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Molecular approaches for characterization and use of natural disease resistance in wheat

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Abstract Wheat production is threatened by a constantly changing population of pathogen species and races. Given the rapid ability of many pathogens to overcome genetic resistance, the identification and practical implementation of new sources of resistance is essential. Landraces and wild relatives of wheat have played an important role as genetic resources for the improvement of disease resistance. The use of molecular approaches, particularly molecular markers, has allowed better characterization of the genetic diversity in wheat germplasm. In addition, the molecular cloning of major resistance (*R*) genes has recently been achieved in the large, polyploid wheat genome. For the first time this allows the study and analysis of the genetic variability of wheat *R* loci at the molecular level and therefore, to screen for allelic variation at such loci in the gene pool. Thus, strategies such as allele mining and ecotilling are now possible for characterization of wheat disease resistance. Here, we discuss the approaches, resources and potential tools to characterize and utilize the naturally occurring resistance diversity in wheat. We also report a first step in allele mining, where we characterize the occurrence of known resistance alleles at the wheat *Pm3* powdery mildew resistance locus in a set of 1,320 landraces assembled on the basis of eco-geographical criteria. From known *Pm3 R* alleles, only *Pm3b* was frequently identified (3% of the tested accessions). In the same set of landraces, we found a high frequency of a *Pm3* haplotype carrying a susceptible allele of *Pm3*. This analysis allowed the identification of a set of resistant lines where new potentially functional alleles would be present. Newly identified resistance alleles will enrich the genetic basis of resistance in breeding programmes and contribute to wheat improvement.

Keywords Allele mining · Genetic diversity · *Pm3* · Wheat powdery mildew

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Introduction

Wheat is globally one of the three most important food crops, the other two being maize and rice. Wheat

diseases cause severe yield losses and often reduce grain quality. Some of the most important fungal diseases of wheat include three rust species (stripe, leaf and stem rust), powdery mildew, fusarium, septoria, mycosphaerella and tan spot. To achieve sufficiently high resistance to fungal pathogens is an ongoing challenge for wheat breeding. Various aspects such as understanding pathogen biology, characterization of pathogen avirulence and plant disease resistance genes, and finally the search for new resistance sources, all contribute to the development of wheat with increased resistance to various diseases.

Wheat (*Triticum aestivum*) is an allopolyploid species featuring three distinct homoeologous genomes A, B and D. As the wheat genome is large (16×10^9 bp) and the major fraction consists of repetitive sequences, the molecular cloning of genes, for which only genetic information is available, remains a challenge (Keller et al. 2005). Therefore, the first efforts to characterize loci of disease resistance at the molecular level concentrated on the development of molecular markers linked to these important traits.

The availability of molecular markers linked to specific resistance genes and of information on their genetic location has supported resistance breeding by simplifying the detection of specific genes in breeding material. This makes the selection process faster and more cost effective. In addition, different genes can be combined in a pyramiding strategy for resistance breeding. Despite these efforts, the genetic base of disease resistance in wheat remains dangerously narrow and adaptation of pathogens is always a threat, challenging the resistance of existing elite material. Currently, the emergence and spread of the new virulent stem rust race *Ug99* is considered to be a potential threat to wheat production worldwide. The *Ug99* race has overcome the major stem rust resistance gene *Sr31* (http://www.ars.usda.gov/Main/docs.htm?docid=14649). Previously, there was no report of virulence against *Sr31*, a gene which is widely used in India, China, Europe and South America. This makes it imperative to identify new sources of resistance which are thought to exist in germplasm collections and then to make combinations with existing sources to develop more durable types of resistance.

The introduction of resistance genes from landraces, traditional varieties and wild relatives, e.g. the diploid and tetraploid progenitors of hexaploid wheat, has been successful in broadening resistance (Miranda et al. 2006; Liu et al. 2002; Rong et al. 2000). Over thousands of years, landraces of hexaploid wheat have developed under a variety of different edaphic and climatic environments. This has resulted in the evolution of a large number of ecotypes adapted to specific local environments. Thus, the genetic collections available in gene banks are expected to provide a rich resource to identify new functional genes or alleles of resistance genes. The molecular changes underlying this adaptation are mostly unknown and this diversity is largely unused and uncharacterized at the molecular level. Traditionally, resistance genes in wild relatives of wheat have been introgressed by complex breeding schemes involving irradiation and chromosomal translocations (Baum et al. 1992). This has resulted in the introgression of large chromosomal segments, often carrying negative breeding traits (linkage drag). The molecular isolation of the underlying genes and their use through transgenic technologies will contribute to an efficient future use of resistance genes from wild grasses.

