Molecular Basis of Host Pathogen Interaction

Rizwan Rashid1, J. A Bhat2, Ajaz Malik1, Z. A. Bhat3, W. A. Dar4, S. A. Untoo4 and M Y Ganaie3

1Department of Vegetable Crops, Punjab Agricultural University, Ludhiana 141004, India
2Department of Plant Pathology, Punjab Agricultural University, Ludhiana 141004, India
3Department of Horticulture (Pomology) Punjab Agriculture University Ludhiana -141004, India
4Department of Plant Pathology, Skuast, Shalimar Srinagar 190001, India

Abstract

Plant disease presents a constant threat to agriculture around the world. Understanding how plant and pathogen interact with each other is crucial for developing a sustainable agriculture. During the long history of co-evolution between host and pathogens, plant immune response has culminated in a highly defense system that is able to resist potential attack by microbial pathogens. As plants lack mobile defender cells and somatic adaptive systems, they must be relying on the innate immunity of each cell and on systemic signal emanating from infection sites. The perception of the pathogen is achieved through receptors, a surveillance system capable of recognizing both conserved molecular patterns and specific effector proteins and activation of the corresponding defenses. The primary immune response is referred as PAMP triggered immunity (PTI) and has to evolve to recognize the common features of microbial pathogens. At the same time, pathogens acquired the ability to deliver effector proteins to the plant system to suppress PTI allowing pathogen growth and disease. In response to this, plants acquired surveillance proteins (R proteins) to either directly or indirectly monitor the presence of pathogen effector proteins which leads to the activation of defense cascade. The new insights in the discovery of molecular basis of host pathogen interaction are changing the way as we think about pathogenesis and the treatment of various plant diseases. An integrated approach based on the combined knowledge of the molecular basis of ‘defense systems’ used by the plant and the ‘assault systems’ used by pathogens will help us to design novel methods for plant disease management.

Keywords: Molecular basis, pathogens, plant diseases, host pathogen.
Introduction

Being sessile organisms, plants are often exploited as a source of food and shelter by a wide range of parasites including viruses, bacteria, fungi, nematodes, insects and even other plants. During the long history of co-evolution between host and pathogens, plant immune response has culminated in a highly defense system that is able to resist potential attack by microbial pathogens (Allard “et al”). However, they have developed remarkable strategies to adapt to environmental changes by using a range of constitutive or inducible biochemical and molecular mechanisms. They exhibit both long- and short-term defense responses to immediate challenges such as pathogen attacks (Nurnberger “et al”). Nevertheless, a synergic effect of many stresses represents the primary cause of crop loss. The appropriate response of plant emerges from the perception of an extracellular signal and its transduction between and within plant cells. Interactions between disease resistance (R) genes in plants and their corresponding pathogen avirulence (Avr) genes are the key determinants of whether a plant is susceptible or resistance to a pathogen attack. Evidence has emerged that these gene-for-gene interactions in the perception of pathogenic invasions and development of acquired resistance in plants involve different molecular and hormonal transduction pathways, which are still poorly understood. It has become apparent that plants actively produce several phytohormones such as ethylene, jasmonate, salicylic acid, and reactive oxygen intermediates prior to up-regulation of R genes. The physiological role of these molecules in plant resistance to pathogens is beginning to attract attention (Montesano “et al”). The use of transgenic plants in recent attempts, including development of mutants with altered R genes, has provided new insights into the mechanisms involved in pathogen perception, signal transduction and subsequent resistance to disease in plants. Specificity of the interactions between plants and pathogens is still an incomprehensible phenomenon with a complicated hierarchy of biological organization. Elucidation of this phenomenon represents an important task of contemporary plant pathology. However, tremendous opportunities for crop improvement are likely to arise, as the complete sequencing of Arabidopsis plant genome, which is a molecular research model, become a reality. Many R genes, which confer resistance to various plant species against a wide range of pathogens, have been isolated.

