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REVIEW

The Plant – Pathogen Interactions

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Interaction between plants and their pathogens is complex, involving multifaceted recognition of pathogens by the plants and, on the other hand, subtle evasion from the pathogens. Plants perceive pathogens through direct recognition of common molecular patterns in microbes and direct recognition of effectors or their perturbation on cellular components by the pathogens. Recognition of microbe- or pathogen-associated molecular patterns triggers innate immunity that renders plants resistant to most potential microbial pathogens. Recognition-dependant immunity in plants largely relies on polymorphism of resistance gene products that confer specificity towards host-specialised pathogens, which, in turn, induces more specific resistance that is effective against host-specialised pathogens. The deployment of effective resistance involves signalling of pathogen recognition through complex signalling cascades, transcriptional reprogramming, and defence-related genes, which all contribute to an arrest of pathogen growth. Our current insights into effector biology and to which the plants respond, provide a detailed information on the evolutionary arms race between plants and their pathogens. These will lead to an improvement of current strategies for crop improvement and protection.

Key words: innate immunity, resistant, effectors, microbe- or pathogen-associated molecular patterns

Disease in plants is rare because most plants are resistant to most pathogens. However, in extensively cultivated genetically identical plants, coevolutionary arms race between pathogens and plants is not uncommon. Plants are constantly challenged by a battery of potential pathogens ranging from fungi, oomycetes, bacteria, insects, nematodes, and viruses. Unlike animals, plants are sessile, unable to escape pathogen attack. Plants lack mobile cells able to deliver somatically generated, adaptive immune responses to sites of infection. Nevertheless, every plant cell is independently able to mount defence responses against microbial attack. Moreover, in order for potential pathogens to become actual pathogens in plants, they must overcome multifaceted defence mechanisms, from physical barrier, preformed antimicrobial compounds, and recognition-dependent immunity mediated by resistance (*R*) genes (Dangl and Jones 2001). Pathogens able to breach beyond the passive lines of defence layers may seek persuasive and subtle relationships with the host to dodge the surveillance system. Interaction between plants and their pathogens seems to be an intricate relationship involving subtle evasion (in pathogens) and recognition (in plants) mechanisms. Host plant immunity to pathogen attack largely relies on the polymorphic capacity of *R* gene products that perceive specific elicitors produced by pathogens (Dangl and Jones 2001; Jones and Dangl 2006; Bent and Mackey 2007). In theory, any pathogen-derived molecule could act as an elicitor for re-programming the transcriptional or physiological states of plants into defence mode. On the other hand, pathogens continue to evolve their virulence machinery to subvert host defence responses. Here, we review both virulence system in pathogens and resistance system in plants in turn, the evolutionary arms race involving both pathogen and host, and we present updates to the field based on recent findings on molecular plant-pathogen interactions.

The Plant Surveillance System. There are two branches of the plant immune system (Jones and Dangl 2006). The first system uses transmembrane pattern recognition receptors (PRRs) that perceive slowly evolving microbial - or pathogen-associated molecular patterns (MAMPs or PAMPs) and the other uses R proteins that perceive effectors produced by host-specialised pathogens (Fig 1). PAMPs or MAMPs can trigger initiation of PAMP-triggered immunity (PTI) (Nürnberger *et al.* 2004), which is probably the first perception-based response against microbial infection that makes most plants immune to most potential pathogens. PTI contributes to the so-called non-host resistance or basal resistance. However, host-specialised pathogens, particularly biotrophic microbes, have evolved the ability to suppress or interfere with PTI by releasing effector proteins into the plant apoplast or the plant cell cytosol.

The second and more elevated deployment of host immune response is initiated by recognition of specific elicitors encoded by pathogen avirulence (*Avr*) genes and hence, designated as effector-triggered immunity (ETI). An avirulence protein refers to the recognised effector protein that renders pathogens avirulent in their host plants. *Avr* genes and their cognate host resistance (*R*) genes, contribute to the basis of the so-called gene-for-gene model of host–pathogen incompatibility. A plant is resistant if it carries cognate *R* genes to the corresponding *Avr* genes in the interacting pathogens. Alternatively, if either is inactive or absent, disease results. Thus, gene-for-gene interaction between *R* and *Avr* determines the outcome of plant-pathogen relationship: incompatible (resistant plant, avirulent pathogen) or compatible (susceptible plant, virulent pathogen) interactions.

Surprisingly, *R*-mediated resistance and basal resistance give similar defence responses (Tao *et al.* 2003). Here, we define basal resistance as a form of immunity in susceptible

