Chapter 15
Identification and Implementation of Resistance: Genomics-Assisted use of Genetic Resources for Breeding Against Powdery Mildew and Stagonospora Nodorum Blotch in Wheat

Liselotte L. Selter, Margarita Shatalina, Jyoti Singla and Beat Keller

Abstract Wheat belongs to the three most important cereal crops of the world and is grown under a wide variety of climatic and agricultural conditions. Fungal pathogens represent the most relevant biotic stresses for wheat. These include different rust species, powdery mildew, leaf spots, as well as a number of other diseases that result in reduced grain yield and quality. Recently developed genomic tools allow new approaches to improve breeding for resistance to these pathogens based on a more efficient use of genetic resources. In this chapter, we will focus on the powdery mildew and Stagonospora nodorum blotch diseases and discuss the successful identification of wheat genes determining the outcome of pathogen-host interaction and the development of perfect markers for them. Genomic approaches, including gene cloning, allele mining, transcriptomics and comparative genomics have greatly changed and improved our understanding of molecular wheat-powdery mildew interactions. For the necrotrophic pathogen *Stagonospora nodorum* much of the interaction was found to be based on pathogen toxins and host susceptibility genes. The work on specific gene-for-gene interactions opened new possibilities for more efficient resistance breeding. In addition, the molecular identification of quantitatively acting resistance loci in wheat has made important progress, although only few such genes have been cloned, only one of them each against mildew and Stagonospora nodorum blotch. However, even at this early stage it can be foreseen that the new knowledge might revolutionize breeding for durable resistance in the near future. The progress made towards a whole genome sequence of wheat together with ongoing developments of high throughput techniques provides a completely new perspective on resistance breeding against these two diseases.

B. Keller (✉) · L. L. Selter · M. Shatalina · J. Singla
Institute of Plant Biology, University of Zurich, Zollikerstrasse 107,
8008 Zurich, Switzerland
e-mail: bkeller@botinst.uzh.ch

L. L. Selter
e-mail: liselotte.selter@gmx.net

M. Shatalina
e-mail: margarita.shatalina@access.uzh.ch

J. Singla
e-mail: jyoti.singla@uzh.ch

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15.1 Introduction

15.1.1 Emerging Challenges for Wheat Resistance Breeding Against Powdery Mildew and Stagonospora Nodorum Blotch: Changes in Agricultural Practice, Climate Change and Pathogen Adaptation

The powdery mildew disease occurs in crop growing regions worldwide. Before the green revolution, powdery mildew was found predominantly in regions with a cool, humid and semi-continental climate. However, with the introduction of new agricultural practices and intensified crop production during the last decades, powdery mildew has gained importance also in the more arid crop growing regions. Today, economically relevant powdery mildew epidemics cause serious yield losses in the cool and humid areas of China, North and South America, Northern Europe as well as in North and East Africa. The widespread use of irrigation systems and nitrogen fertilizers for yield improvement has created favourable conditions for this particular pathogen in additional agro-ecosystems. Wheat farmers can considerably influence powdery mildew epidemics by adapting appropriate agricultural practices, such as choosing the right sowing period, lower population densities or lower use of fertilizers. Breeding for genetic resistance to powdery mildew is nevertheless considered the most effective disease control strategy and will be discussed in detail below. Cultivar mixtures and low density planting are good strategies to slow disease development, but both have their specific problems and have been employed only occasionally so far. The application of foliar fungicides is often chosen as a last strategy if cultural practices are not able to control powdery mildew development. However, an intense use of fungicides can lead to fungicide resistance in the pathogen. This has become a major concern in Europe (Wolfe 1984).

Stagonospora nodorum blotch (SNB) affects wheat grown under humid conditions and mild temperatures in Europe, South America, Central Asia and North Africa. In North America, China and Europe, the genetic diversity of *S. nodorum* populations is very high. It was shown that populations in Europe, North America and China have no or relatively little subdivision and serve as donors for disease distribution to other continents (Stukenbrock et al. 2006). Therefore, the suggested center of origin for *S. nodorum* in the Fertile Crescent coincides with the center of origin for wheat (Balter 2007; Burger et al. 2008). The distribution of *S. nodorum* is mainly human-mediated, which is the main way of disease transport from North America, Europe and China to other parts of the world (Stukenbrock et al. 2006). A wide range of fungicides are efficiently applied in the areas with SNB infections. Reports about fungicide-resistant isolates of *S. nodorum* are very rare. However, the possibility of their emergence remains a threat in regions with extensive fungicide application (Oliver et al. 2012).

Modern bread wheat is a temperate crop adapted to regions with annual rainfall between 30 and 90 cm. It is nowadays cultivated on both hemispheres under a wide range of climatic conditions and different soils, making up 17 % of all crop acreage. Although the impact of climate change on crops shows complex regional patterns, significant yield losses have been predicted using the worst case CO_2 emission scenario of the Intergovernmental Panel on Climate Change (Luck et al. 2011). Climate change will not only differentially affect wheat cultivars in their geographic distribution and their growth, but also pathogens. Biotrophic pathogens such as powdery mildews, highly depend on the plant's health and its water and nitrogen status (Olesen et al. 2000). As plant disease development and spreading is influenced predominantly by increased atmospheric CO_2 levels, heavy rains, increased humidity, drought and warmer winter temperatures (Cannon 1998; Chakraborty et al. 2000; Pimentel et al. 2001; Berry et al. 2002; Anderson et al. 2004) we can expect that the lifecycle of some pathogens will be limited by increasing temperatures, while other pathogen species might respond positively to the same climatic changes. Changes in global minimum temperatures and rainfall patterns will presumably cause shifts in growing seasons of certain wheat cultivars and alter the land use of specific crops. This might then in turn lead to the occurrence of novel plant-pathogen interactions through the introduction of new host genotypes, new pathogens or both to a specific agro-ecosystem. In addition, temperature changes in critical periods of host infection might reduce the effectiveness of resistance genes, as it has been shown that some *R* genes against powdery mildew are known to be temperature-sensitive (Ge et al. 1998).

Clearly, based on the considerations described above, we can expect that climate change will have multiple, highly complex effects on plant disease epidemiology and the consequences on yield are difficult to predict. As today's agriculture primarily aims at crop yield improvement and breeding programs mainly focus on cultivars adapted to longer growth periods, drought and stress tolerance, it is of great importance to establish efficient disease screening methods which allow to monitor changing disease epidemics. This is because pathogens are not only important yield-reducing factors, but due to their short generation times also act as early indicators of environmental changes (Newton et al. 2011). Intensifying the research of climate change effects on plant-pathogen systems will certainly allow an improvement of the disease management practices necessary for a sustainable agriculture.

