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Minireview

Map-based isolation of disease resistance genes from bread wheat: cloning in a supersize genome

BEAT KELLER[1]*, CATHERINE FEUILLET[2] AND NABILA YAHIAOUI[1]
[1] *Institute of Plant Biology, University of Zurich, Zollikerstrasse 107, 8008 Zurich, Switzerland*
[2] *UMR INRA-UBP ASP Clermont-Ferrand, 234 av. du Brézet, 63039 Clermont-Ferrand Cedex 2, France*

Summary

The genome of bread wheat is hexaploid and contains $1{\cdot}6 \times 10^{10}$ bp of DNA, of which more than 80% is repetitive sequences. Its size and complexity represent a challenge for the isolation of agronomically important genes, for which we frequently know only their position on the genetic map. Recently, new genomic resources and databases from genome projects have simplified the molecular analysis of the wheat genome. The first genes to be isolated from wheat by map-based cloning include three resistance genes against the fungal diseases powdery mildew and leaf rust. In this review, we will describe the approaches and resources that have contributed to this progress, and discuss genomic strategies that will simplify positional cloning in wheat in the near future.

1. Introduction

Bread wheat (*Triticum aestivum* L.) is one of more than 10 000 species in the grass family *Poaceae*. It is the staple food for 35% of the world's population and therefore contributes substantially to human nutrition (http://www.cimmyt.org/Research/Wheat/map/developing_world/wheat_developing_world.htm). Wheat is attacked by a large variety of pathogens, mostly of fungal origin. Some lines, landraces, wild ancestors and inter-crossable wheat relatives are resistant against some diseases. The genetic basis of this resistance has been studied in great detail and two genetically distinct mechanisms have been found. First, there is monogenic resistance for which many different genes with similar effects are present in the gene pool. For example, there are more than 50 *Lr* (leaf rust) resistance genes against the fungal leaf rust disease (McIntosh *et al.*, 1995). Second, and of increasing economic importance, there is quantitative resistance based on an average of two to five genes with additive effects. An economically relevant level of resistance, although never complete, can be achieved by combining genes with minor effects. Single monogenic resistance genes are frequently not durable and new, virulent pathogen races may emerge after a few years. Therefore, sustainable breeding today concentrates on achieving quantitative resistance and on the combination of monogenic resistance genes. No gene involved in quantitative resistance has yet been isolated from wheat, but in several studies disease resistance quantitative trait loci (QTLs) with large effects have been identified and mapped in populations of recombinant inbred lines. These include a QTL for leaf rust resistance (*QLrP.sfr-7DS*; Schnurbusch *et al.*, 2004) explaining between 39% and 59% of the observed phenotypic variance, depending on the population studied. In addition, the *Fusarium* head blight resistance QTL *Qfhs.ndsu-3BS* (Liu & Anderson, 2003) contributed 55% to the phenotypic variance observed for this disease whereas a glume blotch resistance QTL (*Qsnb.sfr-3BS*; Schnurbusch *et al.*, 2003) explained 31% of the phenotypic variance. The LOD scores for these three QTLs vary from 18 to 47 and their isolation is a major goal in wheat research in the coming years. In this minireview, we focus on the recent successful isolation of the first monogenic disease resistance (*R*) genes in wheat.

The grass genus *Triticum* includes diploid, tetraploid and hexaploid wheat species. Bread wheat (*Triticum aestivum* L., 2n = 42, AABBDD) is an allohexaploid plant carrying three different subgenomes (called A, B and D) which are shown schematically in Fig. 1. There are two different A genomes in the wheat species: the A^u genome in *T. urartu* and the closely related A^m genome in *T. monococcum* (Dvorak *et al.*,

