisted breeding, including gene cloning. These efforts have resulted in the development of extensive genomic resources, including high-quality genome sequences, high-throughput genotyping platforms, and several rapid gene-cloning strategies. The results of these achievements have already become apparent when looking at the increasing rate at which disease-resistance genes are isolated in wheat (**Figure 1**). Here, we review these recent advances in the genomics of wheat and its pathogens and discuss implications for wheat disease-resistance breeding.

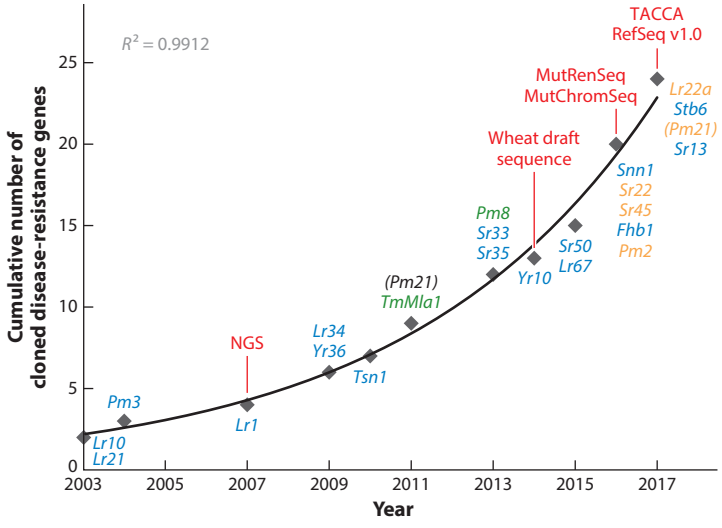

Figure 1

Genomic advancements and their implications for the cloning of disease-resistance genes in wheat. Key advances in genomics, such as the introduction of next-generation sequencing (NGS) technologies in 2007, the release of the wheat draft genome sequence in 2014, and the Chinese Spring reference sequence (RefSeq v1.0) in 2018, are indicated in red. In addition, novel gene-cloning approaches such as MutRenSeq, MutChromSeq, and TACCA are shown. Genes isolated by map-based cloning are shown in blue, genes isolated through homology-based cloning are shown in green, and genes isolated with novel gene-cloning approaches are shown in yellow. The trend line corresponds to an exponential growth curve. Abbreviations: MutChromeSeq, mutagenesis, chromosome flow sorting, and sequencing; MutRenSeq, mutant resistance gene enrichment sequencing; TACCA, targeted chromosome-based cloning via long-range assembly.

ADVANCES IN WHEAT GENOMICS: IMPLICATIONS FOR THE GENETIC AND MOLECULAR UNDERSTANDING OF DISEASE RESISTANCE

Gene-Cloning Strategies in the Prereference Genome Era

The cloning or isolation of a gene describes the process of pinpointing it to its exact location within the genome, which ultimately reveals its nucleotide sequence and the protein it encodes. The cloning of disease-resistance genes forms the basis for many important breeding objectives: (*a*) It allows the design of perfect gene-based molecular markers for marker-assisted selection; (*b*) it enables the rapid transfer of cloned genes through transgenesis or their modification through genome editing; (*c*) it enables diagnostic applications to, e.g., determine which gene is present in which wheat cultivar; and (*d*) it forms the basis for unraveling the molecular function of plant-pathogen interactions, knowledge that will ultimately help to protect wheat from pathogens. However, gene cloning from the vast wheat genome has presented a great challenge in the past. The majority of all wheat disease-resistance genes cloned so far (**Table 1**) were isolated through map-based cloning (**Figure 1**), an approach that aims to narrow down the location of a gene stepwise by making use of genetic recombination in biparental populations that segregate for the gene of interest. A critical unit in this process is the size of DNA fragments that are easily accessible to sequencing. Because it was not possible until recently to sequence whole wheat genomes, wheat DNA was fragmented and tiny segments of the wheat genome were randomly

Table 1 Cloned disease resistance genes in wheat. The table lists the approach that led to the cloning of the respective gene, the family of proteins to which the encoded gene product belongs, the donor accessions that were used, and the disease the gene is effective against

Gene	Cloning strategy	Protein encoded by gene	Donor accession	Resistance to disease	Reference
Lr10	Map-based cloning	NLR	Hexaploid wheat line Thatcher*Lr10*	Leaf rust	28
Lr21	Map-based cloning	NLR	*Aegilops tauschii* accessions TA1649 and TA1599	Leaf rust	37
Pm3	Map-based cloning	NLR	Hexaploid wheat landrace Chul	Powdery mildew	105
Lr1	Map-based cloning	NLR	Hexaploid wheat breeding line 87E03-S2B1	Leaf rust	21
Lr34/Yr18/ Sr57/Pm38	Map-based cloning	ABC transporter	Hexaploid wheat lines Thatcher*Lr34*, Avocet*Lr34*, Forno, Chinese Spring	Leaf rust, stripe rust, stem rust, powdery mildew	55
Yr36	Map-based cloning	Kinase-START	Wild emmer wheat accession FA15-3	Stripe rust	30
Tsn1	Map-based cloning	S/T protein kinase–NLR domain containing protein	Durum wheat cultivar Langdon	Stagonospora nodorum blotch, tan spot	27
TmMla1	Homology-based cloning	NLR	*Triticum monococcum* line DV92	Powdery mildew	51
Pm21[a]	Expression level–based candidate gene identification	S/T protein kinase	*Haynaldia villosa*	Powdery mildew	16
Pm8	Homology-based cloning	NLR	Rye line Petkus, wheat line Kavkaz/4*Federation	Powdery mildew	39
Sr33	Map-based cloning	NLR	*A. tauschii* accession RL5288	Stem rust	73
Sr35	Map-based cloning	NLR	*T. monococcum* line DV92	Stem rust	82
Yr10	Map-based cloning	NLR	Hexaploid wheat cultivar Moro	Stripe rust	57
Sr50	Map-based cloning	NLR	Rye cultivar Imperial, wheat introgression line Gabo 1BL.1RS	Stem rust	62
Lr67/Yr46/ Sr55/Pm46	Map-based cloning	Hexose transporter	Hexaploid wheat line Thatcher *Lr67*	Leaf rust, stripe rust, stem rust, powdery mildew	69
Snn1	Map-based cloning	Wall-associated kinase	Chinese Spring–Hope 1B	Septoria nodorum blotch	87
Sr22	MutRenSeq	NLR	Hexaploid wheat cultivar Schomburgk	Stem rust	91
Sr45	MutRenSeq	NLR	Hexaploid wheat introgression line CS1D5406	Stem rust	91
Fhb1	Map-based cloning	Pore-forming toxin-like protein	Hexaploid wheat cultivar Sumai 3	Fusarium head blight	78

