

Deciphering plant–pathogen communication: fresh perspectives for molecular resistance breeding

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Activation of local and systemic plant defences in response to pathogen attack involves dramatic cellular reprogramming. Over the past 10 years many novel genes, proteins and molecules have been discovered as a result of investigating plant–pathogen interactions. Most attempts to harness this knowledge to engineer improved disease resistance in crops have failed. Although gene efficacy in transgenic plants has often been good, commercial exploitation has not been possible because of the detrimental effects on plant growth, development and crop yield. Biotechnology approaches have now shifted emphasis towards marker-assisted breeding and the construction of vectors containing highly regulated transgenes that confer resistance in several distinct ways.

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Abbreviations

Avr	avirulence protein
CC	coiled coil
eLRR	extracellular leucine-rich repeat
ET	ethylene
GM	genetically modified
ISR	induced systemic resistance
JA	jasmonic acid
LRR	leucine-rich repeat
MAPK	mitogen-activated protein kinase
NB	nucleotide binding
R	resistance protein
RNAi	RNA interference
SA	salicylic acid
SAR	systemic acquired resistance
TIR	Toll and Interleukin-1 receptor
VIGS	virus-induced gene silencing

Introduction

Plants routinely combat pathogenic microbes and survive. Different layers of plant defence have been uncovered by genetic, genomic and biochemical analyses. Plants

possess many preformed barriers, but also activate species level (non-host) resistance, race-specific and race non-specific resistance, as well as basal defence (Table 1). Triggering of local responses can also induce systemic immunity that primes tissues against subsequent attack. This article focuses on recently identified pathogen signals that are deciphered by the plant's surveillance systems and the plant genes required for local and systemic biotic defence. We also aim to highlight new technologies that are now setting the pace of plant and pathogen gene discovery. Finally, we discuss several realistic approaches to achieve durable disease control using genetically modified (GM) or non-GM options. For the most part, the discussion is limited to pathosystems involving fungi, *Oomycetes* and bacterial pathogens.

Plant–pathogen recognition complexes

Plant resistance (R) proteins recognise pathogen avirulence (Avr) determinants and in turn trigger signal transduction cascades that lead to rapid defence mobilisation [1]. The pace of R gene discovery in crop plants, such as barley, rice, maize and tomato, has accelerated over the past five years owing to impressive developments in high-throughput molecular mapping, sequencing and gene isolation technologies (Table 2). These advances already allow a reasonable assessment to be made of the potential R gene complement in a given species and provide useful sources of R gene variants. Comparison can also be made with recognition and resistance signalling components identified in the smaller genome of *Arabidopsis* [2]. Striking similarities are found in the structures of R proteins from monocotyledonous and dicotyledonous species, implying that fundamental modes of recognition and defence signalling have been retained through plant evolution and diversification. The most prevalent class of functionally defined R genes encode intracellular nucleotide-binding/leucine-rich repeat (NB-LRR) proteins with variable N-terminal domains. Less common are the serine/threonine protein kinase class of proteins and extracellular LRR (eLRR) proteins that possess a single transmembrane domain and either a short intracellular C terminus or kinase domain (Figure 1; Table 2).

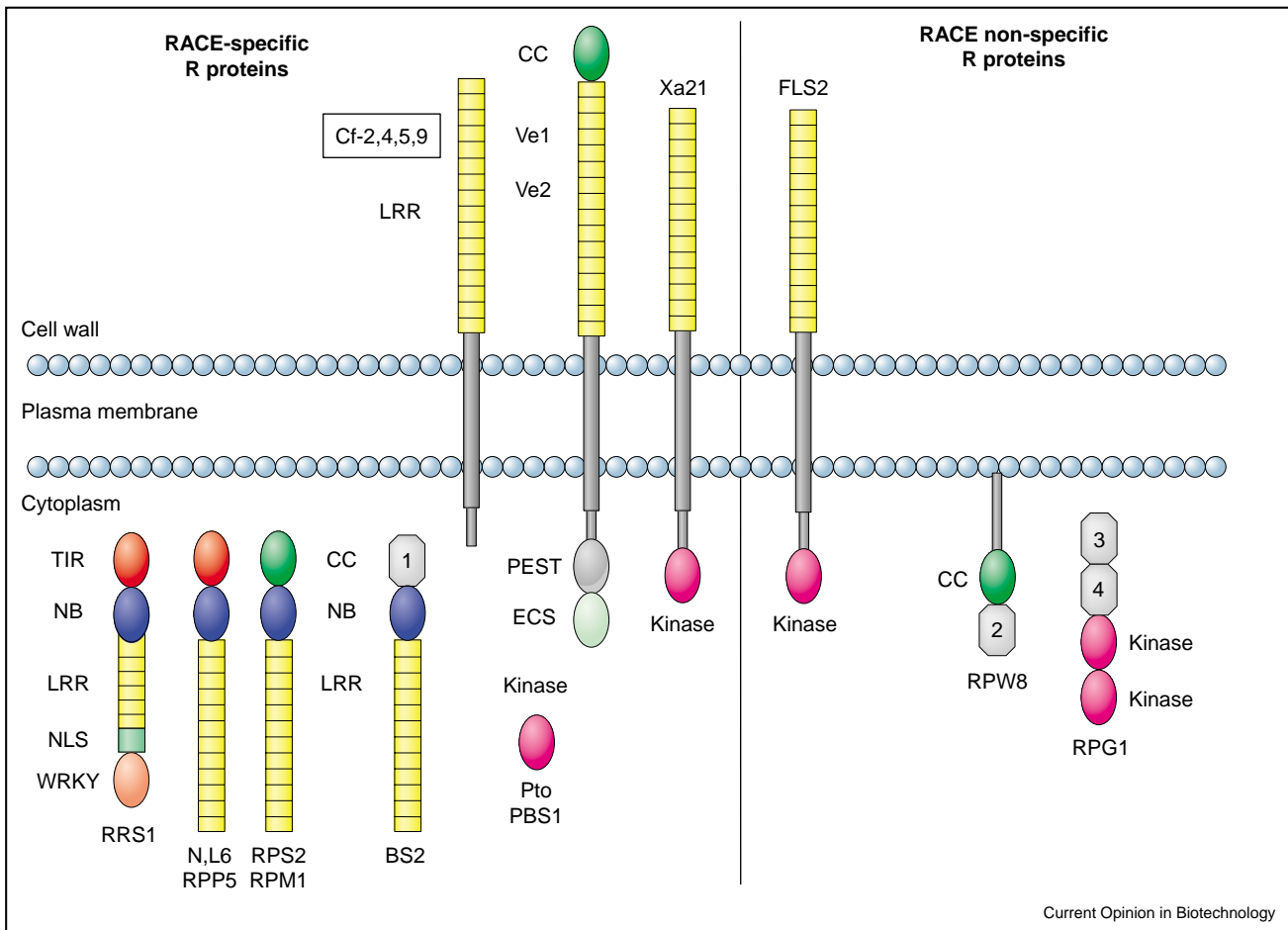
Plant NB-LRR proteins have structural counterparts in animal cells that are involved in innate immunity. Information can now be gained on the intramolecular and intermolecular associations of related protein modules in animal and plant systems [3**]. Analyses reveal that the LRR domains of various plant R proteins contribute to specificity in pathogen recognition [1]. Also, *in vitro*

Table 1

Basic disease resistance concepts.

Term	Explanation	Outcome
Non-host resistance	Disease resistance operating between species Effective against all known isolates of the pathogen	No disease
Race non-specific resistance	Disease resistance operating within a species Effective against all known isolates of the pathogen. R-protein-mediated	Only some plant genotypes fully resistant
Race-specific resistance	Disease resistance that varies within a species Effective only in plants with R proteins that correspond to elicitors produced by specific isolates of the pathogen	Each plant genotype exhibits differential disease resistance and susceptibility to a single isolate
Basal defence	Activated in susceptible genotypes of a host plant species	Disease severity varies between susceptible plant genotypes

Figure 1



Schematic representation of the predicted domains of R proteins which confer either race-specific or race non-specific resistance. Further details for each named R protein are given in Table 2. The two Ve proteins differ slightly in protein structure. Ve1 contains a putative CC domain but no PEST sequence in the C terminus, whereas Ve2 lacks the CC domain at the N terminus but contains a C-terminal PEST sequence. Four protein domains are indicated (grey; labelled 1–4) that lack significant homology to known proteins. BS2, bacterial speck resistance 2; Cf-2,4,5,9, resistance to *Cladosporium fulvum* races 2, 4, 5 and 9; ECS, endocytosis signal; L6, flax rust resistance 6; NLS, nuclear localisation sequences; PEST, Pro-Glu-Ser-Thr-like sequence; PBS1, resistance to *Pseudomonas* bacterial speck expressing avrPphB; Pto, *P. syringae* pv. tomato resistance; RPG1, resistance to *Puccinia graminis* f.sp. *tritici* 1; RPM1, resistance to *P. syringae* pv. *maculicola* expressing avrRPM1 or AvrB; RPP5, resistance to *Peronospora parasitica*; RPW8, resistance to powdery mildew; RRS1, resistance to *Ralstonia solanacearum* 1; Xa21, resistance to *Xanthomonas oryzae* p.v. *oryzae*.