* Corresponding author. e-mail: bkeller@botinst.unizh.ch

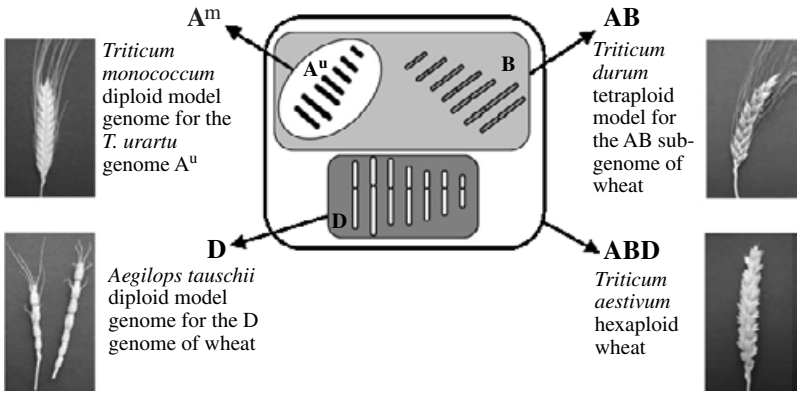

Fig. 1. The subgenome cloning strategy in wheat. For physical mapping, lower-ploidy wheat species are used as models for the three different hexaploid wheat subgenomes A, B and D.

1988). Modern hexaploid wheat resulted from two independent hybridization events. The first step combined the A^u genome of the wild diploid wheat *Triticum urartu* (2n = 14) and the B genome of an unknown species closely related to *Aegilops speltoides* (Dvorak & Zhang, 1990; Huang *et al*., 2002). This 'recent' event probably occurred between 0·5 and 3 million years ago (Huang *et al*., 2002; Wicker *et al*., 2003) and gave rise to the tetraploid ancestor of modern *Triticum* species: *Triticum turgidum* (2n = 28, genome AABB). The cultivated *Triticum turgidum* subsp. *durum* is derived from this tetraploid ancestor. *Triticum turgidum* and the diploid donor of the D genome (*Aegilops tauschii*) hybridized approximately 8000 years ago resulting in hexaploid bread wheat *Triticum aestivum* L. (Feldman *et al*., 1995).

Members of the grass plant family have widely differing genome sizes ranging from 450 Mb for rice to 2500 Mb for maize, 5000 Mb for barley and 16 000 Mb for hexaploid wheat (Arumuganathan & Earle, 1991). This genome size variation is partly caused by differences in ploidy level, but is mainly due to differences in the amount of repetitive DNA. The size of the bread wheat genome, being 5 times larger than the human genome, is a considerable challenge for map-based cloning. The large amounts of repetitive DNA and the presence of three homologous genomes result in major difficulties during chromosome walking. This requires time-consuming approaches and a variety of genomic resources. Despite these problems, the first disease resistance genes have recently been cloned from hexaploid wheat.

2. Resources for high-density genetic mapping in wheat

The first step in map-based cloning is the development of a high-density genetic map covering the target locus. In wheat, given the size of the genome, genetic maps are far from being saturated. In 1989, an international effort (International Triticeae Mapping Initiative) was launched to develop and share molecular markers and genetic maps in genomes of the *Triticeae* (wheat, barley and rye). After a first effort of anchoring mainly restriction fragment length polymorphism (RFLP) markers on a common mapping population referred to as the 'ITMI population' (synthetic wheat Altar84/*Ae. squarrosa* × Opata M 85), it became clear that additional markers were needed to increase the densities of wheat genetic maps. Consortia were built nationally and internationally to develop and map microsatellites and EST expressed sequence tag markers. In 1998, only six *Triticeae* ESTs (wheat, rye and barley) were present in the public databases. An international effort (ITEC: http://wheat.pw.usda.gov/genome/) was then launched to produce more ESTs and, to date, 612 568 ESTs from wheat species (587 628 from *T. aestivum*, 9973 *T. monococcum*, 8714 *Triticum turgidum* subsp. *durum*, 4315 *Ae. speltoides*, 1938 *T. turgidum*) have been released and are publicly available. Recently, 7104 unique ESTs were used to assign 16 099 loci (at an average of 2·8 loci per EST) onto a chromosome bin map using Southern hybridization with a set of euploid and aneuploid wheat lines (Qi *et al*., 2004). Together with the existing 5537 RFLP loci, 1620 microsatellites, 2049 protein loci and genes controlling phenotypic traits (McIntosh *et al*., 2003) that were already present on wheat genetic maps, these data have made the wheat genome one of the most densely mapped plant genomes for which no genome sequence is available (see the Graingene web site, http://wheat.pw.usda.gov/GG2/index.shtml, for additional information).