Hybridization and introgression of chromosomal segments, marker-assisted selection and the breeding of synthetic hexaploid wheat (Zhang et al. 2005) are well established methods for broadening the genetic diversity of disease resistance in wheat. Still largely unexplored, a new approach of 'allele mining' has recently become available and shows promise for the more efficient use of genetic diversity (to be discussed in detail later in this paper). The first wheat disease resistance genes have been cloned at the molecular level (Huang et al. 2003; Feuillet et al. 2003; Yahiaoui et al. 2004; Srichumpa et al. 2005; Yahiaoui et al. 2006; Cloutier et al. 2007). The sequence information of these cloned genes facilitates the rapid analysis of the genetic diversity at these loci over a wide range of germplasm and the subsequent identification of new alleles through allele mining. Molecular tools that specifically access the existing genetic diversity at a particular locus provide a promising approach for utilising the diversity maintained in the gene banks globally. In this paper, we discuss the molecular approaches available for detecting and using genetic diversity for improving disease resistance in wheat. We also describe an allele mining strategy for new resistance specificities at the wheat resistance locus *Pm3* against the powdery mildew pathogen (*Blumeria*

graminis f.sp. *tritici*) applied to 1,320 wheat landraces from different geographic origin.

Molecular markers in the characterization of wheat disease resistance diversity

Molecular markers play a significant role in the process of identification and introgression of natural resistance into economically important but susceptible breeding material. The range of molecular markers used for this purpose includes restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), sequence tagged sites (STS), simple sequence repeats (SSR)/microsatellites, expressed sequence tags (ESTs) and RGAs (resistance gene analogues). RFLPs were the first molecular markers to be used in wheat in the early 1990s. There are several reports on the essential role of RFLPs in marker-assisted selection, genome mapping as well as characterization and isolation of various disease resistance genes in wheat (Lagudah et al. 2006; Feuillet et al. 2003). Because of lower costs and time requirements, PCR-based markers have a higher potential for applications in the genetic characterization of wheat germplasm than RFLPs. AFLPs combine the simplicity of the RAPDs and robustness of RFLPs. Therefore, they have been frequently used to investigate biodiversity in several crops. Diagnostic markers for different resistance genes in wheat have been developed by using the AFLP technique (William et al. 2003; Adhikari et al. 2003).

STS markers have been highly useful tools for the screening of natural genetic variation as well as for tagging of resistance genes and QTL in wheat (Lagudah et al. 2006; Tyryshkin et al. 2006; Liu et al. 2002). Given the vast molecular information available on various plant disease resistance genes, RGAs have been extensively used. For instance, RGA markers linked to resistance genes *Yr5*, *Pm21* and *Pm31* (Yan et al. 2003; Chen et al. 2006; Xie et al. 2004) have been identified. As RGA sequences are very common in the plant genomes, they can be considered as a type of random marker.

SSR/microsatellite markers are highly polymorphic in wheat and are now widely used in wheat genetics for diversity studies, genotype identification (Prasad et al. 2000), marker-assisted selection, mapping, identification and tagging of disease resistance genes (Chagué et al. 1999; Miranda et al. 2006; Liu et al. 2002; Adhikari et al. 2003). Recently, microsatellite markers have been found linked to leaf rust resistance gene *Lr34* (Bossolini et al. 2006) supporting the identification of wheat genotypes with *Lr34*. With the increasing availability of molecular maps based on SSR markers, the identification and cloning of important genes is predicted to become more straightforward. Multiallelism, chromosome-specificity, even distribution in the genome and the possibility of high-throughput fingerprinting of large numbers of accessions are the properties that make SSR markers a good choice for detection of genetic polymorphism and diversity among wheat lines. However, microsatellite markers are often not suitable to define homoeologous loci, thus limiting their use in intraspecific and intra-genomic studies (Gupta et al. 1999). This also complicates the use of SSR markers for introgression studies involving wild relatives of wheat. Table 1 gives a summary of some molecular markers currently used for tagging different disease resistance genes in wheat.