(*Continued*)

Table 1 (*Continued*)

Gene	Cloning strategy	Protein encoded by gene	Donor accession	Resistance to disease	Reference
Pm2	MutChromSeq	NLR	Hexaploid wheat line CI12632/8*Chancellor	Powdery mildew	85
Lr22a	TACCA	NLR	*A. tauschii* accession RL5271, Hexaploid wheat line CH Campala *Lr22a*	Leaf rust	97
Sr13	Map-based cloning	NLR	Durum wheat cultivars Langdon and Kronos	Stem rust	106
Stb6	Map-based cloning	Wall-associated kinase	Hexaploid wheat cultivars Flame, Chinese Spring and Cadenza	Septoria tritici blotch	81

[a]References 33a and 104a suggest that *Pm21* encodes an NLR protein.
Abbreviations: MutChromSeq, mutagenesis, chromosome flow sorting, and sequencing; MutRenSeq, mutant resistance gene enrichment sequencing; NLR, nucleotide binding-site–leucine-rich repeat; S/T, serine/threonine; TACCA, targeted chromosome-based cloning via long-range assembly.

cloned into bacterial artificial chromosomes (BACs). Each BAC clone can accommodate a 100–200-kb DNA piece, which corresponds to as little as 0.001% of a hexaploid wheat genome. Hence, approximately 500,000 BAC clones are necessary to obtain a representative library of a complete wheat genome (60). To delimit a gene of interest to an attainable piece of DNA, the gene needs to be mapped to a very small genetic interval (<0.5 cM) that corresponds to one or a few overlapping BAC clones. Large mapping populations consisting of several thousand plants were required to achieve the necessary genetic resolutions. For example, the *Lr10* leaf rust resistance gene was fine-mapped to a 0.13-cM genetic interval by using a mapping population consisting of 3,120 F2 plants (90), which allowed narrowing down *Lr10*'s location to a physical interval of 450 kb that was spanned by four overlapping BAC clones. Although map-based cloning works well in telomeric regions with high-recombination rates, cloning genes from centromeric regions that often span megabases devoid of recombination is nearly impossible using map-based cloning. For similar reasons, the cloning of genes from regions that were introgressed into wheat from wild grasses is very challenging. Below we highlight and discuss recent key advances in genomics (6) that have had and will have significant impact on the speed, cost, and precision at which wheat genes can be isolated in the future.

Sequencing the Wheat Genome: The Rocky Road Toward a High-Quality Reference Sequence

The wheat genome has long been considered to be too large and too complex to be sequenced at high quality. First, whole-genome shotgun assemblies of the hexaploid bread-wheat landrace Chinese Spring and the wild diploid wheat relatives einkorn (*Triticum urartu)* and goatgrass (*Aegilops tauschii*) were generated using short-read Roche 454 and Illumina sequencing (10, 48, 56). Although these assemblies provided a first snapshot of the wheat genome and its gene space in particular, they are still highly fragmented and consist of tens of thousands of unordered scaffolds. The scaffold N50 lengths of these early assemblies were in the range of a few kilobases. In 2014, the International Wheat Genome Sequencing Consortium (IWGSC) adopted a novel strategy by generating a whole draft genome sequence of Chinese Spring from single isolated chromosome arms (44). This approach was based on the ability to isolate single wheat chromosome arms from aneuploid Chinese Spring lines by flow cytometry (23), which allowed unambiguous assignment of

sequence scaffolds to individual chromosome arms. The order of the genic sequences was inferred through a method named genome zipper, which makes use of the fact that gene order is highly conserved among different grass species (64). Thus, the gene order from grass species with completed high-quality genome sequences such as rice (43), sorghum (71), and *Brachypodium distachyon* (96) was used to infer the gene order in wheat. However, this approach did not allow the capture of large structural rearrangements between wheat and other grasses and is also prone to errors in regions in which gene order is poorly conserved or that contain large chromosomal translocations. In the same year as the Chinese Spring draft genome sequencing, a high-quality genome assembly of the 1-Gb Chinese Spring chromosome 3B was reported (19). For this assembly, a physical map was initially produced by painstakingly fingerprinting and ordering 67,968 BAC clones (72, 80). The resulting sequence assembly had a scaffold N50 of 892 kb, which provided a much improved contiguousness compared to the whole-genome shotgun assemblies that were published around the same time (44).

The End of the Rocky Road: Chromosome-Scale Assemblies of Wheat Genomes

A quantum leap toward the improvement of cereal genomes was recently achieved through several technological and conceptual advancements like integrating whole-genome shotgun libraries of various insert sizes (35), the use of long-read sequencing technologies (49) and chromosome contact maps (Hi-C) (63), and the development of better assembly algorithms (20). The first high-quality genome sequence of a wheat relative was generated from the wild emmer (*Triticum turgidum* ssp. *dicoccoides*) accession Zavitan (4). The sequencing of several whole-genome shotgun libraries resulted in a 10.1-Gb assembly with a scaffold N50 of nearly 7 Mb (i.e., 50% of the assembly is represented in sequence scaffolds of at least 7 Mb), which is 50-times longer than a large BAC clone and represented an improvement by a factor of a hundred to a thousand over the previous whole-genome assemblies. Integration of additional Hi-C chromosome conformation capture sequencing allowed assembly of these scaffolds into 14 pseudomolecules matching the 14 chromosomes of tetraploid wild emmer.