Interactions between host and pathogen

Resistance is the most common response of plants to pathogens and susceptibility is the rare event. Plants have evolved to develop effective mechanisms of defense and resist the attack of microbes that are constantly in contact with their potential host. To establish disease, pathogens need to face and neutralize different obstacles on their way into the plant tissue. The first barrier is the plant cell surface. Penetration could occur through natural openings like stomata, through wounds, or by direct penetration using enzymes and/or mechanical forces. Once pathogens gain access by penetrating the plant cuticle, they face the second obstacle, the plant cell wall. After cell wall penetration, the pathogen is separated from plant cytoplasm just by the plasma membrane (Chisholm “et al”). Plasma membranes contain specialized proteins,
extracellular surface receptors, which are involved in the detection of pathogen associated molecular patterns (PAMPs) to trigger immune responses. Chitin is a component of cell walls that is considered one of the major fungal PAMPs. Plants are equipped with both constitutive and inducible defense mechanisms. The former includes constitutive properties such as the strength and thickness of cell walls and the presence of pre-formed antimicrobial compounds such as polyphenol. On the other hand, induced resistance involves newly toxic chemicals and physical barriers. On pathogen recognition, regulatory genes initiate a multicomponent defense response whose elements are activated in a highly controlled temporal and spatial manner. Typical components of such responses include the production of phytoalexins, accumulation of (hydrolytic) pathogenesis-related (PR) proteins, induction of signal pathways, reinforcement of the cell wall, production of reactive oxygen species, and finally programmed cell death and systemic acquired resistance (SAR). In compatible interactions, these defense responses are not initiated or are activated only at later stage and allowing the pathogen to exert its negative impact on growth and development of the plant.

**How Pathogen sense plants?**

Like human beings, plants have not any sensory organs. But it is quite surprising that pathogens have also ability to sense plants by various root exudates and volatile secretions. Zoospores propagate in soil water are attracted to the elongation and differentiation zones of plant. *P. parasitica* spores apparently do not have plant species-specific root preferences, thus contrasting to *P. sojae* zoospores, which are attracted specifically to roots exuding the isoflavones daidzein and genistein. *P. parasitica* zoospores can also be transported passively by splash water to leaf surfaces, where they are attracted to wound sites before encystment and germination. Spores of the oomycete have been shown to secrete PcVsv1, a protein containing multiple thrombospondin type 1 repeats. This protein, as well as mucin-like glycoproteins and other surface-binding proteins that have been identified in pre-infection stages of *P. infestans* and *P. parasitica*, are presumed to assure host adhesion of the zoospores. Encysted spores on both roots and leaves then attract further zoospores to form clumps of cysts. This phenomenon of self-attraction appears to be a strategy to increase the likelihood of infection. It is known as the “homing response” and is probably due to chemical signaling involving calcium. Once encysted on plant surfaces, Ca2+-dependent signaling triggers germination of *P. parasitica* spores to initiate infection. Germ tubes then enter directly into the intercellular spaces through wound openings on leaves, or form swellings that allow penetration between epidermal cells on root surfaces (Allrad “et al”).

**Attachment of the Pathogen to host**

Attachment of the pathogen takes place through adhesion of spores by various mechanisms. The propagule of pathogen have on their surface or tips mucilaginous substances that consisting of mixtures of water-insoluble polysaccharides,
glycoproteins, lipids and fibriller materials which when moistened, become sticky and help the pathogen to adhere to the plant. In some fungi, hydration of the spore by the moist air or dew causes the extrusion of preformed mucilage at the tip of the spore that serves for immediate adherence of the spore to the hydrophobic plant surface and resistance to removal by flowing water. However, in powdery mildew, which do not require free water for infection, adhesion is accomplished by release from the spore of the enzyme cutinase, which makes the plants and spore areas of attachment more hydrophilic and cements the spore to the plant surface. In some other cases, propagule adhesion requires on the spot synthesis of new glycoproteins and it may not reach maximum level until 30 minutes after contact. In some fungi causing vascular wilts, spore fails to adhere after hydration but become adhesive after they are allowed to respire and to synthesize new proteins [5].

Spore germination and perception of host surface
Spore germination is mainly triggered by contact with the host surface, hydration and adsorption of low molecular weight ionic materials from the host surface, and availability of nutrients. Once the stimulation of germination has been received by the spore, the latter mobilized its stored food reserves and directs them towards the rapid synthesis of cell membrane and cell wall towards the germ tube formation and extension. The perception of signals from plant surfaces by pathogenic fungi seems to be result of signaling pathways mediated by cyclic adenosine monophosphate (MAPK), which is implicated in the regulating the development of infection related phenomenon in many different fungi.

