

8

# Breeding Strategies for Developing Disease-Resistant Wheat: Present, Past, and Future

Anuj Choudhary, Antul Kumar, Harmanjot Kaur, Vimal Pandey, Baljinder Singh, and Sahil Mehta

#### Abstract

Since its origin in Southeast Turkey, wheat (*Triticum aestivum* L. AABBDD; Family Poaceae) has been a prime dietary cultivated cereal that is consumed worldwide by nearly 20% of the world population. However, there are a wide plethora of biological variables that seriously threaten production around the world. Among the biological stresses, phytopathogens are considered the most serious threat to yield. This can be further elaborated by the fact that since the nineteenth century, more than 30 diseases have been reported to have had a drastic impact as epidemics, including karnal bunt, smut, mildew, blight, rust, etc. So far, in response, various landraces and several wild-related genera (such as Thinopyrum, Triticum, Hordeum, Aegilopsis, Elymus, and Leymus) represent the different gene pools that have been utilized in developing disease-resistant varieties. With the emergence of advanced molecular markers, whole genome sequences, and new genomic approaches, there are multiple ways and tools for researchers to enhance durability and wide-range disease resistance in a short period. The present documentation of trait introgression offers an effective option to narrow down the cost of unsustainable fungicides. Therefore, the current

A. Choudhary · A. Kumar · H. Kaur

Department of Botany, Punjab Agricultural University, Ludhiana, India

V. Pandey

Department of Botany, Kalinga Institute of Social Sciences, Bhubaneswar, Odisha, India

B. Singh National Institute of Plant Genome Research, New Delhi, India

S. Mehta (⊠) School of Agricultural Sciences, K.R. Mangalam University, Gurugram, Haryana, India

137

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chapter is an attempt to incorporate various successful reports regarding the development of more resistant wheat cultivars using new breeding strategies.

#### **Keywords**

Wheat · Diseases · Productivity · Fusarium · Spot blotch · Lr9 gene

#### Abbreviations

AgRenSeq	Associated genetics R gene enrichment sequencing
Cas9	CRISPR-associated protein 9
CRISPR	Clustered regularly interspaced palindromic repeats
dsRNA	Double-stranded RNA
EMS	Ethyl methanesulfonate
FHB	Fusarium head blight
GE	Genome editing
GWAS	Genome-wide association sequences
LRR	Leucine-rich repeat proteins
MAPK	Mitogen-activated protein kinase
miRNA	MicroRNA
MNs	Meganucleases
MutChromSeq	Mutant chromosome sequencing
NBS	Nucleotide-binding site
NLR	Nucleotide-binding and leucine-rich repeat
PGT	Puccinia graminis f. sp. tritici
PST	Puccinia striiformis f. sp. tritici
PT	Puccinia triticina
QTL	Quantitative trait locus
R gene	Resistance gene
siRNA	Small interfering RNAs
SSNs	Sequence-specific nucleases
TACCA	Targeted chromosome-based cloning via long-range assembly
TAL	Transcription-activator-like
TALENs	Transcription activator-like effector nucleases
ZFNs	Zinc-finger nucleases

# 8.1 Introduction

With the doubling of the human population, the past decade has witnessed significant growth in cereal production, resulting in a remarkable reduction in global food hunger (FAO STAT 2018; Liu et al. 2018; Grote et al. 2021; Jiang et al. 2020; Liu et al. 2021; Li et al. 2020a, b, c; Singh et al. 2021a). Despite no exaggeration, the

level of global poverty is currently lower than any recorded in modern times. Nonetheless, the goal of "zero hunger" is not achieved and requires significantly increased efforts (Mujeeb-Kazi et al. 2019; Shakeel et al. 2021; Singh et al. 2021b). However, more than one in seven individuals did not fulfill the prerequisite of a complete diet, and a higher number experienced different forms of malnutrition. However, demand for food surges exponentially, causing the continuous rise in population. As a result, qualitative and quantitative food production must be done in a remarkable two-fold manner that is both socially and environmentally sustainable (Hickey et al. 2019). The average grain production has increased from 1.35 tons/hectare (1961) to 3.35 tons/hectare (2007) and is expected to reach 4.8 tons/hectare by 2040. Recently, the agriculture area has been shrinking, with overarching issues like a serious threat of climate change, posing issues of how adaptation and mitigation mechanisms may impact food supply (Singh et al. 2022; Choudhary et al. 2021a; Paul et al. 2021).