Similar strategies recently allowed generation of a high-quality genome assembly of the hexaploid wheat landrace Chinese Spring (45). The 21 resulting pseudomolecules have a cumulative length of 14.1 Gb and thus represent near-complete chromosomes. The total size of the assembly is close to previous estimates of the hexaploid wheat genome size, which were based on cytometric measurements and ranged from 15.9 Gb (3) to 17.3 Gb (5). The difference from previous estimates is likely due to the presence of highly repetitive sequences (e.g., ribosomal DNA clusters), which were collapsed during the assembly, and possibly also due to an overestimate in the original numbers (45). The quality of the new Chinese Spring assembly surpasses that of previous assemblies. The new assembly has a scaffold N90 of 4.1 Mb (i.e., 90% of the assembly is represented in scaffolds of at least 4.1 Mb). This near-complete assembly allowed for the first time a genome-wide assessment of genic and repetitive regions. Gene content of the three subgenomes was found to be very similar, with each containing approximately 34,000 to 35,000 genes. Furthermore, gene order and spacing are well conserved between subgenomes. Nevertheless, approximately 15% of the homologous triads (i.e., groups of orthologs across the A, B, and D subgenomes) showed duplications of genes in at least one subgenome, indicating that many gene loci are prone to copy number variation.

In the near future, a high-quality assembly of the tetraploid durum wheat cultivar Svevo will become available (International Durum Wheat Sequencing Consortium, unpublished data). Together with genome sequences of diploid wheat progenitors such as the one of *A. tauschii* (59), future comparative analyses can be performed across genomes of three ploidy states. Furthermore,

an effort is underway to sequence a total of ten wheat genotypes at the same level of quality as the IWGSC reference sequence for Chinese Spring (**http://www.10wheatgenomes.com**). Comparative analysis across these ten genotypes will provide insight into differences in gene content, large-scale chromosomal rearrangements, and haplotype diversity.

Whole-Genome Sequences Assist Identification and Cloning of Disease-Resistance Genes

The availability of near-complete chromosome sequences marked a milestone for gene cloning because it became possible to navigate within wheat genomes and to get insight into large contiguous genomic regions. This will make map-based cloning much simpler and faster in the future. Furthermore, the best markers identified in genome-wide association studies (GWASs) can be rapidly anchored to large contigs, allowing the definition of candidate genes for the trait of interest. Finally, these assemblies allow rapid identification of potential disease-resistance genes across entire genomes based on homology with already known disease-resistance genes. For example, many disease-resistance genes encode NLRs (22). Based on this knowledge, Steuernagel et al. (91) developed an in silico NLR-Annotator algorithm that allows detection of NLR genes based on the presence of conserved motifs that are typically found in NLRs. A systematic evaluation of the Chinese Spring reference sequence (45) revealed the presence of 3,400 NLR loci in the Chinese Spring genome, serving as potential candidates for disease-resistance genes (**Figure 2**). NLR density was higher toward the telomeres and rapidly decreased toward the centromeric regions. Interestingly, a literature survey with known and mapped race-specific leaf and stem rust resistance loci revealed that many of them coincided with regions that contained only a few NLR genes in Chinese Spring (B. Steuernagel, personal communication) (**Figure 2**). These results show the enormous value

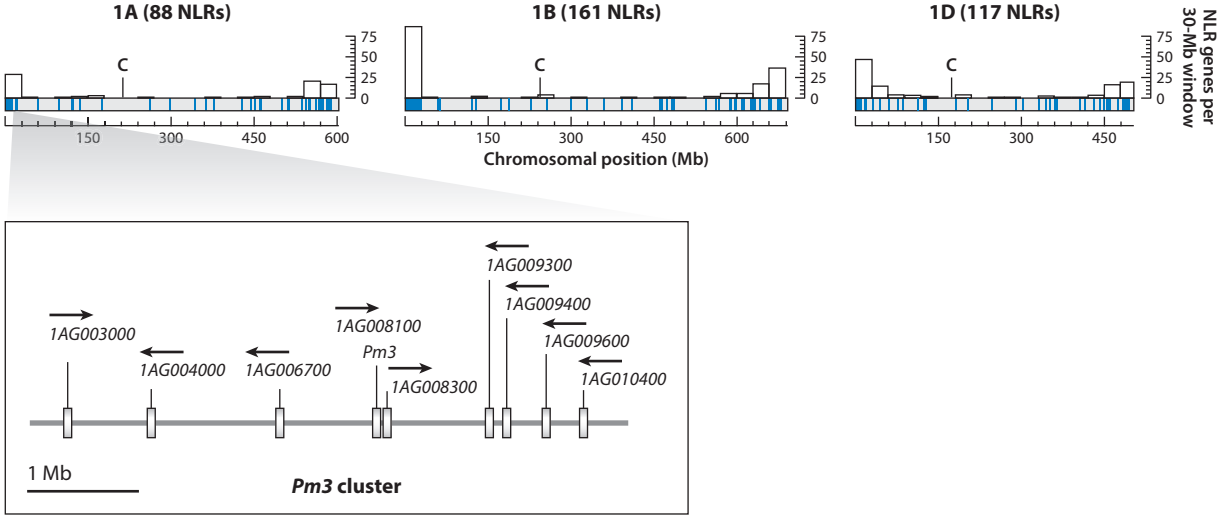

Figure 2

Distribution of nucleotide binding-site–leucine-rich repeat (NLR) genes across wheat chromosomes. Shown are group 1 chromosomes as representatives of the whole genome. NLR genes are enriched in distal regions of chromosomes. Locations of individual genes are indicated with blue lines. The density of NLR genes is indicated as a bar plot in windows of 30 Mb. The inset shows the structure of the *Pm3* powdery mildew resistance locus on chromosome 1A. The gray boxes indicating the genes are not drawn to scale. Abbreviation: C, centromere.

and potential of high-quality wheat genome sequences for gene cloning and a rapid increase of the number of cloned disease-resistance genes can be expected in the near future. Furthermore, it has been found that orthologous genes can have a conserved function in disease resistance across different species. An example is the mildew A (*Mla*) locus that was originally identified in barley, where it confers resistance against the fungal barley powdery mildew disease (*Blumeria graminis* f. sp. *hordei*). The *Mla* locus consists of multiple copies of paralogous NLR genes (101), but the allelic series of the active *Mla* resistance gene in different genotypes is derived from only one of them (86). Subsequent to the identification of *Mla* in barley, orthologous wheat and rye genes of *Mla* were identified that confer resistance against wheat powdery mildew (*B. graminis* f. sp. *tritici*) and stem rust (*P. graminis* f. sp. *tritici*) (51, 62, 73). The availability of high-quality wheat reference genomes greatly simplifies the identification of orthologs of known resistance genes from other cereals. The identification of resistance gene orthologs is of great relevance for practical breeding, as was shown by studies on a specific wheat suppressor of the rye *Pm8* powdery mildew resistance gene (39, 40). In this work, the wheat ortholog *Pm3CS* (a susceptible allele of the *Pm3* allelic series of resistance genes) of *Pm8* was shown to suppress *Pm8*. Resistance gene suppression has been observed frequently in wheat resistance breeding, and homologous or orthologous suppressor genes might be quite common. Such putative suppressor genes can now be identified and functionally tested in the transient *Nicotiana benthamiana* system (40), and they could be eliminated by gene editing.

