Pathogen Resistance Signalling in Plants

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Within natural ecosystems, most plants are resistant to most pathogens. At a fundamental level, this seemingly simply truth may hold the key to our understanding of how plants have evolved to survive under a myriad of environmental conditions and their associated stresses. Indeed, in defining how plants evolve, adapt and maintain broad spectrum resistance to most pathogens – typically referred to as non-host resistance – we may not only reveal the mechanisms that underpin plant resistance signalling but also the precise manner in which plants regulate these processes under various environmental conditions. Herein lies the greatest challenge and unanswered question in the field of agriculture today: How do we feed 9 billion people by the year 2050? To address this, one of the first hurdles that must be overcome is a full understanding of the processes that regulate stress (i.e. abiotic and biotic) signalling in plants, as well as the processes that define pathogen and host specificity, including the performance of these processes under rapidly changing environmental conditions. In the case of pathogen infection, plants utilise a broad suite of innate and inducible mechanisms to resist invasion. In large part, these processes are governed by the activity of resistance (R) proteins, which are evolutionarily conserved and highly evolved proteins that function not only in pathogen recognition but also in the activation of the cellular processes necessary to defend against proliferation and the elicitation of disease. Furthermore, recent data supports the hypothesis that numerous processes, such as the balance between growth and defence, also contribute to the host resistance and pathogen virulence.

Plant Disease, Pathogen Virulence, Environmental Change and Human Influence

The Green Revolution, led by the pioneering work of Norman Borlaug in the mid-1960s, yielded increases in crop production around the world, and as a result, saved more than a billion people from starvation. Over the past 50 years, world population growth has more than doubled, and current estimates suggest that numbers may reach as high as 12 billion people by the year 2100 (Gerland et al., 2014). To keep pace with this increasing population growth, it is estimated that significant improvements in agriculture are needed to meet a demand of an approximate 50% increase in food consumption. As a critical component in meeting this challenge, translational research in plant stress tolerance represents one of the primary areas of focus; this includes plant
protection against pathogen infection, rising temperatures, flooding, drought, freezing tolerance and protection from insect pests. As each of these environmental stresses contributes to significant decreases in crop production, losses due to biotic stress alone are estimated to impact global crop production by 15% annually (Dangl et al., 2013). These estimated impacts further support the need for advances in biotic stress research in plants, as well as translational applications towards the generation of improved breeding lines.

To understand and define what plant immunity is, and moreover, how plant immunity functions, it is important to first identify the underlying genetic basis that defines how plants and microbes interact. Plant disease epidemics are rare within natural ecosystems; heterogeneity has a demonstrable positive impact on plant performance and survival (Pagan et al., 2012; Johnson and Thielges, 2010; Roscher et al., 2007). In recent decades, the impact of climate change, combined with changes in modern agricultural practices (e.g. cropping intensification and chemical management), have had significant impacts on crop production, particularly with regard to the incidence of disease severity and pathogen adaptation to biotic and abiotic stress. Indeed, while agricultural disease epidemics are historically rare, recent disease outbreaks in a number of key staple crops around the world indicate that a number of challenges and opportunities still remain in terms of meeting the global demands related to food production. Historically, the ‘Disease Triangle’ model has been used to describe how plants, pathogens and the environment interact to drive resistance and susceptibility (Scholthof, 2007). Within this basic framework, however, are a multitude of complex interactions that influence host performance during pathogen infection. Modern agricultural practices, including disease management through pesticide application, high-density monoculture production and the repeated use of crops containing selected R genes collectively impose a number of strong selective pressures which ultimately drive enhanced pathogen virulence. This results in a ‘defeat’ in R gene efficacy, which in turn leads to increases in disease outbreaks (Figure 1).

