

Putting knowledge of plant disease resistance genes to work

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Plant disease resistance genes trigger defence mechanisms upon recognition of pathogen compatibility factors, which are encoded by avirulence genes. Isolation of the barley powdery mildew resistance gene *Mla* opens the door to understanding the extensive allelic diversity of this locus. Completion of the *Arabidopsis* genome sequence enables the analysis of the complete set of *R*-gene homologues in a flowering plant. A new *R* gene, *RPW8*, conferring resistance in *Arabidopsis* to powdery mildew, reveals a new class of protein associated with pathogen recognition. New prospects for using *R*-gene polymorphism in agriculture are becoming apparent.

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Abbreviations

<i>Avr</i>	avirulence
BRI1	BRASSINOSTEROID INSENSITIVE 1
<i>Bs2</i>	<i>Bacterial spot resistance 2</i>
CC	coiled coil
<i>Cf-9</i>	<i>resistance to Cladosporium fulvum-9</i>
<i>eds1</i>	<i>enhanced disease susceptibility1</i>
LRR	leucine-rich repeat
GFP	green fluorescent protein
Mi	Meloidogyne incognita resistance
<i>Mla</i>	<i>Mildew resistance a</i>
<i>mlo</i>	<i>mildew resistance o</i>
NB	nucleotide-binding
Pto	<i>Pseudomonas tomato resistance</i>
<i>R</i>	resistance
<i>Rar1</i>	<i>Required for mla-dependent resistance 1</i>
<i>Rp1</i>	<i>Resistance to Puccinia sorghi 1</i>
<i>Rpm1</i>	<i>Resistance to Pseudomonas syringae ssp. maculicola 1</i>
<i>RPP7</i>	<i>Resistance to Peronospora parasitica 7</i>
<i>Rps2</i>	<i>Resistance to Pseudomonas syringae 2</i>
TIR	Toll and Interleukin-1 receptor
<i>Xa21</i>	<i>Xanthomonas campestris resistance 21</i>

Introduction

From studies of flax and its interaction with flax rust, Flor [1] originally defined ‘gene-for-gene’ disease resistance in plants. He showed that for a plant to be resistant to a particular parasite race, there must be matching pairs of resistance (*R*) genes and avirulence (*Avr*) genes in host and parasite, respectively. This led to the prediction that, directly or indirectly, *R* genes must encode receptors for parasite, *Avr*-gene-specified, ligands. During the 1990s, many *R* genes were isolated and nearly all were found to belong to one of four main classes [2–5]. Most encode members of the nucleotide-binding (NB)-leucine-rich repeat (LRR) class. The NB-LRR proteins are cytoplasmic, and can be further divided into those that carry amino-terminal homology to the Toll and Interleukin-1 receptor (TIR) genes (the TIR:NB:LRR family) and those that

don’t, most of which carry a putative coiled coil (CC) at their amino terminus (the CC:NB:LRR family). Numerous reviews have assessed this knowledge of *R* genes in some detail. Table 1 summarises the major *R*-gene classes. The purpose of this review is to reflect on recent advances in the field, and evaluate the ways in which our expanding knowledge of *R* genes and resistance mechanisms might be put to use in crop protection.

Mla, a new member of the NB:LRR gene family

To geneticists, one of the most fascinating aspects of *R* genes is the existence of extensive allelic series of resistance specificities at some *R* loci. This has long provoked curiosity about the molecular and evolutionary mechanisms that create and sustain this diversity. The flax *L* locus has been a focus for particular attention; it is a single-copy gene that can encode at least 10 distinct recognition specificities for different flax rust *Avr* genes. The *Rp1* (*Resistance to Puccinia sorghi 1*) locus of maize, also encoding rust resistance, carries at least 14 recognition specificities that occur as different haplotypes. The isolation of *Rp1* has provided the springboard from which to analyse the molecular basis of this diversity [6], and the results of this analysis are keenly awaited.

This year, barley *Mla* (*Mildew resistance a*), the last of the well characterised allelically diverse *R* loci, was defined [7•,8•]. There are 28 *Mla* alleles, each recognising a different spectrum of races of powdery mildew, and by implication, different *Avr* genes. A 240-kilobase region that is linked to *Mla* carries multiple NB-LRR genes, that fall into three distinct (non-cross hybridising) NB:LRR families: within the *Mla* family of NB:LRRs the *Mla1* and *Mla6* haplotypes each carry two *Mla1* homologues. The functional homologue was identified by an elegant transient transformation assay in which an *mlo* (*mildew resistance o*) mutant (*mlo* mutants are derepressed in disease resistance mechanisms [9]) was co-bombarded with a candidate *Mla* gene, a functional *Mlo* gene and green fluorescent protein (GFP). The bombarded leaves were then infected with mildew spores. In the absence of an active *Mla* transgene, the bombarded cells were rendered susceptible to mildew by the dominant *Mlo* gene and expressed GFP from the reporter gene. Functional *Mla* candidates caused the co-bombarded cells to be resistant to mildew and prevented GFP expression because this resistance is associated with cell death.