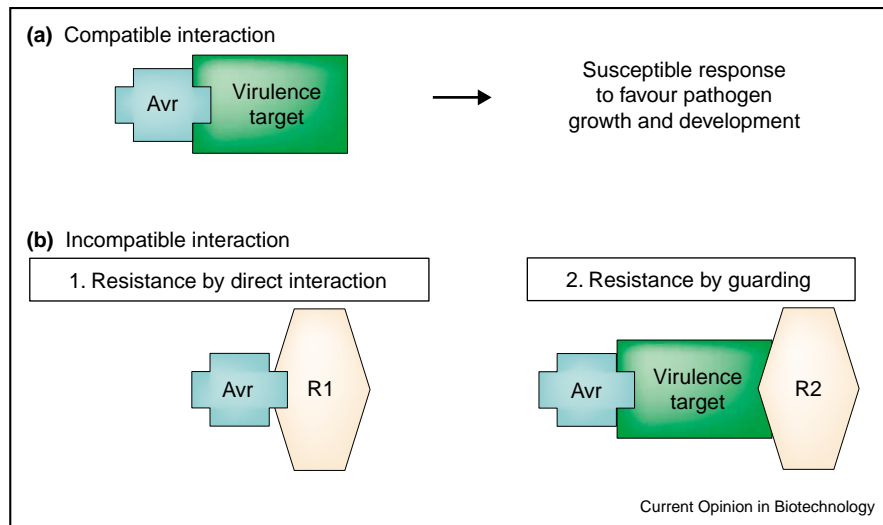
Table 2

The major classes of cloned plant disease resistance genes*.

Class	Gene	Plant	Pathogen	Infection type/organ attacked	Predicted features of R protein	Race-specific	Year isolated	References
1	<i>Hm1</i>	Maize	<i>Helminthosporium maydis</i> (race 1)	Fungal necrotroph/leaf	Detoxifying enzyme HC toxin reductase	Yes	1992	†
2	<i>Asc-1</i>	Tomato	<i>Alternaria alternata</i> f.sp. <i>lycopersici</i> (<i>AAL toxin</i>)	Fungal necrotroph/leaf	TM helix-LAG1 motif	No	2000	[66]
3A	<i>Pto</i>	Tomato	<i>Pseudomonas syringae</i> p.v. <i>tomato</i> (<i>avrPto</i>)	Extracellular bacteria/leaf	Intracellular serine/threonine protein kinase	Yes	1993	†
3B	<i>PSB1</i>	<i>Arabidopsis</i>	<i>Pseudomonas syringae</i> p.v. <i>phaseolicola</i> (<i>avrPphB</i>)	Extracellular bacteria/leaf	Different subfamily	Yes	2001	[27]
4A	<i>RPS2</i>	<i>Arabidopsis</i>	<i>Pseudomonas syringae</i> p.v. <i>maculicola</i> (<i>avrRpt2</i>)	Extracellular bacteria/leaf	CC-NB-LRR Intracellular protein	Yes	1994	†
	<i>Mla1/Mla6</i>	Barley	<i>Blumeria graminis</i> f.sp. <i>hordei</i> (resp. race 1, race 6)	Biotrophic intracellular fungus with haustoria/leaf		Yes	2001	[58]
	<i>R1</i>	Potato	<i>Phytophthora infestans</i> (race 1)	Biotrophic intracellular <i>Oomycete</i> with haustoria/leaf and tuber		Yes	2002	[67] [68]
	<i>RPP8</i>	<i>Arabidopsis</i>	<i>Peronospora parasitica</i>	Biotrophic intracellular <i>Oomycete</i> with haustoria/leaf		Yes	1998	†
4B	<i>N</i>	Tobacco	Mosaic virus	Intracellular virus/leaf and phloem	TIR-NB-LRR Intracellular protein	Yes	1994	†
	<i>RPP4</i>	<i>Arabidopsis</i>	<i>Peronospora parasitica</i>	Biotrophic intracellular <i>Oomycete</i> with haustoria/leaf		Yes	2002	[69]
4C	<i>Bs2</i>	Pepper	<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i> (<i>avrBs2</i>)	Extracellular bacteria/leaf	NB-LRR Intracellular protein	Yes	1999	†
	<i>Dm3</i>	Lettuce	<i>Bremia lactuca</i>	Biotrophic intracellular <i>Oomycete</i> with haustoria/leaf		Yes	2002	[70]
4D	<i>RRS-1</i>	<i>Arabidopsis</i>	<i>Ralstonia solanacearum</i> (race 1)	Extracellular bacteria/leaf	TIR-NB-LRR-NLS-WRKY	Yes	2002	[5]
4E	<i>Pi-ta</i>	Rice	<i>Magnaporthe grisea</i> (<i>avrPita</i>)	Hemibiotrophic intracellular fungus without haustoria/leaf	NB-LRD	Yes	2000	†
5A	<i>Cf-9</i>	Tomato	<i>Cladosporium fulvum</i> (<i>Avr9</i>)	Biotrophic extracellular fungus without haustoria/leaf	eLRR-TM-sCT Extracellular protein with single membrane-spanning region and short cytoplasmic C terminus	Yes	1994	†
5B	<i>Ve1</i> <i>Ve2</i>	Tomato	<i>Verticillium albo-atrum</i>	Extracellular vascular wilt fungus without haustoria/root and stem	CC-eLRR-TM-ECS eLRR-TM-PEST-ECS	Yes	2001	[6]
6	<i>Xa-21</i>	Rice	<i>Xanthomonas oryzae</i> p.v. <i>oryzae</i> (all races)	Extracellular bacteria/leaf	eLRR-TM-kinase	Yes	1995	†
	<i>FLS2</i>	<i>Arabidopsis</i>	Multiple bacteria (flagellin)	Extracellular bacteria/leaf		No	2000	†
7	<i>RPW8.1</i> <i>RPW8.2</i>	<i>Arabidopsis</i>	Multiple powdery mildew species	Biotrophic intracellular fungus with haustoria/leaf	Small, probable membrane protein with CC domain	No	2001	[18]
8	<i>Rpg1</i>	Barley	<i>Puccinia graminis</i> f.sp. <i>tritici</i>	Biotrophic intracellular fungus with haustoria/stem	Receptor kinase-like protein with 2 tandem kinase domains	No	2002	[19]

*Only new *R* genes conferring resistance to bacteria, fungi or *Oomycete* pathogens are listed. Details are also given of the first *R* genes isolated in each class. †The references for *R* genes isolated prior to 2001 and those conferring resistance to plant viruses, insects and nematodes are summarised elsewhere [28]. ECS, endocytosis signal; LAG1, longevity assurance gene; LRD, leucine-rich domain; PEST, Pro-Glu-Ser-Thr; sCT, single cytoplasmic tail; TM, transmembrane.

Figure 2



Guarding of pathogen virulence targets by plant R proteins. **(a)** A virulence target exists in a susceptible host plant. Upon pathogen infection, the Avr factor binds to its cognate virulence target resulting in modifications to the target. These modifications lead to pathogen virulence and host susceptibility, thereby generating a compatible interaction. **(b)** Incompatible interactions conferred by a resistant host plant can arise in two ways.

(1) The R1 protein directly recognises the Avr factor itself. This situation is now considered to occur only rarely. An example of this type of interaction occurs between Avr-Pita and Pi-ta. (2) The R2 protein is a guard protein, recognising the modified plant virulence target caused by the earlier binding of the Avr factor. An example of this type of interaction occurs between AvrB/AvrRpm1 (Avr), RIN4 (the virulence target) and RPM1 (R2). See Table 2 and Figure 1 for further details. Direct recognition in situation 1 can be circumvented by alterations of the Avr factor without modifying its virulence function. By contrast, in situation 2 recognition mediated by the guarding R2 protein cannot be circumvented by alterations of the Avr factor without affecting its virulence function.

data show that the NB domains of the tomato Mi-1 and I-2 NB-LRR resistance proteins can bind and hydrolyse ATP [4]. These results reinforce the opinion that the NB domain functions in signal transduction.

Several distinct *R* gene sequences were discovered more recently which have extended the repertoire of potential motifs. For example, *Arabidopsis* RRS1, conferring resistance against the bacterium *Ralstonia solanacearum*, is an NB-LRR protein with an additional C-terminal WRKY domain [5]. WRKY proteins are plant-specific zinc-finger transcription factors that are frequently induced during defence responses and bind *cis*-acting elements in multiple pathogen-induced promoters. Thus, RRS1 may bind DNA and thereby activate other genes conferring the resistance phenotype. By contrast, two tomato *Ve* resistance genes independently confer resistance to the fungus *Verticillium albo-atrum*. Each encodes an eLRR protein with a cytoplasmic domain possessing sequences that in mammalian receptors stimulate their endocytosis and degradation. In *Ve1* an N-terminal coiled coil (CC) domain precedes the LRR domain while a PEST (Pro-Glu-Ser-Thr) sequence is present at the C terminus of *Ve2* (Figure 1). PEST sequences are often involved in protein ubiquitination, internalisation and degradation and therefore suggest a short R protein half-life once cytoplasmic localisation occurs [6].

Although direct association between at least two R–Avr protein pairs has been demonstrated *in vitro*, consistent with a receptor–ligand model, current data suggest that most R–Avr protein interactions are not direct but instead involve perception of pathogen-derived proteins within a complex [7,8–10]. One favoured model (the ‘Guard’ hypothesis) places R proteins as molecular ‘antennae’ that register interactions between pathogen avirulence factors and their host targets (Figure 2) [9,11]. In the absence of the host R protein, the pathogen avirulence protein could interfere with a positive plant defence regulator or promote a plant defence suppressor. This may be crucial to successful pathogen proliferation [7,12].

Components of non-host resistance

Of particular interest to plant breeders are processes that control non-host (or species level) resistance (Table 1). Unlike race-specific resistance, which is often rapidly overcome by pathogen isolates that evolve matching virulence, non-host resistance exerts a robust, durable barrier to a broad range of pathogens. Emerging evidence suggests that non-host resistance is the composite of several overlapping mechanisms and this may, at least in part, account for its durability. Individual genes have been identified by mutational analysis in *Arabidopsis* that contribute to non-host resistance, such as *NHO1* (*Non-Host1*) against *Pseudomonas syringae* pv. *phaseolicola* [13]

and the *PEN1* and *PEN2* genes that prevent penetration by the barley powdery mildew fungus (P Schulze-Lefert, personal communication). Mutations in *PEN2* cause constitutive alterations in the plant cell-wall architecture, consistent with evidence that cell-wall-associated structures are a first important barrier to pathogen invasion [14]. It is unclear to what extent proteins engaged in other structural cellular modifications governing race-specific resistance have roles in non-host resistance. However, similarities exist between the predicted structures of eLRR resistance proteins and animal Toll-like receptors, which recognise invariant pathogen-derived determinants (so-called pathogen-associated molecular patterns or PAMPs). Both trigger innate immune responses and suggest that non-host resistance may in certain plant-pathogen combinations be exerted by R-like proteins targeting indispensable pathogen molecules [15*,16*]. An important discovery is that *Arabidopsis* FLS2 (Figure 1; Table 2), which controls perception of a portion of the highly conserved bacterial flagellin protein, is an eLRR receptor kinase [17]. The search for further PAMP receptors in plants is likely to provide good targets for engineering durable resistance control.