How pathogens recognize host plant?
In general, the plant response to a pathogen is based on its ability to recognize signature molecule produced by the pathogen. These molecules are termed elicitors and mostly originate from the pathogen (exogenous elicitors) and include peptides, glycoprotein, lipids and oligosaccharides. In addition, endogenous elicitors are released from the plant’s surface during pathogen attack. Race- and cultivar specific elicitors are encoded by the avirulence gene of the pathogen [6]. Most, but not all, proteins encoded by R gene recognize Avr proteins through leucine rich repeat (LRR) domains and initiate signaling through kinase domains or interactions with kinases. A signal transduction pathway for bacterial flagellin includes a LRR receptor kinase as well as a mitogen activated proteins (MAP) kinase cascade that activates defined transcription factors (Asai “et al”).According to their structural characteristics they have been classified into different categories, including receptor-like protein kinases (RLKs), histidine kinase receptors and receptors with different numbers of transmembrane domains. Of special interest in pathogen perception are the RLKs; there are at least 340 genes encoding putative RLKs in the Arabidopsis genome. RLKs are characterized by an extracellular domain which is probably involved in signal perception, a transmembrane domain and a cytoplasmic kinase domain, which may initiate a signal transduction cascade into the cell. All plant RLKs identified are
serinethreonine kinases and based on the structural characteristics of the extracellular domain they have been divided into different categories (Montesano “et al”).

**Elicitors- weapons of plant pathogen**

Plant cell-surface receptors are key components that perceive extracellular stimuli from the environment, including both general and race specific elicitors. Evolutionary ancient innate immunity, the ability to discriminate between self and nonself, is a quality of both animals and plants. It relies on the detection of pathogen-associated molecular patterns (PAMPs) characteristic of a whole class of potentially harmful microbial organisms (Nurnberger “et al”). Pathogen-derived molecules of diverse nature, including lipopolysaccharides), flagellins, glucans, and chitins, serve as general elicitors that trigger basal defense responses independently of the genotype of the individual pathogen For example, flagellin, the protein subunit of the bacterial surface structure flagellum, acts as a PAMP in both animals and plants. General elicitors are usually molecules that are indispensable in the lifestyle of microbes and thus provide a fitness penalty for the pathogen if recognized by the plant surveillance system. Endogenous plant cell wall-derived structures released by the hydrolytic enzyme activities of invading microbes can also act as general elicitors (Nürnberger “et al”).

During evolution some pathogens have developed to overcome the PAMP-triggered basal resistance by acquiring the ability to deliver effector proteins into plant cells (Chisholm “et al”). These effector proteins interfere with, manipulate, or suppress disease signaling, hereby enhancing pathogen growth and disease development. In response, during co-evolution plants have adapted to detect these specific pathogen-derived molecules. This cultivar-specific, gene-for-gene disease resistance system is determined by pathogen-encoded effector proteins and the corresponding plant derived R proteins [6]. Many Gram-negative bacterial pathogens possess the hypersensitive response and pathogenicity (hrp) gene cluster that encodes the type III secretion system (TTSS). TTSS is utilized by the bacteria for injection of the effector proteins into plant cells. (Zhang “et al”) demonstrated that P. syringae effector proteins AvrRpt2 and AvrRpm1 suppress PAMP-triggered defense responses in Arabidopsis by inhibiting flagellin-induced accumulation of callose. Moreover, another P. syringae effector, AvtPto, suppressed Arabidopsis genes encoding secreted cell wall and defense proteins. Some avirulence factors act by suppressing HR response, which is central in activating certain plant defense responses. Although many effector proteins have been cloned, the biochemical function of most remains unknown. AvrPtoB has been shown to have ubiquitin ligase activity in vivo. Deletion of key residues from this protein eliminated ubiquitin ligase activity and the capability of AvrPtoB to inhibit cell death. Thus, this effector was suggested to act by targeting proteins responsible for regulation of programmed cell death to degradation mimicking ubiquitin ligase of the host (Chisholm “et al”). However, if the effector protein meets a matching R gene in the plant, it becomes a specific elicitor and the plant defense system is activated by the R protein.
Molecular basis of Host Pathogen recognition
Plants, unlike mammals, lack mobile defender cells and a somatic adaptive immune system. Instead, they rely on the innate immunity of each cell and on systemic signals emanating from infection sites. But, many plant R proteins might be activated indirectly by pathogen-encoded effectors, and not by direct recognition. This ‘guard hypothesis’ implies that R proteins indirectly recognize pathogen effectors by monitoring the integrity of host cellular targets of effector action. The concept that R proteins recognize ‘pathogen-induced modified self’ is similar to the recognition of ‘modified self’ in ‘danger signal’ models of the mammalian immune system. It is now clear that there are, in essence, two branches of the plant immune system.