Plant Recognition of Pathogens

Plants rely on a suite of fundamentally complex, highly conserved mechanisms to detect changes in their environment, including the perception of potential pathogenic microorganisms. In many cases, the mechanisms that underpin abiotic and biotic stress signalling share many signalling components. For example, a number of studies have shown that plant response to drought and pathogen infection converge at a core set of signalling pathways (Nakashima et al., 2014); the same is true for the convergence of pathogen perception and response to temperature (Hua, 2013), light (Sano et al., 2014) and touch (Braam, 2005). As a first barrier to pathogen infection, and as an initial stimulus required for the activation of robust immune signalling, plants have evolved mechanisms to recognise the most conserved features of a wide range of potential biotic threats. Highly conserved regions associated with a wide variety of microorganisms have been termed pathogen-associated molecular patterns (PAMPs). These molecules are essential to the function of the organism and represent the most evolutionarily conserved components of the organism; as such, they are typically indispensable for survival. Moreover, and as an illustration of their functional specificity, PAMPs are further defined as uniquely ‘non-self’, meaning that these molecules are not found in plants. Examples of PAMPs include features such as the bacterial flagellum, components of the fungal cell wall and features functionally associated with organismal lifestyle (Table 1). In addition to PAMPs, some molecules function as a signal of warning for surrounding cells; such is the case of damage-associated molecular patterns (DAMPs) – plant-derived compounds involved in the signalling of invading insects or wounding damage. Like PAMPs, DAMP perception initiates signalling associated with the production of important hormones involved in plant defence and immune signalling (Tintor et al., 2013; Zipfel, 2014). Once recognised by the host, PAMPs/DAMPs initiate a series of general signal transduction cascades within the host cell to prevent proliferation of the invading pathogen. This first layer of plant immunity, termed pathogen-associated molecular pattern-triggered immunity (PTI; Chisholm et al., 2006), is best defined as a low-amplitude defence response that does not result in localised cell death, but rather initiates a highly regulated response that is effective in blocking most pathogens from proliferating and causing disease (Figure 2).

To further illustrate the significance and highly conserved function of PTI, it is noteworthy that a number of advances in human pathogen and disease research were preceded by the discovery and characterisation of PAMP receptors (e.g. pattern recognition receptors; PRRs) in plants (Gomez-Gomez and Boller, 2000) (Figure 3). This work, supported by the most expensive plant genome initiative to date – The Arabidopsis Genome Initiative (2000) – resulted in the discovery of the largest class of recognisable transmembrane (TM) sensors, or PRRs, by identifying more than 300 receptor-like kinases (RLKs). Generally, PRRs described to date possess an extracellular leucine-rich repeat (eLRR; Figure 3) and a cytosolic kinase domain that functions in downstream signalling following elicitor binding. Of the most studied PRRs in plants is the Arabidopsis thaliana receptor-kinase protein FLS2 (FLAGELLIN SENSITIVE2, Gomez-Gomez and Boller, 2000; Zipfel et al., 2004), which binds a fragment of bacterial flagellin. Once bound to its ligand, FLS2 initiates a series of signal transduction cascades, including activation of the mitogen-activated protein kinase (MAPK) pathway, concomitant changes in gene expression and the induction of localised changes in cellular organisation, all of which function to specifically counter further infection. Similar responses have also been characterised following the activation of additional plant PRRs via PAMP perception, including the activation of EFR (EF-Tu RECEPTOR) by the bacterial elicitor EF-Tu (Kunze et al., 2004; Zipfel et al., 2006) and the activation of CEBiP (CHITIN ELICITOR BINDING PROTEIN) and OsCERK1 (CHITIN ELICITOR RECEPTOR KINASE) by fungal-derived chitin during infection of rice (Kaku et al., 2006; Shimizu et al., 2010). In all instances, ligand–receptor association results in the activation of functionally similar highly conserved mechanisms whose cellular responses have evolved to prevent pathogen infection.
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Human influence

Adaptation

Disease management

Modern breeding and monoculture

Selection

Environment

Host

Pathogens

Figure 1 Illustration of the interactions between plants, pathogens, humans and the environment. As an expansion of the 'Disease Triangle' paradigm, human influence on modern agricultural practices has significantly impacted not only the performance of cropping systems as a function of biotic stress performance but also the inadvertent selection of enhanced, often hyper-virulent pathogen races. As environmental conditions and abiotic stressors become more taxing on both plants and their pathogens, the influence of high temperatures, for example, has opposing effectors. For example, at high temperatures, the growth and selection of microbes with enhanced virulence is often observed. Conversely, at high temperatures, plant performance is often negatively affected, with plant resources and signalling processes shifted to abiotic stress tolerance/response signalling. As a result, plants are ‘forced to choose’ between survival due to abiotic stress and to allocate signalling to support immunity and defence signalling. As a key feature of the interaction of plants with environmental and microbes, human influence on this process is also becoming more evident. As populations increase, there is also an increase in the need to produce crops that generate higher yields, as well as perform under what are typically limiting environmental conditions (i.e. high temperature and drought). As a result, plant breeders must often choose between a balance of phenotypic traits and genotypes to introduce lines with elite performance under one or the other conditions, including the introduction of lines with enhanced nutritional or horticultural qualities. As a function of disease management, chemical treatment of crops with pesticides/fungicides influences not only plant performance under biotic stress conditions but can often times have a negative – unintended – influence on the selection and propagation of hyper-virulent, pathogenic microbes. As a combination of each of these factors, we present an updated model of the classical 'Disease Triangle' to highlight the influence of humans on agricultural systems and plant performance: the 'Disease Diamond'. Image reproduced from Hammond-Kosack and Kanyuka (2007).

