

# Allele mining and sequence diversity at the wheat powdery mildew resistance locus *Pm3*

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## SUMMARY

The production of wheat is threatened by a constantly changing population of pathogen races. Considering the capability of many pathogens to overcome genetic resistance, the identification and 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. Here, we discuss the allele mining approach to characterize and utilize the naturally occurring resistance diversity in wheat. This study is a large scale systematic allele mining, including 1320 hexaploid wheat landraces selected on the basis of eco-geographical parameters favouring growth of powdery mildew. The landraces were infected with a set of differential powdery mildew isolates, which allowed the selection of resistant lines. The molecular tools derived from *Pm3* haplotype studies were applied to study the genetic diversity at this locus. From the known *Pm3* *R* alleles, *Pm3b* was the only one frequently identified. In the same set, we also found a high frequency of landraces carrying a susceptible haplotype. This analysis allowed the identification of candidate resistant lines that were further tested for the presence of new potentially functional alleles. Based on transient expression assays as well as Virus Induced Gene Silencing (VIGS), we conclude that we have identified at least two new functional *Pm3* alleles. The new interesting and functional alleles can be transferred to susceptible but economically important wheat varieties as single genes or *R*-gene cassettes to achieve efficient control of mildew. This study contributes to targeted use of genetic diversity resources for research and breeding.

## INTRODUCTION

An advantage of diversity studies in wheat 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.

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; Cloutier *et al.*, 2007). The sequence

information of these genes allows 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. The wheat powdery mildew resistance gene *Pm3* exists in 10 different alleles (*Pm3a-Pm3j*) as identified by classical genetic studies. 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 contain additional resistance genes.

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. The development of molecular tools to specifically access the existing genetic diversity at particular loci facilitates 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. The strategy of finding valuable, unknown alleles at a known locus is referred to as 'allele mining'.

There are reports about the use of allele mining strategy in several cereal species to isolate alleles of target genes (barley, Malysheva *et al.*, 2004; rice, Latha *et al.*, 2004). In this paper, we discuss an allele mining strategy for finding new resistance specificities *Pm3* locus against the powdery mildew pathogen (*Blumeria graminis* f.sp. *tritici*) that was applied to 1320 wheat landraces.

## FIGS APPROACH AND ALLELE MINING AT *PM3* LOCUS

We focused on the *Pm3* resistance locus as there is extensive sequence information available for targeted allele cloning in a diverse germplasm. A subset of bread wheat landraces were selected for the study using the FIGS (Focused Identification of Germplasm Strategy) system (Mackay, Street *et al.*, manuscript in preparation. Also see [www.figstraitmine.com](http://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 finally includes landraces originating from Turkey (419), Iran (391), Afghanistan (292), Pakistan (133), Armenia (34), Turkmenistan (16), Russia (9), India (6), Azerbaijan (1) and Uzbekistan (1).

## SCREENING FOR IDENTIFICATION OF RESISTANT LINES

For characterization of the “FIGS powdery mildew set” we used a combined strategy of screening with molecular markers and classical pathogenicity tests. The entire ‘FIGS powdery mildew set’ was screened with a set of four powdery mildew isolates to select a subset of resistant landraces for molecular analysis. The choice of the isolates was based on the pattern of their pathogenicity on 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 scoring was done after 9-10 days of infection, on a 1 to 100% susceptibility scale. This screening led to identification of 211 resistant or intermediate resistant lines to at least one of the four mildew isolates tested.

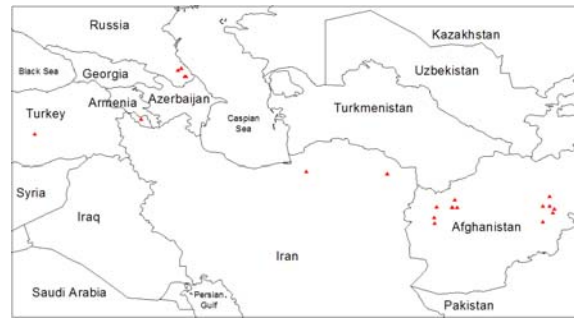
## PCR BASED CHARACTERISATION OF PM3 ALLELES

We first tested a subset of 295 AWCC landraces for the detection of the *Pm3* gene. An STS marker obtained from haplotype studies at the *Pm3* locus (Yahiaoui *et al.*, 2004; Srichumpa *et al.*, 2005) was used. This *Pm3* haplotype marker amplifies a 946bp fragment originating from the 5’ non-coding region of *Pm3b* which is diagnostic for the presence of a *Pm3* gene.

Unexpectedly high frequency of the *Pm3* haplotype was observed 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%). Given this high percentage we checked this subset for the presence of the already known alleles (*Pm3a-Pm3g*) using *Pm3* allele specific markers (Tommasini *et al.*, 2006). 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 are not the known resistance alleles. The infection data obtained from the powdery mildew infection (as described above) showed that only 40 out of 295 AWCC lines were resistant or intermediate resistant to at least one of the isolates while the other 255 lines were susceptible to the tested isolates. This indicated that susceptible alleles of *Pm3* are present in at least 86.4% of the lines and are therefore expected to be widespread. Concerning the

resistant lines containing a *Pm3* type gene, it cannot be ruled out that resistance to powdery mildew is not 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. Hence, in the particular case of *Pm3* allele mining, the strategy of screening the lines with different mildew isolates before sequencing was chosen. However, for other genes and traits, sequencing the complete set of germplasm without prior phenotypic analysis might be an alternative strategy.