Map-based cloning of disease resistance genes in wheat

To design precise and targeted molecular tools for diversity analysis based on allele mining, knowledge of the DNA sequence at a particular resistance locus becomes important, requiring the molecular cloning of genes. DNA sequence information is essential to devise rapid and inexpensive PCR strategies to isolate alleles of identified resistance genes from a wide range of cultivars, landraces and related species.

In map-based cloning, saturation of the genomic region of interest is greatly supported by genomic information and markers obtained from the grass model species rice and brachypodium (Griffiths et al. 2006). In addition, there are now a number of BAC libraries available from diploid, tetraploid and hexaploid wheat species (Keller et al. 2005) supporting map-based cloning strategies of wheat genes. Until now, three leaf rust resistance genes (*Lr*) and one allelic series of a powdery mildew (*Pm*) resistance gene have been cloned from wheat: *Lr21* (Huang et al. 2003), *Lr10* (Feuillet et al. 2003), *Lr1* (Cloutier et al. 2007) and the *Pm3* alleles (Yahiaoui et al. 2004; Srichumpa et al. 2005; Yahiaoui et al. 2006).

Table 1 Examples of molecular markers used for characterization and genetic mapping of different disease resistance genes in wheat

Disease	Resistance gene/genomic region	Marker used for identification and/or mapping	Reference
Leaf rust, yellow rust and stem rust	*Lr34/Yr18*	SSR, STS	Bossolini et al. (2006), Lagudah et al. (2006)
	Lr9, Lr19 and *Lr24*	STS	Tyryshkin et al. (2006)
	Lr46	AFLP	William et al. (2003)
	Lr47	RFLP/CAPS	Helguera et al. (2000)
	Yr15	SSR	Chagué et al. (1999)
	Yr5	RGA	Yan et al. (2003)
	Sr30	AFLP, RFLP	Bariana et al. (2001)
	Sr36	SSR	Bariana et al. (2001)
Powdery mildew	*Pm3*	STS	Tommasini et al. (2006)
	Pm21	RGA	Chen et al., (2006)
	Pm26	RFLP	Rong et al. (2000)
	Pm30	SSR	Liu et al. (2002)
	Pm31	RGA	Xie et al. (2004)
	Pm34	SSRs	Miranda et al. (2006)
Septoria leaf blotch	*Stb8*	AFLP and SSRs	Adhikari et al. (2003)

The first cloned wheat disease resistance gene, *Lr21,* was incorporated into bread wheat cv. Thatcher from the diploid wheat ancestor *Ae. tauschii*. A diploid/polyploid shuttle mapping strategy was deployed for map-based cloning of *Lr21* (Huang et al. 2003). There, the genetic analysis was done in hexaploid wheat but the large insert cosmid library was developed from the diploid donor. *Lr21* was chosen for cloning because of its location in a gene rich region and the extensive allelic diversity at this locus in natural populations of *Ae. tauschii* (Huang and Gill 2001).

Feuillet et al. (2003) isolated the leaf rust resistance gene *Lr10,* located on chromosome 1AS in hexaploid wheat by combining subgenome map-based cloning (Stein et al. 2000) and haplotype studies in the genus *Triticum*. The chromosome walking was performed on BAC clones of the diploid wheat *T. monococcum* DV92 (A genome) which had an *Lr10* haplotype, while the genetic map was constructed on the basis of genetic data from a hexaploid wheat population segregating for the *Lr10* resistance.