In addition to markers developed from closely related species, a number of sequences from other grass genomes can now be used to saturate wheat genetic maps. In particular, the recent sequencing of the rice genome (Goff *et al*., 2002; Yu *et al*., 2002) has provided researchers in cereal genetics with thousands of potentially new markers for increasing genetic map densities (see below).

3. Resources for chromosome walking in wheat: BAC libraries

After the establishment of a high-density genetic map and the identification of markers near the gene of interest (<2 cM), a physical map spanning the target gene must be developed using large insert genomic libraries. Until recently, the large size and complexity of the wheat genome has hampered the construction of such libraries from wheat species. The first two wheat bacterial artificial chromosome (BAC) libraries were constructed in 1999 from two diploid species: *Triticum monococcum* (Lijavetzky *et al*., 1999) and *Aegilops tauschii* line AUS18913 (Moullet *et al*., 1999). Their genomes are related to the A and D genome of hexaploid wheat, respectively. In 2003, the first BAC library from tetraploid wheat (*T. turgidum* subsp. *durum* with the A, B genomes) was constructed to support positional cloning of a gene involved in high grain protein content (Cenci *et al*., 2003). The *T. monococcum* DV92 library has been used to isolate *VRN1*, a gene controlling vernalization response in *T. monococcum* (Yan *et al*., 2003) as well as a candidate gene for the *Q* locus that confers the free-threshing character to domesticated wheat (Faris *et al*., 2003). In addition to direct isolation of genes, these libraries have been used successfully in sub-genome map-based cloning strategies that have led to the isolation of the fungal disease resistance genes *Lr10* and *Pm3b* from hexaploid wheat (Feuillet *et al*., 2003; Yahiaoui *et al*., 2004). Improvement of the quality and efficiency of BAC cloning (Chalhoub *et al*., 2004) has recently allowed the construction of high-genome-coverage BAC libraries from the large hexaploid wheat genome (Allouis *et al*., 2003). In addition, a number of unpublished BAC libraries have been produced or are under construction in different laboratories (for an overview see the ITMI web site: http://wheat.pw.usda.gov/ITMI/). For example, *Ae. tauschii* BAC/BIBAC libraries (302 976 clones) representing the wheat D genome at 8·5 × coverage have been developed within the framework of a project led by Dr J. Dvorak (UC Davis) aimed at assessment of the insular organization of this genome by physical mapping (http://wheat.pw.usda.gov/PhysicalMapping/).

The development of BAC libraries in the last 5 years has been a major breakthrough in wheat genetics and genomics, but these resources remain limited with regard to the future isolation of genes of agronomic interest in wheat. Indeed, in the final steps of map-based cloning it is desirable to use a genomic library of the cultivar or line that contains the gene of interest, as BAC libraries of related species or lines from the same species might not contain the gene. This problem has been highlighted by recent findings showing high diversity and disruption of colinearity in allelic regions in maize and barley (Fu & Dooner, 2002; Song *et al*., 2002; Scherrer *et al*., 2005). However, given the number of clones required (approximately 1 million clones for a 6 × coverage of the hexaploid wheat genome) and the associated cost, BAC libraries cannot be constructed for every wheat genotype of interest. One relatively rapid and reasonably low cost alternative consists in the construction of pooled non-gridded BAC libraries from the relevant genotype. Such strategies have been used in mouse (Pierce *et al*., 1992), soybean (Salimath & Bhattacharyya, 1999) and more recently in hexaploid wheat (Liu *et al*., 2000; Ma *et al*., 2000) and barley (Isidore *et al*., 2005), and will certainly be used in the future to develop additional BAC library resources in wheat. In addition, recent progress in wheat chromosome sorting has allowed generation of BAC libraries from single chromosomes and chromosome groups (Janda *et al*., 2004; Safar *et al*., 2004).