Rapid Gene-Cloning Approaches from Nonreference Wheat Cultivars

Despite the progress in generating high-quality reference genomes, one particular problem remains; the gene of interest might only occur in a very specific wheat cultivar or landrace from a genebank collection and might be absent from the reference genotypes. It is known that sequence variation between different wheat cultivars can be extensive, including large structural rearrangements (50, 61). In particular, regions harboring disease-resistance genes have been found to be rapidly evolving and diverse (46). Thus, although high-quality reference sequences of particular genotypes are invaluable resources to define and characterize a region of interest, it might not be possible to identify the actual coding sequence of a specific gene from the reference cultivars. One exception is the durable multipathogen resistance gene *Lr34*, which is present in Chinese Spring and codes for an ABC transporter protein (55). The *Lr34* gene was cloned in 2009 by mapping the gene to a 0.15-cM genetic interval. A considerable amount of time was spent in assembling the corresponding 370-kb physical interval from four overlapping BAC clones. With the availability of the Chinese Spring reference sequence, the same task could now be completed within a few minutes instead of several months. Chinese Spring also contains the *Zymoseptoria tritici* resistance gene *Stb6* that was recently found to encode a wall-associated kinase (WAK)-like receptor-like kinase (81). To establish the physical interval of the *Stb6* target region, the chromosome survey sequences of Chinese Spring were used, which allowed direct identification of candidate genes (81). However, these two examples are exceptions and most disease-resistance genes are not present in Chinese Spring or the wild ancestors that have been sequenced at high quality.

As described above, map-based cloning approaches in wheat have been slow and tedious in the past. Several recent strategies have been developed to overcome the limitations associated with classical map-based cloning (98). In addition, these approaches aimed to facilitate gene cloning directly from the cultivar of interest to eliminate the problem of genotype-to-genotype variation. One of these approaches, mutant resistance gene enrichment sequencing (MutRenSeq), makes use of the fact that disease-resistance genes often encode NLR proteins. Based on this knowledge, an exome capture approach was developed that allowed enrichment of NLR-specific wheat sequences (91). The enrichment library was based on 60,000 NLR baits that were designed on publicly

available wheat and barley genome sequences. As a proof-of-concept, MutRenSeq was applied to rapidly clone the two wheat stem rust resistance genes *Sr22* and *Sr45* (91). To isolate *Sr22*, captured NLR short-read sequence contigs of six ethyl methanesulfonate (EMS)-derived *Sr22* loss-of-function mutants in the genetic background of the *Sr22*-carrying Australian wheat cultivar Schomburgk were compared to the respective wild-type contigs, which revealed a single contig with independent mutations in five of the six loss-of-function mutants. A similar strategy was used to isolate *Sr45* from the wheat line CS1D5406. The great advantages of MutRenSeq are that it does not require fine-mapping and that it can be applied to any wheat cultivar. However, as the molecular identification of *Stb6* gene showed (81), not all (race-specific) disease-resistance genes encode NLR proteins and this must be considered when applying MutRenSeq.

Other approaches utilized genome complexity reduction through the isolation of individual wheat chromosomes. For example, the race-specific wheat powdery mildew resistance gene *Pm2* was cloned by isolating and sequencing chromosome 5D of a *Pm2*-carrying wheat line and six independent EMS-derived *Pm2* loss-of-function mutants using flow cytometry and short-read sequencing. Comparison of sequence contigs of the six mutants to the *Pm2* donor line allowed delimitation of the causal mutations to a single gene encoding an NLR protein. This approach that combines EMS mutagenesis, chromosome flow sorting, and short-read sequencing was named MutChromSeq (**Figure 3*a***) (85). A third rapid gene-cloning approach used chromosome flow sorting in combination with long-range scaffolding to rapidly generate high-quality assemblies of target intervals identified through genetic mapping (**Figure 3*b***). By using this approach, the leaf rust resistance gene *Lr22a*, previously mapped to chromosome 2D (34), was isolated. To sidestep the tedious process of assembling the *Lr22a* target region through multiple rounds of BAC library screening, chromosome 2D from the *Lr22a*-containing wheat line CH Campala *Lr22a* was isolated and the corresponding physical interval was rapidly established by using a combination of short-read sequencing with chromosome contact maps of chromosomes reconstituted in vitro (Dovetail Genomics Chicago method) (76, 97). The resulting assembly had a scaffold N50 of 9.76 Mb. In other words, this approach generated scaffolds of the target region that were 50–100-fold longer than a BAC clone. *Lr22a* was found on a single scaffold of 6.39 Mb that contained both *Lr22a* flanking markers. Using five independent EMS-derived loss-of-function mutants, *Lr22a* was shown to encode an NLR protein (97). Hence, this approach, named targeted chromosome-based cloning via long-range assembly (TACCA), eliminates the need to construct BAC libraries for gene cloning. MutRenSeq, MutChromSeq, and TACCA provide a significant reduction in the time and costs needed to isolate genes. Although in the past, it was not unusual to spend 5–10 years on a gene-cloning project, these novel approaches allow isolation of genes in fewer than two years.

Other recent developments allow reduction of the time and effort needed to validate the phenotypic effects of specific candidate genes. For example, the exome-captured coding sequences of 2,735 EMS mutagenized plants, 1,535 in the background of the tetraploid durum variety Kronos and 1,200 in the genetic background of the UK hexaploid wheat cultivar Cadenza, were recently sequenced and deposited in a public database. This work revealed more than 10 million mutations in 48,172 and 73,895 gene models in Kronos and Cadenza, respectively (54). This resource was already successfully used to validate candidate genes in the *Stb6* target region, which helped to determine the WAK-like receptor-like kinase as the *Stb6* gene (81).