15.1.2 Wheat as the Host Plant for Blumeria graminis f.sp. tritici and Stagonospora nodorum

Bread wheat (*Triticum aestivum*, $2n = 6x = 42$, AABBDD) belongs to the four most important cereal crops in modern agriculture (http://www.FAOSTAT.org). The FAO estimates that 682.5 million t of wheat was harvested in the year 2011. Bread wheat accounts for approximately 20 % of the totally consumed human food calories and provides the major staple food for 40 % of the human population, predominantly

in Europe, North America and the western and northern parts of Asia (Peng et al. 2011). The origin of modern bread wheat lies in a region of the Near East known as the Fertile Crescent which covers parts of south-eastern Turkey, Israel, Syria, Iraq and Jordan. There, wild wheat progenitors such as Einkorn or Emmer (He et al. 2009), were among the first cereals subjected to human selection 10,000 years ago (Charmet 2011). Hexaploid bread wheat originated approximately 9,000 years ago from a hybridization event between the allotetraploid domesticated Emmer wheat (*T. turgidum spp. dicoccoides* (2n = 4x = AABB) and the diploid wild goatgrass *Ae. tauschii* (2n = 2x = DD). Bread wheat and its wild progenitors were selected by the first farmers for agriculturally advantageous traits in the specific agroecological system where domestication occurred. These traits also included disease resistance to fungal pathogens.

15.1.3 Characteristics of Powdery Mildew and Stagonospora Nodorum Blotch Diseases in Agricultural Systems

The powdery mildew pathogen of barley, *Blumeria graminis* f.sp. *hordei,* was found to have evolved on wild grasses in the Middle East (Koltin and Kenneth 1970; Wolfe 1984). Comparative genome analysis of wheat and barley powdery mildew revealed that these two *formae speciales* diverged about 10 million years ago, after divergence of their respective hosts (Oberhaensli et al. 2011). This suggests that the wheat powdery mildew, *Blumeria graminis* f.sp. *tritici*, originates from an ancestral pathogen which initially colonized ancestors of both wild wheat and barley. There is evidence that wheat powdery mildew originated and co-existed with wild wheat long before their domestication (Wicker et al. 2013).

Wheat yield losses caused by the two wheat fungal pathogens powdery mildew and *Stagonospora nodorum* are difficult to estimate. In controlled experimental environments, it is feasible to measure yield losses, but on farmer's fields, crop health and actual losses are significantly different from experimental calculations. Oerke et al. (1994) estimated that collectively all wheat diseases cause annual grain losses of about 12.4 %, including all developed and developing countries. Disease epidemics of the two described wheat pathogens of this chapter, powdery mildew and SNB, depend mostly on three factors: prevalence of inoculum, the genetic constitution of grown cultivars, and to a large extent on environmental conditions (Duveiller et al. 2007). The changes in agricultural practices during the last decades have led to changes at the microclimate level in wheat growing areas. In order to increase productivity, genetically uniform varieties are planted in dense stands. These genotypes contain often semi-dwarf varieties and have a high tillering density, thus increasing the humidity within the crop canopy. In addition, the regular application of nitrogen fertilizers and irrigation creates a microclimate which is highly favourable for the spreading of biotrophic fungal diseases (Sharma et al. 2004).

Systematic reports on SNB epidemics are lacking from most of the wheat growing areas. The most complete dataset is available from Rothamsted Broadbalk experiment archive (UK). There, wheat leaf samples have been collected for nearly 160 years (from 1844 to 2003) and used to estimate the epidemics of SNB and Septoria tritici blotch (STB) caused by *Mycosphaerella graminicola* (Bearchell et al. 2005; Shaw et al. 2008). The predominance of SNB was shifted towards *M. graminicola* after 1970. Bearchell et al. (2005) linked this shift in predominance of STB over SNB to the decrease in SO_2 emissions after 1970. Another suggested reason is that before 1970 the widely used cultivars had good partial resistance to STB, but not to SNB. Later, large efforts were made to introduce SNB resistance in newly released cultivars (Arraiano et al. 2009). Additionally, in Western Australia in regions where SNB dominates SO_2 pollution is very low. Therefore, the shift between SNB and STB epidemics is likely caused by a combination of factors (Oliver et al. 2012).

Breeding for resistant wheat varieties is the most effective strategy to counteract fungal diseases. Despite an overall good success of resistance breeding, changes in wheat genotypes as well as pathogen races are frequent, making breeding for disease resistance a continuous task. It is obvious that a better understanding about the molecular basis of disease resistance in wheat can contribute significantly to improve strategies in achieving resistance and to make resistance breeding faster and more efficient. Importantly, this can be achieved through the use of recently developed genomic tools such as high-throughput platforms for molecular marker analysis and genotyping in combination with classical breeding methods and increasing knowledge on the genomes of wheat and its relatives. Various genomics-assisted breeding approaches such as marker-assisted selection (MAS), association mapping, QTL identification and MAS for them, as well as genome -wide association studies have been successfully utilized in modern plant breeding for the development of improved crop varieties. The limited number of molecularly cloned resistance genes/QTL in wheat can be explained by the genetic complexity observed in this species. The large wheat genome size and the high amount of repetitive DNA (80 %) makes map-based cloning in wheat a challenging task. Nevertheless, some disease resistance genes (*Lr1*, *Lr10*, *Lr21, Lr34/Yr18/Pm38, Yr36*, *Pm3b and Tsn1*) have been cloned from hexaploid wheat (Feuillet et al. 2003; Huang et al. 2003; Yahiaoui et al. 2004; Cloutier et al. 2007; Qiu et al. 2007; Fu et al. 2009; Krattinger et al. 2009, Faris et al. 2010) using sub-genome chromosome walking techniques and comparative genomics.

In this chapter we will describe how genomic approaches for wheat resistance breeding against powdery mildew and Stagonospora nodorum leaf blotch have been used in the last years: the work discussed includes classical map-based cloning approaches but also new strategies such as allelemining and the use of transcriptomics and finally the new and exciting field of transgenic use of modified resistance genes.