Certain wheat diseases prominently contribute to losses by pathogens including viruses, bacteria, and fungi responsible for blight, scab, rust, smut, blotches, and blast diseases (Kumar et al. 2022a). Better management of fungal diseases is the need of the hour, which results in a 15–20% yield loss of wheat per year. Rust fungi are obligate biotrophic organisms that belong to the family Basidiomycete, which means they are dependent on the living cells of plants for growth and reproduction. Stem, stripe, and leaf rust are mainly three types of wheat rust diseases. Although the causative agent of black rust disease (wheat stem rust), Puccinia graminis sp. tritici, is widely distributed throughout the world, it is uncommon in comparison to other rust diseases. Although rust diseases are controlled in yield in most parts of the world, there are still global losses estimated at about 6.2 million metric tons per year (Pardey et al. 2013; Figueroa et al. 2018; Kumar et al. 2021b). There is a reduction in grain size along with the lodging of plants due to rust diseases (Miedaner and Juroszek 2021). Thus, the emergence of recent fascinating approaches, including clustered regularly interspaced palindromic repeats (CRISPR), CRISPR-associated protein 9 (Cas9), genome-wide association sequences (GWAS), transcription activator-like effector nucleases (TALENs), transcription-activator-like (TAL), Meganucleases (MNs), and zinc-finger nucleases (ZFNs), etc., helps to overcome the biotic and abiotic challenges in wheat (Mehta et al. 2020; Dilawari et al. 2021; Chattopadhyay et al. 2022; Schenke and Cai 2020; Razzag et al. 2021). The understanding of plant-pathogen interaction and the advancement of new approaches or molecular techniques including speed breeding, genome editing, CRISPR/Cas9 (Cluster Regularly interspaced Palindromic Sequences/CRISPRassociated protein 9), RNA interface (RNAi) Silencing, etc. are being harnessed for gene editing or alteration of traits (Chattopadhyay et al. 2022; Schenke and Cai 2020; Paul et al. 2021; Zhang et al. 2017a, b; Kis et al. 2019; Verma et al. 2021). Presently, conventional breeding approaches help to manage disease-free, highly productive, nutritious, and safe crops. It also includes interspecific hybridization, pure line selection, backcross, and pedigree methods (Kaiser et al. 2020).

In the present document, we highlight the significant role of emerging breeding techniques in the introgression of the novel resistance gene. We see advanced breeding strategies as an affordable and efficient way forward to overcome the consequences of climate change through the development of new resilient varieties. There are many strategies like integrated or multidisciplinary includes in agronomy pathology, seed production, pathology, postharvest methods, and extension (Raffan et al. 2021; Li et al. 2018, 2020a, b).

# 8.2 Disease's Epidemics and Their Impact on Productivity

Wheat is the most essential staple crop that impregnates the human diet with protein and calories (Rasool et al. 2021; Kumar et al. 2021b). The genetic diversity in the wheat gene pool has been statistically increased, offering the most promising possibilities to combat pathogen emergence meant to reduce the threat of diseases to global wheat production (Kumar et al. 2022b). The preliminary step has been integrated with traditional as well as advanced breeding tools to repair signaling loops that effectively combat a variety of pathogens.

Rust pathogens have a long history dating back to the domestication of crops. They have a good image in the hindrance of global wheat production. The global losses due to wheat rust pathogens are estimated in the range of 4.3-5 billion US dollars annually (Pardey et al. 2013; Tehseen et al. 2021). These are the obligatory biotrophic pathogens that have completed their life cycle for nutritional resources (Różewicz et al. 2021). Globally, there are three well-known rust diseases of wheat caused by genus Puccinia (belongs to family Basidiomycetes), stem rust caused by Puccinia graminis sp. tritici (PGT), stripe rust caused by Puccinia striiformis sp. tritici (PST), and leaf rust caused by Puccinia triticina (PT) (Różewicz et al. 2021). Wheat stem or black rust usually prevails in moist and warm conditions and materializes as red brick urediniospores on the stem, sheath, leaf, awns, and glumes of susceptible cultivars (Kolmer 2005; Gupta et al. 2017). However, Leonard and Szabo (2005) reported that the yield losses are due to the lodging of plants and grain size reduction in the infected cultivars. Stem rust epidemics have historically affected all major wheat-producing regions, and disease control was one of the major milestones in the development of stem rust-resistant high-yielding wheat cultivars during the green revolution (Figueroa et al. 2016).

According to forecasting models, the average loss is 6.2 million metric tons annually during serious epidemics in the absence of durable, resistant varieties (Pardey et al. 2013). The emergence of a new PGT population poses a threat on a global scale, such as the Ug99 race in Uganda (1998), which expanded within Africa, towards the Middle East, and was reported as Ug99 variants, showing the immense threat to the wheat crop (Pretorius et al. 2000; Singh et al. 2015). It has been estimated that about 90% of wheat varieties are prone to the Ug99 attack (Singh et al. 2011). The 'Digalu' race became an epidemic in 2014 in Ethiopia and was also observed in Germany (Olivera Firpo et al. 2015, 2017). Similarly, a "broadly" disease race was reported as the Sicily wheat outbreak in 2016 (Bhattacharya

2017). Subsequently, it was reported in Bangladesh in Asia and Zambia in Africa. Researchers have warned that there may be a possible expansion of disease to other continents as well (Tembo et al. 2020).