Genomics of Genebanks: Unlocking the Treasure of Diversity to Increase Disease Resistance

A greatly underutilized resource for the improvement of disease resistance consists in the hundreds of thousands of wheat accessions that are stored and maintained in genebanks worldwide

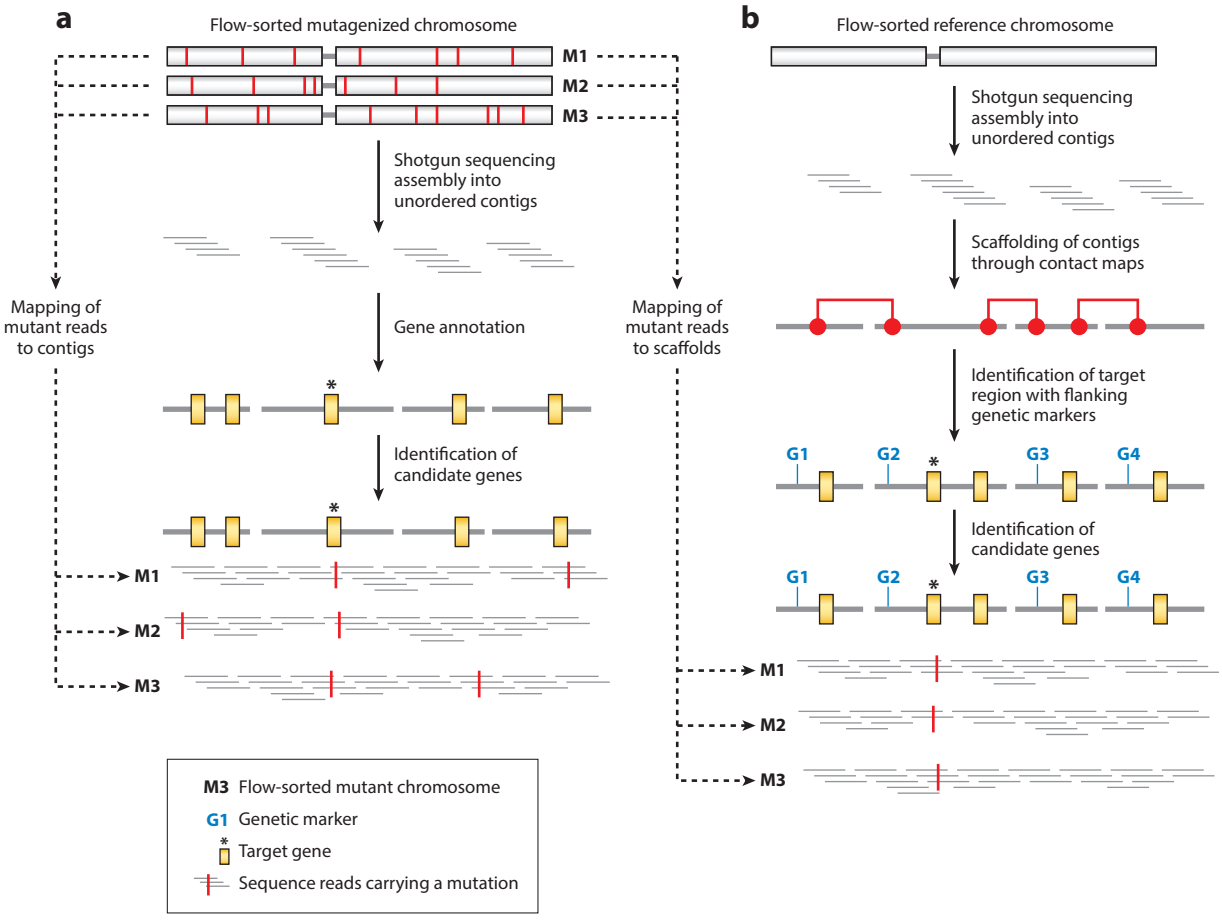

Figure 3

Cloning of target genes using next-generation sequencing technologies. (*a*) Chromosomes from individual mutagenized wheat plants are isolated via cytometric flow-sorting. Illumina shotgun sequences are then assembled into (usually several thousand) sequence contigs. The target gene is identified by mapping of Illumina reads of the individual mutants onto the sequence contigs (i.e., the target gene carries mutations in all independent mutants). (*b*) The chromosome containing the target gene is isolated via flow-sorting and sequenced with Illumina technology. Contact maps (e.g., through reconstituted chromatin) are used to construct megabase-size scaffolds. The target interval on a single scaffold is identified by mapping genetic markers flanking the gene onto the assembly. The target gene is identified by mapping of Illumina reads of the individual mutants onto the sequence scaffolds.

(7, 58). These collections hold an enormous richness in genetic diversity, novel disease-resistance genes, and alleles. Wheat breeders have used old landraces and wild-wheat progenitors to increase the diversity of elite wheat genepools, for example through the generation of synthetic wheat or through alien introgressions of chromosome fragments from wild grass species into cultivated wheat. These attempts aim to compensate for the loss of diversity that went along with domestication and modern breeding (94). One of the most extensive synthetic wheat breeding programs has been performed at the International Wheat and Maize Improvement Center (CIMMYT), where more than 1,000 synthetic wheat lines from more than 900 different *A. tauschii* accessions have been produced since the 1990s (24). One of the major drawbacks of using old landraces and wild-wheat progenitors in modern breeding is that undesired traits that result in reduced

yield or quality are often cointroduced when landraces or wild progenitors are crossed with elite wheat cultivars. The use of genomic information for marker-assisted selection allows breakage of this linkage drag and the specific introduction of desired genes. However, even with the recent advances in genome sequencing, the generation of whole-genome assemblies from thousands of wheat accessions is not yet feasible. Hence, to assess the genetic variation in large collections, different genotyping methods are required. For example, several array-based SNP platforms have recently been developed that allow rapid and cost-effective genotyping of many different wheat accessions using a predefined set of markers. These arrays contain between 9,000 and 819,571 SNP markers and are available from different SNP genotyping platforms such as the Affimetrix's Axiom or Illumina's Infinium technology (2, 17, 77, 99, 103). For example, the wheat 9K iSelect Beadchip Assay was used to genotype 2,994 diverse hexaploid wheat accessions, including landraces and modern cultivars (17). The so-called wheat breeders' array containing 35,143 SNP markers was tested on a set of 1,779 hexaploid wheat accessions (2). Other approaches to rapidly genotype many wheat accessions include exome capture (52) and genotyping-by-sequencing (74). Current projects aim to obtain a comprehensive genomic insight into entire cereal genebank collections. For example, the goal of the BRIDGE (biodiversity informatics to bridge the gap from genome information to educated utilization of genetic diversity hosted in Genebanks) project is the genotyping of 20,000 barley accessions held in the IPK genebank using genotyping-by-sequencing (**http://apex.ipk-gatersleben.de/apex/f?p=164:1**). Similarly, the Seeds of Discovery project of CIMMYT aims to genotype wheat accessions of the CIMMYT genebank and to complement these data with phenotypic information. The combination of these genotypic data with phenotypic information allows the discovery of marker-trait associations through GWASs and the uncovering of novel disease-resistance genes. Whereas in the past, producing the genotypic information might have been the bottleneck, the limitation in the number of accessions that can be evaluated at present comes from the space and effort needed for accurate phenotyping.