The wheat powdery mildew resistance gene *Pm3*, a dominant gene on chromosome 1AS, exists in ten different alleles (*Pm3a–Pm3j*) as identified by classical genetic studies. These alleles are predicted to confer resistance to specific races or isolates of the powdery mildew pathogen. Yahiaoui et al. (2004) used the combined analysis of genomes from wheat species with different ploidy levels for positional cloning of the *Pm3b* allele of *Pm3* in bread wheat. This represented the first molecular isolation of a powdery mildew resistance gene from wheat and a breakthrough for further analysis of diversity and evolution of this important locus. Based on the identification of a specific *Pm3* haplotype and using molecular markers derived from the *Pm3b* locus, additional known *Pm3* alleles (*Pm3a, Pm3b, Pm3c, Pm3d, Pm3e, Pm3f, Pm3g)* were isolated from different wheat lines (Srichumpa et al. 2005; Yahiaoui et al. 2006). Interestingly, it was also found that the three alleles *Pm3h*, *Pm3i*, *Pm3j* are actually identical to *Pm3d*, *Pm3c* and *Pm3b* respectively (Yahiaoui et al. 2006), suggesting that the lines in which the *h* to *j* alleles were identified contained additional resistance genes.

Quantitative resistance is often assumed to be more durable than single-gene resistance. Therefore, the improvement of quantitative resistance through tagging and cloning of QTL is of increasing importance in several wheat research and breeding programmes. A few QTL have been cloned in plants mainly by positional cloning (Salvi and Tuberosa 2005). In wheat, no QTL for disease resistance have yet been cloned but there are many ongoing projects with this goal. These include projects aimed at the isolation of rust resistance

loci such as *Lr34/Yr18* (Bossolini et al. 2006; Lagudah et al. 2006; Spielmeyer et al. 2005) and *Lr46/Yr29* (William et al. 2003; Rosewarne et al. 2006), *Sr2* (Kota et al. 2006) and a major QTL for Fusarium head blight (FHB) resistance (Liu and Anderson 2003). Mardi et al. (2005) reported the tagging of QTL responsible for FHB resistance with SSR markers and suggested that the SSR markers linked to the QTL would facilitate marker-assisted selection for FHB resistance in wheat. QTL tagging and cloning provide tools to the breeder for marker-assisted selection of complex disease resistance traits. It should also help to understand the respective roles of specific resistance loci versus partial resistance genes and the interactions between the genes and the environment.

Genetic resources for improvement of wheat disease resistance

A big advantage of diversity studies in wheat, compared to model plants such as *Arabidopsis*, is the existence of large collections of wild and cultivated diploid, tetraploid and hexaploid species secured in gene banks. However, at the molecular level this diversity remains largely unexplored due to a lack of fast and efficient tools to identify and study potentially useful new alleles. In addition to wheat landraces, wild relatives of wheat have been always explored and exploited as sources of new resistance genes. For example, a number of *R* genes originate from wild wheat relatives: the stem rust resistance gene *Sr39* was transferred from the wild relative *Aegilops speltoides* to bread wheat cv. Thatcher, leaf rust resistance gene *Lr24* from *Agropyron elongatum*, *Lr47* and *Pm32* from *Aegilops speltoides*, *Pm6* from *T. timopheevi* (Allard and Shands 1954), *Pm26* and *Pm30* from *T. turgidum* var. *dicoccoides* and *Pm34* from *A. tauschii* (Miranda et al. 2006; Liu et al. 2002; Rong et al. 2000). Common wheat has also been genetically improved for many decades through the introgression of rye chromatin. The rye chromosome arm 1RS is the most widely incorporated alien variation in the wheat genome. To give an example, wheat cv. Amigo carries the powdery mildew resistance gene *Pm17* on its introgressed 1RS chromosome arm (Forsström and Merker 2001).

As each of these *R* genes usually act only against a subset of the existing pathogen races, combinations of genes as well as the identification of new resistance genes/alleles are essential. Classically, identification of new resistance genes or of new alleles at already known loci is achieved by infection experiments on landraces or wild relatives of wheat followed by crosses necessary to determine if the resistance is due to a single gene and if the gene is a new allele at a known locus or represents a new locus. As resistance may be lost with rapid emergence of new pathogen strains, it becomes a continuous task to identify new resistance genes and to transfer these genes into common wheat if they are present in wild relatives or related species.

Molecular tools for screening the diverse germplasm: allele mining in cereals

Tools to access the existing genetic diversity at specific loci facilitate the rapid analysis of allelic diversity in the gene pool of wheat and its relatives. This in turn allows the molecular isolation of new alleles with potential agronomical relevance and a more efficient and targeted use of genetic resources for research and breeding. The strategy of finding valuable, unknown alleles at a known locus is referred to as 'allele mining.' In allele mining, the sequence of a target gene is used to develop specific markers to amplify, isolate and sequence new alleles at that particular locus. It seems to be a promising, although largely untested method to unlock the diversity in the collections of genetic resources in the world genebanks.