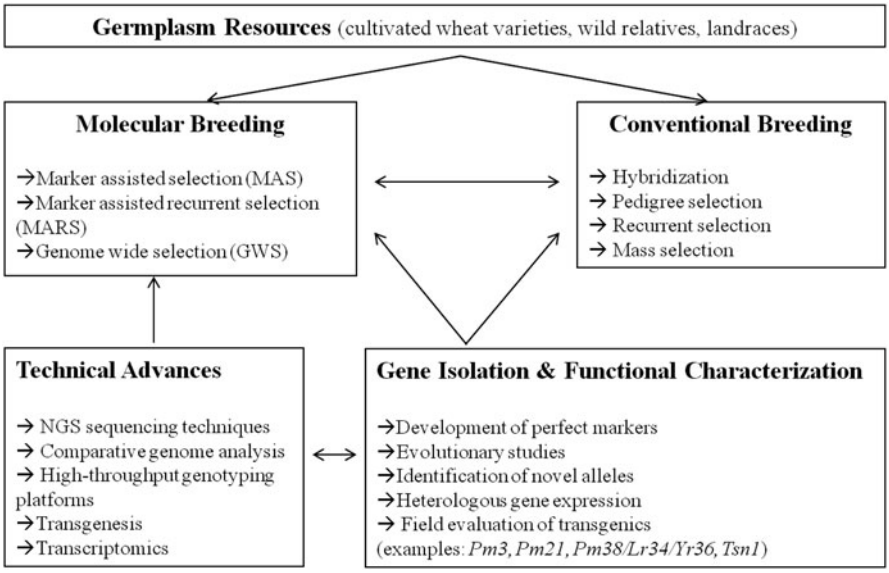

Fig. 15.1 Scheme of genomics-assisted breeding

15.2 Genomics-Assisted Breeding by Cloning of Major Resistance Genes

Major race-specific resistance genes can provide plants with a level of disease resistance which is close to immunity. However, fungal pathogens are fast evolving pathogens, which under selection pressure can rapidly adapt to overcome plant resistance mechanisms. Thus, there is a strong need to (i) identify new and durable sources of genetic resistance in order to avoid an erosion of the current pool of agriculturally important resistance genes and to find (ii) new and innovative ways to use the known resistance genes in a more durable way (Fig. 15.1).

15.2.1 Map-Based Cloning of Powdery Mildew Resistance Genes

15.2.1.1 Wheat Powdery Mildew: A Strictly Biotrophic Pathogen

Blumeria graminis f.sp. *tritici*, the causal agent of wheat powdery mildew is a highly specific pathogen which grows only on wheat species. It belongs to the obligate biotrophic pathogens which fully depend on the integrity of the invaded host plant cell (Horbach et al. 2011) to accomplish all important stages of pathogenesis such as attachment, host recognition, penetration and proliferation (Mendgen and Hahn 2002). The infection process starts when a spore lands on a leaf surface, germinates

and forms a primary and appressorial germ tube. The appressorium penetrates the cell wall using mechanical force and cell walldegrading enzymes, and invaginates the plant cell by forming a special feeding structure—the haustorium, which is surrounded by an extrahaustorial plasma membrane. The haustorium is not only required for nutrient supply, but is also important for signalling, communication and prevention of recognition by the host (Perfect and Green 2001; Horbach et al. 2011). Since it is essential for the pathogen to keep the host cell alive, biotrophic fungi suppress the programmed cell death induced at the infection site—a defense response known as hypersensitive reaction (HR). This defense suppression is possibly the result of the release of effector proteins during the penetration process (Panstruga 2003). Only very recently, the genome sequence of the barley powdery mildew became available (Spanu et al. 2010) and a genome sequence of the wheat powdery mildew can be expected in the near future (Wicker et al. 2013). This genomic sequence information, provides an extremely valuable tool to gain a better understanding of the biology of the powdery mildew pathogen, the factors required for biotrophy as well as virulence determinants. In addition, comparative genome analysis between the wheat and barley powdery mildews will allow an improvement of our understanding of host specialization in these diseases. Only by improved knowledge on all the components of the host-pathogen interaction, we will be able to develop rational resistance improvement based on molecular interactions in the future.

To date, 61 powdery mildew resistance genes including three recessive genes (*Pm5*, *Pm9* and *Pm26*) have been genetically described. They confer resistance against specific races of the pathogen and have been identified and mapped to 46 loci in the wheat genome (He et al. 2009; Hua et al. 2009; Luo et al. 2009; Huang et al. 2012). Out of these, Lillemo et al. (2008) identified two race non-specific genes, *Pm38* and *Pm39* which confer partial resistance. Among these 61 genes, only *Pm3b* (Yahiaoui et al. 2004), *Pm21* (Cao et al. 2011) and *Pm38* (Krattinger et al. 2009) have been cloned so far.

15.2.1.2 Wheat Genomics at Different Ploidy Levels Allows the Isolation of the *Pm3* Powdery Mildew Resistance Alleles in Wheat

A map-based cloning approach was used to isolate the *Pm3b* gene that controls powdery mildew resistance in the hexaploid wheat landrace Chul. The powdery mildew resistance gene was mapped genetically to the distal end of the short arm of chromosome 1A in 1,340 plants of a Chul x Frisal derived F2 population. Physical mapping was performed by using BAC libraries developed from the diploid wheat *T. monococcum* cv. DV92 and the tetraploid *T. durum* cv. Langdon wheat. By using these BAC libraries, Yahiaoui et al. (2004) proved the usefulness of exploiting wheat genomes with different ploidy levels, and combined sub-genome chromosome walking with haplotype analysis. The sub-genome chromosome walking between the three wheat species revealed dissimilarities in the haplotype structures at the *Pm3* locus. Haplotype similarity was found between the *durum* wheat cv. Langdon and the susceptible hexaploid parent Frisal, whereas only partial similarity between the haplotypes of

T. monococcum cv. DV92 and the resistant parent Chul was observed. This led to the isolation of the *Pm3b* gene from the hexaploid wheat donor line by deriving low copy probes from the conserved resistance-gene-like sequences in both genomes using long-range PCR (Yahiaoui et al. 2004). Validation of the candidate gene was done by γ- irradiated mutant analysis. Molecular analysis of 13 independent mutants showed six different deletion patterns. One mutant without any major deletion at the *Pm3* locus showed a single base pair deletion in the coding region of the candidate gene resulting in loss of expression as demonstrated by RT-PCR. Hence, the candidate gene for *Pm3b* could be confirmed.

The rapid resistance response occurring in leaf epidermal cells in the case of an incompatible interaction between wheat and powdery mildew leads to a termination of pre-haustorial fungal growth. This hypersensitive response provides the basis to study resistance gene function by a particle bombardment based, transient transformation of leaf epidermal cells. Using the GUS reporter gene (Schweizer et al. 1999; Douchkov et al. 2005), transformed cells undergoing an active defense response can be identified by co-bombarding the candidate gene with the GUS reporter plasmid. Thus, this functional assay does not require the time consuming procedure of generating stably transformed wheat plants. With this transient transformation assay *Pm3b* was functionally validated and assigned to the biggest class of R gene family, the CC-NBS-LRR proteins. It encodes a domain with 28 well conserved LRR domains and a protein of 1,415 amino acids. Subsequently, additional 7 *Pm3* alleles (*Pm3a-3 g*) were identified from other genetic backgrounds providing race-specific resistance to a different subsets of powdery mildew isolates, and used to develop functional allele-specific markers for germplasm screening (Tommasini et al. 2006) (Table 15.1).