Two other newly discovered R proteins appear to confer resistance to multiple pathogen races. *Arabidopsis* RPW8, which recognises all *Arabidopsis*-infecting powdery mildew isolates tested [18], carries a putative signal anchor at the N terminus and possesses a single CC domain. RPG1, which controls stem rust in barley [19], has tandem protein kinase domains at its C terminus and appears not to be membrane bound. It will be interesting to see if RPW8 and/or RPG1 act as receptors for pathogen-encoded determinants or function further downstream in the defence response.

Pathogen effectors detected by the plant's surveillance system

Each plant cell has the capacity to recognise non-self. While the majority of plant pathogenic bacterial components detected, now commonly referred to as effectors, have been characterised on the basis of avirulence activity, an expanding body of evidence points to primary roles of Avr proteins as virulence factors that are then engaged by plant R proteins to activate defences (see above) [7]. Bacteria multiply in the plant intercellular spaces and utilise a type III secretion system conserved in plant and animal bacterial pathogenesis to deliver effectors into the host cell [7,12]. Thus, bacterial effectors gain access to intracellular compartments in which potential host virulence targets and R proteins reside [9*,20]. Several elegant immunolocalisation studies have provided direct evidence for the translocation of bacterial effectors into plant cells via the bacterial pilus [21*-24*]. Insights to the biochemical activities of some bacterial effectors are also beginning to emerge, revealing similarities between plant and animal pathogenesis. Animal and plant homologues

of the *Yersinia* effector family, YopJ and YopT, act as cysteine proteases that disrupt vital host cellular processes [25**,26**]. DNA sequence analysis identified AvrPphB from the plant pathogen *P. syringae* as a YopT homologue. Importantly, its cysteine protease activity is required for autocatalytic processing of an AvrPphB precursor and for eliciting the plant's hypersensitive response. These studies strengthen the argument that bacterial virulence and avirulence functions reside in the same molecules. A possible plant target of AvrPphB could be the PBS1 protein kinase, which was found in *Arabidopsis* mutational studies to be required specifically for recognition of AvrPphB mediated by the CC-NB-LRR protein RPS5 (resistance to *P. syringae* pv. *phaseolicola*) (Figure 1; Table 2) [27]. By using other bacterial effectors it should now be possible to isolate and characterise additional host virulence targets.

The repertoire of identified fungal- and *Oomycete*-derived signals that activate R-protein-mediated plant defences remains small. It is also currently biased towards small secreted molecules, because these have been the easiest to isolate (Table 3) [28,29]. In contrast to true fungi, recent map-based cloning experiments indicate that some *avr* genes may be clustered in the *Oomycete* pathogen *Phytophthora infestans* [30]. This finding should aid *avr* sequence discovery. The *avr1b* gene has been cloned from the haustorium-forming *Oomycete*, *Phytophthora sojae*. Avr1b is a small, secreted protein with a sequence unrelated to several well-studied sterol-binding elicitors produced by many *Phytophthora* species [31]. It is not yet known whether the Avr1b protein is secreted into the extracellular haustorial matrix. Eagerly awaited are the results from other map-based cloning experiments to isolate *avr* genes from different haustorial pathogens, for example, *Blumeria*, *Bremia* and *Peronospora* species. Intriguingly, evidence has been reported to suggest that the fungal necrotroph *Septoria lycopersici* destroys the pre-formed antimicrobial compound tomatine present in the host and uses the tomatine degradation products to interfere with induced defence responses [32**]. The pathogen enzyme tomatinase is, therefore, required for a two-component attack process leading to disease susceptibility.

Successful pathogen invasion, multiplication, growth and development can also heighten the plant cell's awareness of non-self. Defence inducers generated include pathogen cell-wall fragments, degraded plant polymers, detoxified plant xenobiotics, as well as changes in the host tissue carbon, nitrogen and hormone status caused by pathogen nutrition (Table 3). Many of these cues appear to heighten basal defences in susceptible plant genotypes (Table 1), thereby reducing the damaging effects of disease.

Plant defence signalling networks

Plant-pathogen recognition causes the rapid activation of appropriate defences (Figure 3). The discovery of new

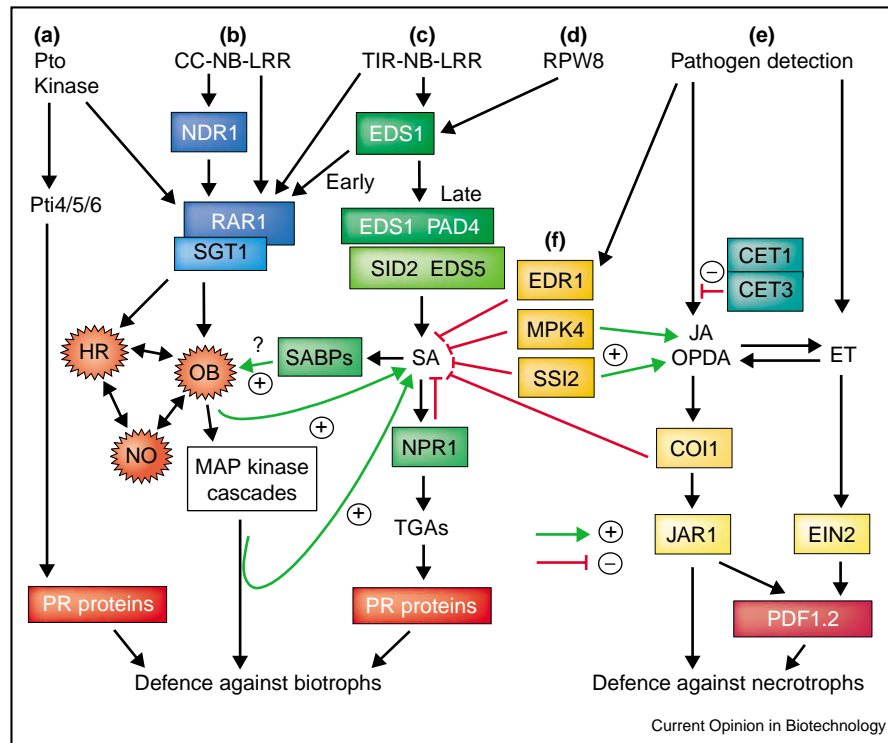
Table 3

Essential pathogen components detectable by plants*.

Organism and component type	Protein or molecule	Example of signal type	Species	Potential plant receptor/interactor
Bacteria				
Structural components	Subunit of flagellum	Flagellin	All pathogenic bacteria	PM-bound FLS2
	Subunit of pilus	HrpA HrpY	<i>Pseudomonas syringae</i> <i>Ralstonia solanacearum</i>	?
Quorum sensing	Subunit of T-pilus	VirB1, VirB2, VirB5, HcrC, HrpT (outer membrane proteins)	<i>Agrobacterium</i> spp.	?
	Subunit of type III secretion apparatus		All pathogenic bacteria	?
	Secreted autoinducer	N-Acyl homoserine lactone	All Gram(-) pathogenic bacteria, <i>Erwinia</i> spp., <i>Ralstonia</i> spp.	?
Non-host specific toxins	Secreted modified oligopeptide	3-Hydroxyl-palmitic acid-methylester	<i>R. solanacearum</i>	
	Exopolysaccharides	Multiple related types		
Hrp and Hrc proteins	Lipopolysaccharides	Multiple related types		
	Non-proteinaceous	Coronatine	<i>P. syringae</i>	
Hormones		Tabtoxin	<i>P. syringae</i> pv. <i>tabaci</i>	Glutamine synthase
		Phaseolotoxin	<i>P. syringae</i> pv. <i>Phaseolicola</i>	OCTase
Hrp and Hrc proteins	Delivered by type III secretion	AvrBs2	<i>P. syringae</i> pv. <i>vesicatoria</i> <i>P. syringae</i>	?
	Regulator of Hrc/H gene expression	HrpD, HrpO, HrpP AvrRXv (YopJ family) PrhA (OM siderophore receptor)	<i>R. solanacearum</i>	MAPK kinases Siderophores/cell wall factor
Hormones	Auxin	Indole-3-acetic acid	Gall forming <i>Pseudomonas</i> sp. <i>Agrobacterium</i> sp.	IAA receptor
		Indole-3-acetic acid		
Fungi and Oomycetes				
Structural components	Cell-wall chitin	Nascent synthesis at hyphal tip	All fungal species	Soluble chitinases extracellular
	Appressorium	Cell-wall melanin MPG1 hydrophobin	Many species <i>Magnaporthe grisea</i>	? ?
Degradation of plant polymers	Cell wall glucans (β 1-3, β 1-4 type)	Nascent synthesis at hyphal tip	All Oomycete species	Soluble glucanases
	Cell walls	pelA and pel D, pectate lyase	<i>Fusarium solani</i> f. sp. <i>pisi</i>	
Overcoming plant defences	Cuticle	AcpG1 endopolygalacturonase Cut A cutinase	<i>Alternaria citri</i> <i>Fusarium solani</i> f. sp. <i>pisi</i>	PGIPs
	Tomatinase	Saponin degradation products	<i>Septoria lycopersici</i>	?
Nutrition	Membrane transporter	ABC1, secretion of plant xenobiotics	<i>M. grisea</i>	?
	Oxalic acid	Oxalic acid or low pH	<i>Sclerotinia sclerotiorum</i>	Apoplastic oxalate oxidase
Secreted toxins	Amino acid transporters	Influx of essential amino acids	Many fungal species	Amino acid sensor
	Proteinaceous, host-specific	ToxA for HC toxin	<i>Cochliobolus carbonum</i>	Membrane channels?
Secreted peptides	Non-proteinaceous	Ptr toxin	<i>Pyrenophora tritici-repentis</i>	?
		AAL toxin	<i>Alternaria alternata</i> f.sp. <i>lycopersici</i>	TM helix-LAG1 motif
	DON	<i>Gibberella zeae</i>	Peptidyl transferase	
	ECP2	<i>Cladosporium fulvum</i>	Cf-ECP2	
	INF1	<i>Phytophthora infestans</i>	?	
	Pep13	<i>Phytophthora sojae</i>	PM receptor	
	cgDN3	<i>C. gloeosporioides</i>		
Hormones	Gibberellins	Avr-Pita (zinc protease) (essential?)	<i>Magnaporthe grisea</i>	Pi-ta protein
		PLW2	<i>Magnaporthe grisea</i>	PM-receptor?
		Elicitins (essential?)	Many <i>Phytophthora</i> species	PM receptor for endocytosis
		GA3	<i>Gibberella fujikuroi</i>	GA receptor