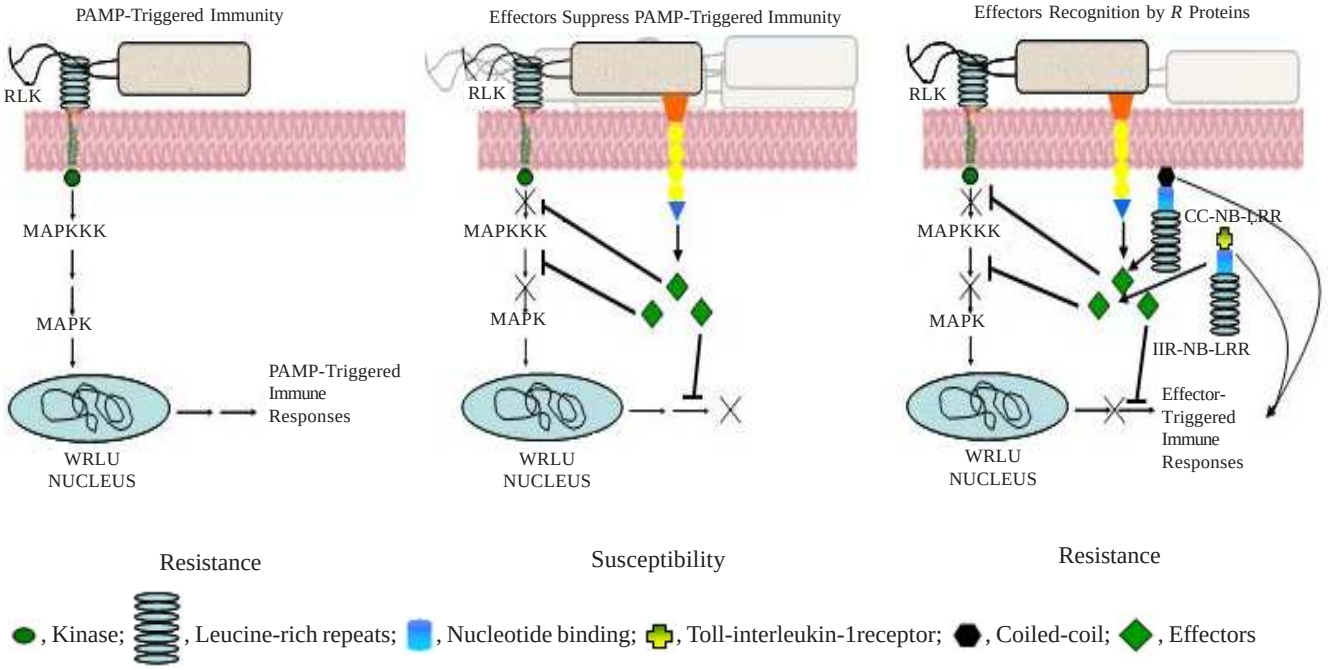

Fig 1 Model for the plant immune system for bacteria. Left to right, the evolution of bacterial resistance through recognition of pathogen-associated molecular patterns (such as bacterial ûagellin) by extracellular receptor-like kinases (RLKs) that triggers the deployment of basal immunity that requires MAP kinase signalling cascades and transcriptional reprogramming in the nucleus mediated by WRKY transcription factors. This type of immunity confers the plant resistant to most potential pathogens. Some pathogenic bacteria have evolved the machinery to dodge the recognition by RLKs by releasing effector proteins that suppress the basal immunity. In the absence of cognate plant resistance proteins, plants are susceptible and therefore the bacteria proliferate in the apoplast. Plants, on the other hand, have evolved to produce resistance proteins that recognise the effectors either directly or indirectly. In the figure, the resistance proteins are represented by CC-NB-LRR and TIR-NB-LRR (see text). These proteins recognise the activity of the effectors and trigger the so-called effector-triggered immunity (ETI) that is efficient to limit the growth of the bacteria.

hosts challenged by virulent pathogens. This similarity in defence output suggests that ETI is an accelerated and magnified version of basal resistance (Wise *et al.* 2007). However, whether the *R*-mediated resistance and basal resistance superimpose similar signalling cascades or not is not yet understood. In addition, even though the gene-for-gene relationship may imply a simple ligand-receptor interaction between Avr and R proteins, there are, in other cases, demonstrations in which R proteins recognise Avr proteins indirectly (Mackey *et al.* 2002; Axtell and Staskawicz 2003; Mackey *et al.* 2003; Shao *et al.* 2003; Rooney *et al.* 2005). The notion of indirect recognition of Avr proteins by R proteins implies that R proteins monitor the structural integrity of plant proteins that are the nominal targets of Avr proteins. Thus, a third component is required in this model, which could be the virulence target of Avr proteins. This model is known as "guard hypothesis", denoting R proteins guard the "guardee", the molecular target of effector proteins.

All functional *R* genes known so far confer resistance to fungal, bacterial, viral, and even insect and nematode pathogens with very different lifestyles. Despite the ability to confer resistance against a broad diversity of pathogens, *R* genes encode only five classes of proteins. The largest class of R proteins contains a "nucleotide binding site and leucine-reach repeat" (NB-LRR) domains and thought to be cytoplasmic (although they could be membrane-associated). Based on the N-terminal structures, the NB-LRR class can be subdivided into two subclasses: one that has homology to the intracellular signalling domains of the *Drosophila* Toll and mammalian interleukin (IL)-1 receptors (TIR-NB-LRR), and the other consists of coiled-coil domain (CC-NB-LRR). To a lesser extent, the other classes of R proteins consist of receptor-like kinases (RLKs), membrane-anchored receptor- like proteins (RLPs), and serine-threonine protein kinases that are mostly transmembrane or membrane-associated. The NB-LRR proteins function so far exclusively in disease resistance. Plant NB-LRR proteins share broad similarity with mammalian CATERPILLER/nucleotide oligomerisation domain (NOD)/NOD-like receptor (NLR) proteins family (Ting and Davis 2005), and STAND ATPases (Leipe *et al.* 2004). The most common feature of most R proteins is the presence of an LRR domain in variable length. In other proteins, LRR domains function in protein-protein interaction, protein-ligand binding, and carbohydrate-protein interaction (Kajava 1998). Indeed, domain swapping experiments suggest that LRR domain play a role for R proteins – effectors interaction specificity (Ellis *et al.* 1999; Luck *et al.* 2000). In addition, comparative sequence analyses predicted that LRR is solvent-exposed and is under diversification pressure from highly evolving pathogen effectors (Michelmore and Meyers 1998; Kobe and Kajava 2001). For instance, the flax *L* alleles *L2* and *L10*, differ in amino acid sequences of their LRR domains and recognise different effectors. Swapping the LRR of *L10* with that of *L2* changed its specificity in effector recognition (Ellis *et al.* 1999). However, analyses of flax L proteins suggested that besides of LRR, TIR domain is also under diversification pressure and this domain determines the specificity of L proteins with the corresponding Avr proteins of *Melamspora lini* (see below) (Luck *et al.* 2000).