15.2.1.3 Cloning of *Pm21*: Integration of Map-Based Cloning and Gene Expression Analysis to Isolate the *Pm21* Gene from a Non-Recombining Genetic Region

Gene expression analysis has complemented map-based cloning approaches and helped to identify a second powdery mildew resistance gene from wheat. Cao et al. (2011) used a high-throughput strategy of GeneChip microarray analysis in combination with genetic mapping to isolate *Pm21*, an important source of durable and broad spectrum resistance to wheat powdery mildew. *Pm21* was originally transferred from the short arm of chromosome 6V of the wild wheat relative *Haynaldia villosa* ($2n = 2x = 14$) to cultivated wheat by the development of a 6VS.6AL translocation line. Approaches to isolate *Pm21* by map-based cloning using this translocation line were unsuccessful due to the low chromosome pairing frequency and suppressed recombination between the 6VS chromosome from *H. villosa* and chromosome 6AS from wheat. A GeneChip approach was therefore applied to identify genes that are up-regulated upon *Bgt* infection in *H. villosa* compared to the mock control. Among the 196 differentially expressed genes, four resistance gene analogs (RGAs) were

Table 15.1 A success story of genomics-assisted breeding: the isolation and functional characterization of the wheat powdery mildew resistance gene *Pm3*

Step	Approach	Achievements	Reference
1	Identification of the genetic basis of resistance in a specific background	Chromosomal location of a powdery mildew resistance gene in wheat cv. Amigo	Heun et al. 1990
2	Identification of additional alleles of the resistance gene	Identification of other powdery mildew resistance genes/alleles at the *Pm3* locus in hexaploid wheat	Zeller et al. 1993
3	Development of molecular markers linked to the locus	Development of molecular markers for the different *Pm3* alleles	Hartl et al. 1993 Ma et al. 1994 Bougot et al. 2002
4	Gene isolation and functional validation	Map-based cloning of *Pm3b* from hexaploid wheat using genome analysis at different ploidy levels	Yahiaoui et al. 2004
5	Isolation of additional alleles	Identification of the *Pm3* allelic series in hexaploid wheat	Srichumpa et al. 2005
6	Development of functional allele-specific markers	Development of functional markers specific for the seven *Pm3* resistance alleles and their validation in the bread wheat gene pool	Tommasini et al. 2006
7	Evolutionary studies on the resistance genes/allelic sequences	Studies on the evolution of the *Pm3* alleles	Yahiaoui et al. 2006
		Comparative analysis on the evolution of the *Pm3* locus in three different wheat species and rice	Wicker et al. 2007
		Comparative analysis on the evolution of functional *Pm3* alleles from wild and cultivated wheat	Yahiaoui et al. 2009
8	Identification of novel alleles using additional genetic resources (wild relatives, landraces)	Identification of novel functional *Pm3* alleles using extended wheat genetic resources	Bhullar et al. 2009
		Large scale allele mining of wheat gene bank accessions for novel *Pm3* alleles	Bhullar et al. 2010
9	Functional gene characterization using transgenic technology	Intragenic allele pyramiding using the transgenic technology combines different specificities of wheat *Pm3* resistance alleles	Brunner et al. 2010
10	Field assessment of transgenic plants	Transgenic *Pm3b* wheat lines show resistance to powdery mildew in the field	Brunner et al. 2011
		Transgenic *Pm3* multilines of wheat show increased powdery mildew resistance in the field	Brunner et al. 2012

identified which were selected for further investigation. Using a series of alien deletion and translocation lines these genes were cytogenetically mapped by *in situ* hybridization (FISH). Only one RGA, a putative serine/threonine protein kinase (Stpk-V), was found to localize on chromosome 6VS of *H. villosa*, thus making this the best candidate gene for the *Pm21* resistance activity. Expression of Stpk-V was suggested to alter the function of target proteins by phosphorylation of serine or threonine residues. A significant decrease in the haustorial index was observed when epidermal cells were co-transformed with the *GUS* and the *Stpk-V* gene, in comparison to cells only transformed with the *GUS* gene. Also, transgenic plants expressing the *Stpk-V* gene showed an increased broad-spectrum powdery mildew resistance compared to the controls. Further validation of this gene was provided by virus-induced gene silencing (VIGS), where increased susceptibility was observed in *Stpk-V* silenced wheat and its wild relative. The isolation of *Pm21* sets a promising example for future efforts to identify potentially useful genetic sources from wild species by integration of cytogenetic, molecular and transcriptomic methods.

As discussed above for the *Pm21* gene, until recently high-throughput analysis of transcriptomes relied on the microarray technology (Varshney et al. 2009). Microarray based expression profiling has been successfully used to investigate and compare the transcript patterns in various cell types and organisms, however, tracking genetic diversity at the transcript level using the microarray technology has some limitations: Firstly, microarray technology is limited to already existing sequence information of genomes and their annotation. Thus, the gene content available on the array restricts the expression data which can be collected. Further, sensitivity and specificity can be low. The recent development of next generation sequencing (NGS) techniques allows sequencing of the entire transcriptome at a much higher coverage. Compared to the microarray technology, RNA sequencing also has the advantage of providing an unbiased representation of all transcripts. In addition, rare transcripts or alternative splice variants can be detected, as well as allele specific expression and expressed single nucleotide polymorphisms. Sequence variation at RNA levels is therefore more likely to be detected using next generation transcriptomics. Thus, NGS techniques combined with classical cloning methods serve as potentially useful tools to isolate additional disease resistance genes from wheat in the near future.

15.2.1.4 Allele Mining as a Strategy to Identify Additional and Novel Resistance Sources

The identification of genetic resistance sources in wheat and their combination and accumulation in particular cultivars has greatly contributed to the progress in resistance breeding. Nevertheless, we can presume that a huge portion of beneficial resistance genes in the wheat gene pool remains unexploited (Kumar et al. 2010). Several studies have found that resistance in cultivated wheat could be significantly improved by introducing novel alleles from wild relatives. It was further observed that expression of novel alleles or combinations thereof can vary tremendously depending on the genetic background (McCouch et al. 2007; Cao et al. 2011). Thus, a

great potential exists in finding new resistance sources by re-investigating the large germplasm material of wild progenitors or landraces and expressing the new genes in different genetic backgrounds. With the recent development of NGS technologies, sequence information from several crop species has greatly improved and made publicly available to the research community. Although this will presumably accelerate resistance gene discovery in wheat, our current knowledge about resistance genes is still very limited. Thus, it is even more important to use the existing knowledge on cloned resistance genes and exploit the genome information from germplasm resources in order to identify novel, potentially functional alleles. The cloning of the wheat *Pm3* gene and the molecular characterization of its alleles, together with the development of allele-specific markers, allowed an in-depth investigation of a large set of wheat landraces, aiming at the identification of new, potentially functional *Pm3* alleles (Kaur et al. 2008).