*Reviewed [28,29,31,71]. AAL, *Alternaria alternata* f.sp. *lycopersici*; DON, deoxynivalenol; ECP2, extracellular protein 2; IAA, indole-3-acetic acid; LAG1, longevity assurance gene; OCTase, ornithine carbonyltransferase; OM, outer membrane; PGIPs, polygalacturonase inhibitor protein; PLW2, pathogenicity toward weeping lovegrass; PM, plasma membrane; TM, transmembrane.

Figure 3



Overview of the local signalling networks controlling activation of local defence responses. The relative positions of many of the mutants described in Table 4 have still to be confirmed and therefore were not included in this figure. Five main signalling cascades are shown as (a), (b), (c), (d) and (e), but considerable evidence exists for both positive and negative regulatory steps and feedback loops to create a highly interactive signalling network. R-protein-dependent activated signalling cascades are revealed for four different classes of R proteins, namely (a) Pto serine-threonine protein kinase, (b) CC-NB-LRR, (c) TIR-NB-LRR and (d) RPW8. (a) Pto-kinase-mediated resistance involves both RAR1 and direct interaction with the Pti4/5/6 transcription factors to activate directly pathogenesis-related (PR) protein gene expression. The protein Prf is required downstream of Pto, but its precise position in the defence pathway remains unclear. (b,c) Most CC-NB-LRR-type R proteins require NDR1, whereas TIR-NB-LRR proteins are dependent on EDS1. (d) RPW8 operates through EDS1 and SGT1. A possible convergence point of the four R-protein-triggered pathways is at RAR1/SGT1, both operating upstream of the hypersensitive response (HR) and oxidative burst (OB). Another early defence signal generated is nitric oxide (NO), which can potentiate both the HR and OB. Activation of later potentiating defence responses by TIR-NB-LRR proteins involves the combined actions of EDS1 and PAD4, EDS5, SA and NPR1. EDR1, MAPK4 and SSI2 can each repress activation of the SA pathway, while various SA-binding proteins (SABP) located in distinct cellular compartments may modulate the local concentrations of available SA signal. The OB can potentiate SA-mediated signalling directly and via the induction of various MAPK cascades, for example, SIPK. NPR1 is required downstream of SA, which also stimulates NPR1 translocation into the nucleus where it interacts with TGA transcription factors and induces the expression of PR genes. The signalling cascades (a), (b), (c) and (d) are important for resistance biotrophic pathogens. A different signal transduction network (e) leads to the activation of parallel JA and ET signalling cascades. Steps upstream of JA and OPDA are negatively regulated by CET1 and CET3, while downstream, CO1 and JAR1 are required sequentially to activate resistance to necrotrophic pathogens. Transduction of the ET signal requires EIN2 and leads to expression of the PDF1.2 defence marker gene. (f) The signalling proteins EDR1, MPK4 and SSI2 have roles in communication between the SA and JA/ET signalling networks. CET1/CET3, constitutive expression of thionin 1/3; CO1, coronatine insensitive 1; EDR1, enhanced disease resistance 1; EIN2, ethylene-insensitive 2; NDR1, non-race specific disease resistance 1; OPDA, 12-oxophytodienoic acid; PAD4, phytoalexin-deficient 4; PDF1.2, plant defensin 1.2; Pti4/5/6, Pto-interacting 4, 5 and 6; SID2, SA induction deficient 2; SSI2, suppressor of salicylate insensitivity of NPR1-5.

genes or mutants allows further dissection of local and systemic signalling networks and begins to highlight the complex interplay between defence molecules such as salicylic acid (SA), nitric oxide, reactive oxygen intermediates (ROI), jasmonic acid (JA) and ethylene (ET) [33]. Information derived from studies of *Arabidopsis* mutants or transgenic plants is presented in Table 4. Although this list emphasises a potentially huge resource for manipulation of plant disease resistance pathways, it also reveals enormous gaps in our knowledge of particular gene products and their impact on interactions with a

range of pathogen types. Recent studies revealed two proteins, RAR1 (required for Mla-dependent resistance 1) and SGT1 (suppressor of G2 allele of SKP1), to be regulators of R-gene-mediated resistance in plants as diverse as *Arabidopsis*, barley and tobacco [34^{**},36^{**},37,38]. RAR1 encodes a small zinc-binding protein that interacts with SGT1 in barley and tobacco extracts [34^{**},36^{**}]. In yeast cells, SGT1 is essential for the function of Skp1-Cullin-F-box protein (SCF) E3 ubiquitin ligase complexes that target proteins for degradation by the 26S proteasome. The ubiquitin-proteasome

Table 4

List of *Arabidopsis* mutants and transgenes known to compromise or enhance plant defence without causing spontaneous cell death/lesion formation.

Mutant	Wild-type gene product/function	Defence phenotype					Morphological traits
		Basal defence		R-protein-mediated defence	Systemic		
		Biotrophs	Necrotrophs		SAR	ISR	
Mutations and transgenes that suppress defence							
axr1	Auxin and JA signalling		+				
coi1	SCF-mediated ubiquitination, JA signalling		+				Male sterile
dir1	Apoplastic lipid transfer protein					+	
dth9	SA-independent regulator, auxin sensitivity	+			+	+	
eds1	Lipase-like protein, SA signalling	+			+		
eds4	JA and ET signalling	+					+
eds5/sid1	MATE transporter, SA biosynthesis	+			+	+	
eds7		+					
eds8	JA signalling	+					+
eds10	JA and ET signalling	+					+
eds11		+					
eds12	SA response regulator	+				+	
eds13		+					
eds14	SA signalling/biosynthesis?	+					
eds15	SA signalling/biosynthesis?	+					
eds16/sid2*	Isochorismate synthase, SA biosynthesis	+			+	+	
ein2	Nramp metal-ion transporter, ET signalling	t	t				+
							Taller plants, more seed
esa1			+				
eto1	ACC synthase regulator, ET production	+					
etr1	ET receptor, ET sensitivity	+					+
isr1	ET signalling in ISR						+
							Unidentified floral fungus
jar1	JA adenylation, JA signalling		+				
nahG [†]	SA hydroxylase, no inducible SA	+			+	+	
ndr1	SA/ROI response regulator				+	+	
nho1		+			+		
npr1/nim1	Ankyrin repeat protein, SA and JA/ET response regulator	+			+	+	+
pad2	Camalexin/SA signalling	+					
pad3	P450 cytochrome monooxygenase; camalexin synthesis		+				
pad4	Lipase-like protein, SA signalling	+			+		
pbs1	Serine-threonine kinase, RPS5-AvrPphB recognition				+		
pbs3		+			+		
phyA-phyB	phytochrome perception	+			+		
rar1/pbs2	Zinc-binding protein, SGT1 interactor				+		
ref8	P450-dependent monooxygenase, lignification						Unidentified floral fungus
rhd1	ET modulator	+					
sgt1b/edm1	SCF-mediated ubiquitination, RAR1 interactor				+		
Mutations and transgenes that activate defence							
cev1	Cellulose synthetase, JA/ET signalling	+					Reduced plant size
cet 1	JA/ET signalling					+	Reduced plant size
cir1	Modulator of SA, JA/ET and ROI levels	+					
cpr1	SA accumulation	+				+	
cpr6	SA accumulation, SA/JA pathway interplay	+				+	Reduced plant size
dnd1	Cyclic nucleotide-gated ion channel, SA accumulation	+				+	Reduced plant size
edr1	MAPKKK (CTR1 type), negative regulator of SA signalling	+					
ERF1 [†]	ET response factor 1		+				
fbr1, fbr2	Resistance to <i>Fusarium</i> Fumonisin B1 toxin	+					
JMT [†]	Carboxymethyltransferase, JA biosynthesis		+				
mpk4	MAPK 4, SA—JA pathway interplay					+	Extreme dwarf

Table 4 Continued

Mutant	Wild-type gene product/function	Defence phenotype					Morphological traits
		Basal defence		R-protein-mediated defence	Systemic		
		Biotrophs	Necrotrophs		SAR	ISR	
MYB30 [†]	R2R3 Myb transcription factor, extent of HR	+		+			
NPR1/NIM1 [†]	Ankyrin repeat protein, SA and JA/ET response regulator	+					
pmr1		+					
pmr2		+					
pmr3		+					
pmr4		+					
pmr6	Pectate lyase, cell-wall composition	+					
psi2	Phytochrome signalling	+		+			
Pti4 [†]	ERF transcription factor, Pto interactor	both + and t					Reduced plant size
rin4*	RPM1, avrRPM1 and AvrB interactor	+					
snc1	RGA within <i>RPP5</i> locus, suppressor of NPR1	+					Reduced plant size
sni1	Plant-specific novel protein, negative regulator of SAR	+				wt	
son1	F-box protein, negative regulator of SA-independent pathway	+				-	
ssi2	Stearoyl-ACP desaturase, 18:0 fatty acid synthesis	+	-				Spontaneous lesions
TGA5*	bZIP transcription factor, NPR1 interactor	+				-	
WRKY18 [†]	Zinc-finger transcription factor	+					Reduced plant size