Wheat stripe or yellow rust is prevailing in the cool and wet conditions of temperate regions (Chen et al. 2014; Jamil et al. 2020). PGT is efficiently declining the wheat yield by affecting nearly 100% of the susceptible cultivars. It has been targeting 88% of the wheat varieties globally and losing 1 billion US dollars per year (Wellings 2011; Beddow et al. 2015). Moreover, Murray and Brennan (2009) reported 127 million AU dollar losses from stripe rust in Australia. In the last 50 years, PST has affected nearly 60 countries (Beddow et al. 2015). Since 2000, PST virulence races have been spread to the non-affected regions of the world by adapting to the higher temperatures of climates (Ali et al. 2014). The clonal distribution of PST in Australia, North America, and Europe showed a significant level of genetic diversity in the populations of pathogens (Chen et al. 2014). The variants were also found in Central Asia and Western China, as well as the Himalayas and their surrounding areas (Ali et al. 2014). Other race groups that originated in the Himalayan regions (Hovmøller et al. 2015) also appeared and spread in 2011, 2012/2013, and 2015 throughout Europe. Recent studies regarding P. striiformis concluded that most of the recombinant population structure and the highest levels of genetic diversity come from the Himalayan and its nearby regions, which shows that this may be the area of its center of origin and diversity (Sheikh et al. 2021).

Leaf rust is a well-known, common, and more widely distributed condition with a prevalence in moist and mild temperature conditions (Bolton et al. 2008). The yield losses are associated with the reduction in grains per head and the kernel weight. About 350 million US dollars in losses have been estimated from the period of 2000 to 2004 in America (Huerta-Espino et al. 2011). There were total losses estimated to be 12 million AU in Australia (Murray and Brennan 2009). The upper hand of the leaf rust is due to high diversity in the pathogen population and emerged strains showing wider adaptability too in a wide climatic range (Huerta-Espino et al. 2011; McCallum et al. 2016).

Blotch diseases including *Septoria nodorum*, blotch tan spot, and *Septoria tritici* blotch are caused by the *Pyrenophora tritici-repentis*, *Parastagonospora nodorum*, and *Zymoseptoria tritici*, *respectively*. *Septoria tritici* blotch is the leaf disease of wheat flourishing in the temperate regions. It is causing a primary threat to the wheat yield at the cost of 280–1200 EU € annually in Europe (Fones and Gurr 2015). This disease is causing 20 million AU\$ losses in Australia, annually (Murray and Brennan 2009). Tan spot disease is found in most wheat-growing regions such as North America, Australia, and Europe. The yield losses are due to reducing the grains per head and kernel weight (Shabeer and Bockus 1988). The yield losses are 200 million AU\$ in Australia due to this disease annually (Murray and Brennan 2009).

Interestingly, *Septoria nodorum* blotch was fully replacing the *Septoria tritici* blotch in the UK in the 1980s. The disease has been reported to be prevalent in France and Scandinavian countries. The disease has a high prevalence in Australia,

causing 100 million AU\$ annually (Murray and Brennan 2009). There are three resistant alleles of tan spot disease investigated from germplasm present on chromosomes *3AS*, *3AL*, *3BS*, and *6AL* along with genes *tsn1* and *tsc2* (Simón et al. 2021; Kokhmetova et al. 2021).

Fusarium head blight, or scab disease, or ear blight, or wheat scab, is caused by Fusarium graminearum (belongs to Ascomycetes). The pathogen is causing premature senescence of wheat heads and, in combination with other Fusarium species, is inducing severe epidemics (Brown and Proctor 2013). The disease onset rate is every fourth to fifth year in the USA, EU, UK, Brazil, Africa, and China. Hence, the disease is of prime concern and most hazardous. The yield losses in the USA were 3 billion US dollars between 1990 and 2008 due to fusarium head blight (Schumann and D'Arcy 2009). During the anthesis stage, the disease is infecting the wheat crop under the prevailing rain conditions. Grain quality, grain yield, and aggregation of type B toxin deoxynivalenol (sesquiterpenoid trichothecene mycotoxin) reduce the overall harvest of crop production and market value. The toxin poses a health risk to humans, animals, and natural ecosystems. The legal limit has been set for the permitted level of mycotoxins. For instance, permitted levels are 1250-2000 ppb in the EU and 200-1000 ppb for the finished product in the USA (http://scabusa. org). In North America, the Fg strain has been reported to produce two novel types, NX-2 and NX-3 (trichothecene mycotoxins) (Varga et al. 2015).

*Bipolaris sorokiniana* causes spot blotch to have foliar and root damage. The disease has a major impact and is reported in the eastern Gigantic plains, specifically in India, Nepal, and Bangladesh (Duveiller and Sharma 2009). Significant losses have been observed in South America under warm and humid climatic conditions (Duveiller and Sharma 2012). *Magnaporthe oryzae* is another Triticum pathotype causing wheat blast and recognized by head disease. The symptoms have appeared as elliptical lesions to entire bleaching as well as empty spikes (Igarashi et al. 1986). Warm (25 °C) and humid (10-h wetting period) conditions are the prerequisites for the development of wheat blasts (Cardoso et al. 2008). It was first observed in the Paraná state of Brazil in 1985, followed by dissemination to Paraguay, Bolivia, and Argentina (Igarashi et al. 1986). Previously, these pathogens were restricted to regions of South America. However, they were discovered in 2016 in Bangladesh and followed by India (Islam et al. 2016; Bhattacharya 2017).