Based on animal model, the TIR and CC domains from plants are suggested to be involved in protein-protein interactions that recruit host signalling partner proteins. Recent work suggests the role for TIR domain in stabilisation of R-Avr interaction (directly or indirectly) and defence signalling based on observation in *Nicotiana* N protein that associates with the Tobacco mosaic virus (TMV) – derived effector protein, p50 (Burch-Smith and Dinesh-Kumar 2007; Burch-Smith *et al.* 2007). The N's TIR domain is necessary for N – p50 association, and the TIR domain itself is sufficient to associate with p50. This domain interacts with p50 indirectly and requires other yet unknown proteins for N activation and defence signalling to ensue. The central domain of R proteins (the NB domain) has a nucleotide-binding pocket and modular functions for ATPase activity to hydrolyse ATP *in vitro* (Tameling *et al.* 2002). Mutations in the conserved motifs of NB domain impair its ATPase activity, but not its binding capacity, and cause autoactivation leading to pathogen-independent hypersensitive response (HR) induction *in planta* (Tameling *et al.* 2006). The *Lr10* leaf rust resistance gene of wheat is unusual in a way that the N-terminus of the CC domain is under strong diversifying selection (Caroline *et al*. 2009). Moreover, resistance conferred by Lr10 requires two related, yet unidentified CC-NB-LRR proteins.

Very little is known about the mechanism of NB-LRR protein activation. Activation of these proteins involves intra- and intermolecular conformational changes and seems to be under negative regulation. Mutation in the LRR domain or NB domain can lead to autoactivation and trigger auto-defence responses, suggesting that intramolecular twist or domain interaction negatively regulates NB-LRR activation (Bendahmane *et al.* 2002; Shirano *et al.* 2002). Heat shock protein 90 (HSP90) and other receptor cochaperones are involved in NB-LRR folding, rendering it signal-competent prior recognition of pathogen effectors or their targets (Fuente *et al.* 2005). Activation of NB-LRR involves Avr-dependent release of ATPase domain from inhibition by LRR followed by multimerisation that forms a complex, which recruits additional proteins to the N terminus for signalling (DeYoung and Innes 2006).

Microbial Pattern Recognition Triggers PTI. In plants, distinguishing between self and non-self through perception to widely conserved microbial molecules is perceived by pattern-recognition receptors (PRRs) that are mostly receptor-like kinases (RLKs). RLKs are transmembrane proteins consisting of divergent extracellular domains involved in ligand interaction and intracellular kinase domains that modulate downstream signalling. Microbial patterns perception subsequently prompts PTI. Although the complete mechanism of PTI has not been elucidated, studies on bacterial flagellin, a subunit protein of flagella, have provided invaluable information on the induction of PTI. Induction of PTI involves mitogen-activated protein (MAP) kinase signalling cascade, expression of defence-related proteins, reactive oxygen species (ROS) burst, and cell wall strengthening or callose formation, all of which contribute to an arrest of pathogen growth (Nürnberger *et al.* 2004). In addition to flagellin, Gram-negative bacteria also contain lipopolysaccharide, which can also trigger PTI. Similarly, chitin, glucan, and ergosterol, which are major constituents of fungal cell wall, are also elicitors of PTI.

The N- and C-termini of flagellin are more conserved among bacteria compared with the central part. A 22-amino-acid peptide (flg22) from the N-terminus of flagellin is sufficient to induce activation of many cellular responses in *Arabidopsis thaliana* (Felix *et al.* 1999). flg22 is perceived by FLAGELLIN SENSING 2 (FLS2), a receptor-like kinase (RLK) that contains extracellular LRRs and intracellular serine/threonine kinase domain (Gómez-Gómez and Boller 2000). FLS2 binds to flg22 and acts in early stages of pathogen invasion (Chinchilla *et al.* 2006). Early activation of FLS2 is inferred from mutants lacking *fls2* that are more sensitive to flagellin from *Pseudomonas syringae* pv. *tomato* DC3000 in spray application, but not in syringe infiltration into the leaf apoplast (Zipfel *et al.* 2004; Sun *et al.* 2006). A rapid deployment of defence responses following perception of flg22 by FLS2 requires SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK)3/brassinosteroid-associated kinase (BAK)1, which is also an RLK (Chinchilla *et al.* 2007; Heese *et al.* 2007). SERK3/BAK1 links signal perception and transduction in PTI, not only to flg22-dependent PTI, but also to some unrelated PAMPs-dependent PTI, suggesting a role for this RLK in integrating diverse PAMP perceptions into downstream responses. Downstream of flagellin perception by FLS2 is the activation of MAP kinase cascade and WRKY transcription factor that culminate in induction of defence responses. However, *Arabidopsis* plants overexpressing WRKY showed enhanced resistance to both bacteria and fungi, suggesting that resistance mechanisms following flagellin perception are not specific to bacteria. Alternatively, plants respond to PAMPs and activate defence responses using diverse MAP kinase cascade components and transcription factors that converge to signal multiple PAMPs perception (Asai *et al.* 2002).