Mla1 and *Mla6* encode highly homologous NB-LRR genes with a putative coiled-coil amino-terminal domain. The most remarkable finding of the recent work lies in the differing requirements of different *Mla* alleles for the *Rar1* (*Required for mla-dependent resistance 1*) gene. The *rar1-1* and *rar1-2* mutations were originally isolated as suppressors of *Mla12* [10]. *RAR1* encodes a novel zinc-finger motif

Table 1

The major *R*-gene classes.

<i>R</i> -gene class	Member	Protein structure	<i>Arabidopsis</i> homologs
LRR kinase	<i>Xa21</i> Rice gene for bacterial-blight resistance	Transmembrane protein; extracellular LRRs, cytoplasmic protein kinase domain	~174
eLRRs	<i>Cf-9</i> Tomato gene for <i>Cladosporium</i> resistance	Transmembrane protein with extracellular LRRs	~30
<i>Pto</i>	<i>Pto</i> Tomato gene for <i>Pseudomonas syringae</i> resistance	Serine/threonine protein kinase with myristoylation site	~100
TIR:NB:LRR	<i>N</i> Tobacco gene for tobacco mosaic virus (TMV) resistance	Cytoplasmic protein with homologies to Toll cytoplasmic domain, apoptotic ATPases CED4 and Apaf1, and carboxy-terminal LRRs	~100
CC:NB:LRR	<i>RPS2</i> <i>Arabidopsis</i> resistance to <i>P. syringae</i>	Cytoplasmic protein with homologies to CC, apoptotic ATPases CED4 and Apaf1, and carboxy-terminal LRRs	~65
SA:CC	<i>RPW8.1</i> , <i>RPW8.2</i> <i>Arabidopsis</i> resistance to powdery mildew	Putative signal anchor (SA) for membrane insertion, and putative CC domain	5

called CHORD (cysteine- and histidine-rich domain) [11]. *Mla6* is suppressed by *rar1* mutations, but *Mla1* is not. However, the *Rar1*-requiring allele, *Mla6*, and the *Rar1* non-requiring allele, *Mla1*, were found to encode 91% identical proteins. Why two such similar alleles should differ in their requirement for *Rar1*, which probably encodes a protein that participates in signalling by regulating protein turnover through the proteasome, is currently mysterious. However, neither *Rar1* mutant allele has been proved to confer complete loss of function; conceivably, a full loss-of-function *Rar1* mutation would suppress both *Mla1* and *Mla6*.

An orgy of swapping

The availability of cloned genes has permitted investigation of which domains contribute to function. Several papers published this year report novel insights obtained using *in vitro* recombination, either using classical restriction-enzyme-mediated cut and paste techniques or gene shuffling.

The *Cf-9* (resistance to *Cladosporium fulvum-9*) gene of tomato encodes a transmembrane protein that has extracellular LRRs and a short cytoplasmic domain [12]. The *Cf-4* gene lies at an orthologous position and encodes a protein with approximately 90% amino-acid sequence identity to *Cf-9* [13]. The Avr proteins that correspond to these genes, Avr9 and Avr4, are well characterized [14,15]. Using a combination of transient transformation assays in *Nicotiana benthamiana* and stable transformation in tomato, two groups have attempted to define the amino-acid differences between Cf-4 and Cf-9 that are most crucial for their distinct recognition capacities. They concluded that it is the amino acids in LRRs 10–18 that are most important [16*,17*]. The gene-shuffling approach appears to be particularly powerful for generating a library of recombinants,

and might find future use in the production of libraries of potential synthetic *R* genes.