## 8.3 Genepools Contribution in Disease Management

Race-specific resistance or qualitative or seedling resistance is conferring the 150 genes for rust resistance reported in local wheat varieties or their wild cousins. Almost 50 genes are nominated for stem rust resistance genes against the reactions of PGT. *Sr31* is widely known for race-specific resistance against the PGT (Singh et al. 2004). However, *Sr31* also led to the emergence of Ug99; besides this, resistance due to *Sr38*, *Sr36*, *Sr24*, *Sr21*, and *SrTmp* has also been conquered by Digalu and Ug99 races (Jin et al. 2008; Pretorius et al. 2010; Olivera Firpo et al. 2015).



Fig. 8.1 Illustration showing overview on major fungal diseases in wheat and associated genes/ QTLs for imparting resistance

*Sr50*, *Sr45*, *Sr35*, *Sr33*, *Sr25*, *Sr23*, and *Sr2* are the most important genes against the recently emerged races (Singh et al. 2015). Over 70 genes are nominated against the yellow rust disease rust diseases (Jamil et al. 2020). Dakouri et al. (2013) studied about 68 genes including the most common *Lr20*, *Lr10*, *Lr3*, and *Lr1* widely used against the leaf rust in global wheat cultivars. Similarly, *Lr22*, *Lr21*, *Lr10*, *Lr1*, *Sr50*, *Sr45*, *Sr35*, *Sr33*, *Sr22*, and *Yr10* are the 10 race-specific genes of wheat encoding the nucleotide-binding site (NBS) leucine-rich repeat (LRR) proteins (Mago et al. 2015; Thind et al. 2017) (Fig. 8.1). Therefore, the resistance is conferred by the indirect or direct recognition of alike Avr factors.

More than 24 major genes have been addressed against the resistance of *Septoria tritici* blotch (Brown et al. 2015). One hundred and sixty seven genomic regions are anchoring the quantitative trait loci (QTL) providing genetic resistance against the *Zymoseptoria tritici*. The phenotyping study has been displaying the role of QTLs against the sporulation, latency, and necrosis of different disease progression stages.

Against the *Fusarium* head blight, a few moderately resistant sources such as Fontana from Brazil and Sumai-3 from China have been recognized. Several major and minor QTLs have conferred the resistance to *Fusarium* head blight linked with yield penalty or fitness cost (Gilbert and Haber 2013). More precisely, two of the commercially important types of resistances, viz., Type I and type II, are considered such as resistance to initial infection and resistance to spreading of *Fusarium* head blight inside the host (Cuthbert et al. 2006).

The resistance to spot blotch and *Helminthosporium* leaf blight is quantitatively conquered in wheat (Singh et al. 2016). Wheat germplasm from China, Zambia, and Brazil has resistance to both diseases including synthetic hexaploids, wide cross derivatives, and Chinese materials. Association mapping and QTL are displaying the involvement of several genes for resistance (Singh et al. 2016). Several genes such as *Rmg8*, *Rmg7*, *Rmg3*, and *Rmg2* might show promising results, but required field confirmations for effective controls (Ahn et al. 2015).

Adult plant resistance or non-race-specific has conferred the resistance against the rusts in wheat (Periyannan et al. 2017). Several genes such as *Lr68*, *Lr67*, *Lr46*, *Lr34*, *Sr2*, and *Yr36* are potential members in resistance (Ellis et al. 2014). Among them, *Yr36*, *Lr67*, and *Lr34* encode for cytoplasmic protein kinase, hexose transporter, and ATP-binding cassette transporter, respectively, which are directly involved in facilitating resistance (Fu et al. 2009; Dodds and Lagudah 2016).

# 8.4 New Breeding Tools to Attain Higher Disease Resistance

#### 8.4.1 Pathogen-Resistant Germplasm

The adoption of monoculture and high-yield crops has been reducing the diversity positioning and crop genetic diversity in modern crops at a high risk of disease epidemics. The wild, landraces, or progenitor species are excellent sources of R genes for effective pathogen control against the dominant pathogen races. Several R genes have been introgressed successfully from the wild progenitor or landraces/ local varieties. For instance, Fhb7 (Fusarium head blight) has been introgressed from the wild relatives of wheat to confer resistance against the Fhb (Wang et al. 2020). Hence, the wild relatives and landraces are favorable mines for mining the new R genes for the improvement of modern wheat cultivars (Dwivedi et al. 2016). The identification of R genes requires efficient field trials for resistance evaluation for utilization in breeding programs. Natural nursery-based selection should be set up for pandemic pathogens in the diverse screening of highly resistant germplasm. Under high selection pressure in natural nurseries, plants are under a mixed and continuous type of infection in all growth stages.

Therefore, exclusive plasma member-anchored pattern recognition receptors (for pathogen triggered immunity) and nucleotide-binding leucine-rich repeat proteins (for effectors triggered immunity) will be identified to confer the broad-spectrum resistance. A study was conducted in the Huang Huai-Hai region of china where 146 wheat entries were inoculated with races of PST, FHB, and BGT. *Yr15*, *Yr18*, *Pm21*, and *Fhb1* are recommended for breeding programs in combination with other effective genes for broad-spectrum and durable resistance, whereas *Yr10*, *Yr9*, *Yr26*, and *Yr17* were ineffective against the PST races (Ma et al. 2021).