Plant perception of bacterial elongation factor and cold shock proteins (EF-Tu) activates similar defence responses to flg22 (Kunze *et al.* 2004; Zipfel *et al.* 2006). In *Arabidopsis*, EF-Tu is perceived by another RLK protein called EFR. The first 18-amino-acids of EF-Tu are sufficient to induce expression of genes that are also activated upon flg22 recognition (Zipfel *et al.* 2006). Moreover, flg22 can activate transcription of *EFR*. Hence, it is likely that similar defence responses comprising PTI following PAMP recognition converge on common signalling pathways. However, *Arabidopsis* double mutant lacking *fls2* and *efr-1* is still able to induce PTI after treatment with *Agrobacterium* cell extracts (Zipfel *et al.* 2006). It hints a possibility that there should be additional PAMPs and corresponding receptors. There are over 600 RLKs genes in *Arabidopsis thaliana*, and it is possible that many are PAMP receptors function to recognise a broad range of PAMPs.

PTI Suppression and Host Resistance. Logically, it would be surprising if pathogens were to carry *Avr* genes without any other functions other than to allow recognition by the corresponding *R* genes. One possible explanation is that Avr proteins often act as virulence factors in the absence of R proteins partner (susceptible plants). In contrast to most

R genes, effector genes are remarkably diverse (Luderer and Joosten 2001). An individual strain of a bacterial pathogen delivers effectors into its hosts ranging from 20 to nearly 100 effectors. They are also diverse in their biological functions that all contribute to the virulence of pathogens in plants. Compared with PAMPs, which conceptually are surface-derived structural molecules, effectors are relatively high-evolving molecules that often function as virulence factors.

Plant pathogenic microbes release effectors to attain a subtle evasion to host plant defence responses. Effectors from bacteria are delivered into host cell cytoplasm or apoplast through their type III secretion system (TTSS) machinery (Petnicki-Ocwieja *et al.* 2002; Alfano and Collmer 2004). Some pathogenic fungi and oomycetes use specialised feeding structures called haustoria to facilitate intimate interface with their hosts. Both systems deliver effectors that function in virulence, either by inhibiting or mimicking cellular process in plants. To achieve this, most effectors have function in suppressing PTI. For example, resistance to non-host *Pseudomonas* requires *NONHOST1* (*NHO1*) that is activated upon recognition of flagellin. However, *NHO1* is ineffective against virulent *P. syringae* DC3000. DC3000, as many other strains has flagellin that is potent in triggering PTI, but this form of immunity fails to halt DC3000 growth because it produces effectors that contribute to PTI suppression. At least nine effectors were defined to be capable of suppressing *NHO1* expression (Li *et al.* 2005). PTI suppression is also evident in bean deploying more rapid and elevated defence responses against *P. syringae* mutant strain that is unable to inject any type III-effectors compared with bean treated with the isogenic wild-type strain (Wei *et al.* 2007). Hence, the diminishing of PTI allows pathogens to achieve successful colonisation in host plants. Plants have evolved R proteins as a surveillance system for effector traffic in host plants. *R*-mediated responses (ETI) are typically characterised by host programmed cell death (PCD), a form of hypersensitive response that is usually restricted to the site of pathogen ingress, leaving the surrounding cells unaffected (Hofius *et al.* 2007). ETI is aimed to restrict the fitness of successful pathogen capable of suppressing PTI. Although the mechanism of arresting pathogen growth by HR is unclear, at least in pathosystems undergoing R-Avr interaction, HR is the most common and effective defence response against pathogens. Moreover, HR is not always observable, nor it is *per se* a requirement for ETI. Thus, it is unclear what actually retards the growth of pathogens in resistant host plants.