*Antisense. [†]Over-expression. t, tolerance — equivalent pathogen biomass to that occurring on a susceptible line, but lower amounts of visible disease symptoms. The full details of the references cited in the table are give at the website, <http://www.iacr.ac.uk/ppi/staff/khkReviews.html>. Table 4 will be regularly updated on the website. ACC synthase, 1-aminocyclopropane-1-carboxylate synthase; CTR1, constitutive triple response 1; ERF, ethylene responsive element binding factor; MATE, multidrug and toxin extrusion; MAPKK, MAPK kinase kinase; Nrapm, natural resistance-associated macrophage protein; RAR1, required for Mla-dependent resistance 1; RPM1, resistance to *Pseudomonas syringae* pv. *maculicola* expressing avrRMP1 or avrB; RPS5, resistance to *Pseudomonas syringae* expressing AvrPphB; ROI, reactive oxygen intermediates; SCF, Skp1-Cullin-F-box protein. The minus symbol in the table indicates an impaired response.

pathway is therefore likely to be an important modulator of *R*-gene-triggered resistance. Further studies reveal a role for SGT1 in non-host resistance in tobacco and provide additional indications that *R*-gene-mediated and non-host resistance may engage similar mechanisms [39,40]. Defence signal regulation by EDS1 (enhanced disease susceptibility 1) is also wider than previously thought. The broad-spectrum mildew R protein RPW8 (Figure 1; Table 2) operates through both EDS1 [18,41] and SGT1 (S Xiao, personal communication). These findings point to a mechanistic overlap between species-level and *R*-gene-specified defence signalling (Figure 3).

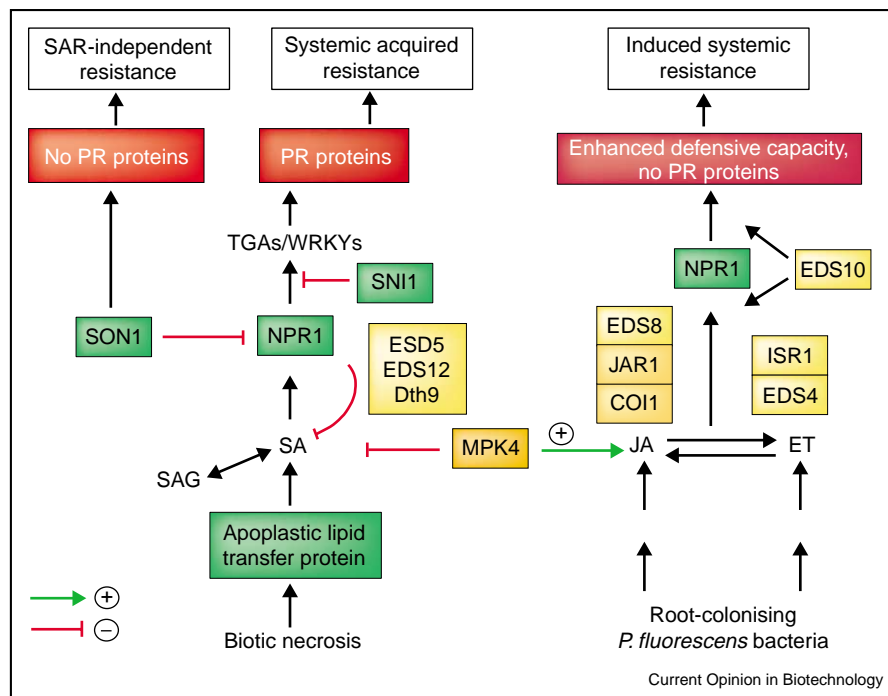
Other important defence regulators to emerge are components of mitogen-activated protein kinase (MAPK) cascades that constitute functionally conserved eukaryotic signal relay systems in response to various environmental stresses [16,40,42]. Importantly, the MAPK kinase kinase, EDR1, negatively regulates SA-inducible defences [43], whereas MAPK 4 appears to differentially regulate SA and JA signals [44]. These findings strongly implicate MAPK modules in molecular communication between different plant defence pathways (Figure 3). Unlike MAPK proteins, a large family of calcium-dependent protein kinases (CDPKs) appears to be specific to plants and protozoa [42]. At least one CDPK member,

NtCDPK2, is essential for *Cf-9*-specified resistance to the *Cladosporium fulvum* Avr9 peptide and has a role in perception of abiotic stresses in tobacco plants [45]. The emergence of light-signalling pathways and processes within the chloroplast as modulators of plant defence extends the idea that signal interplay between different biotic and abiotic environmental detection systems is key to the proper mobilisation of defences. Besides being a major generator of redox-active molecules, the chloroplast is a site for SA synthesis by the enzyme isochorismate synthase [46] and the chloroplast stroma contains a potential SA effector-binding protein, carbonic anhydrase (CA) [47]. Moreover, an intact phytochrome pathway is required for full expression of SA-mediated responses and a pathogen-triggered hypersensitive response (HR) [48].

Distinct systemic responses can be activated simultaneously

It is significant for plant resistance breeders that two distinct systemic defence mechanisms — systemic acquired resistance (SAR, dependent on SA) and induced systemic resistance (ISR, independent of SA but reliant on JA and ET signalling) (Figure 4) — can be simultaneously activated and thus potentially enhance plant protection against diverse pathogen types [49]. The

Figure 4



Systemic defence signalling networks. Systemic acquired resistance (SAR) occurs when pathogens induce localised plant necrosis (biotic necrosis) during initial infection. This then activates defence responses in the distal uninfected plant tissues to prime plants against subsequent attack. A mobile lipid signal arising from the initial site of pathogen infection may interact with an apoplastic lipid transfer protein DIR1 (defective in induced resistance 1) to trigger SA and SAG accumulation throughout the plant. The NPR1 signalling protein is required downstream of the SA signal. NPR1 relocates from the cytoplasm to the nucleus, binds with various TGA class transcription factors and results in the expression of several classes of PR genes. WRKY transcription factors are also activated during SAR. SNI1 is a negative regulator of SAR that functions downstream of NPR1. The proteins EDS5, EDS12 and Dth9 also play a role in SAR, but their exact location in the pathway is uncertain. Induced systemic resistance (ISR) is caused by soil-inhabiting non-pathogenic rhizobacteria colonising plant roots. ISR requires both JA- and ET-mediated signalling as well as the NPR1 protein. EDS8, JAR1 and COI1 proteins are required for ISR and function between JA and NPR1, whereas ISR1 and EDS4 operate between ET and NPR1. EDS10 is also required for ISR and acts either upstream or downstream of NPR1. ISR does not involve the accumulation of PR proteins or require SA. Both SAR and ISR can be simultaneously activated. MAPK4, enhances JA-mediated signalling and suppresses SA signalling. A third type of systemic response is termed SAR-independent resistance (SIR). This phenomenon which does not involve PR protein or SA accumulation arises through the negative regulation of a novel defence response pathway by the SON protein. SON was initially isolated as a suppressor of NPR1. Dth9, detachment 9; ISR1, induced systemic resistance 1; JAR1, JA resistance 1; SAG, salicylic acid glucoside; SNI1, suppressor of NPR1-1 inducible; SON1, suppressor of nim1-1.

ankyrin repeat protein, NPR1 (non-expressor of PR1), initially identified as an SA response regulator, is required for both SAR and ISR. NPR1 is therefore competent, in some way, to gauge different input signals [50]. The addition of SA to *Arabidopsis* seedlings promotes movement of NPR1 to the nucleus [51^{*}] where it is able to bind several TGA (TGACG DNA motif) class transcription factors, conferring a possible direct route to defence gene induction [52^{*},53^{*}]. It will be interesting to see whether or not a related process is involved in NPR1 participation in the ISR response. Little is known about the nature of mobile signals in SAR or ISR. Identification of an apoplastic lipid transfer protein, DIR1, as an inducer of long-distance defence signalling in SAR suggests that lipid-derived molecules may have a role [54^{*}].

Unexpectedly, the *son1* mutant delivers systemic resistance without the customary pathogenesis-related protein

induction. This phenomenon has been termed SAR-independent resistance or SIR [55]. Several new *Arabidopsis* mutants were defined with compromised SAR and/or ISR systemic responses. These included some of the already known *eds* mutants required for local basal defence against biotrophic pathogens (Table 4; Figure 4).

Emerging technologies

Table 5 summarises the most important technologies that are driving the rate of discovery in plant-pathogen interactions. Numerous new gene sequences can now be correlated with plant disease resistance, either through homology searches, transcript profiling, protein interaction or map-based cloning approaches. Similarly, novel mutants either compromised or enhanced in defence are being identified through increasingly sophisticated screening and selection strategies. For example, mutagenised transgenic *Arabidopsis* populations can be monitored for

Table 5

Emerging technologies applicable to detailed pathosystem exploration.