There are reports about the allele mining strategy in several cereal species to isolate alleles of target genes. In barley, an evaluation of cultivated germplasm was carried out to detect the presence of thermostable alleles of β-amylase (*Bmy-Sd2H* and *Bmy-Sd3* alleles) that improve the fermentability during brewing (Malysheva et al. 2004). The study was carried out on 891 accessions originating from different geographic regions worldwide. This led to the identification of 166 accessions with superior alleles, suggesting that the improvement of malting quality in barley could be achieved by introducing these alleles into breeding programmes. Latha et al. (2004) used the rice calmodulin genes and a salt-inducible rice gene for allele mining of stress tolerance genes on identified accessions of rice and related germplasm. They examined the feasibility of

allele mining using PCR primers based on the 5′- and 3′-untranslated regions of genes and found that these primers were sufficiently conserved to be effective over the entire range of germplasm in rice. The new HMW-glutenin alleles encoded by the *Glu-R1* locus of *Secale cereale* (rye) have been analysed and characterized (De Bustos and Jouve 2003) from different rye cultivars and their most closely related wild subspecies. Primers designed from a nucleotide sequence of the allele *Glu-Dly10*, which recognised the upstream and downstream flanking positions of the coding regions of the genes, were used in the study. Thus, allele mining supports the discovery of new alleles of target genes. However, the limitation of this approach in wheat lies in the fact that very few genes of agronomical importance have yet been cloned. This is particularly true for genes involved in disease resistance.

Ecotilling (Comai et al. 2004) represents a specific approach to allele-mining and refers to a high-throughput screening technique for the discovery of polymorphisms in natural populations. It can serve as a cheaper alternative to full DNA sequencing when searching for rare polymorphisms, but similar to the other allele-mining strategies it still requires specific sequence information for the target gene. Ecotilling can also be used for mapping, association analysis, mutational profiling and biodiversity studies. It has been successfully used in *Arabidopsis* (Comai et al. 2004) where 55 haplotypes of five genes have been discovered after screening of more than 150 individuals. The polymorphisms discovered were confirmed by sequencing, and base pair changes, insertions, deletions and variation in microsatellite number were observed.

Focused identification of germplasm strategy (FIGS) and allele-mining for molecular diversity at the *Pm3* locus

To test a strategy of allele mining in wheat using a large set of diverse germplasm, we focused on the *Pm3* resistance locus as there is extensive sequence information available for targeted allele cloning. A subset of bread wheat landraces were selected for the study using the FIGS system (Mackay, Street et al. (unpublished). Also see www.figstraitmine.com). In this case, the eco-geographic profile of 400 accessions, from the USDA-ARS National Small-Grains Collection, with known powdery mildew resistance was identified. This profile was then used as a template to identify environmentally similar collection sites from the FIGS database of nearly 17,000 landraces. Individual accessions were selected using multivariate statistical procedures that determined how eco-geographically similar the collection site of a given accession was to the resistant set template. The FIGS powdery mildew set of accessions now includes 899 landraces from ICARDA (International Centre for Agricultural Research in the Dry Areas, Syria), 295 landraces from AWCC (Australian Winter Cereals Collection) and 126 landraces from VIR (N.I. Vavilov Research Institute of Plant Industry, Russia), making a total of 1,320 landraces. These originate from Turkey (419), Iran (391), Afghanistan (292), Pakistan (133), Armenia (34), Turkmenistan (16), Russia (9), India (6), Azerbaijan (1) and Uzbekistan (1).