What biochemical functions do effectors govern to achieve successful colonisation in plants? Most pathogen effectors that have been characterised biochemically exhibited proteolytic activity, suggesting that virulence mainly involves host protein degradation. However, the enzymatic activities of many effectors remain obscure since their sequence and structure predictions do not give clues to their cellular functions. Some effectors have unique characteristics and are likely to act in a diverse fashion for virulence. Here, we highlight only some new examples. HopU1 from the bacterial pathogen *P. syringae* is a mono-ADP-ribosyltransferase (ADP-RT) that suppresses plant immunity by targeting RNA-binding proteins that possess RNA-recognition motifs (RRMs) (Fu *et al.* 2007). ADP-ribosylation of RNA-binding proteins affects RNA metabolism and plant defence transcriptome, and eventually suppresses plant immunity. Another mechanism of virulence in *P. syringae* is that this bacterium impedes host cell vesicle transport. The *P. syringae* HopM effector manipulates ARF-EGF proteins likely to be involved in host vesicle transport (Nomura *et al.* 2006). In fungi, exocytosis is an important step during infection as a mean of delivering a cargo of molecules required for pathogenicity including effectors. In *Magnaporthe grisea*, an integral membrane P-type ATPase, MgAPT2, is required for exocytosis during infection in plants (Gilbert *et al.* 2006). *M. grisea* mutants lacking MgAPT2 are defective in the ability to secrete several extracellular enzymes and form abnormal Golgi-like cisternae. The enzyme is also required by the fungus for successful foliar and root infection, but in incompatible interaction, it triggers a rapid induction of plant defence responses. Plant pathogens may also exploit the "compatibility factor" in plants for their virulence. For instance, PthXo1, a type III effector from *Xanthomonas oryzae* strain PXO99A targets *Os8N3*, a dominant susceptibility gene in rice that is induced during disease development (Yang *et al.* 2006). PthXo1 is a member of transcription activator-like (TAL) effector family. RNA-mediated silencing of *Os8N3* leads to resistance against PXO99A infection, but not to other strains. In addition to *Os8N3*, PXO99A affects the expression of two additional plant genes, *OsTFX1* and *OsTFIIAγ1* (Sugio *et al.* 2007). *OsTFX1* encodes a bZIP transcription factor, whereas *OsTFIIAγ1* encodes a subunit of the transcription factor IIA that resides in chromosome 1 of rice, respectively. Expression of both genes is dependent on two type III effectors, pthXo6 and pthXo7. An interesting evidence is found in interaction between *LOV1* in *A. thaliana* and victorin from *Cochliobolus victoriae*. *LOV1* confers susceptibility in *A. thaliana* to *C. victoriae* (Lorang *et al.* 2007). Surprisingly, *LOV1* encodes a CC-NB-LRR protein that shares extensive similarity to the *RPP8* resistance gene family. *C. victoriae* produces victorin, a host-selective toxin (HST), necessary for the fungus to cause Victoria blight disease in oats. Although *LOV1*, in the presence of victorin, confers susceptibility to *C. victoriae*, it also induces the production of defence-related proteins similar to resistance-like physiology. Nonetheless, alterations in known defence response pathways do not alter susceptibility to *C. victoriae*, suggesting that these defence response pathways are dispensable for susceptibility towards *C. victoriae*. These demonstrate that a plant NB-LRR gene can confer both resistance and susceptibility.

Some pathogens are shown to be able to subvert plant defence by suppressing *R*-mediated host resistance. Interaction between *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) and tomato plants is race-cultivar specific. Races of *Fol* are historically named according to the *R* gene that counters them in order of their discovery. *Fol* race 1 is countered by the *I* (Immunity) gene and the unlinked *I-1* gene; *Fol* race 2 is virulent towards *I* and *I-1* tomato plants, but is impeded by *I-2*; and *Fol* race 3 is virulent on *I*, *I-1*, and *I-2* tomato plants, but is stopped by *I-3* (Fig 2). Surprisingly, *Avr1* that is only present in *Fol* race 1 and recognised by the