#### 8.4.2 Identifying New R Genes Using High-Throughput Genomic Approaches

Recent advances in genomic sequencing and bioinformatics have accelerated approaches to improving R gene cloning. Sequencing-based mapping is regarded as a potential tool in the mapping and cloning of R genes in plants (Wulff and Moscou 2014; Mascher et al. 2014). With the aid of a GWAS, the genetic architecture of many economically important crops, including wheat, has been studied with the aid of a GWAS (Huang et al. 2010; Li et al. 2019; Lin et al. 2020). Kumar et al. (2020) have been conducting the GWAS on spring wheat panels for leaf rust, stem rust, and stripe rust. A total of 16, 18, and 27 QTLs have been discovered for resistance against stripe rust, leaf rust, and stem rust, respectively. In seedling and adult plant responses, a number of these regions were annotated with ABC transporter protein, E3ubiquitin-protein ligase, and NB-LRR. According to Jupe et al. (2013), resistance gene enrichment gene sequencing is another powerful tool to identify newly NLR-like genes from landraces or wild species.

Steuernagel et al. (2016) demonstrated that MutRenSeq (combined approaches of EMS and RenSeq mutagenesis) is used to identify NLR genes and used in isolating Sr22 and Sr45 (stem rust-resistance genes) in wheat. Thind et al. (2017) also investigated how the TACCA method was used to isolate Lr22a (R gene) from wheat polyploidy genomes. MutChromSeq (a combined technique of high-throughput sequencing, chromosome flow sorting, and EMS mutagenesis) was used to identify the Pm2 gene (Sanchez-Martın et al. 2016). Similarly, AgRenSeq (combining association genetics with RenSeq) was used to exploit the pan-genome variations for the cloning of R genes from the diverse panels of germplasm in wheat, such as SrTA1662, Sr46, Sr45, and Sr33 (Arora et al. 2019). Allele mining is a simple and effective approach for the identification of elite alleles of R genes from wild germplasm and landraces (Ashkani et al. 2015). In a study in which wild germplasms of wheat were studied for resistance against the powdery mildew, Pm3 alleles were observed in wild *T. dicoccoides* accessions (Kaur 2008).

#### 8.4.3 Expanding NLR Recognition Specificity Through BSR Genes Engineering

The period of resistance the R resistance gene induces is shortened by the adapted virulence of the pathogen (McDonald and Linde 2002). This bottleneck can be overcome with the aid of genetic engineering of NLR variants where engineered NLR can respond to numerous pathogen effectors. According to Segretin et al. (2014), different conserved domains and integrated domains of NLRs can be altered to attain the new capability to progress in disease resistance against different pathogens and strains. A few nucleotide differences among the coding regions of genes, prime genome editing technology, and CRISPR-mediated homology direct repair can be practiced to produce new R alleles with a broad resistance spectrum (Lin et al. 2020). For example, using CRISPR/Cas9, *EDR1*, which acts as a negative

regulator in defensive responses against powdery mildew, was knocked out to generate powdery mildew-resistant wheat plants (Zhang et al. 2017a, b). Similarly, random elimination in the start codon containing a sequence of the TaHRC gene in the Bobwhite (wheat variety cultivar) confirmed resistance against the Fusarium head blight (Su et al. 2019). Modifications in the decoys or integrated domains of NLRs can be helpful in the expansion of effector recognition specificity (Maqbool et al. 2015; Kim et al. 2016). Therefore, various R variants can be produced for the selection of required wide-range resistance in the crop by using CRISPR/Cas9 technology (Fig. 8.2).

#### 8.4.4 GWAS: A Step Ahead Toward Wheat Breeding

GWAS is currently known as the most common approach for decoding the genotype-phenotype association in crop plants (Liu and Yan 2019). GWAS is the more statistical strategy for mapping QTL to coordinate the desired phenotype with the genotypes on the significance of historic linkage disequilibrium. GWAS can increase the likelihood of identifying loci linked to crop domestication, crop improvement, and grain yield (Li et al. 2019; Lujan Basile et al. 2019; Hao et al. 2020). Re-sequencing and GWAS studies on 145 elite wheat cultivars in China help in the discovery of genomic regions integrated with crop improvement as well as domestication, providing genetic resources for wheat improvement programs (Hao et al. 2020). The study was conducted on 175 winter wheat genotypes from NordGen and GWAS analysis was done. The phenotypic data indicated a significant variation between genotypes in disease resistance response to Septoria tritici blotch as well as powdery mildew. The genomic-assisted germplasm selection with superior alleles for disease resistance in wheat could be then integrated into active breeding programs (Alemu et al. 2021).

## 8.4.5 Speed Breeding

Generally, breeders take 8–10 years to develop novel wheat cultivars. Therefore, novel elite crop development of wheat is a difficult task in terms of time consumption and laboriousness. Speed breeding is one of the possible solutions to overcome this prolonged time barrier. It involves specific growing conditions, including optimal temperature and light intensity, photoperiod requirement, premature seed harvesting, and shortening of generation time by up to 8–10 weeks. Speed breeding was successfully deployed to obtain six generations in one year for bread wheat. The attempt was made by Alahmad et al. (2018) to *Triticum durum* Desf. with the key traits' involvement, such as phenotyping for resistance to leaf rust, tolerance to crown rot, seminal root angle, seminal root number, and plant height.