Screening and identification of powdery mildew resistant lines

For characterization of the FIGS powdery mildew set we used a combined strategy of screening for genetic diversity with molecular markers and classical pathogenicity tests. The entire FIGS powdery mildew set was screened with a differential set of powdery mildew isolates to select a subset of resistant landraces for molecular analysis. The detached leaf segments from seven day-old plants were placed on phytagar media and subjected to infection with four different isolates of powdery mildew (Fig. 1). The choice of the isolates was based on the pattern of their avirulence/virulence to the known alleles of *Pm3*. The four isolates used were 96224, 98275 and 96244 (avirulent on most known *Pm3* alleles) and 2000.15.Syros (virulent on all the known *Pm3* alleles). The phenotypes were grouped in three categories: resistant (R), intermediate (I) and susceptible lines (S). The experiment was scored 9–10 days after inoculation using a 1 to 100% susceptibility scale, i.e. the leaf area covered with mildew was ranked phenotypically where lines with 100% leaf area covered with mildew were considered fully susceptible and 0% marked complete resistance. This screening led to the selection of 211 resistant or intermediate resistant lines to at least one of the four mildew isolates used in the screen.

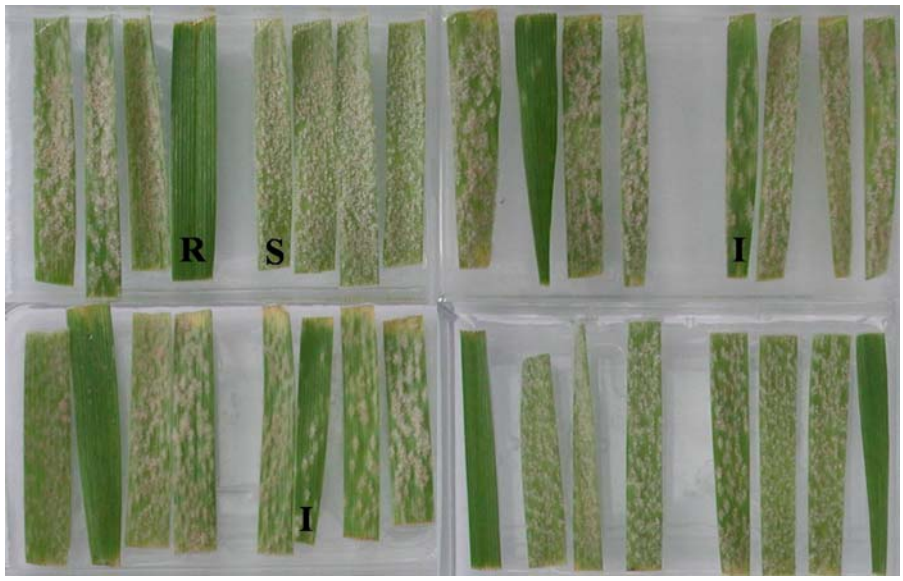

Fig. 1 Phenotypic assay of wheat landraces for powdery mildew resistance by infection with powdery mildew isolate 96244. Powdery mildew resistant (R), Intermediate (I) and Susceptible leaves (S) are marked

PCR-based approach for characterization of *Pm3* alleles

Initially we tested the molecular tools available for the detection of the *Pm3* gene in a subset of 295 AWCC landraces. We used an STS marker obtained from haplotype studies at the *Pm3* locus (Yahiaoui et al. 2004; Srichumpa et al. 2005). This *Pm3* haplotype marker amplifies a 946 bp fragment originating from the 5′ non-coding region of *Pm3b* which is diagnostic for the presence of a *Pm3* gene (Fig. 2).

The *Pm3* haplotype was present at an unexpectedly high frequency in the subset of the FIGS powdery mildew set tested. In the 295 AWCC landraces, amplification of the *Pm3* STS marker was found in 257 lines (87.1%). This high percentage prompted us to check this subset for the presence of the already known alleles (*Pm3a–Pm3g*) using *Pm3* allele-specific markers. These markers were developed in our laboratory (Tommasini et al. 2006) based on the specific nucleotide polymorphisms of coding and adjacent non-coding regions of each of the *Pm3* alleles. We found that the *Pm3b* allele was the only known functional *Pm3* allele present in the subset. It was detected in seven lines. This demonstrated that most of the alleles of *Pm3* in the subset do not correspond to known resistance alleles. The infection data obtained from the powdery mildew infection described above showed that only 40 out of 295 lines were resistant or intermediately resistant to at least one of the isolates while the