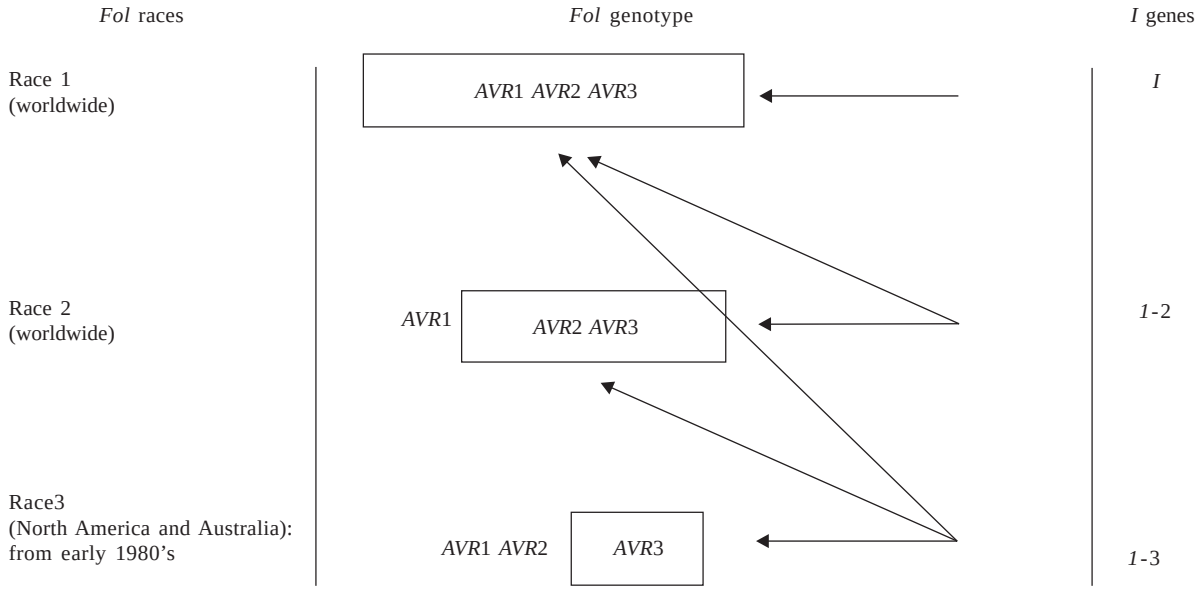

Fig 2 he gene-for-gene interaction between *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) with tomato plants. The interaction between *Fol* with tomato plants is race-cultivar specific. Arrows indicate the resistance in tomato plants conferred by the resistance genes against their corresponding avirulence genes in *Fol* races. *Fol* race 1 that carries all the *AVR1*, *AVR2*, and *AVR3* is countered by the *I* (Immunity) gene and the unlinked *I-1* gene; *Fol* race 2 is virulent towards *I* and *I-1* tomato plants, but is impeded by *I-2*; and *Fol* race 3 is virulent on *I*, *I-1*, and *I-2* tomato plants, but is stopped by *I-3*. However, some natural *Fol* race 1 isolates are known to be virulent on *I-2* and/or *I-3* plants, and introduction of *AVR1* in race 2 and race 3 renders them gaining virulence on *I-2* and *I-3* plants, while removal of *AVR1* in race 1 leads to loss of virulence towards *I-3* plants. *AVR1* that is present only in *Fol* race 1 and recognised by the *I* and *I-1* genes, suppresses the *I-2* and *I-3*-mediated resistance.

I and *I-1* genes, suppresses the *I-2* and *I-3*-mediated resistance (Houterman *et al.* 2008). Some *Fol* race 1 strains are known to be virulent on *I-2* and/or *I-3* plants (Mes *et al.* 1999; Rep *et al.* 2005), and introduction of *Avr1* in race 2 and race 3 renders them gaining virulence on *I-2* and *I-3* plants, while removal of *Avr1* in race 1 leads to loss of virulence towards *I-3* plants. Suppression of *R*-mediated resistance has also been observed in bacteria (Janjusevic *et al.* 2006; Rosebrock *et al.* 2007) and oomycetes (Dou *et al.* 2008).

Molecular events following pathogen recognition by R proteins involve ion fluxes, activation of MAP kinase and other protein kinases accompanied by production of reactive oxygen intermediates (ROIs) (Ligterink *et al.* 1997; Romeis *et al.* 1999) and nitric oxide (NO) (Delledonne *et al.* 1998; Durner *et al.* 1998; Delledonne *et al.* 2001), and transcriptional reprogramming of genes mostly involve in defence responses (Kan *et al.* 1992). Biochemical changes ensued upon elicitation by non-specific and race-specific elicitors are rapid. Ion fluxes occur within minutes, ROIs and NO are subsequently produced, and protein kinases pathways are activated. Downstream of these pathways is activation of defence genes function in induction of hormonal signalling molecules, cell wall strengthening, production of antimicrobial compounds, and programmed cell death as a form of hypersensitive response, all of which contribute to resistance (Hammond-Kosack and Jones 1996; McDowell and Dangl 2000). Non-specific, systemic acquired resistance (SAR) establishment is also preceded by these events (Durrant and Dong 2004). The deployment of defence responses is dependent on salicylic acid (SA), jasmonic acid (JA), and ethylene that act as regulators of signalling pathways mediating plant responses to pathogens with different lifestyles. SA mediates defence responses against biotrophic pathogens, whereas JA activates defence responses to necrotrophic pathogens. In compatible interaction, SA- and JA-dependent pathways are mutually antagonistic in a way that activation of SA as a response to a biotrophic pathogen renders a plant more susceptible to a necrotrophic pathogen when both pathogens are in close proximity (Spoel *et al.* 2007). However, the trade-off control is weak in spatial inoculation of both pathogens and absent in incompatible interaction. The latter suggests a mechanism of plants to prevent the growth of necrotrophic pathogens in cells undergoing programmed cell death. It seems that plants tightly regulate the tradeoffs between SA and JA to prevent unfavourable signal interactions and maximise their versatility to defend themselves from multiple attackers. Programmed cell death is typically accompanied by systemic acquired resistance, which is broad spectrum and long lasting. JA, but not SA, acts as a mobile signal in transmitting long-distance information and mediates the systemic acquired resistance (SAR) (Truman *et al.* 2007). Transcriptional signature of JA-mediated SAR overlaps with local basal defence and wounding, as well as herbivory responses, indicating that JA-dependent signalling pathways are evolutionarily conserved that mediate biotic and abiotic responses. SA is known to regulate plant defence responses mediated by *R* genes. Interplay between SA-mediated responses and MAMP-triggered responses involve overlapping functions of two SA signalling components *SID2* and *PAD4*, which in *Arabidopsis*, disruption of these components affects MAMP-triggered responses to flg22 of *Pseudomonas syringae* pv. *tomato* DC3000 (Tsuda *et al.* 2008).

Recognition of Pathogen Effectors. Sequence analyses of many effectors usually do not give clues on their cellular

functions (Kamper *et al*. 2006; Does and Rep 2007). Nonetheless, some can be identified as having enzymatic activities or other cellular functions. Those that have cellular functions would logically target host proteins for virulence, and R proteins would recognise these perturbations on host proteins. Plants recognise pathogen effectors either through direct R-Avr interaction or indirectly through recognition of changes in structural integrity of host proteins following their interaction with Avr proteins (Jones and Dangl 2006). The simplest model for R-Avr interaction would be R proteins, specified by the LRR domain, recognise Avr proteins directly (the receptor-ligand model). Domain swapping and mutational analysis revealed that the LRR domain govern recognition specificity in R proteins. However, as will be described herein, only few demonstrations of direct R-Avr interaction have been described. Direct R-Avr interaction is possibly best exemplified by the flax L5, L6, and L7 R proteins with the corresponding AvrL567 protein in flax rust fungus, *Melampsora lini* (Dodds *et al.* 2006). The *L* loci encode NB-LRR proteins with different specificities to Avr proteins in various strains of *M. lini*. In yeast, L5, L6, and L7 R proteins interact physically with AvrL567 protein with specificities consistent with responses observed *in planta*, indicating that recognition specificity between these proteins is based on direct interaction. Amino acid sequence differences in AvrL variants render different recognition in both flax and yeast, and variation in specificity is associated with several polymorphic sites.