One uses transmembrane pattern recognition receptors (PRRs) that respond to slowly evolving microbial- or pathogen-associated molecular patterns (MAMPs or PAMPs), such as flagellin. The second acts largely inside the cell, using the polymorphic NB-LRR protein products encoded by most R genes. They are named after their characteristic nucleotide binding (NB) and leucine rich repeat (LRR) domains. NB-LRR proteins are broadly related to animal CATERPILLER/NOD/NLR proteins and STAND ATPases. Pathogen effectors from diverse kingdoms are recognized by NB-RR proteins, and activate similar defence responses. NB-LRR-mediated disease resistance is effective against pathogens that can grow only on living host tissue (obligate biotrophs), or hemibiotrophic pathogens, but not against pathogens that kill host tissue during colonization (necrotrophs) (Munch “et al”). The mechanism of this interaction of the plant immune system with pathogen can be represented as a four phased ‘zigzag’ model [10]

Modes of plant pathogen interactions
Direct Interaction
The “gene-for-gene” model was proposed to explain genetically the high specificity of plant-pathogen interaction and it predicts that there is a receptor/ligand-like interaction between plant R gene products and the corresponding pathogen-derived Avr gene products [11], [12] and [13]. Direct interaction between Avr proteins and R proteins was indeed demonstrated in a few cases, i.e. Pto-AvrPto (Tang “et al”), Pita-AvrPita (Jia “et al”) and RRS-1R and popP2 (Deslandes “et al”).

In a susceptible plant, RIN4 negatively regulates basal defense which is activated by PAMPs via the corresponding receptors (e.g. FLS2 for flagellin). The effector AvrRpm1 or AvrRpt2 is injected into an Arabidopsis cell via the TTSS. Binding of AvrRpm1 to RIN4 induces RIN4 phosphorylation, which could augment the ability of RIN4 to suppress basal defense, via an unknown mechanism. AvrRpt2 cleaves RIN4. It is not known how cleavage of RIN4 would enhance the suppressor function, unless the cleavage products are more potent suppressors AvrRpt2 targets multiple host proteins, so its function in defense suppression might be exerted via additional mechanisms (Nomura “et al”).
Indirect interactions (Guard Hypothesis)

Until recently, the only example of direct interaction between an NBS-LRR protein and a pathogen Avr determinant was the interaction described between the LRR domain of the rice Pi-ta CC-NBS-LRR protein and the Avr-Pita protein of the rice blast fungus *Magnaporthe grisea*. However, the absence of evidence for direct interaction in many other systems examined led to the formulation of the ‘guard’ hypothesis, which proposes that the interaction between an R protein and its cognate Avr determinant is mediated by a host protein that is the target for the effector function of the Avr determinant on the one hand, and under R-protein surveillance for such interference on the other. The examples showing strong support for the ‘guard’ hypothesis are protein RIN4 (RPM1 interacting) from Arabidopsis. RIN4 mediates interactions between the RPM1 (resistance to *P. syringae pv. maculicola*) CC-NBS-LRR protein and the AvrB and AvrRpm1 type III effector proteins from the leaf speck *bacterium P. syringae* on the one hand and between the RPS2 (resistance to *P. syringae pv. tomato*) CC-NBS-LRR protein and the AvrRpt2 type III effector protein also from *P. syringae* on the other. RPM1 and RPS2 form a complex with RIN4. AvrB and AvrRpm1 cause the phosphorylation of RIN4 and the consequent activation of RPM1 and AvrRpt2 causes the degradation of RIN4, thereby interfering with RPM1 function, but activating RPS2 [18].