4. Isolation of fungal disease resistance genes in wheat: subgenomic chromosome walking and haplotype analysis as substitutes for whole-genome resources

Due to the complexity and the size of the wheat genome, the isolation of genes by positional cloning greatly benefits from the use of smaller and simpler model genomes for physical mapping. As the rice genome is of limited value for the cloning of resistance genes from wheat (see below), an alternative is to use diploid or tetraploid wheat genomes that are closely related to the genomes of modern hexaploid wheat (Fig. 1). Thus, for the A genome of hexaploid wheat, either the A^m genome of the diploid wheat *T. monococcum* or the A genome of tetraploid wheat can be used, whereas for the D genome of hexaploid wheat, the genome of the diploid donor species *Ae. tauschii* is closely enough related to be useful. This subgenomic strategy originally suggested by Gill *et al*. (1991) was an essential breakthrough in map-based cloning in wheat. Based on this strategy, three disease resistance genes have been isolated recently: the leaf rust resistance genes *Lr21* (Huang *et al*., 2003) and *Lr10* (Feuillet *et al*., 2003), and the *Pm3b* powdery mildew resistance gene (Yahiaoui *et al*., 2004).

The cloning of the *Lr21* leaf rust resistance gene was simplified by its location on the D genome of wheat. In fact, the *Lr21* gene itself was originally introgressed from *Ae. tauschii* (Rowland & Kerber, 1974). This allowed direct identification of the resistance gene from the diploid donor line using a cosmid library and a diploid/polyploid shuttle mapping strategy (Huang *et al*., 2003). Genetic mapping was done in the hexaploid recipient crop using RFLP markers, and a probe co-segregating with *Lr21* in hexaploid wheat was identified. This probe was then used to screen a cosmid library constructed from the

Lr21 diploid donor line *Ae. tauschii* TA1649. A cosmid containing the co-segregating probe was isolated and only one coding sequence identified on this cosmid was similar to resistance-gene-like sequences. An intragenic recombination event resulted in a susceptible allelic variant that encodes a 151 amino acid long, truncated protein (Huang *et al.*, 2003). Finally, resistant plants were obtained after transformation of the candidate gene on a cosmid clone into a susceptible wheat cultivar, demonstrating definitively the identity of the gene.

The *Lr10* and *Pm3* genes are located on wheat chromosome 1AS and no diploid relatives are known to carry these genes. Therefore, the *T. monococcum* cv. DV92 BAC library was chosen as a model for physical mapping of both genes. This subgenomic strategy was very effective for the *Lr10* gene as strong conservation of the haplotype around the *Lr10* gene was found between *T. monococcum* cv. DV92 and the hexaploid wheat donor line Thatcher*Lr10* used for genetic mapping (Stein *et al.*, 2000; Scherrer *et al.*, 2002). The physical map in *T. monococcum* and the genetic map in hexaploid wheat were highly colinear. Two candidate *R* genes were found on the *T. monococcum* cv. DV92 BAC contig (Wicker *et al.*, 2001) spanning the hexaploid wheat *Lr10* locus and orthologues of both genes could be isolated from hexaploid Thatcher*Lr10* (Feuillet *et al.*, 2003). One of the two candidate genes was subsequently identified as *Lr10* by transformation of a susceptible cultivar and confirmed by the isolation of three independent, susceptible EMS mutants in the gene (Feuillet *et al.*, 2003).