The dissection of naturally occurring variation at a known candidate gene locus is also referred to as "allele mining", a strategy taking advantage of an overall high sequence conservation at a specific locus (Kumar et al. 2010). Initial allele-mining studies focused on identification of sequence variation in coding sequences of important loci. However, with increasing evidence for non-coding regions having large effects on transcript and trait expression, mining for sequence variation in regulatory regions of resistance loci is relevant, too. In "promoter mining", promoter regions instead of gene coding sequences are investigated for sequence variation. Both allele and promoter mining have several important applications in resistance breeding. Superior and novel alleles can be identified, new markers can be developed to allow rapid identification of different haplotypes in marker-assisted selection, and evolutionary studies can be performed as well as expression studies. However, there are major considerations for a successful and efficient allele mining approach: besides the requirement of sufficient genome sequence information, there should be high-throughput techniques available to generate allelic data and efficient bioinformatic tools to identify nucleotide variation. Once novel alleles have been identified, a reliable and rapid system for functional validation of the novel alleles is desired. Besides these technical considerations, the foremost challenge in allele mining is the selection of a manageable and sensible number of genotypes capturing the highest possible sequence variation at a specific locus. One possible strategy is the Focused Identification of Germplasm Strategy (FIGS) which allows the identification of trait-specific sets of accessions with maximum diversity. Assuming that the frequency of the trait of interest is strongly influenced by the environment and is based on adaptive selection processes, accessions are selected according to eco-climatic parameters of their original collection sites (Endresen et al. 2011). To date, FIGS has been successfully used to identify new genetic diversity for resistance against abiotic and biotic stresses and specifically also in the case of *Pm3* based resistance (Bhullar et al. 2009). There, 1,320 accessions from 323 geographic sites with potentially high selection pressure for powdery mildew resistance were selected from a virtual collection of 16,089 accessions, and tested against different powdery mildew isolates (Kaur et al. 2008). Among them, 211 accessions which showed complete or intermediate resistance were further analyzed at the molecular level. 111 landraces which

were positive for a *Pm3* diagnostic fragment, but did not amplify specific markers for the known *Pm3a-Pm3g* alleles, were selected as candidates for potentially new functional *Pm3* alleles. Functional analysis of these 111 candidates used a combination of pathogenicity assays and virus-induced gene silencing (VIGS), and resulted in the identification of seven new functional alleles (*Pm3l-Pm3r*) in addition to previously described alleles. As the FIGS screening set contained accessions from a limited geographic area (with a strong focus on the Near East), a new set including accessions from more diverse locations was screened to investigate *Pm3* diversity in more depth. From a collection of an additional 733 wheat accessions eight new *Pm3* sequences were isolated. From these, two additional novel alleles, originating from Nepal *(Pm3s)* and China *(Pm3t)* respectively could be functionally validated (Bhullar et al. 2010). Thus, the large genebank collections comprising germplasm of wild wheat relatives and landraces provide a great potential to identify new resistance resources. In the case of the *Pm3* alleles, out of 30 different countries most of the functional alleles were isolated from accessions originating from Turkey, Afghanistan, Turkmenistan, China and Nepal (Bhullar et al. 2010). The germplasm derived specifically from these countries therefore has a great potential for further exploration specifically for powdery mildew resistance.

15.2.1.5 Field Assessment of Wheat Lines Carrying a Transgenic *Pm3* Resistance Gene

The molecular isolation of the two powdery mildew resistance genes *Pm3* and *Pm21* also provided the opportunity to modify their expression and investigate their efficiency under natural field conditions using transgenic approaches. This has been described in some detail for the *Pm3* resistance alleles. The question was if transgenic genes, under the control of a constitutive promotor would result in improved resistance, and if mixtures of genotypes with the same genetic background, but containing different *Pm3* alleles (so called multilines) would show enhanced resistance due to a mixture effect.

In order to test the transgenic use of race-specific *R* genes for their effectiveness in the field, transgenic wheat lines over-expressing *Pm3a*, *Pm3b*, *Pm3c*, *Pm3d*, *Pm3f* or *Pm3 g* were analyzed during one to three field seasons. All 12 tested transgenic lines were significantly more resistant than their respective non-transformed sister lines but the *Pm3* lines showed differences in the level of powdery mildew resistance. These differences were possibly caused by the differences in frequency of virulence to the particular *Pm3* allele in the powdery mildew population, *Pm3* expression levels and most likely also allele-specific properties. Half of the transgenic lines revealed additional phenotypes in the field, which were not visible under greenhouse conditions. Besides an increased powdery mildew resistance, three of four independent transgenic events carrying *Pm3b*, two *Pm3f* lines and a *Pm3g* line exhibited a leaf chlorosis phenotype, reduced fertility or a reduced plant height (Brunner et al. 2011). High *Pm3* gene expression levels or PM3 protein accumulation were the most likely,

but not all-embracing explanation for these phenotypes. This work showed the importance of field trials for assessment of agronomically relevant disease resistance. It further showed that the success of a transgenic use of *R*-genes in the field critically depends on optimization of expression levels, for example by using tissue and/or development-specific promoters.

To improve the durability of major *R* genes such as *Pm3*, the multiline strategy has been proven to be effective in small grain crops (Zhu et al. 2000; Mundt 2002). Multilines are seed mixtures of agronomically uniform lines that differ only in a specific trait, mostly disease resistance. Brunner et al. (2012) could show in a multiline field experiment that two-way seed mixtures between transgenic lines carrying *Pm3a*, *Pm3b* or *Pm3d* significantly increased the powdery mildew resistance when compared to the mean of the pure component lines alone. This demonstrates that diversity in a single *R* gene is sufficient to improve resistance levels when used in multilines, most probably through host-diversity effects.

15.2.1.6 The Use of Natural Variation to Make Artificial Resistance Genes with Broadened Specificity

The durability of major *R*-genes can possibly be improved by designing artificial resistance genes exhibiting broadened specificity. A successful example of this strategy was provided by Brunner et al. (2010) where they investigated in detail the powdery mildew isolate recognition spectra of different *Pm3* alleles and identified some alleles with enlarged resistance spectra compared to others. Sequence analysis of the natural variation occurring in the *Pm3* alleles exhibiting broad or narrow resistance spectra, allowed to propose hypotheses on the functional roles of individual protein subdomains. Domain-swap experiments revealed for example that the NB-ARC domain is also playing a role in resistance specificity, although pathogen recognition specificity is mostly determined by the LRR-domain. A chimeric, artificial PM3 protein combining different polymorphic residues of the functional alleles proved that intramolecular pyramiding of different *R*-gene recognition specificities is possible and a new resistance gene with a broader specificity can be made.