At the flax *L* locus, 11 alleles condition recognition of a different spectrum of races of flax rust. Although many studies on different *R* genes have suggested that the R-protein LRR domain makes the major contribution to the unique recognition capacity of individual *R* genes, recent analysis of the *L* allelic series has shown that the TIR domain can also contribute [18**]. For example, when the TIR domain encoded by the *L6* allele is replaced by that of *L2*, the *L6* protein converts its recognition specificity to that of *L7*. Consistent with this, an excess of non-synonymous over synonymous nucleotide substitutions on certain codons in the TIR domain suggests that these genes are the subjects of divergent selection. It also appears that the amino-terminal and carboxy-terminal regions of NB-LRR proteins need to coevolve to generate functional proteins, as some domain exchanges between functional genes were non-functional.

A similar conclusion emerges from another study. The *Mi* gene that provides resistance to the nematode *Meloidogyne incognita* in tomato (*Mi-1.2*) has a closely linked homologue (*Mi-1.1*) that does not confer nematode resistance. Domain swaps between the two homologous proteins were constructed and examined in *Agrobacterium rhizogenes* transformed roots and also in transient transformation assays in *N. benthamiana* [19*]. Replacing the *Mi-1.2* LRRs with those from *Mi-1.1* led to loss of *Mi-1.2* function, but the reciprocal swap provoked a lethal phenotype that was consistent with constitutive activation of the defence response. The researchers who carried out this study interpreted these and their other data to mean that the LRRs play a role in signalling [19*]. An alternative interpretation is that the amino-terminus and the carboxy-terminal LRR

regions coevolve, and that the LRRs are negative regulators of a lethality imposed by the signalling domains at the amino-terminus. Putting the Mi-1.2 LRRs onto Mi-1.1 creates a combination that fails to repress the Mi-1.1 amino-terminus, leading to lethality. Further work is needed to distinguish between these two possibilities.

The distinct signalling and recognition components of the rice *Xa21* gene for *Xanthomonas* resistance, were nicely visualised in a domain swap with BRI1 (BRASSINOSTEROID INSENSITIVE 1), another transmembrane LRR kinase, which is required for brassinosteroid perception [20**]. In this work, He *et al.* made chimeric genes in which the BRI1 LRRs were fused to the protein kinase domain of Xa21, and assayed the fusions in rice suspension-culture cells. One such fusion, which retained all of the extracellular amino acids of BRI1 plus the transmembrane domain and 65 amino acids of the intracellular BRI1 protein, activated defence responses upon treatment with brassinosteroid. One implication of this result is that BRI1 and Xa21 recognise their distinct ligands and initiate signalling through similar mechanisms, and that the protein kinase domain specifies which specific signal transduction pathway ensues. This remarkable result opens the way to attempts to understand the responses triggered by the large number of LRR kinase orphan receptors in the *Arabidopsis* genome; their protein kinase domain could be fused to the BRI1-recognition domain, brassinosteroid added and the consequences studied.

A genome-wide survey of *R* genes: lessons from the *Arabidopsis* genome

'How many *R* genes does a plant need?' is a question that arose from the earliest isolation of *R* genes. With the *Arabidopsis* genome sequence now available, we can answer this question definitively [21**]. Annotation of the ten *in silico* pseudomolecules that correspond to each of the arms of the five *Arabidopsis* chromosomes revealed 150 sequences that have homology to the NB-LRR class of *R* genes. Not all of these appear intact. Despite the fact that many previously isolated *R* genes appear to reside in local multigene families, there are 46 singleton *Arabidopsis* *R*-gene homologues, as well as 25 doubletons, seven loci with three *R*-gene copies and individual loci with four, five, seven, eight and nine NB-containing genes. In recent months, the *RPP7* (*Resistance to* *Peronospora parasitica*7) family has now been defined as an additional cluster of approximately 14 copies of Col-0 on chromosome 1 (E Holub, personal communication). An excellent and continuously updated annotation of *Arabidopsis* *R*-genes by Blake Meyers and colleagues can be found at URL http://pgfsun.ucdavis.edu/niblrrs/At_RGenes/.

The high frequency of multigene family *R* genes so far identified from *Arabidopsis* and other species suggests that such loci may be, on average, more likely to generate genetic variation for resistance than loci in which only a few gene copies are present. Extensive allelic series should be more