Technique	Application	Advantages	Current disadvantages	Examples
Sequence acquisition				
Genome and EST sequencing	Comparative genomics Source of sequences for arrays	Routine	High cost	http://cogeme.ex.ac.uk/
Transcriptome analyses				
EST and oligo microarrays	Links phenotype to transcriptome	Expression profile of thousands of gene sequences simultaneously compared. Unannotated sequence expression can be related to annotated genes	High cost. Multiple dataset comparisons difficult because of lack of central repository of datasets. Only correlative data generated	[72]
cDNA-AFLP, differential display SAGE (serial analysis of gene expression)	Links phenotype to transcriptome Most useful when applied to fully sequenced genomes	Lower cost than array technology Quantitative analysis	Output dependent on the restriction enzymes used High cost	[73] http://www.sagenet.org
Forward genetics				
Promoter luciferase reporter constructs	Identification of mutants from mutagenised populations by image screening	Semi-automatic non-destructive screening		[74] [75]
Reverse genetics				
Tilling (targeting induced local lesions in genomes)	Links locus to phenotype	A non-GM route to identify and exploit variant gene alleles	<100 plant tissue samples can be bulked for screening in each PCR-based assay	[56]
	Identification of mutant alleles, generated by EMS mutagenesis. Screening for natural diversity in gene sequences	Missense, single base pair and gene knockouts identified. Small population size required		
Fast-neutron deletion mutagenesis	Links locus to phenotype	A non-GM route, greater genome coverage than insertional or EMS mutagenesis. Applicable to multiple species	Lethal gene deletions are difficult to evaluate. May affect >1 gene. Considerable genomic sequence around the gene required	[76]
Gene shuffling	Deletion mutants identified in pools of DNA samples >2500 Gene sequence-to-phenotype Generation of chimeric gene sequence via PCR	High-throughput. Large numbers of variant alleles generated	Requires some knowledge of protein domains	[77]
Transient gene function evaluation				
Virus-induced gene silencing (VIGS)	Systemic gene function assays on leaves and roots	High-throughput Dicots and monocots	Limited range of plant species Mild viral symptoms in some test systems	[39*]
Agro-infiltration, gene overexpression and RNAi	Transient gene function assay on leaves and roots	High-throughput	RNAi often only causes partial inactivation	[77]
RNA viral vectors	HR/defence gene induction	Dicots and monocots Intra- or extracellular targeting	Only for small proteins (<300 amino acids)	[78]
Single-cell particle bombardment	Identification of novel <i>R</i> genes A cell autonomous, quantitative gene function assay	Cosmid DNA or cDNA evaluation GFP co-bombardment marks the transformed cells	Applicable to superficial cell layers (e.g. epidermis) or those exposed by cutting plant tissue	[58]

Table 5 Continued

Technique	Application	Advantages	Current disadvantages	Examples
Others				
Protein fragment complementation assays	Direct visualisation of protein interactions in plant cells	Biochemical pathway mapping		[53*]
Chimeric eLRR-kinase receptors	Discovery of ligands for the LRR kinases	Screening for small molecules modulating protein interactions High-throughput High sensitivity. Use of Xa21 multiple based defence responses to indicate receptor activation	Anticipate some chimeras may be non-functional due to protein structure constraints	[79]
Avr-Cya fusion protein	Biochemical test for bacterial type III secretion via monitoring cAMP levels	Kinetic studies on effector protein delivery into plant cells possible		[22*]
Computer software				
Numerical simulation of plant signalling networks	Qualitative description of signal transduction processes using Boolean (digital, numerical) language	Signalling evaluated within networks	Only qualitative analysis possible	[80]
GenomePixelizer	Visualisation tool for location of specific sets of genes within the genome	Integration of multiple data types (e.g. transcriptome, proteome, genetics). Simple computer simulations possible Analysis of gene duplication events within and between species Data input via spreadsheets (e.g. MS Excel)		*

*http://niblr.ucdavis.edu/GenomePixelizer/GenomePixelizer_Welcome.html. AFLP, amplified restriction fragment polymorphism; EMS, ethylmethane sulphonate; EST, expressed sequence tag; GFP, green fluorescent protein; HR, hypersensitive response.

aberrant activity of a defence component by use of a promoter fused to a luciferase reporter (Tables 4 and 5).

The prolific accumulation of data raises the question of how to remove spurious associations from the genuine? Besides mutant screens, two techniques, virus-induced gene silencing (VIGS) and RNA interference (RNAi), are proving valuable in this context. Both provide robust, high-throughput transient assays to test for the functions of single genes or gene families. As only ~300 bp of sequence is needed to induce gene silencing, VIGS and RNAi are highly compatible with all gene sequence discovery routes.

Gene and protein structure/function studies have been pioneered by two technologies based on the polymerase chain reaction (PCR) (Table 5). Firstly, DNA shuffling can provide many variant sequences for subsequent functional evaluation in transient or stable expression systems. Secondly, gene sequence variants that occur naturally in wild germplasm collections or are created by chemical mutagenesis can be identified using the tilling (targeting induced local lesions in genomes) technique [56]. Specific gene variants may then be linked to a desired phenotypic trait.

To complement the experimental approaches, specific databases and software programmes have been developed

to assist *R* gene sequence comparisons (e.g. GenomePixelizer). Increasingly complex datasets associated with the defence signalling networks can be interpreted using Boolean language and to simplify the information flow between pathosystems (e.g. the COGEME database) (Table 5).

Fresh approaches to molecular resistance breeding

GM crops are deployed globally to control various insect pests and virus diseases. By contrast, the development of GM crops to resist fungal and bacterial diseases has been a failure. Hindsight suggests our initial expectations were unrealistic. For example, why should overexpression of a single defence protein from the innate, multicomponent defence response lead to high efficacy control of multiple pathogens? Why would constitutive activation of plant defence signalling components not lead to reduced growth rates, altered plant development and lower yields? Moreover, localised activation of the hypersensitive defence responses to control biotrophic pathogens needs to have an 'off' switch to prevent run-away cell death. Many promising approaches have also failed protein toxicity, digestibility and allergenicity tests. What these early attempts to engineer plant disease control have done, however, is highlight the complexity of pathosystems.

Where should the emphasis now be placed? Most of the major *R* genes used by plant breeders were isolated and sequenced in the past 10 years (Figure 1; Table 2). Interest has now turned to the identification and exploitation of the natural diversity at *R* loci. Gene sequence diversity searches, achieved through techniques such as PCR tilling [56] (Table 5), may be targeted to the entire *R* protein or to specific *R* domains known to be required for pathogen recognition. Diversity searches will also allow us to define truly novel *R* sources so that effective *R* gene pyramiding can be implemented [57]. *R* gene sequences can be used to create precise ‘within the gene’ (WTG) molecular markers, thereby obviating the need for pathogen testing during introgression breeding.

Searches for resistance gene analogues (RGAs) in the syntenic regions between closely related plant species are now a realistic option. For example, the barley *Mla* locus, encoding 28 resistance specificities to powdery mildew, when mapped onto the wheat genome, is tightly linked (0.7 cM) to the highly polymorphic *Pm3* locus conferring resistance to wheat powdery mildew [58].

The simple deployment of variant race-specific *R* and RGA loci will probably not provide durable resistance and therefore must be integrated with other measures. For example, to control the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* on rice crops, the pathogen population is examined each season to monitor *avr* modifications that may give rise to virulence. Only *R* genes that imposed a high fitness penalty to the pathogen for adaptation to a virulent race have proven durable in this six-year field study [59]. The pathogen’s mode of dissemination, reproduction and population size also has an immense bearing on which *R* gene deployment strategy should be selected to attain durable control [60].

Identification of functional homologues of durable race non-specific *R* genes in other plant species is an attractive approach to disease control. The current *R* gene search list includes *RPW8* for powdery mildew control, *Rgp1* for stem rust, and *FLS2* for bacterial diseases (Table 2).

GM approaches are also becoming more sophisticated and have a conceptual basis [61]. Some of the focus has shifted to exploiting ‘master-switch’ defence signalling proteins. Such proteins appear to function at taxonomically greater distances than *R* proteins [62], can activate multiple component defence to provide broader spectrum pathogen control, and ideally would be key components of non-host defence.

Another GM strategy has concentrated on direct debilitation of the pathogen. By using DNA shuffling techniques (Table 5), variant chitinases with 30–50-fold enhanced activity can be deployed in combination with antimicrobial proteins. The chitinase activity slows hyphal tip

growth and increases the efficacy of other antimicrobial transgenes (M Müller, personal communication). For bacterial control, the flagellin-derived peptide was identified as a ubiquitous and indispensable pathogenicity component, which is absent from symbiotic bacterial species. In *Arabidopsis*, recognition of flagellin is controlled by a single eLLR-kinase receptor protein, *FLS2* [17] (Figure 1). Additional microbial pathogenicity factors are keenly sought that are either membrane-localised or secreted and would therefore provide accessible targets for plant recognition by *R* proteins or disruption by plant-delivered transgenic proteins (Table 3).

To minimise the cost of defence activation on plant yield a new repertoire of pathogen-inducible plant promoters is required. These promoters should restrict transgene expression solely to pathogen infection sites. Plant promoters activated by biotrophic fungal species have been reported for the first time [63,64]. Using this promoter type it should now be feasible to test rigorously the R–Avr two-component defence system previously described [61].

Chemical activation of innate immunity

Integrated disease management is now considered the best control strategy for most crops. In this context, two recent reports are significant. *Arabidopsis* plants overexpressing the signalling protein NPR1 exhibit an enhanced response to the defence-activating compound benzothiadiazole, which also increased the efficacy of three fungicide treatments [65]. Therefore, low doses of plant defence-activating compounds and/or fungicides applied to plants expressing the ‘master’ regulators may enhance defences to a commercially workable level.

Conclusions

Plant protection delivered via the seed through germplasm modifications provides one of the simplest solutions to disease control. It also reduces the use of chemical pesticides, thereby conserving energy, and provides growers with novel options for sustainable disease control. Fresh insights into the activities of *R* and *Avr* proteins, defence signalling networks and microbial pathogenicity factors should provide a range of materials to design new and effective disease control strategies. A key challenge now is to harness their collective potential. It is also important to elucidate the molecular processes underlying pathogen adaptation to new plant germplasm or chemical applications. Monitoring the losses to pathogen fitness associated with their resistance-breaking ability will undoubtedly contribute to achieving durable disease resistance in a, so far, unproven AgBiotechnology sector.