#### 8.4.6 Genome Editing (GE)

GE involves sequence-specific nucleases (SSNs) for desired gene modification via introgression of selectable traits into a target crop in a transgenic-free selected genome. SSNs induce specific alteration at the chromosomal level, leading to insertion, substitution, or deletion of undesired sequence from a particular position (Mehta et al. 2020; Dilawari et al. 2021; Chattopadhyay et al. 2022). Several SSNs types are used, such as the CRISPR/Cas, TALENs, and ZFNs system are particularly used for genomic modification. Such target genomic alteration has become a distinct genetic tool for the introduction of disease resistance genes against different pathogenic diseases (Jamil et al. 2020; Shakeel et al. 2020). Indeed, crop susceptible genes are eliminated, edited, or restructured in such a manner to change them into tolerant genes.

For instance, in *T. aestivum*, CRISPR/Cas9 has exhibited complete resistance against powdery mildew by developing mutants like *TaEDR1* by continuous editing of *TaEDR1* along with other homolog sequences. However, CRISPR/Cas9 was significant for developing transgenic cultivars against fungal pathogens via deletion (Jamil et al. 2020). In another study, successful editing of various genes has been done using CRISPR/Cas9 such as *TansLTP9*, *TaNFXL1*, and *TaABCC6*, with protoplasm fusion in wheat for stimulation of resistant mechanism toward *Fusarium* head blight (FHB). Additionally, there are various reports on rust-resistant using CRISPR/Cas9. Several reports have been published on stripe rust resistance gene introgression into cultivated wheat (For detailed extension see Tables 8.1 and 8.2).

#### 8.4.7 RNA Interface (RNAi) Silencing

RNAi silencing is a highly conserved process that mediates gene silencing or restricts the functional mechanism of a selected gene of virulence pathogens. The gene silencing RNAi involves double-stranded RNA (dsRNA), a homologous gene of interest. The silencing process offers dsRNA cleavage into small RNA (21–26 nucleotide long), which are microRNA (miRNA) and small interfering RNAs (siRNA). These miRNA or sRNA possibly stimulate the various cascade, viz., regulating RNA stability, processing of signals, and response to a different pathogen in crop plants.

In *stripe mosaic virus*, the *Pst* from PR genes has been silenced that acts as a vector for dsRNA homologous expression to *Pst* target gene (Qi et al. 2019; Jamil et al. 2020). The transcription factor-like mitogen-activated protein kinase (MAPK) stimulating gene (*FUZ7*), which is the crucial pathogenic factor of *Pst* mediating fungal hyphal morphology and infection and triggering pathogenesis in the host plant, was eliminated using RNAi. However, in the transgenic wheat line, RNAi prepares *Afuz7* targeting of *Pst* which was significantly expressed and strongly confirmed the durable resistance against pathogenic strains. On contrary, another *CPK1* was eliminated in transgenic wheat lines with the help of RNAi. Moreover, *Pst* knockdown uses different transgenic wheat lines that are *PstGSRE1* and *PsHXT1* genes (hexose transporter) (Qi et al. 2018; Satheesh et al. 2019; Ahmad et al. 2020; Chang et al. 2020).

Gene	Techniques used	Resistant against	Reference
mtlD	Plasmid-mediated gene transfer	Mosaic virus (Aceria tosichella)	Abebe et al. (2003)
pac1	Agrobacterium- mediated gene transfer	Barley yellow dwarf virus (Cereal aphids)	Yan et al. (2006)
$\beta$ -1,3-glucanase	Agrobacterium- mediated gene transfer	Powdery mildew (Blumeria graminis)	Zhao et al. (2006)
TiERF1	Biolistics method	Sharp eyespot ( <i>Rhizoctonia cerealis</i> )	Liang et al. (2008)
TaPIMP1	Agrobacterium- mediated gene transfer	Root rot (Bipolaris sorokiniana)	Zhang et al. (2012)
TiMYB2R-1	Biolistics method	Take-all disease (Gaeumannomyces graminis)	Liu et al. (2013)
TaCLP1	Biolistics method	Stripe rust (Puccinia striiformis)	Zhang et al. (2013)
SN1	Biolistics method	Take all disease (Gaeumannomyces graminis)	Rong et al. (2013)
Bt	Agrobacterium- mediated gene transfer	Armyworm (Spodoptera frugiperda)	Huang et al. (2014)
TaERF3	Virus-induced gene silencing	Stripe mosaic virus (Hordeivirus)	Rong et al. (2014)
NIb8	Biolistics method	Yellow mosaic virus (Polymyxa graminis)	He et al. (2015)
Ta-Mlo RC24	Agrobacterium- mediated method	Powdery mildew (Blumeria graminis)	Acevedo-Garcia et al. (2017)
viviparous 1	Agrobacterium- mediated gene transfer	Rust (Puccinia triticina)	Kocheshkova et al. (2017)
KN2	Biolistics method	Powdery mildew (Blumeria graminis)	Zhang et al. (2017a, b)
Mrl40	Agrobacterium- mediated method	Powdery mildew (Blumeria graminis)	Tang et al. (2018)
BADH	Particle bombardment method	Smut (Ustilago tritici)	Khan et al. (2019)
GhDREB	Plasmid-mediated gene transfer	Rust (Puccinia triticina)	Andersen et al. (2020)
TaNAC21	Agrobacterium- mediated method	Stripe rust (Puccinia striiformis)	Feng et al. (2014)
TaNAC069	Agrobacterium- mediated gene method	Leaf rust fungus ( <i>Puccinia</i> <i>triticina</i> )	Zhang et al. (2021)