common in loci that carry multiple *R* genes if multicopy loci are more able to generate diversity on which selection could act. It's worth noting that both *Rps2* (*Resistance to Pseudomonas syringae* 2) and *Rpm1* (*Resistance to Pseudomonas syringae ssp. maculicola* 1) were originally defined by the ethane-methyl-sulphonate (EMS) mutagenesis of an *R* gene that confers recognition of an *Avr* gene isolated from a *Pseudomonas syringae* race that does not infect *Arabidopsis*. Subsequently, *Arabidopsis* landraces were identified in which *Rps2* and *Rpm1* function is absent. Both inverted and direct repeats of *R* genes, at a ratio of about 3:2, are observed in the *Arabidopsis* genome. There are more TIR-NB-LRR genes (approximately 60%) than CC-NB-LRR genes (approximately 40%). The largest clusters are at the *RPP5* locus, which carries the Col-0 *RPP4* gene and seven other *RPP5* homologues, and at a complex locus on chromosome 1 that is linked to the *RPP7* gene and carries multiple distinct CC:NB:LRR genes. It is conceivable that there remain a few more such clusters, as the presence of so many repeats made sequence determination in these regions difficult, requiring the participation of workers involved in cloning *RPP4/5* and *RPP7*.

R homologues are unevenly distributed among chromosomes, with 49 on chromosome 1, 2 on chromosome 2, 16 on chromosome 3, 28 on chromosome 4 and 55 on chromosome 5. Some *R* homologues also have unexpected structures. Two *R* genes carry, in addition to a TIR-NB-LRR structure, a WRKY domain that is likely to confer DNA-binding capacity. Furthermore, one TIR-NB-LRR gene has been annotated to carry not only a WRKY but also a protein kinase domain. Reverse transcription (RT) polymerase chain reaction (PCR) experiments are required to verify these annotations experimentally. The identification of truncated forms of both CC-NB and TIR-NB genes that lack the LRRs is of considerable interest. It remains to be determined whether these are simply the unpurged debris of past mutation events or encode important and functionally redundant adaptor molecules that play a role in signalling, as does MyD88 in TIR signalling in animals [22]. MyD88 encodes a protein with a TIR domain and a death domain that recruits Interleukin-1-associated kinase (IRAK) to Toll-like receptors or to the Interleukin-1 receptor.

Arabidopsis carries homologues of other *R*-gene classes, including 174 homologues of genes encoding the Xa21 class of LRR kinases, many of which are likely to play a role in development rather than in defence. There are also 30 genes that resemble *Cf-9* and *CLAVATA2* in that they carry extracellular LRRs and a short cytoplasmic domain. Finally, there are more than 50 genes encoding protein kinases that are strongly homologous to Pto (for *Pseudomonas* tomato resistance), the tomato serine/threonine protein kinase that confers recognition of *P. syringae* strains that carry AvrPto.

It must be remembered that the Col-0 genome sequence is no more than that, and comparison to other landraces

will require additional work. For example, *Rpm1* is absent from landrace Nd-0 [23]; there may be Nd-0 *R* genes that are not found in Col-0, as turns out to be the case for the newly isolated *RPW8* class of *R* gene (see below). The *RPP8* gene family is 'one copy' in Col-0, and 'two copy' in Landsberg erecta (Ler) [24]. In addition, as stated earlier, one of the most interesting features of *R*-gene loci is their striking level of polymorphism. Further insight into this polymorphism requires extensive analysis by gel-blot hybridisation and perhaps by DNA sequencing of homologues from multiple landraces. This will be a great deal of work but should provide significant insights into *R*-gene evolution [25].

It is perhaps surprising that *Arabidopsis* carries only approximately 100 different non-cross-hybridising types of NB-LRR *R* genes, about half of which are singletons. This could mean that many of these non-cross-hybridising types recognise conserved pathogen molecules, and will turn out to be of ancient origin, as recently shown for tomato *Pto* [26]. If this is the case, the idea that *R*-gene polymorphism to provide different recognition specificities is important for restricting disease in wild populations becomes more plausible (see below).