Along with SNP genotyping platforms, approaches like the NLR capture arrays can be used to assess the diversity of NLR genes in genebank collections. Because this approach allows reduction of the size and complexity of the wheat genome, the sequencing of the NLRome from a single wheat cultivar becomes relatively cheap and can be applied to many wheat accessions.

GENOMICS OF WHEAT PATHOGENS: IMPLICATIONS FOR RESISTANCE BREEDING AND DISEASE CONTROL

The increasing pace of genome-based molecular identification of disease-resistance genes in wheat is complemented by an increased understanding of the molecular components from the pathogen involved in the resistance interactions. The combination of these different fields of research and a complete molecular comprehension of pathosystems will allow breeders to integrate pathogen-based information into the development of sustainable strategies for the use of resistance genes. The major wheat pathogens are fungal organisms and some of their genomes have been completely sequenced. Depending on the technology used and the genome size of the respective pathogen, the available genome sequences are of different quality in terms of number and contiguity of fragments: the complete genomes of *Stagonospora nodorum* (33), wheat stem rust (*Puccinia graminis* f. sp. *tritici*) (25), *Zymoseptoria tritici* (95), wheat stripe rust (*Puccinia striiformis* f. sp. *tritici*) (15), wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*) (102), and wheat leaf rust (*Puccinia triticina*) (53) and the transcriptome of wheat blast (47) have been determined. These genome sequences have been complemented for some pathogens by other genomic tools such as BAC libraries and physical maps (70, 102), and all these resources have been used for the development of pathogen-based strategies to improve resistance. Here, only a few selected studies are presented and discussed in more detail to demonstrate the applications of such knowledge for resistance breeding.

The molecular analysis of wheat-pathogen interaction has focused mostly on the host, but a more complete understanding of the pathosystems depends on the identification of the molecular factors from the pathogen recognized by the immune receptors encoded by wheat resistance genes. The molecular identification of such recognized pathogen factors, also called avirulence (Avr) proteins, has lagged behind in wheat pathogens. Only recently, the new genomic tools have allowed the isolation of a number of avirulence genes. GWASs have been successful in the identification of virulence/avirulence genes in surprisingly small populations (31, 75), and population genomics of fungal wheat pathogens is a highly productive area of research (32).

In wheat powdery mildew, two *Avr* genes have been isolated using map-based cloning approaches (8) or combined methods integrating GWASs (75). The activity of the cloned genes as specific *Avr* genes was confirmed in transient expression assays in Nicotiana, which result in cell death when corresponding NLR and *Avr* genes are coinfiltrated. This transient assay will also be of great importance for future identification of many additional *Avr* genes. In the near future, it should be possible to identify all *Avr* genes for which the corresponding immune receptor gene has been cloned from the wheat genome. Interestingly, for wheat powdery mildew, it was found that a suppressor gene is also involved in recognition specificity (8, 9). Thus, the pathogen can hide the presence of an *Avr* gene by expressing a suppressor gene and thus avoid recognition. If the corresponding immune receptor is not present in a host, the pathogen can easily regain the Avr function (possibly an effector function increasing virulence) by removing the suppressor gene by recombination.

The *AvrPm2* gene is recognized by the wheat *Pm2* resistance gene. It was found that not only is *AvrPm2* present in wheat mildew but that a highly similar gene is also present in rye powdery mildew, and the product of this rye mildew gene is recognized by the Pm2 immune receptor. This provides a general explanation for the successful introgression breeding of resistance genes from wheat relatives into wheat such as the wheat-rye translocations: A subset of *Avr* genes might be present in mildews specialized on different hosts, and, therefore, *NLR* resistance genes are active in different hosts against different host-specialized mildews (66, 75).

The recent identification of the *AvrSr35* and *AvrSr50* genes in wheat stem rust (18, 84) and *AvrStb6* in *Zymoseptoria* (107) represents important steps forward in our understanding of these host-pathogen interactions. These discoveries were all based on whole-genome analysis and describe the first *Avr* genes from these species. In *Zymoseptoria*, the identification of *AvrStb6* complements the molecular identification of the *Stb6* resistance gene (81). Very interestingly, *AvrStb6* encodes a secreted, small effector protein, very similar to the fungal Avr proteins recognized by intracellular immune receptors of the NLR class (8, 75). However, as described above, the corresponding *Stb6* gene encodes a receptor-like kinase with an extracellular WAK-like domain. From work in the model plant *Arabidopsis thaliana*, it has previously been established that WAK domains generally bind oligosaccharides (12). If we assume a direct interaction between Stb6 and AvrStb6, their interaction occurs in the apoplastic space, and Stb6 thus perceives a protein ligand rather than an oligosaccharide. The molecular details of this putative interaction remain to be determined and represent a challenging novel frontier in disease-resistance biology. Clearly, it is highly important to further study WAK resistance genes, given that there are several hundred WAK encoding genes in the wheat genome, with *Stb6* being the only member with an identified function (45). Also, WAKs were recently found to be key players in disease resistance against fungal pathogens in other cereals, including rice and maize (36, 41, 108).

The molecular identification of genes encoding avirulence as well as suppressor genes results in the development of specific markers for them, allowing easy determination of their presence and evolution in pathogen populations. This simplifies the analysis of pathogen avirulence patterns over time and their distribution and frequency in specific agricultural regions, which contributes

to the rational resistance gene deployment in agriculture. Furthermore, molecular knowledge of recognized Avr proteins might allow the molecular breeder to select combinations of genes with complementary specificities and lower risks for pathogen adaptation.