Direct R-Avr interaction is also observed in the rice Pi-ta protein and the Avr-Pita protein from *Magnaporthe grisea* (Jia *et al*. 2000). Based on yeast two-hybrid system and *in vitro* binding assays, Avr-Pita binds specifically to LRR domain of Pi-ta. Another example is the RRS1-R protein that interacts with a nucleus-targeted type III effector of *Ralstonia solanacearum*, PopP2 (Deslandes *et al.* 2003). RRS1-R, a member of TIR-NB-LRR is an unusual R protein because it has the WRKY motif characteristic of some plant transcriptional factors. Physical interaction between RRS1-R and PopP2 was observed in yeast although the domains in RRS1-R responsible for the interaction could not be determined. Instead, a full-length RRS1-R protein is required for such interaction. Nuclear localisation of RRS1-R requires interaction with PopP2, and indeed, both proteins are colocalised in the nucleus of protoplasts. Interestingly, RRS1-S, a protein with high similarity to RRS1-R but present in susceptible ecotype also binds to PopP2 in yeast and is colocalised into the nucleus. However, in many cases, simple physical interaction does not necessarily imply the detection of effectors by R proteins *per se*. As mentioned above, pathogen effectors may be recognised by R proteins indirectly.

Observation in many other pathosystems failed to detect direct R-Avr interaction, and this led to the formulation of "guard hypothesis", which proposes that an R protein detects the perturbation of a host protein by the cognate Avr determinant. The host protein is the molecular target for the virulence function of the Avr determinant and, on the other hand, the surveillance target of the cognate R protein for such interference. One host protein may be guarded by more than one R proteins and may be the target of several pathogen effectors as well. RIN4 (RPM1 interacting 4) is required for RPM1-mediated resistance in *Arabidopsis* against *P. syringae* carrying two unrelated type III effectors, AvrRpm1 and AvrB (Mackey *et al.* 2002). RIN4 also mediates interaction between the RPS2 (resistance to *P. syringae* pv. *tomato* 2) and the *P. syringae* AvrRpt2 type III effector (Axtell and Staskawicz 2003; Mackey *et al.* 2003). As in the case of RRS1-S and PopP2 interaction (see above), RPS2 co-immunoprecipitates with AvrB and produces nonproductive complex (Leister and Katagiri 2000). The corresponding R protein for AvrB is RPM1. Such overlapping interactions suggest that either direct or indirect interaction of R/Avr does not necessarily imply R proteins *per se* confer gene-for-gene specificity. Other component(s) are required to form active and specific complexes of effector recognition.

RIN4 is a 211-acylated-amino acid that possesses plasma membrane-associated domain, which is by far known to be manipulated by three different bacterial type III effectors and "guarded" by two NB-LRR proteins. AvrRpm1 and AvrB both interact and phosphorylate RIN4, and such modification induces activation of RPM1 (Mackey *et al.* 2002). The *P. syringae* AvrRpt2 is a cystein protease that is autoprocessed and activated inside host cells, which then it destroys RIN4 by cleaving it at two sites (Kim *et al.* 2005). This cleavage has two consequences: activation of RPS2 on one hand, and interference of RPM1 function on the other hand. Besides RIN4, additional host proteins may also be the targets of AvrRpm1 or AvrRpt2 since the elimination of RIN4 did not abolish the virulence to a weakly pathogenic strain possessing these effectors in susceptible plants (*rin4 rpm1 rps2*) (Belkhadir *et al.* 2004). RIN4 interacts with NDR1 (non-race specific disease resistance 1), a GPI-anchored protein that is required for the activation of both RPM1 and RPS2 (Day *et al.* 2006). In Solanaceae, the mechanism of RIN4 degradation overlaps between tomato and *Nicotiana benthamiana* (Luo *et al.* 2009). This degradation is induced by an effector AvrPto from P. syringae and dependent on resistance proteins Pto and Prf in tomato and *N. benthamiana*. Analysis on effector secretome in *P. syringae* reveals other effectors, besides of AvrRpt2, which can also elicit RIN4 proteolysis. This suggests that RIN4 could be a common target for several effectors from *P. syringae*.

Another example of indirect interaction has been shown in the interaction between the RPS5, a CC-NB-LRR protein in *Arabidopsis* and the AvrPphB, a type III effector from *P. syringae* (Shao *et al.* 2003). RPS5 is NDR1-independent, and its activation requires PBS1 (AvrPphB susceptible 1), which is a serine-threonine protein kinase that is cleaved by AvrPphB. Both kinase activity and cleavage of PBS1 are required for RPS5 to function, suggesting that cleaved PBS1 retains its enzymatic activity and contributes to the activation of RPS5. Similar to AvrRpt2, AvrPphB is a cysteine protease that is self-cleaved and activated inside host cells and is nonetheless able to cleave PBS1 at a site in the kinase activation region homologous to its own cleavage site. Direct and indirect recognition of effectors by R proteins will result in different consequences on how pathogens modify their effectors in order to evade the surveillance system in their hosts. Effectors that are recognised directly will undergo diversification but retain their virulence capacity. Whereas

effectors that are recognised indirectly, they will be discarded by the pathogens. Although discarding such effectors might be such a significant penalty for the pathogens, evidence indicated that the polymorphism in such effectors is presence or absence.