***Arabidopsis* powdery mildew resistance genes *RPW8.1* and *RPW8.2*: a new *R*-gene class**

Studies on obligate biotrophs of *Arabidopsis* were pioneered by Holub and coworkers, who focussed on the oomycete parasites downy mildew (*Peronospora parasitica*) and white blister (*Albugo candida*). More recently, isolates of *Erysiphe orontii*, *E. cichoracearum* and *E. cruciferarum* have been identified that parasitise *Arabidopsis* [27–30]. Genetic variation has been identified at *Arabidopsis* loci that confer *Erysiphe* resistance [31]. Landrace Ms-0 carries the *RPW8* locus on chromosome 3, and the corresponding locus has now been characterised at the molecular level [32•]. Of five diverged homologues at the Ms-0 *RPW8* locus, two (*RPW8.1* and *RPW8.2*) confer *Erysiphe* resistance, though these two genes are only 50% identical at the amino-acid-sequence level. In Ler and RLD, there are also five *RPW8* homologues, but in Col-0, there are just two. The *RPW8* genes encode relatively short proteins that carry a putative signal anchor and then a coiled-coil domain. Despite its lack of resemblance to known resistance genes, *RPW8* provides resistance that is associated with a hypersensitive response, is compromised in a strain expressing *nahG* (the bacterial salicylate hydroxylase enzyme, which removes salicylic acid), and is even compromised in the presence of a homozygous *eds1* (*enhanced disease susceptibility1*) mutant allele. The *EDS1* gene, encoding a lipase homologue, has hitherto only been shown to be required for signalling from the TIR-NB-LRR class of *R* genes [33–35]. The *EDS1* requirement of *RPW8* is particularly surprising given that the only other homology detected to the *RPW8* coiled-coil domain is found at the amino terminus of a CC-NB-LRR gene on chromosome 5. On overexpression, the *RPW8*

gene leads to plant growth inhibition and other phenotypes that are consistent with the constitutive activation of the defence pathway.

***R* genes in populations; the significance of polymorphism**

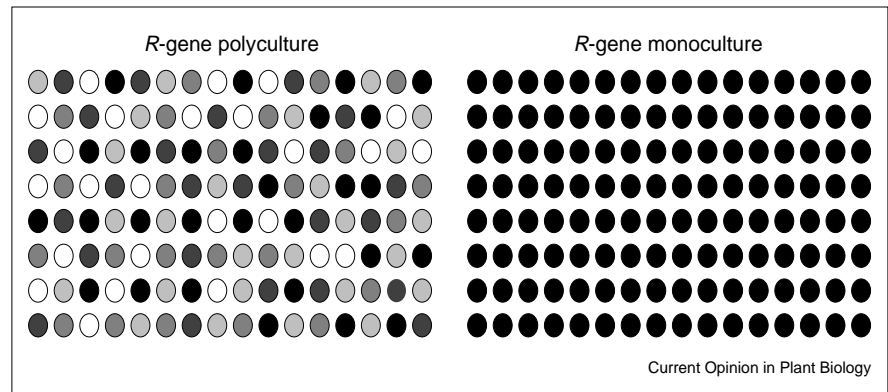
Natural selection has generated wild populations that are polymorphic at *R*-gene loci over much longer periods than those during which plant breeders have been recruiting such diversity for crop improvement [36,37]. It is clear from many studies that some, though not all, mutations in *Avr* genes lead to reduced pathogen fitness. However, there are also data that suggest that *R* genes could lead to reduced plant fitness in the absence of a pathogen, presumably because activating the defence response consumes resources that in the absence of pathogens are best devoted to growth and reproduction. For example, high-level expression of *Rps2* results in lethality [38]. As it has been lost so frequently in independent accessions, *Rpm1* might confer a fitness cost in the absence of pathogen [39]. Thus, though the experiment is undoubtedly worth attempting, the stacking or 'pyramiding' of *R*-gene alleles from one species into a single genotype might not provide durable resistance, will select pathogens that can tolerate the loss of multiple compatibility factors, and may lead to yield penalties.

In an important paper [40], the population geneticist Bill Hamilton presented the idea that the main purpose of out-crossing was to reduce parasite pressure by sustaining polymorphism at loci that contribute to parasite recognition. According to this model, if a host population is extremely heterogeneous in its recognition capacity, then most isolates of the parasite will not be able to grow on most hosts. In the absence of out-crossing, such polymorphism would be more likely to be lost, unless it is maintained by selection. Furthermore, if sexual recombination among parasites leads to exchange of dominant avirulence genes, then most progeny of most parasites will not be able to find a host. There is still debate about whether such frequency-dependent selection, in which rare resistance (recognition) specificities are less likely to be overcome by the parasite, is the main explanation for the enormous diversity of human haplotypes at the major histocompatibility (MHC) locus. An alternative model proposes that this diversity can be explained by overdominance (i.e. heterozygote advantage), through which heterozygotes have twice the recognition capacity and resistance of any homozygote [41]. Many plant species, including *Arabidopsis*, reproduce by self-fertilisation, and overdominance cannot explain the extreme polymorphism of *R* loci compared to other loci in such species (see [38,39]).