15.2.1.7 Molecular Analysis of Quantitative Resistance Against Wheat Powdery Mildew

Race-specific powdery mildew resistance genes based on a gene-for-gene interaction with the corresponding pathogen avirulence genes confer strong and effective resistance. Thus, there has been an extensive use of these race-specific *R* genes during the past decades. In the natural situation, the gene-for-gene relationship reflects a co-evolution between the pathogen and the host, where advantageous polymorphisms for either host resistance or pathogen virulence are balanced and stable. If the factors important for this balance are lost, as it is the case in modern agricultural systems, parasite evolution becomes instable and pathogens evolve at much higher

rates. Thus, host-pathogen dynamics resemble more an arms race and this type of resistance becomes of short duration only (Brown and Tellier 2011). It is therefore of great importance to reduce the opportunities for a pathogen to adapt to crop resistance, for example by increasing the genetic diversity of crops or by taking advantage of resistance genes interacting with costly pathogen avirulence genes. Most importantly, exploring durable or quantitative sources of resistance with a combination of several minor genes can greatly help to control powdery mildew diseases in a durable way. Quantitative resistance, also referred to as slow-mildewing or partial resistance is controlled by several genetic loci. It is also known as adult plant resistance (APR) due to the compatible interaction at all stages of growth combined with low infection frequency, prolonged latency period and reduced sporulation at adult plant stage. A series of studies has been conducted on the identification and mapping of quantitative loci involved in disease resistance in the past few years. The development of reliable selection tools has greatly helped to include APR genes in wheat breeding programs. APRs for powdery mildew have been mapped to all homeologous chromosomes of the wheat genome (Sharma et al. 2011). However, to date, there are very few success stories on the molecular isolation of quantitative resistance genes in plants, one being the isolation of the resistance gene, *Lr34/Yr18/Pm38* in wheat (Krattinger et al. 2009). *Lr34/Yr18/Pm38* presents one of the most important durable, race non-specific, adult plant resistance (APR) gene resources which was first identified in Canada by Dyck et al. (1966). Besides providing resistance to leaf rust, it also confers resistance against stripe rust (*Yr18*) (McIntosh 1992), powdery mildew (*Pm38*) (Spielmeyer et al. 2005; Lillemo et al. 2007), stem rust (Dyck 1987) and tolerance to barley yellow dwarf virus (*Bdv1*) (Ayala et al. 2002). Being an APR in nature, *Lr34/Yr18/Pm38* is most effective in the flag leaves of adult plants which also develop necrotic leaf tips, a morphological marker known as leaf tip necrosis (*Ltn*) associated with the presence of *Lr34/Yr18/Pm38* (Dyck 1991; Singh 1992).

The consensus genetic map of three *Lr34/Yr18/Pm38*– based high resolution mapping populations, marked the target interval of 0.15 cM for the *Lr34/Yr18/Pm38* locus. The complete sequencing of a 363 kb physical target interval from the *Lr34/Yr18/Pm38* containing Chinese Spring cultivar revealed eight open reading frames as candidate genes. These open reading frames shared homologies to a hexose carrier, an ATP-binding cassette (ABC) transporter, two cytochromes P450, two lectin receptor kinases, a cysteine proteinase and a glycosyl transferase (Krattinger et al. 2009). Sequence analysis of the candidate gene coding regions from the parental alleles as well as the *Lr34* mutants identified several sequence polymorphisms in the ABC transporter gene leading to either splice site mutations, amino acid changes, frame shift mutations or pre-mature stop codons, thus confirming the ABC transporter gene as the *Lr34/Yr18/Pm38* gene providing durable resistance against leaf rust (Krattinger et al. 2009). Thus, the *Pm38* gene is the first cloned quantitatively acting disease resistance gene against powdery mildew and was also reported in the cultivars Fukoho-Komugi and Saar from Japan and CIMMYT, respectively (Liang et al. 2006; Lillemo et al. 2008). As discussed above, there are many additional quantitative trait loci (QTL) involved in powdery mildew resistance. Keller et al. (1999) identified 18 QTLs against powdery mildew in a segregating wheat x spelt

(*Triticum spelta*) population explaining 77 % of the phenotypic variation. However, in most of the cases only 1–4 QTLs have major effects. The wheat cultivars Knox (Shaner 1973) and Massey (Griffey and Das 1994) are two cultivars showing effective powdery mildew APR, which presumably is governed by two to three genes only. Similarly, several other QTLs have been identified in different wheat cultivars originating from different countries such as RE 714, Festin, Courtot and RE 9001 from France (Chantret et al. 2001; Mingeot et al. 2002; Bougot et al. 2006), USG3209 from North America (Tucker et al. 2007), Oligoculm from Israel (Liang et al. 2006), Avocet from Australia (Lillemo et al. 2008), Suwon 92 from Korea (Xu et al. 2006) and Bainong64 originating from China (Lan et al. 2009). Once molecular markers for a number of QTL contributing additively to powdery mildew resistance are known, this will allow a very efficient breeding approach to combine such loci and obtain genotypes with sufficient field resistance efficiently.

15.2.2 Basis of Resistance to Stagonospora Nodorum Leaf Blotch in Wheat

Being a necrotrophic fungus, *Phaeosphaeria nodorum* (anamorph *Stagonospora nodorum)* infects and kills wheat leaf tissue and feeds from the organic compounds of the dead cells during its life cycle. To invade wheat leaves, *S. nodorum* produces proteinaceous Host Selective Toxins (HST). These HSTs interact with the plant host in a mirrored gene-for-gene interaction. In the following paragraphs, we will describe the current knowledge on toxin- mediated resistance to Stagonospora nodorum blotch (Oliver et al. 2012).

15.2.2.1 Interactions Between Fungal Toxins and Wheat Sensitivity Genes Cause Susceptibility

According to the classical gene-for-gene model developed by (Flor 1955), a pathogen is only able to invade the host successfully if the plant does not recognize the pathogens virulence factor by a corresponding *R* gene. In the mirrored gene-for gene interaction of Stagonospora nodorum leaf blotch, the infection will be successful only if the wheat cultivar has a corresponding susceptibility gene (Friesen et al. 2007). This type of interaction was identified as the cause of a few additional fungal diseases in different plant species (Table 15.2) (Mengiste 2012).

The recently sequenced genome of *S. nodorum* provided the opportunity to study the genetic basis of pathogenicity together with other features of the fungal lifestyle. The genome size was estimated to be 37.2 Mbp (Hane et al. 2007) and gene predictions and EST library analysis suggested that the genome contains at least 10,762 genes. Interestingly, a large number of identified genes were predicted to encode secreted proteins with no similarity to any known genes. Possibly, new host-selective toxins are among these genes. For instance, the host-selective toxin SnTox1 was identified by screening the whole *S. nodorum* genome for suitable candidates and then testing them in infection experiments (Liu et al. 2012).