**Table 8.1** Overview on the discovery of major disease-resistant genes and techniques used for their introgression

Wild progenitor	Target	Introgression	Pasistanca	Poforonco
Aegilops	Cre2	Recombination	Cyst	Jahier et al. (2001)
Taxodium distichum	Sr2	Spontaneous	Leaf rust	Prins et al. (2001)
Aegilops umbellulata	Lr9	Spontaneous	Leaf rust	Gupta et al. (2005)
Aegilops triuncialis	Lr58	Recombination	Leaf rust	Kuraparthy et al. (2007)
Aegilopss umbellulata	Lr9	Irradiation	Leaf rust	Chhuneja et al. (2007)
Aegilops ventricosa	Rkn2	Recombination	Root-knot nematode	Williamson et al. (2013)
Aegilops speltoides	Sr32	Recombination	Stem rust	Mago et al. (2013)
Africallagma elongatum	Lr19	Irradiation	Stem rust	Worku et al. (2016)
Triticum timopheevii	Sr23	Homoeologous recombination	Powdery mildew	Liu et al. (2017)
Africallagma elongatum	Lr24	Spontaneous	Stem rust	Kumar et al. (2017)
Africallagma elongatum	Sr26	Irradiation	Stem rust	Rai et al. (2017)
Secale cereale	Pm8	Spontaneous	Stem rust	Crespo-Herrera et al. (2017)
Aegilops ventricosa	Yr17	Recombination	Stripe rust	Coriton et al. (2019)
Aegilops ventricosa	Pch1	Recombination	Eyespot	Pasquariello et al. (2020)
Aegilops longissima	Pm66	Spontaneous	Powdery mildew	Li et al. (2020a, b, c, d)
Aegilops tauschii	Dn3	Recombination	Russian wheat aphid	Kisten et al. (2020)

**Table 8.2** Introgression of major disease-resistant genes from wild relative species into a wheat plant

# 8.4.8 CRISPR/Cas9 and Disease Resistance: A Way Forward to More Reliability

CRISPR/Cas9 genome editing is an established mechanism in bacteria that helps protect them from harmful plasmids and bacteriophages. The spacer (a DNA fragment of foreign pathogen and host) acts as a genetic memory for future infection. During similar pathogenic attacks in the future, the CRISPR array gets transcribed and processed, leading to the synthesis of CRISPR RNA fragments (Single Guide RNA) via the activity of endonuclease (CAS9). The advancement of plant genome editing, including CRISPR/Cas9 systems, suggests that this application is more

feasible and reliable. It significantly helps to increase multiple beneficial traits as well as disease resistance in wheat (Langner et al. 2018; Zaynab et al. 2020). However, genes encoding proteins that associate between plants and pathogens have been targeted through CRISPR/Cas9 to explain the underlying genetic pathways of plant–pathogen recognition and to produce investigation systems for disease resistance (Li et al. 2018). Disease caused by viruses, bacteria, and fungi could dramatically decrease the quality and quantity of wheat.

CRISPR/Cas9 has been significantly eliminating disease susceptible genes to produce new resistance wheat cultivars. More likely, loss of function in MLO (Mildew resistance locus) leads to gains of resistance against powdery mildew. Such reports confirm a broad-spectrum range of MLO as a favorable site for CRISPR/Cas9 to reduce susceptibility against powdery mildew (Gil-Humanes and Voytas 2014). According to Wang et al. (2014), CRISPR/Cas9-guided wheat mutant, a TaMLO-A1 (mildew resistance locus) of the homoalleles exhibited enhanced resistance to infection against Blumeria graminis. CRISPR/Cas9 application system to useful fungal pathogens including Trichoderma sp. to increase plant defense system as a biocontrol agent against oomvcetes and fungal is also a promising agent. Certain MLO homo-alleles including TaMLO-B1, TaMLO-A1, and TaMLO-D1 were edited using CRISPR/Cas9 and showed that TaMLO-A1mutagenized wheat plants have confirmed resistance against Blumeria graminis (Tyagi et al. 2021). Such techniques like CRISPR/Cas9-dependent plant-pathogen genome editing will draw much attention as well-adapted to increased pathogenic resistance and transgenic-free plants and will be required for global food demand (Paul et al. 2021).