Haplotype studies at the *Lr10* locus on different hexaploid wheat lines and on diploid A genome lines of *T. urartu* and *T. monococcum* (Scherrer *et al.*, 2002) revealed only two haplotypes at the *Lr10* locus. They differed in the presence or absence of both *Lr10* candidate genes. Therefore, the cloning of the *Lr10* gene was possible because the *T. monococcum* line DV92 from which the BAC library was made has the same haplotype as the hexaploid donor line of *Lr10*. The situation was less clear for the *Pm3b* gene as none of the simpler models available for physical mapping (*T. monococcum* cv. DV92 and *T. durum* cv. Langdon) contained a gene or a haplotype similar to the *Pm3b* donor line. The haplotype in the tetraploid cv. Langdon was very similar to the haplotype in the susceptible hexaploid wheat parent of the mapping population and the diploid cv. DV92 showed only partial haplotype conservation to cv. Chul, the donor line of *Pm3b* (Yahiaoui *et al.*, 2004). However, the partial conservation was sufficient to derive specific markers from a low-copy genomic region carrying a resistance-gene-like sequence that was present in both DV92 and Chul genomes. This finally allowed isolation of the *Pm3b* gene from the

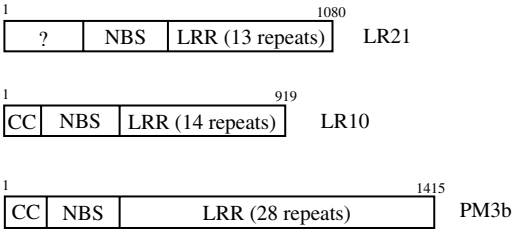

Fig. 2. Schematic representation of the proteins encoded by the three cloned wheat disease resistance genes. The question mark represents an unknown domain in the LR21 protein. CC, coiled coil domain; NBS, nucleotide binding site; LRR, leucine-rich repeat. Numbers indicate amino acids.

hexaploid wheat donor line using long-distance PCR (Yahiaoui *et al.*, 2004). In contrast to leaf rust which attacks mesophyll cells, the interaction between wheat and wheat powdery mildew occurs in leaf epidermal cells. Therefore, the functional analysis and proof of identification of the *Pm3b* gene took advantage of a transient transformation assay in leaf epidermal cells of a susceptible wheat line (Schweizer *et al.*, 1999; Yahiaoui *et al.*, 2004). This transient assay allows rapid functional tests and is not dependent on the fastidious and time-consuming generation of stably transformed wheat plants.

The *Lr10* and *Pm3b* genes encode proteins of 919 and 1415 amino acids, respectively (Feuillet *et al.*, 2003; Yahiaoui *et al.*, 2004). They both show well-conserved motifs of classical coiled-coil, nucleotide-binding-site, leucine-rich-repeat proteins (CC-NBS-LRR; Meyers *et al.*, 1999) (Fig. 2). LR10 is a CC-NBS-LRR protein with 14 LRRs and a C-terminal domain under diversifying selection (Feuillet *et al.*, 2003). PM3b has 28 LRRs that show good conservation to the classical consensus sequence of this domain. *Lr21* encodes a protein of 1080 amino acids with an NBS domain and 13 imperfect LRRs (Fig. 2). It contains a unique domain of 151 amino acids at positions 105 to 255 that is not found in other R proteins and that has unknown properties (Huang *et al.*, 2003). The three cloned genes are all located in the distal region of the short arm of wheat group 1 chromosomes but they do not show any close nucleic acid sequence similarity, indicating ancient divergence.

5. The rice genome as a model for cloning *R* genes in wheat

Comparative genetic mapping revealed a high degree of genetic colinearity between grass genomes, and rice with its small genome was suggested as a model species for other grass genomes (Moore *et al.*, 1995; Devos & Gale, 1997). Therefore, positional cloning projects in wheat could greatly benefit from the recent sequencing of the rice genome (Goff *et al.*, 2002;

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Yu *et al*., 2002). However, the isolation of the three wheat *R* genes did not profit from rice genome information as wheat and rice show very limited co-linearity in the relevant chromosomal regions. The rice genome contains genes homologous to *Lr10* and *Pm3* but at non-orthologous positions, indicating massive genomic rearrangements (Guyot *et al*., 2004; Yahiaoui *et al*., 2004).