Table 15.2 Cloned plant toxin-sensitivity genes which interact with fungal toxins resulting in susceptible disease response

Plant species	Fungal pathogen	Toxin	Susceptibility gene	References
Sorghum (*Sorghum bicolor*)	*Periconia circinata*	PC toxin	*Pc* (NBS-LRR)	Nagy et al. 2007
Arabidopsis thaliana	*Cochliobolus victoriae*	victorin	*LOV1* (NBS-LRR)	Lorang et al. 2007
Wheat (*Triticum aestivum*)	*Stagonospora nodorum*	ToxA	*Tsn1* (NBS-LRR)	Faris et al. 2010

Different strains of *S. nodorum* produce a range of HSTs. Five different toxins SnToxA, SnTox1, SnTox2, SnTox3 and SnTox4 have been identified until now (Liu et al. 2004a; Friesen et al. 2006; Friesen et al. 2007; Abeysekara et al. 2009). The susceptibility genes for all five toxins were mapped to different regions of the wheat genome: *Tsn1* interacts with ToxA and this interaction explains 77 % of the phenotypic variation in the population of cultivars 'BR34' and 'Grandin' (Liu et al. 2006) and 95 % of the phenotypic variation in the LD5B population of tetraploid wheat (Faris and Friesen 2009). The *Snn1* and SnTox1 interaction explains 58 % of variation in the ITMI population (Liu et al. 2004b) and *Snn2* – SnTox2, *Snn3* – SnTox3 and *Snn4* – SnTox4 are responsible for 47, 17 and 41 %, respectively, observed in segregating wheat populations derived from a cross between the hard red spring wheat line BR34 and cultivar Grandin for *Snn2* and *Snn3*, and a RIL population of Arina x Forno for *Snn4* (Abeysekara et al. 2009). Interestingly, each fungal toxin-wheat gene interaction is qualitative, but they contribute to the resistance response in a quantitative manner. For example, SnToxA-*Tsn1* and SnTox2-*Snn2* have additive effects during the infection (Oliver et al. 2012).

15.2.2.2 Quantitative Resistance to SNB

Classical genetic studies suggest that resistance to SNB is complex and in most cases polygenic (Scott et al. 1982; Fried and Meister 1987; Bostwick et al. 1993; Du et al. 1999). Monogenic resistance was also identified in some wheat varieties (Kleijer et al. 1977; Ma and Hughes 1995; Murphy et al. 2000). The resistance responses to SNB on leaves and glumes are genetically independent (Francki et al. 2011). Several QTL controlling partial resistance to Stagonospora nodorum blotch in seedlings were identified on chromosomes 2B, 3B, 5B and 5D using a double haploid population derived from a cross of winter wheat cultivars 'Liwilla' and 'Begra' (Czembor et al. 2003). However, their effect on adult plants was not tested. QTLs for resistance to SNB on the flag leaf might correspond to the loci associated with toxin insensitivity genes in the wheat genome: for example, Francki et al. (2011) discovered three QTLs using a cross of winter wheat 'P92201D5' and spring wheat 'EGA Blanko'. Two of them, located on chromosomes 1BS and 2AS respectively, did not correlate with any known toxin sensitivity genes. In contrast, the third QTL on chromosome 5BL was

associated with *Tsn1*-ToxA insensitivity. Independent genetic control of resistance to SNB in glumes and leaves combined with diverse resistance on different stages of plant growth suggests that the best strategy for breeding is to combine the different genetic loci and take advantage of their additive effects.

15.2.2.3 The SNB Susceptibility Gene Tsn1 Encodes an NBS-LRR Protein

The susceptibility genes have additive effects if multiple compatible interactions are acting at the same time. Therefore, disease resistance to Stagonospora nodorum leaf blotch depends on the presence of susceptibility genes and is quantitatively inherited (Abeysekara et al. 2009). *Tsn1* confers sensitivity to SnToxA and is located on the long arm of chromosome 5B. The *Tsn1* gene was recently cloned using a classical chromosome walking approach after establishing a physical contig of 350 kb containing the flanking markers (Faris et al. 2010). Bioinformatic analysis identified six genes cosegregating with *Tsn1*. An association study on 386 wheat accession narrowed the number of candidates down to four genes. Further validation revealed that *Tsn1* has a resistance gene-like structure consisting of a nucleotide-binding, leucine-rich repeat (NBS-LRR) and a serine/threonine protein kinase (S/TPK) domain. Mutagenesis experiments demonstrated that all three domains are required for disease susceptibility. The analysis of *Tsn1* suggests that the gene originated from a B-genome donor through a gene fusion. The exact mechanism of the HST-gene interaction still remains unknown. The presence of *Tsn1* is required for ToxA recognition, but yeast two-hybrid experiments suggest that the Tsn1 protein does not interact directly with ToxA. It was shown that *Tsn1* transcription is regulated by the circadian clock and light, indicating that the Tsn1-ToxA interactions are linked to photosynthesis processes. Faris et al. (2010) suggested that in the case of *Tsn1*- ToxA interaction, *S. nodorum* may have subverted a wheat defence mechanism based on an NBS-LRR immune receptor that was (and possibly still is) involved in resistance against a different pathogen species.

15.2.2.4 Genomics-Assisted Use of Genetic Resources for SNB Resistance Breeding Based on the Molecular Understanding of the Pathosystem

Based on the recent findings on host-specific toxins in the *S. nodorum*-wheat pathosystem, it is evident that the presence or absence of specific toxin receptors in the widely grown wheat cultivars will have a significant impact on disease prevalence. It was recently shown (McDonald et al. 2013) that there are significant differences between the frequencies of toxin presence in *S. nodorum* isolates originating from different geographical regions. This suggests that the presence/absence of sensitivity genes in the cultivars grown in particular regions has a strong effect: whenever a cultivar contains the sensitivity gene corresponding to a specific toxin, the presence of this toxin will be of selective advantage for the pathogen and races with the toxin will increase in frequency. On the other hand, if the sensitivity gene is absent, there will be no selective advantage for having the toxin and it is likely that the frequency of such races will decrease.

These findings immediately suggest that a breeding strategy which has the goal to eliminate as many relevant susceptibility genes as possible from the germplasm, might be effective (it remains to be determined which ones belong to this group in addition to *Tsn1*). This has not been tried yet but has considerable potential to reduce the problem of SNB based on diagnostic markers for a limited subset of toxin susceptibility genes. The markers would allow the elimination of all breeding material with active susceptibility genes. Clearly, this will only be possible if the molecular differences between susceptible and non-susceptible alleles will be known. At this stage, only the Tsn1 receptor is cloned and more map-based cloning projects are needed to molecularly isolate the other toxin receptor genes. Ideally such an effort to eliminate susceptible lines would be coordinated in large geographical areas to ensure success and reduce the frequency of toxin genes. Such a project is ongoing in Australia to eliminate the *Tsn1* gene from commercial germplasm (Oliver and Solomon 2010; Waters et al. 2011).