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Computer-based sequence analysis was used to identify related members of the YopJ/avrRxv bacterial effector family in animal and plant pathogens. *Yersinia* YopJ and *Xanthomonas* AvrBsT have conserved residues that comprise a cysteine protease catalytic site. These residues are essential for their respective activities in host cells. YopJ processes the conserved, small ubiquitin-like protein SUMO-1 and its protein conjugates. In animal cells YopJ inhibits MAPK and NF- κ B signalling, presumably by disruption of SUMO-1 modification of these regulatory targets.

26. Shao F, Merritt PM, Bao ZQ, Innes RW, Dixon JE: **A *Yersinia* effector and a *Pseudomonas* avirulence protein define a family of cysteine proteases functioning in bacterial pathogenesis.** *Cell* 2002, **109**:575-588.

This paper reinforces emerging evidence [25**] that bacterial effectors operate in a similar manner in animal and plant pathogenesis. *Yersinia* YopT represents a family of cysteine proteases, including AvrPphB from the plant pathogen, *Pseudomonas*. YopT cleaves a post-translationally modified form of Rho GTPase, causing disruption of the actin cytoskeleton in host cells. Importantly, cysteine protease activity is required for autocatalytic processing of an AvrPphB precursor and for eliciting the plant host hypersensitive response. Thus, protease activities appear to be commonly recruited by bacteria to interfere with host cellular functions.

27. Swiderski MR, Innes RW: **The *Arabidopsis* PBS1 resistance gene encodes a member of a novel protein kinase subfamily.** *Plant J* 2001, **26**:101-112.

28. van't Slot KAE, Knogge W: **A dual role for microbial pathogen-derived effector proteins in plant disease and resistance.** *Critical Rev Plant Sci* 2002, **21**:229-271.

29. Idnurm A, Howlett BJ: **Pathogenicity genes of phytopathogenic fungi.** *Mol Plant Pathol* 2001, **2**:241-255.

30. van der Lee T, Robold A, Testa A, van't Klooster JW, Govers F: **Mapping of avirulence genes in *Phytophthora infestans* with amplified fragment length polymorphism markers by bulked segregant analysis.** *Genetics* 2001, **157**:949-956.

31. Tyler BM: **Molecular basis of recognition between *Phytophthora* pathogens and their hosts.** *Annu Rev Phytopathol* 2002, **40**:137-167.

32. Bouarab K, Melton R, Peart J, Baulcombe D, Osbourn A:
 ●● **A saponin-detoxifying enzyme mediates suppression of plant defences.** *Nature* 2002, **418**:889-892.

The results indicate that pathogens can degrade constitutive plant defences and in so doing produce molecules that suppress induced plant defences. *Nicotiana benthamiana* plants were inoculated with a mutant of the fungal pathogen *Septoria lycopersici* that does not produce the tomatinase enzyme required to degrade the preformed antimicrobial saponin called tomatine. Infection by the *S. lycopersici* mutant caused elevated expression of defence-related genes and host tissue penetration failed. When tomatinase, or its hydrolysis product B₂-tomatine, were pre-infiltrated into leaves of *N. benthamiana* plants expressing the race-specific *Pto* resistance gene, this weakened the hypersensitive response to *P. syringae* pv *tabaci* expressing *avrPto* and permitted bacterial numbers to reach a high titer. This data indicate for the first time that pathogen enzymes such as tomatinase have a dual function: detoxification of a preformed phytoanticipin and suppression of activated plant defence responses. It is unclear whether other pathogens use similar mechanisms to attack their plant hosts.

33. Thomma BP, Penninckx IA, Broekaert WF, Cammue BP:
 ●● **The complexity of disease signalling in *Arabidopsis*.** *Curr Opin Immunol* 2001, **13**:63-68.

34. Azevedo C, Sadanandom A, Kitagawa K, Freialdenhoven A, Shirasu
 ●● K, Schulze-Lefert P: **The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance.** *Science* 2002, **295**:2073-2076.

See annotation to [36**].

35. Austin MJ, Muskett P, Kahn K, Feys BJ, Jones JD, Parker JE:

- **Regulatory role of SGT1 in early R gene-mediated plant defenses.** *Science* 2002, **295**:2077-2080.

See annotation to [36**].

36. Liu Y, Schiff M, Serino G, Deng XW, Dinesh-Kumar SP: **Role of SCF ubiquitin-ligase and the COP9 signalosome in the N gene-mediated resistance response to Tobacco mosaic virus.** *Plant Cell* 2002, **14**:1483-1496.

The results of [34**–36**] provide evidence for a role of the ubiquitin-proteasome system in R-gene-mediated plant resistance, implying that degradation of certain regulatory proteins may be crucial to induction of plant defences. RAR1, a Zn²⁺-binding protein, interacts with SGT1 in barley and tobacco leaf extracts [34**,36**]. Plant SGT1 proteins are structurally and functionally conserved with yeast SGT1, which associates with the SCF core component (SKP1) and is essential for SCF E3 ligase-mediated ubiquitination. Both RAR1 and SGT1 act upstream of programmed cell death and its associated oxidative burst. At least two pools of SGT1 are present in plant extracts, one associating with RAR1 and the COP9 signalosome and a second interacting with SKP1 and COP9 [34**]. Transient silencing of *SGT1* or *COP9* in tobacco compromises R gene resistance (see also [39*]). The occurrence of RAR1 and SGT1 in multiple cellular pools is consistent with genetic data in *Arabidopsis* [35**] demonstrating both cooperative and distinct functions of SGT1 and RAR1 in plant defence signalling.

37. Muskett PR, Kahn K, Austin MJ, Moisan LJ, Sadanandom A, Shirasu K, Jones JD, Parker JE: ***Arabidopsis* RAR1 exerts rate-limiting control of R-gene-mediated defenses against multiple pathogens.** *Plant Cell* 2002, **14**:979-992.

38. Tör M, Gordon P, Cuzick A, Eulgem T, Sinapidou E, Mert-Turk F, Can C, Dangl JL, Holub EB: ***Arabidopsis* SGT1b is required for defense signalling conferred by several downy mildew resistance genes.** *Plant Cell* 2002, **14**:993-1003.

39. Peart JR, Lu R, Sadanandom A, Malcuit I, Moffett P, Brice DC,
 ●● Schauser L, Jaggard DA, Xiao S, Coleman MJ *et al.*: **Ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants.** *Proc Natl Acad Sci USA* 2002, **99**:10865-10869.

VIGS technology in *N. benthamiana* plants was used to demonstrate a role for SGT1 (see [34**–36**]) in resistance conferred by three distinct classes of R protein — tobacco N (TIR-NB-LRR), potato Rx (CC-NB-LRR) and tomato Pto (protein kinase) — as well as in a non-host resistance response triggered by the *Phytophthora infestans* elicitor, Inf1. This paper emphasises the power of VIGS technology to test rapidly a protein's function in multiple plant-pathogen interactions.

40. Jin H, Axtell MJ, Dahlbeck D, Ekwenna O, Zhang S, Staskawicz B, Baker B: **NPK1, an MEKK1-like mitogen-activated protein kinase kinase kinase, regulates innate immunity and development in plants.** *Dev Cell* 2002, **3**:291-297.

41. Feys BJ, Moisan LJ, Newman MA, Parker JE: **Direct interaction between the *Arabidopsis* disease resistance signaling proteins, EDS1 and PAD4.** *EMBO J* 2001, **20**:5400-5411.

42. Romeis T: **Protein kinases in the plant defence response.** *Curr Opin Plant Biol* 2001, **4**:407-414.

A comprehensive review of the involvement of the various classes of protein kinases in coordinating both early and late plant defence responses.

43. Frye CA, Tang D, Innes RW: **Negative regulation of defense responses in plants by a conserved MAPKK kinase.** *Proc Natl Acad Sci USA* 2001, **98**:373-378.

44. Petersen M, Brodersen P, Naested H, Andreasson E, Lindhart U, Johansen B, Nielsen HB, Lacy M, Austin MJ, Parker JE *et al.*: ***Arabidopsis* MAP kinase 4 negatively regulates systemic acquired resistance.** *Cell* 2000, **103**:1111-1120.

45. Romeis T, Ludwig AA, Martin R, Jones JDG: **Calcium-dependent protein kinases play an essential role in a plant defence response.** *EMBO J* 2001, **20**:5556-5567.

46. Wildermuth MC, Dewdney J, Wu G, Ausubel FM: **Isochorismate synthase is required to synthesize salicylic acid for plant defence.** *Nature* 2001, **414**:562-565.

See annotation to [47*].

47. Slaymaker DH, Navarre DA, Clark D, del Pozo O, Martin GB,
 ●● Kleissig DF: **The tobacco salicylic acid-binding protein 3 (SABP3) is the chloroplast carbonic anhydrase, which**

exhibits antioxidant activity and plays a role in the hypersensitive defense response. *Proc Natl Acad Sci USA* 2002, **99**:11640-11645.

SA is required for the activation of both local and systemic defence responses (Figures 3 and 4). A novel route for SA biosynthesis in the chloroplast was revealed [46*], whilst [47*] shows that the chloroplast stroma contains an additional SA-binding protein (SABP). These two papers are significant for various reasons. First, early work on SA biosynthesis in tobacco had suggested that plants synthesised SA from phenylalanine; however, when this pathway was inhibited pharmacologically SA synthesis occurred. The authors [46*] recognised that in certain bacteria SA synthesis could occur from chorismate using isochorismate synthase and pyruvate lyase, and went on to show that the same pathway exists in plants. Second, in tobacco, four SABPs have already been identified: catalase, a 25 kDa protein called SABP2, ascorbate peroxidase and aconitase. Carbonic anhydrase (CA) in the chloroplast, with a hitherto undefined biological role, is now revealed to be an additional SABP3 [47*]. Third, yeast overexpression experiments demonstrated that CA/SABP3 has antioxidant activity like catalase and ascorbate peroxidase. The authors suggest that SA ability to bind and inhibit both cytoplasmic and chloroplastic antioxidant enzymes has a role in increasing reactive oxygen intermediate levels, which may activate a positive feedback loop that amplifies SA production and induces defence responses.