In another study, the fusarium head blight, induced by *Fusarium* spp., was managed in CRISPR/CAS9-silenced mutants. Studies demonstrated that RNA interference on trehalose 6-mutant ( $\Delta$ tri 6) of *Fusarium* spp. confirmed lowered disease indices that lie from 40 to 80% in durum wheat (Muñoz et al. 2019). However, two mutants ( $\Delta$ tri1 and  $\Delta$ tri6) of *Fusarium* spp. were incapable of pathogenic response to the inflorescence and also elicited plant defense response. Moreover,  $\Delta$ map 1 mutants of *Fusarium* spp. demonstrated two times reduction in the production of mycotoxins, but was unable to colonize pathogens in other plant parts except for grains. Such competition for nutrients and space between non-virulent and virulent strains could decrease the disease severity, and the field liberation of non-virulent CRISPR/Cas9-mutant strains of *Fusarium* spp. might help to overcome the emerging issues (Zaidi et al. 2020; Zaynab et al. 2020; Zhang et al. 2017a, b; Wang et al. 2020; Verma et al. 2021; Liu et al. 2021).

According to Wang et al. (2014), using CRISPR/Cas9 to mutate wheat cultivars exhibits improved resistance toward powdery mildew resistance, caused by *Blumeria graminis*. Additionally, CRISPR/Cas9 mediated site-specific mutagenesis in springer and wheat varieties (Hahn et al. 2021). Similarly, mutagenization of 3 genes such as *AUR1* (a visual marker), *Tri5* (toxin production and infection), and *MGV1* (required for infection and reproduction) was done by CRISPR/Cas9. More likely, silencing of *Tri5* and *MGV1* could suppress the fungal ability to suppress infection in crops, whereas *AUR1* silencing acts as an effective visual marker during

mutagenesis (Sack 2020). Recently, in wheat a serious fungal pathogen like powdery mildews caused by *Podosphaera xanthii* was enhanced via editing *Mildew Locus O* (*MLO*) gene through CRISPR/Cas9 technique. Knockdown of susceptibility loci becomes highly complicated in wheat like targeting *MLO* homologous genes and *Enhanced disease resistance 1* (*EDR1*) using CRISPR/Cas9 editing (Wang et al. 2020; Verma et al. 2021; Raffan et al. 2021; Li et al. 2018, 2020a, b).

However, various target locus in the *wheat dwarf virus* (WDA) genome was screened using CRISPR/Cas 9 direct sequences enclosing the PAMs motif. Several target positions were designated that demonstrated no specific effect and were efficient in attacking different viral DNA sequences. The single-guided RNA WDA1 (sgRNA WDA1) displays the complementary overlapping in certain coding sites; sgWDA2 targets the Rep/Rep (Shahriar et al. 2021). Moreover, crop engineering using CRISPR/Cas9 like techniques has helped to develop disease-resistant varieties that are more resilient to climate change (Zaidi et al. 2020). Application of CRISPR/Cas9 ultimately helps to improve disease resistance in wheat and other crops and is well-documented (Schenke and Cai 2020; Paul et al. 2021; Zhang et al. 2017a, b; Kis et al. 2019; Verma et al. 2021; Gil-Humanes and Voytas 2014; Tyagi et al. 2021; Duy et al. 2021).

# 8.5 Concluding Remarks

The conventional breeding approaches for disease-free wheat in modern agriculture are transgenic, mutation breeding, and cross-breeding. These are laborious, timeconsuming, and unfocused crop improvement programs that are unable to meet food demand. To cope with these challenges and to increase crop selection ability, transgenic and marker-assisted breeding has been developed, harnessing target traits via introgression into elite wheat varieties. Such advancements in plant breeding are an excellent tool that maintains rapid mutation and can recognize the significant genetic approaches for disease resistance. No doubt, crop breeding strategies are propelled by next-generation breeding methods. New breeding resistant varieties should remain the key focus. Some alternate approaches, viz., shifting plantation date, integrating fungicide, and eradicating volunteer plants, should also be considered. Precise modification of existing allelic diversity via advanced genomic editing is an efficient alternative for accelerating wheat improvement and sustainably increasing wheat production. Although it is convenient to attain precise allele/gene targeting or replacement in different cereal plant species, impressive work has been published in past years in wheat molecular breeding, which eradicates the constraints of the pathogen during crop improvement programs. In precise genome editing, replacement, deletion, site-directed artificial evolution, knockdown module, and insertion of allele/gene will be significantly facilitated by functional genomics. The advancement of the different genome modifications provides ease to gene pyramiding of novel resistance genes in the desired cultivar in a user-derived way immediately, efficiently, and cost-effectively without any linkage drag from undesired genes. More recently, CRISPR/Cas9 like techniques help in the transformation of agriculture via the deletion or addition of alleles. No doubt, such techniques are cost-effective but eco-friendly, thus becoming a reliable trend. However, approaches like GWAS, RNAi silencing, genome edition, speed breeding, etc. will offer a huge amount of genetic information and enhance disease resistance via genomic editing. Genome editing approaches have various advantages over conventional breeding techniques, given their high efficiency, simplicity, amenability to multiplexing, and high specificity. Breeders strongly believe that combining molecular approaches with numerous breeding strategies will underpin an attempt to create super wheat cultivars for sustainable agriculture and ensure food security in an eco-friendly way.

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