In conclusion, based on the molecular advancements in understanding the *S. nodorum*-wheat pathosystem, future resistance breeding efforts will possibly rely more on molecular markers for selecting against susceptibility (receptor) genes and not only depend on phenotyping under field conditions. It will be interesting to see if similar type of genes is responsible for resistance to Stagonospora nodorum glume blotch, the disease on the glume. As resistance in the glume is inherited independently from resistance in the leaf, other genetic factors must be involved (Schnurbusch et al. 2003).

15.2.2.5 Genomics Reveals an Interspecific Gene Transfer and Rapid Virulence Evolution in a Wheat Pathogen

It is assumed that rapid diversification of effectors in pathogens is closely linked to the avoidance of detection by the plant immune system (Dodds et al. 2006). Biotrophs, such as powdery mildew, are seeking for new ways to overcome the resistance genes and colonize the host. In contrast, necrotrophic pathogens benefit from the hypersensitive response and feed from the dead tissue. However, the diversification of effectors plays an important role for necrotrophs as well. It was suggested that the diversity of fungal toxins found in necrotrophs and in particular in *S. nodorum* can be explained by two hypotheses. The first hypothesis postulates that necrotrophs gain evolutionary benefits by tracking the appearance of new sensitivity alleles in the host (Stukenbrock and McDonald 2007). The second hypothesis suggests that the diversification of the toxins allows the pathogen to increase its fitness and aggressiveness (Tan et al. 2012). Effector diversity is the result of recombination and mutation events in the toxin genes, but also of non-vertical genetic exchanges (horizontal gene transfer) known to occur in filamentous fungi. It was found that some genes in the *S. nodorum* genome have no homology to any known genes in closely related fungi. The presence of those genes might indicate that they were acquired by horizontal transfer from another, more distantly related species (Oliver et al. 2012). Recently, Friesen et al. (2006) provided evidence for the gene transfer between the two fungal pathogens *S. nodorum* and *Pyrenophora tritici-repentis*. *P. tritici-repentis* produces the host-selective toxin ToxA. The ToxA gene was cloned

previously by Ciuffetti et al. (1997). Analysis of the sequenced *S. nodorum* genome (Hane et al. 2007) revealed the presence of a close homolog with a similar gene structure consisting of three exons and two introns and sharing 99.7 % identity with the *P. tritici-repentis* ToxA. The high similarity suggests a recent common ancestor gene. Several isolates of *S. nodorum* and *P. tritici-repentis* with different geographical origins were tested for their ToxA sequence diversity. Among 95 *S. nodorum* and 54 *P. tritici-repentis* ToxA amplicons only one haplotype was identified for *P. tritici-repentis* whereas 11 haplotypes were found in *S. nodorum*. This suggests that the ToxA gene was more ancient in the *S. nodorum* genome and was probably introduced only recently in the *P.tritici-repentis* genome. Further analysis of the 11 kb genomic region flanking the ToxA gene in both species revealed a high degree of conservation: 80–90 % in the distal parts and 98–100 % in the middle. Additionally, functional analysis of ToxA-disrupted mutants and their interaction with the wheat *Tsn1* gene indicated a role of ToxA in inducing a susceptible plant response for both *P. tritici-repentis* and *S. nodorum*. This hypothesis is also supported by the fact that tan spot in comparison with *S. nodorum* leaf blotch was described in wheat only. The first records about tan spot as an occasional pathogen of wheat date from 1928. However, only in 1942 the typical necrotic symptoms were described. In contrast, *S. nodorum* leaf blotch was known as an important wheat disease already since 1889. This strongly suggests that an interspecific gene transfer between *S. nodorum* and *P. triticii-repentis* indeed has occurred and it happened most likely around 1942. Analysis of the *S. nodorum* genome sequence shows that interspecific horizontal gene transfer is not a rare and exotic mechanism, but the significant contributor to the pathogen adaptation. Clearly, the application of genomic tools in pathogenomics has resulted in findings highly relevant for wheat resistance breeding.

15.3 Conclusions

Global food security strongly depends on a highly productive and sustainable agriculture. Fungal pathogens can cause severe yield losses in all major crops and are a serious threat for food security, especially in developing countries. Breeding for resistant wheat varieties is the most effective strategy to counteract these diseases, requiring however a better understanding of the molecular basis of disease resistance. The genetic complexity of wheat greatly complicates gene isolation and functional characterization, explaining the limited number of so far characterized resistance genes in wheat. Major race-specific resistance genes can provide plants with a high level of disease resistance. However, biotrophic fungi such as the powdery mildews are rapidly evolving pathogens which are able to overcome these resistance genes. Thus, new sources of genetic resistance have to be identified in order to avoid an erosion of the current pool of agriculturally important resistance genes.

Molecular isolation of the race-specific *Pm3* resistance gene provided highly valuable insights in the diversity and evolution of resistance genes. With the help of developed molecular markers and an established functional validation assay, the

allele mining strategy could be tested for its efficiency to explore genetic diversity and identify new resistance sources. Indeed, this strategy allowed the isolation of ten functional resistance alleles in addition to the seven genetically known *Pm3* alleles, demonstrating the importance of wild landraces and wheat progenitors as valuable genetic resources for resistance as well as the feasibility of the allele mining strategy.

The recent finding that in necrotrophic pathosystems such as *S. nodorum*, an interaction between a pathogen toxin and a susceptibility host component is required for a successful pathogen invasion, influenced research on the isolation of genes providing resistance to necrotrophic diseases and possibly explains the present limited knowledge thereof. Nevertheless, the awareness of susceptibility genes being required for pathogen establishment allows breeding for cultivars which lack these genes and thus provide higher resistance to necrotrophic fungi.

With the emergence of highly virulent pathogen strains which overcome previously effective resistance genes, disease resistance research is currently expanding towards the isolation of quantitative resistance. Although this type of resistance is often only partial, it was shown to be more durable (Kou and Wang 2010). Cloning of *Lr34/Yr18/Pm38* sets a successful example of isolation of quantitative, durable and broad spectrum disease resistance gene. With the isolation of *Pm21*, providing durable and broad spectrum resistance, it will be possible to gain additional insights into the molecular mechanisms of durable resistance, and also, similarly to *Pm3*, expand the variation of functional and durable *Pm21* alleles. In contrast, durable resistance to *S. nodorum* is still only poorly investigated and urgently needs further molecular analysis.

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