Pathogens can rapidly evolve and adapt to disease resistance, particularly in agricultural ecosystems (68). Studies at the genomic level have recently revealed the evolutionary history of two newly emerged pathogens. One of these is the wheat blast pathogen that originated in South America and is now threatening wheat production in parts of Asia. It was found that wheat blast evolved via the functional loss of a host specificity determinant (42).

Around the year 2000, a novel powdery mildew form was discovered that is able to grow on the rye-wheat amphiploid crop species Triticale. It was found that this novel form of mildew was the product of a hybridization event between the mildew forms specialized on wheat and rye. Thus, pathogen evolution mirrored host evolution of Triticale (67). This finding also indicates that such hybridization events among different powdery mildew forms must be considered for successful introgression of resistance genes from wild-wheat relatives into wheat. Obviously, wheat mildew can exchange genetic material with mildews from other grass species, resulting in novel virulence properties and a powerful evolutionary mechanism to overcome specific resistance genes introgressed from wheat relatives into wheat.

Pathogen genomics has also proven to be a powerful tool in the identification of the causal pathogen races of new stripe rust epidemics in the United Kingdom (38). In an approach called "field pathogenomics," transcriptome sequencing was performed on infected wheat leaves from the field. This showed that the pathogen population dramatically changed within a few years and increased in diversity. Field pathogenomics is a very promising way for genotyping a large number of isolates and can rapidly deliver a view on an emerging epidemic. Thus, it has the potential for wide application to many pathogens and at a global scale.

As a final example of pathogen genomics, the global, transcriptional response in pathogens to the presence of the durable, quantitative *Lr34* resistance gene is discussed here (55). As mentioned above, *Lr34* confers resistance against several pathogen species. Transcriptomes of leaf rust and powdery mildew pathogens growing on partially resistant, *Lr34*-containing wheat or barley host plants, respectively, were compared to pathogens growing on (near-)isogenic host genotypes without *Lr34* (93). Surprisingly, there was no transcriptional response in the pathogens to the presence of *Lr34*, despite slower and reduced growth. Thus, pathogens do not react to slower growth caused by *Lr34*, and it is tempting to speculate that this might be a general property of quantitative and partial disease-resistance genes that could be used as a predictive feature of durable resistance. This hypothesis can now be tested for additional quantitative resistance genes for which isogenic lines are available.

The rapid developments in fungal pathogenomics exemplified above complement the molecular analysis on the host side, and breeding strategies can now integrate the knowledge on pathogen evolution and molecular biology. There is a rapidly developing field of research based on pathogen-informed strategies that takes into consideration these findings at the molecular, functional, evolutionary, and population levels for improvement in resistance breeding strategies (13).

GENOMIC-ASSISTED DISEASE-RESISTANCE BREEDING: A LOOK INTO THE NEAR FUTURE

Despite considerable international effort, only approximately 6% of the known and mapped wheat disease-resistance genes have been cloned so far, and this does not include the yet undiscovered genes hidden in genebank collections. With the availability of high-quality wheat reference sequences, high-throughput marker technologies, and several rapid gene isolation strategies, the

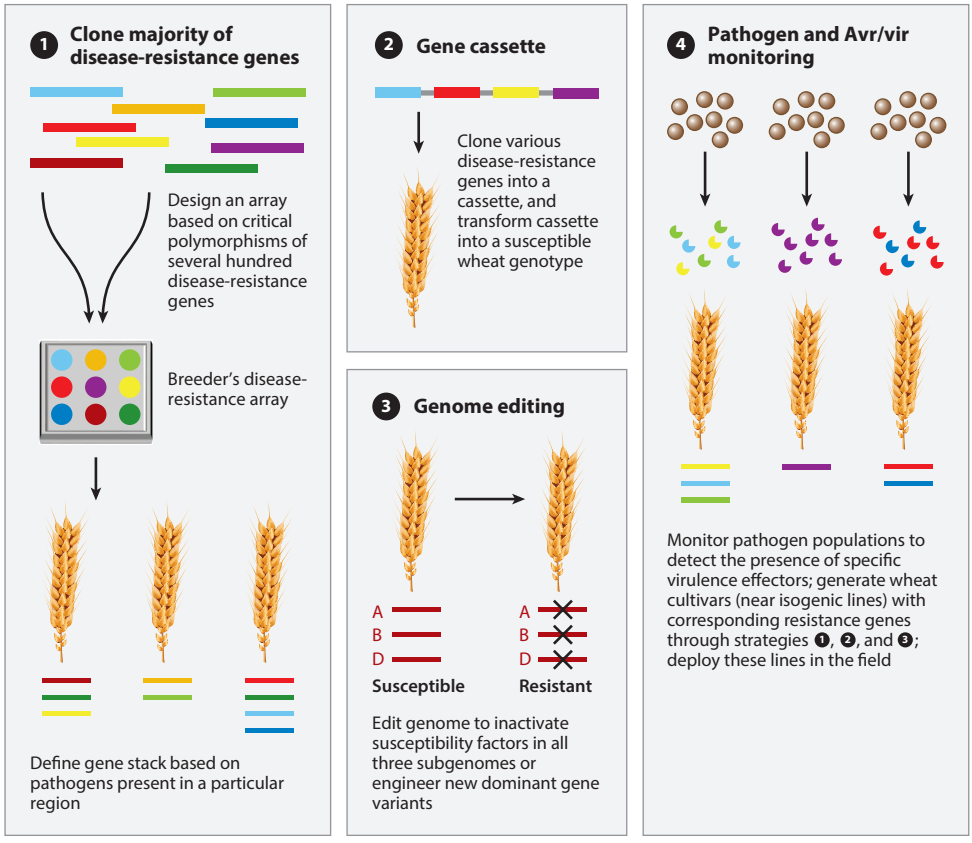

Figure 4

Genomic-informed breeding strategies that could be used to increase disease resistance in wheat. ❶ The cloning of the majority of the described and mapped disease-resistance genes would allow the design of a breeder's disease-resistance array based on highly diagnostic markers. ❷ Gene cassettes with a specific combination of multiple disease-resistance genes could be transformed into susceptible wheat cultivars. ❸ Genome editing could be used to engineer resistance in wheat. ❹ A combination of disease monitoring programs and novel breeding tools allows making informed choices of resistance genes based on the prevalent pathogen races.

