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Diversity, evolution and use of fungal disease resistance genes in wheat

B. Keller, S. Brunner, N. Kaur and N. Yahiaoui
Institute of Plant Biology, University of Zurich, Zollikerstrasse 107, 8008 Zürich, Switzerland

In bread wheat, the *Pm3* locus encodes one of the earliest identified allelic series of resistance genes against a wheat disease. Ten *Pm3* alleles (*Pm3i to j*) have been genetically identified in wheat lines or cultivars originating from four continents (Zeller and Hsam, 1998). Each of them confers race-specific resistance to the fungal pathogen wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*).

For resistance genes defined by classical pathology and plant breeding, there is only genetic map information available and map-based cloning is the only possibility to clone a gene of interest. Given the fact that the wheat genome is large (1.7×10^{10} bp), hexaploid with three homoeologous genomes and contains a high amount of repetitive DNA, comparative genomics is an essential tool for map-based gene cloning in bread wheat. In our group we are using comparative genomic approaches to isolate resistance genes against the fungal diseases powdery mildew and leaf rust.

The first of the *Pm3* alleles isolated at the molecular level was the *Pm3b* allele which was cloned using a positional cloning strategy (Yahiaoui *et al*., 2004). Two wheat species with lower ploidy levels, the diploid wheat *T. monococcum* and the tetraploid *T. turgidum cv. durum*, provided models for the genome of hexaploid wheat and allowed the establishment of a physical contig spanning the *Pm3* locus. Although the haplotypes at the *Pm3* locus differed dramatically between the three levels of ploidy, a large resistance gene-like family was consistently found at the *Pm3* locus and a candidate gene cosegregating with *Pm3b* was identified in a segregating F2 population of 1320 plants. A single mutant showing no obvious large deletion at the *Pm3* locus showed a single base-pair deletion in the coding region of the candidate gene, and no mRNA could be detected from the candidate gene by RT-PCR in this mutant. As powdery mildew is exclusively growing in the epidermal cells of the wheat leaf, a transient assay based on particle bombardment can be used to study gene function (Douchkov *et al*.,. 2005; Schweizer *et al*., 1999; Shirasu *et al*., 1999). Using marker genes such as genes encoding beta-glucuronidase or green fluorescent protein, the transformed cells can be identified. By cobombardment with a candidate resistance gene and subsequent infection with a specific race of the powdery mildew pathogen, transformed cells can be examined for compatible or incompatible interaction with the pathogen. This allows to test whether a candidate gene confers resistance and has been successfully used for the *mlo* gene (Shirasu *et al*.1999) and the *Mla* alleles (Halterman *et al*., 2001; Shen *et al*., 2003; Zhou *et al*., 2001) in barley as well as for the *Pm3* allelic series in wheat (Srichumpa *et al*., 2005; Yahiaoui *et al*., 2006; Yahiaoui *et al*., 2004). In this transient assay for powdery mildew resistance genes, the identity of the candidate gene as *Pm3b* was demonstrated by its race-specific interaction with the powdery mildew pathogen (Yahiaoui *et al*., 2004).

For the isolation of the remaining *Pm3* genes, haplotype analysis of ten lines carrying different *Pm3* alleles was performed. In all these lines a conserved genomic region delimited by markers co-segregating with *Pm3b* was identified, including a structurally very well conserved, *Pm3b*-like gene (Srichumpa *et al*., 2005). Based on this haplotype conservation, six additional *Pm3* resistance alleles (*Pm3a, c, d, e f,* and *g*) were isolated using a PCR-based strategy (Srichumpa *et al*. 2005; Yahiaoui *et al*., 2006). The *Pm3* resistance alleles encode a coiled-coil nucleotide binding site leucine rich repeat type of protein. Haplotype conservation in *Pm3* lines and high sequence conservation (more than 97% sequence identity) observed between the *Pm3* resistance genes indicated that all *Pm3* specificities form a true allelic series. Highly specific functional markers derived from the allelic sequences now allow to detect the individual *Pm3* alleles with complete accuracy in wheat breeding programs, supporting diagnostic applications in current wheat breeding (Tommasini *et al*., submitted). Such markers could also be useful in a future combination of *Pm3* alleles in transgenic wheat. In addition to the functional alleles, a susceptible variant of the *Pm3* genes was identified. The comparison of the sequence of the *Pm3* resistance alleles with the susceptible allele *Pm3CS* provided important information on the evolutionary processes that generated the resistance alleles. One group

of four *Pm3* resistance alleles showed few, clearly delimited, polymorphic sequence blocks of ancient origin, possibly derived from gene conversion events. A second group of three alleles differed from *Pm3CS* by only two to five mutations, all non-synonymous and all in the LRR-encoding region. Transient transformation assays confirmed that these few differences in the LRR domain of PM3 proteins are responsible for the specificity of resistance against different powdery mildew isolates. Moreover, the very high sequence conservation between these alleles and the susceptible *Pm3CS* allele, together with the absence of synonymous mutations between the seven characterized resistance alleles, indicated a recent evolution of the *Pm3* resistance genes probably after wheat domestication 10,000 years ago (Yahiaoui *et al*. 2006).

We have used the molecular tools developed for the isolation of the known alleles to screen for new *Pm3* alleles in the gene pool of tetraploid and hexaploid wheat. From the later, a set of more than 1300 landraces was screened for powdery mildew resistance and the presence of a *Pm3* haplotype. Most lines contained a susceptible *Pm3* allele which is closely related to the functional *Pm3* resistance genes. We have also identified resistant lines with new types of *Pm3* allelic sequences. These new alleles are currently tested for activity as resistance genes. The diversity and functionality of these genes will be presented.

Interestingly, both the *Mla* and *Pm3* genes are located in the distal region of chromosome group 1 (1HS and 1AS, respectively) and both loci are characterized by a large number of functionally different alleles. However, the two loci are not orthologous and they map about 5 cM from each other (Zhou *et al*. 2001 and our unpublished results). In addition, the two genes are not similar at the sequence level (except for the overall domain structure of CC, NBS, LRR domains). We conclude that *Mla* and *Pm3* represent a case of convergent evolution where quite different genes were recruited to confer resistance against very similar pathogens. It is relevant to study at the molecular level the direct or indirect mechanisms of MLA and PM3 protein interaction with fungal avirulence gene products and the subsequent signal transduction. It is also interesting to note that two different genetic loci have evolved into major resistance genes in the two closely related crop species wheat and barley. This strongly suggests that for most resistance loci it will be necessary to isolate them from the crop species where they are active as there is very low functional similarity between orthologous genes even in closely related species. Thus, the isolation and comparison of the *Mla* and *Pm3* genes has resulted in exciting new findings and demonstrates that, in addition to putative practical applications, it is essential to isolate resistance genes also from crop plants instead of limiting this type of work to model species.

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