48. Genoud T, Buchala AJ, Chua NH, Metraux JP: **Phytochrome signalling modulates the SA-perceptive pathway in *Arabidopsis*.** *Plant J* 2002, **31**:87-95.

The phyA- and phyB-deficient *Arabidopsis* plants affected in light perception exhibited a strong reduction in their response to SA. This study used variegated plants to indicate that a signal resulting from both the SA and the phytochrome pathways controls the regulation of the hypersensitive response induced by an avirulent bacteria, whereas expression of pathogenesis-related (PR) genes is not modified by a reduction in chloroplast activity. The authors infer the existence of specific dark-defences that may compensate for the reduced hypersensitive response observed in low light conditions, for example, PDF1.2 defensin gene expression.

49. van Wees SC, de Swart EA, van Pelt JA, van Loon LC, Pieterse CM: **Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*.** *Proc Natl Acad Sci USA* 2000, **97**:8711-8716.

50. Pieterse CMJ, van Wees SCM, Ton J, van Pelt JA, van Loon LC: **Signalling in Rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*.** *Plant Biol* 2002, **4**:535-544.

51. Kinkema M, Fan WH, Dong XN: **Nuclear localization of NPR1 is required for activation of PR gene expression.** *Plant Cell* 2000, **12**:2339-2350.

A functional NPR-GFP fusion protein was used to detect NPR1 accumulation in the plant nucleus in response to several SAR activators. Also, a steroid hormone inducible NPR1 system was used to demonstrate that nuclear localisation of NPR1 is essential for PR gene induction.

52. Fan WH, Dong XN: **In vivo interaction between NPR1 and transcription factor TGA2 leads to salicylic acid-mediated gene activation in *Arabidopsis*.** *Plant Cell* 2002, **14**:1377-1389.

See annotation to [53*].

53. Subramaniam R, Desveaux D, Spickler C, Michnick SW, Brisson N: **Direct visualization of protein interactions in plant cells.** *Nat Biotechnol* 2001, **19**:769-772.

Earlier, yeast two-hybrid analysis revealed NPR1 to interact with several transcription factors of the TGA class including TGA2 (AHBP-1b). By expressing a truncated form of TGA2 in *Arabidopsis* [52*], a dominant-negative effect was created and thereby revealed that TGA2 and NPR1 interact *in planta*. Additional genetic data demonstrated that TGA2 is SA responsive. The studies described also confirmed *in vivo* the NPR1-TGA2 interaction, using a fluorescent technology called the protein fragment complementation assay (Table 5).

54. Maldonado AM, Doerner P, Dixon RA, Lamb CJ, Cameron RK: **A putative lipid transfer protein involved in systemic resistance signalling in *Arabidopsis*.** *Nature* 2002, **419**:399-403.

The *dir1-1* mutant was isolated by transfer-DNA insertion. The *dir1-1* plants exhibit wild type local responses to avirulent and virulent *P. syringae*, but failed to develop the SAR response leading to PR protein accumulation in the distal non-inoculated leaves. Petiole exudate experiments revealed that *dir1-1* mutants were defective in an essential mobile signal emitted from the initially infected leaf. DIR1 encodes a putative apoplast lipid-transfer-like protein (LTP) and contains the eight con-

served cysteine residues found in all LTPs. The authors propose that SAR is mediated by the DIR1 protein interacting with a lipid-derived molecule to promote long-distance signalling. DIR1 was found not to be required for local or systemically induced accumulation of free SA.

55. Kim HS, Delaney TP: ***Arabidopsis* SON1 is an F-box protein that regulates a novel induced defense response independent of both salicylic acid and systemic acquired resistance.** *Plant Cell* 2002, **14**:1469-1482.

56. McCallum CM, Comai L, Greene EA, Henihoff S: **Targeting induced local lesions in genomes (TILLING) for plant functional genomics.** *Plant Physiol* 2000, **123**:439-442.

57. Jones JD: **Putting knowledge of plant disease resistance genes to work.** *Curr Opin Plant Biol* 2001, **4**:281-287.

A comprehensive review of how *R* gene sequence polymorphism can be exploited in agriculture. This article also gives a good summary of the 1970s concept of varietal mixtures, proposed to slow the progress of disease epidemics within single fields, and the rational underlying *R* gene pyramiding.

58. Zhou F, Kurth J, Wei F, Elliott C, Vale G, Yahiaoui N, Keller B, Somerville S, Wise R, Schulze-Lefert P: **Cell-autonomous expression of barley Mla1 confers race-specific resistance to the powdery mildew fungus via a Rar1-independent signalling pathway.** *Plant Cell* 2001, **13**:337-350.

59. Leach JE, Cruz CMV, Bai JF, Leung H: **Pathogen fitness penalty as a predictor of durability of disease resistance genes.** *Ann Rev Phytopathology* 2001, **39**:187-224.

This review surveys the various pathogen fitness components compromised as a result of *avr* gene loss when examined under non-field conditions and also explores how this variation in pathogen fitness is translated within the competitive environment of an agroecosystem. The authors conclude that the appearance of adapted strains should not be considered as an indicator of the lack of usefulness of an *R* gene.

60. McDonald BA, Linde C: **Pathogen population genetics, evolutionary potential, and durable resistance.** *Annu Rev Phytopathol* 2002, **40**:349-379.

This article proposes a framework for predicting the evolutionary potential of pathogen populations based on an analysis of their genetic structure. A matrix containing nine distinct scenarios is presented. Pathogens predicted as the most likely to overcome *R*-gene-mediated resistance are those capable of sexual and asexual reproduction, those with a high potential for genotype flow, with large effective population sizes and a high mutation rate. Many examples of high, intermediate and low-risk pathogens are given. The authors propose general guidelines for a rational approach to breed durable resistance based on the pathogen's evolutionary potential.

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62. Chern MS, Fitzgerald HA, Yadav RC, Canlas PE, Dong X, Ronald PC: **Evidence for a disease-resistance pathway in rice similar to the NPR1-mediated signalling pathway in *Arabidopsis*.** *Plant J* 2001, **27**:101-113.

Arabidopsis NPR1 was overexpressed in rice under the control of either a ubiquitin or 35S promoter. The transgenic NPR1 rice plants displayed enhanced resistance to bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae*. This is the first result to suggest that monocotyledonous and dicotyledonous plants share a conserved signalling pathway involving NPR1.

63. Ayliffe MA, Roberts JK, Mitchell HJ, Zhang R, Lawrence GJ, Ellis JG, Pryor TJ: **A plant gene up-regulated at rust infection sites.** *Plant Physiol* 2002, **129**:169-180.

64. Rushton PJ, Reinstadler A, Lipka V, Lippok B, Somssich IE: **Synthetic plant promoters containing defined regulatory elements provide novel insights into pathogen- and wound-induced signalling.** *Plant Cell* 2002, **14**:749-762.

The WRKY family of transcription factors are plant-specific and bind to a *cis*-active element, the W-box (TTGAC(C/T)). Rushton and co-workers demonstrate that a range of defined synthetic promoters containing tetramers of specific *cis*-acting elements (boxes W, GCC, JERE, S, Gst and D) can mediate plant gene expression after plant attack and/or wounding. By varying the number, order and space of these *cis*-elements these researchers constructed improved synthetic promoters that were rapidly induced locally in response to attack by compatible pathogens as well as incompatible and non-host pathogens. These synthetic promoters may be very useful in restricting transgene expression and thereby minimise the detrimental effects on plant growth,

- development and crop yield frequently associated with constitutive expression of disease control proteins.
65. Friedrich L, Lawton K, Dietrich R, Willits M, Cade R, Ryals J: **NIM1 overexpression in *Arabidopsis* potentiates plant disease resistance and results in enhanced effectiveness of fungicides.** *Mol Plant Microbe Interact* 2001, **14**:1114-1124.
 66. Brandwagt BF, Mesbah LA, Takken FL, Laurent PL, Kneppers TJ, Hille J, Nijkamp HJ: **A longevity assurance gene homolog of tomato mediates resistance to *Alternaria alternata* f. sp. *lycopersici* toxins and fumonisin B1.** *Proc Natl Acad Sci USA* 2000, **97**:4961-4966.
 67. Halterman D, Zhou F, Wei F, Wise RP, Schulze-Lefert P: **The MLA6 coiled-coil, NBS-LRR protein confers AvrMla6-dependent resistance specificity to *Blumeria graminis* f. sp. *hordei* in barley and wheat.** *Plant J* 2001, **25**:335-348.
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 69. van der Biezen EA, Freddie CT, Kahn K, Parker JE, Jones JD: ***Arabidopsis* RPP4 is a member of the RPP5 multigene family of TIR-NB-LRR genes and confers downy mildew resistance through multiple signalling components.** *Plant J* 2002, **29**:439-451.
 70. Shen KA, Chin DB, Arroyo-Garcia R, Ochoa OE, Lavelle DO, Wroblewski T, Meyers BC, Michelmore RW: **Dm3 is one member of a large constitutively expressed family of nucleotide binding site leucine-rich repeat encoding genes.** *Mol Plant Microbe Interact* 2002, **15**:251-261.
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 72. Glazebrook J: **Genes controlling expression of defense responses in *Arabidopsis* — 2001 status.** *Curr Opin Plant Biol* 2001, **4**:301-308.
 73. Durrant WE, Rowland O, Piedras P, Hammond-Kosack KE, Jones JDG: **cDNA-AFLP reveals a striking overlap in race-specific resistance and wound response gene expression profiles.** *Plant Cell* 2002, **12**:963-977.
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 75. Ellis C, Turner JG: **The *Arabidopsis* mutant *cev1* has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens.** *Plant Cell* 2001, **13**:1025-1033.
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