cloning of disease-resistance genes will become easier, quicker, and less costly. Hence, it is now the right time to discuss the cloning of most of the 400 known disease-resistance genes (65) in an international effort and to study the critical sequence polymorphisms that distinguish susceptible from resistant alleles. This work could reveal perfect functional molecular markers of an extensive gene set that could be used for breeding. Wheat breeders could choose their preferred resistance genes based on the diseases prevalent in a particular region and stack these genes in single cultivars through marker-assisted selection (**Figure 4**). A particular challenge, however, is that a considerable number of segregating genes from different sources need to be combined in a single cultivar to achieve durable resistance. These genes then need to be maintained in subsequent crosses to avoid the thinning out of disease resistance. A strategy that could overcome this problem is through the use of gene cassettes, where several disease-resistance genes are cloned into the same vector and jointly transformed into susceptible cultivars (26, 104). The bottleneck of this strategy has so far been limitations in transformation technology because the resulting

constructs would likely be several dozen kilobases in length. As a different approach, disease resistance could also be engineered by the modification of specific genes through genome editing. A relatively simple approach would be to use genome editing to eliminate susceptibility genes. It has been shown that some necrotrophic fungi require host specific susceptibility genes to establish infection (27, 87). These susceptibility genes show a remarkable similarity to genes that confer resistance against biotrophic fungi, and it has been suggested that necrotrophic pathogens can hijack the plant's disease-resistance mechanisms and provoke cell death through hypersensitive response (HR). HR is a common outcome of resistance reactions against biotrophic pathogens that depend on living host tissue, but HR does provide the basis for necrotrophic fungi to proliferate on dead host tissue. For example, the wheat *Tsn1* gene encodes a protein with a leucine-rich repeat domain similar to the one found in NLR proteins. *Tsn1* is required for successful infection by the fungal pathogens that cause Stagonospora nodorum blotch and tan spot (29). These fungi produce the host-selective toxin ToxA, and it has been hypothesized that ToxA perception by Tsn1 triggers HR. Similarly, the wheat *Snn1* gene coding for a WAK has been shown to perceive the toxin SnTox1 of the necrotrophic pathogen *Parastagonospora nodorum* (87). The removal of susceptibility genes like *Tsn1* or *Snn1* through marker-assisted selection or genome editing would permanently increase disease resistance against necrotrophic fungi. However, it is likely that genes like *Tsn1* or *Snn1* may have important functions in wheat plants other than serving as entry points for pathogens. Therefore, whether the systematic removal of susceptibility genes compromises wheat yield or quality needs to be carefully evaluated. Genome editing might also be particularly useful in polyploid wheat species because it allows simultaneous targeting of the homologous gene copies on all three subgenomes. For example, the *mlo* resistance of diploid barley provides one of the most durable sources of resistance against the fungal powdery mildew disease (*B. graminis* f. sp. *hordei*). The *mlo* resistance in barley arose through natural and induced loss-of-function mutations in the *Mildew resistance locus O* (*Mlo*) gene coding for a plasma membrane–localized protein with unknown biochemical function (14). Natural *mlo* resistance is not known in polyploid wheat because it requires simultaneous knockouts in all three homologous *Mlo* copies. However, it was recently shown that targeting the three homologs of *Mlo* through genome editing or EMS mutagenesis resulted in increased resistance of wheat against wheat powdery mildew (*B. graminis* f. sp. *tritici*) (1, 100). Work on the *Pm3* powdery mildew resistance gene of wheat, which codes for an NLR protein, showed that single amino acid polymorphisms can result in an increased resistance spectrum or stronger disease signaling (11, 92). These studies showed that it would be possible to engineer broad-spectrum NLR genes, although the targeted modification of specific amino acids is more difficult to achieve than loss-of-function modifications. Lastly, the emerging field of pathogenomics described above allows precise monitoring of the presence or absence of avirulence genes in pathogen populations. This knowledge can then be used to choose and grow wheat cultivars with disease-resistance genes that are effective against the pathogen populations prevalent in a certain region. Hence, instead of hoping that wheat cultivars will be resistant against the prevalent pathogens, the choices of which disease-resistance genes to deploy can be based on genomic information from pathogens, which greatly increases the effectiveness of disease resistance in the field.

SUMMARY POINTS

1. Because of recently developed genomic resources, gene isolation in wheat has become easier, and there is a rapidly increasing number of cloned disease-resistance genes.

2. The first high-quality reference sequences of wheat genomes are available and reveal a large number of candidate resistance genes of the NLR and WAK classes.
3. New approaches based on sequencing complete, flow-sorted chromosomes, or array-based technologies allow rapid isolation of resistance genes based on mutants. These approaches are independent of the chromosomal position, do not depend on recombination, and can be used in any genotype of interest.
4. Because of pathogenomics, there has been rapid progress in the isolation of avirulence genes in several fungal wheat pathogens. For these genes, the corresponding resistance genes have been isolated and the molecular interactions can be studied.
5. Knowledge on molecularly identified susceptibility or resistance suppressor genes can be used for selection against their presence or for inactivation by gene editing.
6. Genomics on both the host and pathogen sides is providing resistance breeding with novel tools such as perfect markers, isolated genes for gene cassettes, and pathogen-informed breeding strategies.

FUTURE ISSUES

1. Genome sequences from dozens of wheat genomes with a similar quality to the reference genome are needed to define the pangenome for resistance gene loci. This includes genotypes of important wild grass donors of resistance genes.
2. A large international collaboration is needed to isolate all resistance genes described in the wheat gene catalog that are important for breeding. Novel genes identified in genebank material need to be included. Such a goal has become feasible based on novel and future chromosome and genome-wide strategies based on mutants.
3. Quantitative trait loci for durable resistance should be approached in a similar way. This needs the development of near-isogenic lines and high-quality phenotyping to identify mutants in such lines.
4. For each major wheat disease, appropriate sets of lines should be phenotyped and genotyped for GWASs. This allows identification and isolation of novel genes that have not yet been genetically described.
5. For all disease-resistance genes important in breeding, the corresponding avirulence genes should be isolated to get a comprehensive view on the molecular interactions and develop pathogen-informed breeding strategies.
6. Field pathogenomics should be performed for all major wheat diseases in all major growth regions of wheat. This will result in a global view on pathogen diversity and evolution.
7. Research is needed to identify the best strategies for the integration of the rapidly developing knowledge in different fields into efficient regional breeding programs.

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