In plant populations, the guarded effector targets are evolutionary unstable depending on the presence or absence of the *R* gene. Opposing selection forces occur in the guarded effector targets predicted from evidence that many pathogen effectors have multiple targets in the host and most effectors retain their virulence despite the absence of the *R* gene. In the absence of the R protein, the guardee proteins will be under selective pressure to evade manipulation by effectors while, on the other hand, in the presence of R protein the guardee proteins will be under selective pressure to improve perception by the effectors. These conflicting selection forces impose evolutionary constraints on the guarded effector targets that would be relaxed in the presence of a "decoy" that mimics the operative guarded effector targets by acting as a coreceptor regulating *R* gene activation (Hoorn and Kamoun 2008). The decoy would arise from duplication of guarded effector target genes followed by subsequent evolution, or evolve independently by mimicking effector targets. Decoy exclusively functions in effector perception with no association in development, disease, or resistance. Distinctive to the guard hypothesis, decoy does not support pathogen fitness in the absence of *R* gene. Evidence supporting the decoy model is inferred from several cases of effector perception involving RIN4 (Belkhadir *et al.* 2004; Lim and Kunkel 2004; Chisholm *et al.* 2005; Takemoto and Jones 2005), RCR3 (Shabab *et al.* 2008), Pto (Mucyn *et al.* 2006; Xiang *et al.* 2008), and Bs3 (Schornack *et al.* 2008; Zhou and Chai 2008). However, the decoy model still requires experimental evidence, and therefore, it is a challenging platform for our understanding on effector perception.

Conclusion and Conundrum. Immunity of plants to pathogens is largely dependent on the polymorphism capacity of PRR and R proteins that recognise pathogen molecular patterns and Avr proteins or their interference on plant defence system. Recognition of MAMPs/PAMPs is a prerequisite of PTI that confers plants resistant to potential non-host pathogens. PTI seems to be effective in protecting plants against most non-host pathogens but ineffective to host-specialised pathogens. Evidence from studies of bacterial type III effectors suggest that effector proteins contribute to the virulence of pathogens by suppressing PTI. Such interference is countered by plants through R proteins that recognise Avr proteins (direct interaction) or their perturbation on host proteins (indirect interaction). The resulting ETI is highly effective in such it is able to restrict pathogen growth and spread. However, there are still more questions than answers as to our understanding on plant immunity is still very limited.

The response outputs between PTI and ETI are similar. Do the signalling and transcriptional factors between PTI and ETI overlap? Dissecting the interplay between them is pivotal because there, is where pathogens most likely manipulate the plant defence, and on the other hand plants regulate critical molecules that activate and/or switch these defence modes. Some PRR proteins are also involved in plant development as well as response to abiotic stress. Some effectors mimic plant hormones to suppress PTI. Coronatine, a small protein produced by *P. syringae* mimics jasmonic acid that can suppress salicylic acid signalling pathway necessary for defence against biotrophic pathogens (Zhao *et al.* 2003; Brooks *et al.* 2005). Taken together, plants seem to regulate the interplay between PTI, ETI, abiotic stress, and hormonal metabolism to counteract pathogen attack. Certain plant NB-LRR proteins act in the nucleus to trigger downstream signalling and defence pathways. Mildew A (MLA) R proteins in barley function in the nucleus to confer resistance against the powdery mildew fungus. MLA10 R protein recognises the A10 Avr protein, and this recognition induces association of MLA10 and WRKY transcription factors in the nucleus (Shen *et al.* 2007). The WRKY transcription factors suppress the PTI and MLA interferes with the WRKY suppression to de-repress PTI, therefore defence ensues. This evidence suggests a common transcriptional factors function in PTI and ETI as well as an integration of mechanism of defence responses conferred by PRR and R proteins to distinct pathogen signals.

Although in many observations HR is effective in arresting pathogens, knowledge on the mechanism of how it can arrest pathogen growth is lacking. Nonetheless, in some pathosystems, necrotic HR is not involved in arresting pathogen growth. For example, potato Rx protein can mediate an extreme resistance against potato virus X (PVX) without causing a necrotic HR (Bendahmane *et al.* 1999). In other case, resistance to wilt pathogens *Fusarium oxysporum*, which colonises plants in the vascular system, does not involve PCD distinctive to most resistance responses to foliar pathogens. Thus, cell death and pathogen arrest in disease resistance might represent separable mechanisms. Programmed cell death is specific to ETI and is generally not associated with basal defences. Several pathogen effectors can suppress PCD either by altering expression of some crucial plant genes or by interfering key components involved in signalling pathways. Therefore, some pathogens target PCD as a form of HR in ETI for their virulence in host plants. This indicates the evolutionary arms race between host plants and pathogens is extending beyond the first level of ETI.

We need to understand the coevolution of pathogen effectors and *R* genes in plants. Why are most plants resistant to most pathogens? Host specificity seems to be the chosen strategy for pathogens to survive as a consequence of purifying selection to maintain intrinsic function of effectors. Plants, on the counterpart, maintain the stable effector/R interaction to overcome pathogen virulence. The better understanding of *R*/*Avr* coevolution in the biological population levels would help improving strategy to deploy plant immune system more effectively.

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