This idea that *R*-gene polymorphism by itself could restrict parasite populations is attractive, but what data are available that test it? It is well known that any individual *R* gene in a crop monoculture is likely to be eventually overcome by parasite mutations, though there is still hope

Figure 1

In an *R*-gene monoculture, any pathogen race can overcome an *R* gene and cause an epidemic. In an *R*-gene polyculture, there are many fewer plants that are susceptible to a pathogen race with one virulence gene against a particular *R* gene (solid black fill), so epidemics are slower. Mutations that enhance this race so that it can overcome an additional *R* gene will result in the loss of an additional compatibility factor, and reduced fitness against the first *R* gene. High pathogen pressure with avirulent races may trigger systemic acquired resistance. Differently shaded symbols signify different *R* genes, each recognising a different *Avr* gene.



that the *Bs2* (*Bacterial spot resistance 2*) gene [42], which recognises *AvrBs2* [43], will prove durable. *AvrBs2* appears to be indispensable for full virulence in *Xanthomonas campestris*. The analysis of different *Avr* genes for their contribution to virulence continues to be a valuable and interesting goal [44].

The cost to breeders of continually having to create new varieties that carry new and fleetingly unbroken *R* genes is substantial. Are there strategies that could improve the durability of *R* genes? A recent study on rice and rice blast is instructive [45•]. Two varieties of rice, one low yielding and disease sensitive but high quality, and the other high yielding, more disease resistant and low quality, were grown either individually or in mixtures. In a typical mixture, four rows of the disease resistant variety were grown for every one row of the sensitive variety. Blast incidence in the sensitive variety was reduced 25 fold when grown in a mixture as opposed to a monoculture, and there was even a slight reduction of blast incidence in the resistant variety. A similar study suggesting that varietal mixtures can reduce disease incidence has been reported for late-blight resistance in potato [46]. In the 1970s, the concept of varietal mixtures was championed by Martin Wolfe amongst others [47,48]. However, varieties differ not only in *R* genes but also in other characteristics such as time to ripening or malting quality, resulting in the reduced profitability of crops that are grown in mixtures.

As the *Mla* locus is an allelic series, it is essentially impossible to breed varieties that are genotypically identical at every locus except *Mla*. The isolation of the *Mla* gene has changed this. Using transformation, it should be possible to introduce several different *Mla* alleles into a variety, and thereby create plant populations that are heterogeneous for pathogen recognition but homogeneous for commercial traits. The idea is illustrated in Figure 1, in which plants carrying different *R* genes are given a different shading. In a typical monoculture, all of the plants have the same *R* gene. Any *Avr* mutation that enables the pathogen to defeat the *R* gene enables the explosive population growth

of the pathogen, even if the mutation results in slightly reduced virulence. In contrast, a population that is heterogeneous for five different *R* genes has several advantages. First, only 20% of the plants can support the growth of any virulent race, so the rate of increase of the epidemic will be correspondingly reduced. Second, the intense pathogen pressure of avirulent pathogen races may trigger systemic-acquired resistance, which will reduce susceptibility to virulent races. Third, any mutation affecting the virulence of a race that can overcome one *R* gene that enables it to grow on plants that have another *R* gene may result in slightly reduced parasite fitness against the first *R* gene. If there is a cost to virulence, then such mixed-pathogen populations will never completely lose the corresponding *Avr* genes. Hence, a population that is heterogeneous for five different *R* genes has an advantage over a single cultivar that has all five *R* genes. In contrast, the pyramiding of all five genes into a single cultivar could easily lead to selection for suppressor mutations that enable a pathogen to tolerate loss of all five *Avr* genes without too serious a loss of fitness. Given the isolation of *R*-gene allelic series, it is time to give the 'heterogeneous population' approach serious experimental testing.

Conclusions

The best-characterised resistance gene loci are now all isolated. Future work with these loci will focus on the description of the allelic series and on appropriate inferences about the selective forces at work at these loci. The *R*-gene complement of *Arabidopsis* has proved interesting; we keenly await an annotation of the *R*-gene complement of rice. For example, how many *R*-gene homologues are there in rice, and does it really lack the TIR-NB-LRR class as some early analysis has suggested? A major new challenge is to identify the pathogen *Avr* genes from powdery mildew and from various rusts, and to understand these gene-for-gene interactions at the molecular level. It has become increasingly clear that many *R*-gene loci are highly polymorphic; this makes it much harder for pathogens to cause epidemics in wild populations. It will always be easier for a pathogen to discard a compatibility factor encoded by an

Avr gene, than for a plant to create a completely new recognition capacity. A challenge for breeders and seed merchants is how to take advantage of the benefits of *R*-gene polymorphism in the deployment of cloned *R* genes in new varieties.

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