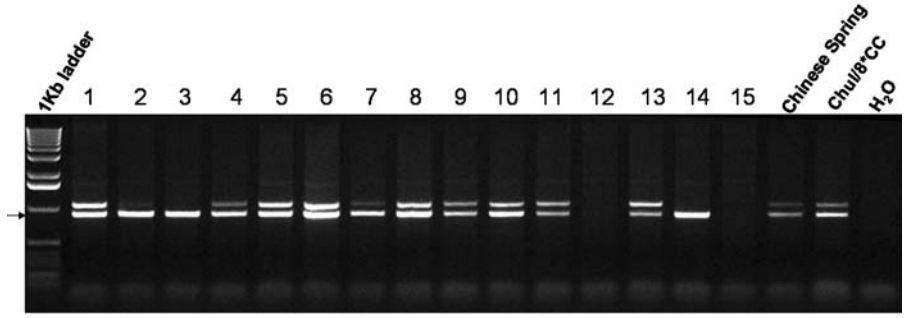

Fig. 2 PCR amplification in the FIGS powdery mildew set of the STS marker specific for the *Pm3* haplotype. The lower band (*see arrow*) corresponding to chromosome 1A is diagnostic for the presence of a *Pm3* like gene. The numbers 1–15 correspond to the tested landraces, Chinese Spring & Chul/8*CC are the positive controls while H_2O serves as the negative control

Table 2 *Pm3* allele mining: the frequency of known *Pm3* resistance alleles found in the FIGS powdery mildew set of 1,320 lines

Specific PCR	Number of lines tested for *Pm3* haplotype and known *Pm3* alleles	Number of lines possessing *Pm3* haplotype and *Pm3* known alleles	Landrace	Origin
Pm3 haplotype	211	145		
Pm3a, Pm3d, Pm3e, Pmf, Pm3g	145	0		
Pm3b	145	15	AUS9943, AUS9948, AUS10003, AUS10033, AUS13239, AUS13297, AUS13306, AUS13307, AUS13311, AUS14504, AUS14532, AUS14840, VIR 45538, VIR 49005, VIR 49006	Afghanistan
		6	IG 122348, IG 122354, IG 122361, IG 122373, IG 122502, VIR 38613	Iran
		2	VIR 16766, VIR 31595	Azerbaijan
		6	VIR 23918, VIR 23922, VIR 34986, VIR 35021, VIR 35030, VIR 34984	Russia
		1	VIR 35203	Turkey
Pm3c	145	3	IG 122491, IG 122372, IG 122346	Iran
		1	VIR 46301	Azerbaijan

Detection was done with the *Pm3* haplotype-specific and *Pm3* allele-specific primers

other 255 lines were susceptible to the tested isolates. This indicated that susceptible alleles of *Pm3* were present in at least 86.4% of the lines and are therefore expected to be widespread among the landraces. This percentage is possibly even higher, given the fact that resistance to powdery mildew might not be due to a gene at the *Pm3* locus but may be caused by any of the known or still uncharacterized resistance genes in the germplasm. Therefore, in the particular case of *Pm3* allele mining, the strategy of screening the lines with different mildew isolates prior to sequencing was chosen. However, for other genes and traits sequencing the complete set of germplasm without prior phenotypic analysis might be a valid alternative strategy.

The 211 intermediate or resistant lines selected during the infection screen were subjected to molecular analysis for the *Pm3* locus. Out of the 211 lines (from 1,320), 145 showed the presence of a *Pm3* haplotype. The search for the seven known *Pm3* resistance alleles in the 145 lines revealed the presence of *Pm3b* and *Pm3c* in 30 and four lines, respectively. Thus, *Pm3b* was the most frequent *Pm3*