Resistance genes

R genes have been defined genetically as the polymorphic component between susceptible and resistant plant genotypes without sequence knowledge of the loci involved. Isolation and characterization of plant R genes represents some of the most exciting discoveries in the field of plant pathology. In the past 15 years, over 60 plant R genes participating in activation of disease resistance to various pathogens ranging from nematodes, fungi, and bacteria to viruses have been cloned. In contrast to the diverse group of avr-encoded proteins, the most important feature of the cloned R genes is that 40 of them encode structurally related proteins with predicted extra cellular or intracellular lucine-rich repeat (LRR) motifs. LRRs can mediate protein-protein interaction and this is consistent with the postulated receptor function of R proteins. R proteins with intracellular LRRs also contain a putative nucleotide-binding (NB) domain that is either associated with an amino-terminal TIR (Toll/interleukin-1-receptor) homologous region or a CC (coiled-coil) domain (Ellis “et al”) as described in Table 1.
Table 1: Classes of Plant R genes according to Function.

<table>
<thead>
<tr>
<th>Class</th>
<th>Function</th>
<th>Example of R gene</th>
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<tbody>
<tr>
<td>I</td>
<td>Encodes a detoxifying enzyme</td>
<td><em>Hm1</em></td>
</tr>
<tr>
<td>II</td>
<td>Encodes a signal-transducing protein kinase</td>
<td><em>PTO</em></td>
</tr>
<tr>
<td>III</td>
<td>Encodes a receptor that transmits signal from protein kinase to phosphokinase for further amplification</td>
<td><em>Xa21</em></td>
</tr>
<tr>
<td>IV</td>
<td>Encodes cytoplasmic proteins that have a nucleotide-binding site and transmit signal to nucleus</td>
<td><em>N’, L6</em></td>
</tr>
<tr>
<td>V</td>
<td>Encodes protein in cytoplasmic membrane that reacts with elicitor product of <em>avr</em> gene</td>
<td><em>Cf9</em></td>
</tr>
</tbody>
</table>

Localized Signaling during Host pathogen interactions

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. 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 dependent on EDS1. RPW8 operates through ESD1 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. These four signaling cascades are important for resistance biotrophic pathogens. A different signal transduction network 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. 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; COI1, 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.

Systemic Signaling during Host pathogen interactions
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. PR1 signaling 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 nonpathogenic rhizobacteria colonizing plant roots. ISR requires both JA- and ET-mediated signaling 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.

Molecular basis of non-host resistance
A potential plant pathogen has to overcome many barriers to become an actual pathogen. The majority of potential pathogens fail to overcome these barriers and are never able to colonise a potential host plant. This non-host resistance may depend on passive preformed barriers, but it often depends on active responses following recognition of the pathogen or its activities as it attempts to penetrate the plant. Induced non-host resistance in plants is comparable to animal innate immunity, which activates pathogen resistance following host recognition of general PAMPs, which are
both indispensable for pathogenicity and unique to pathogens. Surface-derived structural molecules from plant pathogens, such as fungal cell wall constituents (chitin, glucan, protein and glycoprotein), bacterial lipopolysaccharide (LPS) and flagellin, elicit defence responses from a wide range of plant species. and these ‘elicitors’ are conceptually similar to PAMPs. Cell-wall-degrading enzymes, including endopolygalacturonase and xylanase, are ubiquitous as virulence effectors among plant pathogens, but can also function as elicitors. Their enzymatic products, such as plant-cell-wall-derived oligogalacturonides, are also known to induce plant defence responses.

**Conclusion**

In agriculture today, the persistent threat of loss of yield and quality from diseases is one of the most disruptive factors. At present it is being overcome mostly by means of agrochemicals. The isolation and preliminary characterization of R genes provide opportunities for producing plant varieties with disease resistance. To determine the molecular basis of disease resistance to a wide range of phytopathogens, and to determine the mechanisms with which R gene products recognize pathogen elicitors and the plant defense blocks pathogen growth will be future research goals in the field of plant pathology. The knowledge obtained from future research will undoubtedly help to produce durable disease resistance, and will help to reduce the use of environmentally damaging agrochemicals. In nutshell, decoration of plant world with best molecular arsenals who can defend themselves from pathogen attack is possible only by dissecting the molecular basis of plant pathogen interaction.

**References**


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