Information from the rice genome can be successfully used for deriving markers for mapping in wheat, as described for the *Vrn1* vernalization gene (Yan *et al*., 2003). In barley, markers near the *Rpg1* stem rust disease resistance gene were derived from rice–barley gene microcolinearity (Kilian *et al*., 1997; Han *et al*., 1999). The isolation of *Rpg1* by positional cloning in barley later showed that this gene was not present in rice (Brueggeman *et al*., 2002). More recently, synteny-based marker saturation between rice and barley was performed at the barley leaf rust resistance loci *Rph7* and *Rph16* but no obvious candidate gene could be identified in the corresponding orthologous genomic regions in rice (Brunner *et al*., 2003; Perovic *et al*., 2004). Moreover, in several studies disruptions were found in the colinearity between rice and *Triticeae* species. The observed mosaic pattern of conservation was due to translocations, inversions and transpositions at the chromosome or at the gene level (Brunner *et al*., 2003; Guyot *et al*., 2004; Li *et al*., 2004). Thus, the use of the rice genome as a model for gene cloning in wheat is dependent on the level of conservation of the targeted wheat region and its orthologous region in rice. For most of the *R* genes it is expected that the rice genome is a good source of markers to saturate wheat genomic regions but is unlikely to allow the isolation of wheat *R* genes without corresponding physical maps in wheat. For genes that are evolutionarily more conserved, the use of the rice genome seems to be more promising. This has been shown recently with the isolation of the *ror2* gene from barley (Collins *et al*., 2003).

6. The development of more efficient strategies for map-based cloning in wheat

More complete physical maps based on BAC contigs from whole subgenomes of wheat (*Ae. tauschii*, http://wheat.pw.usda.gov/PhysicalMapping/) or single chromosomes (Feuillet *et al*., unpublished work) will become available in the near future. Genetic mapping will be further simplified by the generation of additional microsatellite markers and the development of markers based on single nucleotide polymorphisms. Finally, it is expected that several additional BAC libraries will soon become available from diploid, tetraploid and hexaploid wheat species. This will allow a more targeted approach for chromosome walking and mapping: the BAC library can be selected based on similarity of the haplotype at the genetic locus of interest in the target variety versus the variety from which the BAC library was made. Chromosome walking will become simpler due to the availability of larger physical contigs from wheat genomes. Currently, all these rapid molecular advances are not matched by progress in more classical approaches. Given the molecular resources that can be expected to be available in 5 years, it would be well justified to start coordinated and intensive work to build large populations for all known resistance genes in order to facilitate efficient mapping and finally the isolation of these genes. Thus, in addition to a wheat genome project, there is also a need for high-resolution mapping of traits of interest and, particularly in the case of resistance QTLs, high-quality phenotypic data. An international 'wheat phenome project' might therefore be envisaged to take full advantage of the molecular developments.

7. Outlook

In addition to the genes mentioned in this review, there are a number of other resistance genes for which the isolation is well advanced. These include the *Lr1* gene (S. Cloutier, Winnipeg, personal communication), the *Yr10* yellow rust resistance gene (Frick *et al*., 1998), the *Sr2* stem rust gene (Spielmeyer & Lagudah, 2003) and the *Sr31*/*SrR* stem rust resistance gene introgressed into wheat on a short chromosomal segment from rye (Mago *et al*., 2004). In addition, major QTLs have been narrowed down to relatively small intervals (Liu & Anderson, 2003; Schnurbusch *et al*., 2004) and their cloning can be expected in the near future, possibly by forming international collaborations as is the case for the quantitatively acting *Lr34* gene involved in leaf rust resistance. The gene pool of wheat is an immensely rich resource and has not yet been explored at the molecular level. Such a characterization of wheat genetic resources will greatly contribute to the understanding of the basis of allelic diversity and specificity in resistance as well as the role of resistance genes in natural and agro-ecosystems.

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