The 211 intermediate or resistant lines selected during the infection screen were subjected to molecular analysis for the *Pm3* locus. Of the 211 lines, 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 4 lines, respectively (Kaur *et al.*, 2008). Thus, *Pm3b* was the most frequent *Pm3* 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 4 landraces with *Pm3c* allele originated from Iran (3) and Azerbaijan (1) [see Fig.1 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.1).



**Fig1. Geographic origin of the 30 *Pm3b* lines detected in the ‘FIGS powdery mildew set’. The collection sites are indicated by red triangles.**

These experiments led to the identification of 111 candidate lines (9% of total set) to specifically target for characterization of the allele present at the *Pm3* locus. These candidate lines (i) were resistant or intermediate resistant to at least one of the isolates tested (ii) possess the *Pm3* haplotype and (iii) lack the known *Pm3* alleles. Several new haplotypes of *Pm3* were isolated and sequenced. The newly isolated *Pm3* alleles showed sequence diversity as compared to the known *Pm3* alleles, with the differences mainly lying in the LRR domain. The new alleles were cloned into a plant expression vector under control of the 35S promoter and tested in a transient assay system based on particle bombardment of wheat epidermal cells (for details of this experimental system see Yahiaoui *et al.* 2006). Lines with new functional specificities were further tested using virus-induced gene silencing to find out if

only the *Pm3* allele is involved in the observed resistance, or if other *Pm* genes are additionally present. Based on transient expression assays as well as Virus Induced Gene Silencing (VIGS, Scofield *et al.*, 2005), we conclude that we have identified at least two new functional *Pm3* alleles. While in other cases, the resistant phenotype may be attributed to the presence of other *Pm* genes.

The production of transgenic wheat by introducing newly identified *Pm3* alleles is underway in our lab. Besides these more applied aspects in wheat breeding, the analysis of allelic diversity and availability of different allelic sequences will contribute to a better understanding of the processes involved in resistance gene evolution and 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 isolated sequences. Domain swap experiments are also currently being carried out.

## CONCLUSION

In this study we could demonstrate the successful use of allele mining to isolate active alleles of the wheat powdery mildew resistance locus *Pm3* from landraces. It is essential to efficiently use the genetic diversity in gene banks in order to meet the challenges of wheat breeding now and in the coming decades. However, the use of this diversity is limited due to the resources which are at hand for characterization of all these lines. Therefore, we need to (i) develop strategies to assemble focused sets of material for specific traits based on criteria for selection of the lines but also (ii) to identify genes underlying agronomically important traits and (iii) establish the molecular tools for rapid characterization of new alleles.

## REFERENCES

- Cloutier, S., McCallum, B. D., Loutre, C., Banks, T. W., Wicker, T., Feuillet, C., Keller, B. & Jordan, M. C. (2007) Leaf rust resistance gene *Lr1*, isolated from bread wheat (*Triticum aestivum* L.) is a member of the large psr567 gene family. *Plant molecular biology*, 65, 93-106.
- Feuillet, C., Travella, S., Stein, N., Albar, L., Nublat, A. & Keller, B. (2003). Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 15253-15258.
- Huang, L., Brooks, S. A., Li, W., Fellers, J. P., Trick, H. N. & Gill, B. S. (2003). Map-Based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. *Genetics*, 164, 655-664.
- Kaur, N., Street .K., Mackay, M., Yahiaoui, N., and Keller, B. (2008) Molecular approaches for characterization and use of natural disease in wheat. *Eur J Plant Pathol*, 121, 387-397
- Latha, R., Rubia, L., Bennett, J. & Swaminathan, M. S. (2004). Allele mining for stress tolerance genes in *Oryza* species and related germplasm. *Molecular Biotechnology*, 27, 101-108.
- Malysheva, L., Ganal, M. W. & Röder, M. S. (2004). Evaluation of cultivated barley (*Hordeum vulgare*) germplasm for the presence of thermostable alleles of  $\beta$ -amylase. *Plant Breeding*, 123, 128-131.
- Scofield, S. R., Huang, L., Brandt, A. S. & Gill, B. S. (2005). Development of a Virus-Induced Gene-Silencing system for hexaploid wheat and its use in functional analysis of the *Lr21*-mediated leaf rust resistance pathway. *Plant Physiology*, 138, 2165-2173.
- Srichumpa, P., Brunner, S., Keller, B. & Yahiaoui, N. (2005). Allelic series of four powdery mildew resistance genes at the *Pm3* locus in hexaploid bread wheat. *Plant Physiology* 139, 885-895
- Tommasini, L., Yahiaoui, N., Srichumpa, P. & Keller, B. (2006). Development of functional markers specific for seven *Pm3* resistance alleles and their validation in the bread wheat gene pool. *Theoretical and Applied Genetics*, 114, 165-175.
- Yahiaoui, N., Brunner, S. & Keller, B. (2006). Rapid generation of new powdery mildew resistance genes after wheat domestication. *The Plant Journal*, 47, 85-98.
- Yahiaoui, N., Srichumpa, P., Dudler, R., & Keller, B. (2004). Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. *The Plant Journal*, 37, 528-538.