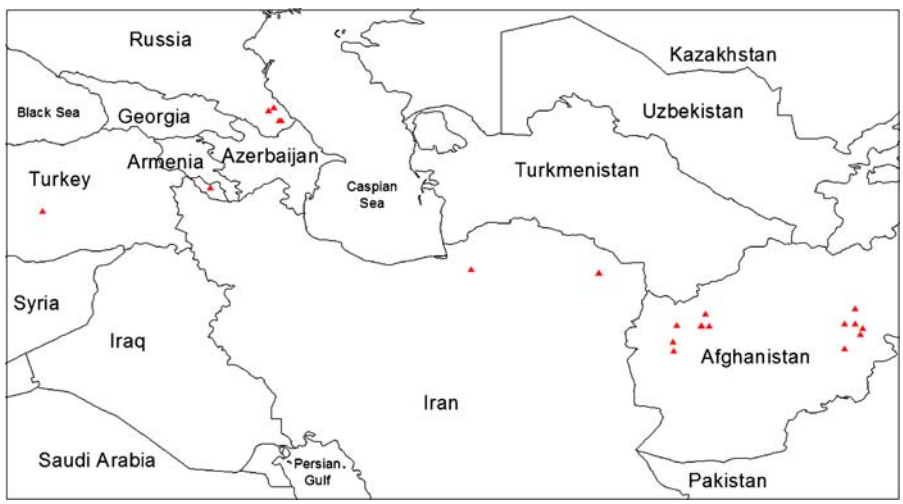

Fig. 3 Geographic origin of the 30 *Pm3b* lines detected in the FIGS powdery mildew set of 1320 landraces. The collection sites are indicated by *red triangles*

allele in the landrace set. It was identified in landraces originating from Afghanistan (15), Iran (6), Russia (6), Azerbaijan (2) and Turkey (1), while the four landraces with *Pm3c* allele originated from Iran (3) and Azerbaijan (1) (see Table 2 for a summary of results and Fig. 3 for geographic distribution of *Pm3b* lines). The first identification of the *Pm3b* allele was in a landrace from Uzbekistan (http://www.ars-grin.gov/npgs/index.html), which is consistent with its frequency and actual geographical distribution, particularly in Afghanistan, a neighbouring country to Uzbekistan (Fig. 3).

To summarize, the strategy for the identification of new *Pm3* alleles described in this paper included phenotypic screening of the FIGS powdery mildew set of landraces for powdery mildew resistance, molecular analysis of *Pm3* haplotype composition and determination of known *Pm3* resistance alleles. This resulted in 111 candidate lines (9% of total set) to specifically target for further characterization of the gene present at the *Pm3* locus. These candidate lines (a) are resistant or intermediately resistant to at least one of the isolates tested (b) possess the *Pm3* haplotype and (c) lack any of the known *Pm3* alleles (145 − 34 = 111). It is predicted that new *Pm3* resistance alleles will be found among these lines, although the presence of susceptible *Pm3* alleles cannot be ruled out (based on the results of AWCC subset, presented earlier in this paper). The resistant phenotype in such cases may be attributed to the presence of other *Pm* genes.

The approach described above is one of the first large-scale attempts of a systematic resistance allele-mining from wheat landraces using molecular tools derived from the target gene sequence. Putative new *Pm3* resistance genes will be tested functionally by using a transient transformation assay (Yahiaoui et al. 2004) and other approaches such as virus-induced gene silencing (VIGS, Scofield et al. 2005). This should reveal whether the newly isolated genes are actually active resistance alleles and whether they confer new resistance specificities to the plant. Finally, the newly identified *Pm3* alleles could be transferred by classical genetic crosses to powdery mildew-susceptible cultivars or alternatively be transformed into susceptible varieties as single genes. In addition, they could be combined as *R* gene cassettes to achieve a disease control which is possibly more durable. Besides these more applied aspects in wheat breeding, the analysis of allelic diversity and accumulation of diverse allelic sequences will contribute to a better characterization of the mechanisms involved in resistance gene evolution.

The identification of new functional *Pm3* alleles from diverse germplasm will also contribute to the molecular understanding of *R* gene function. The comparison of sequences from new alleles can clarify the molecular basis of *Pm3* specificity, e.g. by studying chimeric genes created by domain swap experiments with domains from the newly identified sequences.

Concluding remarks

A more efficient exploitation of the genetic diversity in gene banks is essential for meeting the challenges that wheat breeding is facing in the decades to come. However, the use of this diversity is hampered by the sheer number of accessions available and the limited resources which are at hand for phenotypic characterization of all these lines. Therefore, it is necessary to (a) develop strategies to assemble focused sets of material for specific traits based on rational criteria for selection of the lines but also (b) to identify genes underlying agronomically important traits and (c) establish the molecular tools for rapid characterization of new alleles.

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