Table 1 Pathogen-associated molecular patterns (PAMPs) and plant-derived signals (damage-associated molecular patterns; DAMPs)

<table>
<thead>
<tr>
<th>Elicitor</th>
<th>Pathogen</th>
<th>Receptor</th>
<th>Associated signalling molecules</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flagellin (flg22)</td>
<td>Bacteria</td>
<td>FLS2</td>
<td>BIK1, BAK1, MAPK, CDPK, ROS</td>
<td>Gomez-Gomez and Boller (2000)</td>
</tr>
<tr>
<td>EF-Tu (elf18)</td>
<td>Bacteria</td>
<td>EFR</td>
<td>BIK1, BAK1, MAPK, CDPK, ROS</td>
<td>Kunze et al. (2004)</td>
</tr>
<tr>
<td>Chitin</td>
<td>Fungi</td>
<td>Cerk1, LYK5 <em>Arabidopsis</em>; CEBiP, OsCERK1 (Rice)</td>
<td>MAPK, CDPK</td>
<td>Kaku et al. (2006); Shimizu et al. (2010)</td>
</tr>
<tr>
<td>LPS</td>
<td>Bacteria</td>
<td>Unknown</td>
<td>–</td>
<td>Erbs et al. (2010)</td>
</tr>
<tr>
<td>Coronatine</td>
<td>P. syringae</td>
<td>COI1</td>
<td>Jasmonic acid</td>
<td>Sherd et al. (2010)</td>
</tr>
<tr>
<td>Coat protein</td>
<td>Virus</td>
<td>Unknown</td>
<td>–</td>
<td>Gonsalves, 1998</td>
</tr>
<tr>
<td>Pep1</td>
<td>–</td>
<td>PEPR1</td>
<td>Ethylene</td>
<td>Tintor et al. (2013)</td>
</tr>
<tr>
<td>ATP</td>
<td>–</td>
<td>DORN1</td>
<td>WRKY, CDPK</td>
<td>Choi et al. (2014)</td>
</tr>
<tr>
<td>Oligogalacturonide</td>
<td>–</td>
<td>WAK1</td>
<td>–</td>
<td>Zipfel (2014)</td>
</tr>
</tbody>
</table>
As with most ligand–receptor interactions, additional signalling components have been shown to be required for their full activity. In large part, these signalling components function not only in the activation of resistance but through association with other cellular processes and pathways function to attenuate antagonistic interactions that may impede full immune activation. Of these, a receptor-like cytoplasmic kinase, *BOTRYTIS*-INDUCED KINASE1 (BIK1) and BRI-ASSOCIATED KINASE1 (BAK1) have been shown to associate with PAMP receptor complexes involving FLS2 and EFR, respectively. Once associated, these complexes initiate downstream signalling cascades, in MAPK and calcium-dependent protein kinase (CDPK) signalling (Figure 3; Macho and Zipfel, 2014). In total, the initiation of these processes leads to numerous defence-associated processes, including transcriptional reprogramming, reactive oxygen species (ROS) production and cellular changes resulting in defence against pathogens (Figure 3).

Of direct relevance to crop sustainability and agricultural output, an understanding of the molecular-genetic and biochemical processes described above can have significant, demonstrable impact. For example, translation of fundamental research defining the activity and function of the *Arabidopsis* PAMP receptor EFR has shown promise in improving resistance of tomato plants to the bacterial pathogen *Ralstonia solanacearum*, the causal agent of bacterial wilt. In brief, when susceptible tomato plants are genetically modified (GM) to express the *EFR* gene from *Arabidopsis*, these plants, when infected with *R. solanacearum*, showed reduced bacterial populations and a significant reduction in disease symptoms (Lacombe et al., 2009). Additional studies have demonstrated the broad utility of similar approaches showing that the kinase domain of Xa21, a PRR from rice, is able to functionally complement the *Arabidopsis* PRR EFR (Holton et al., 2014), providing further evidence in support of the need to exploit the conservation of immune signalling in plants as a tool.
to identify new sources of pathogen resistance. It is also noteworthy that the perception of evolutionarily distant pathogens can lead to the resistance of the host plant by pre-activation of the PTI immune response. For example, a recent study shows that the perception of bacterial flagellin by FLS2 can prime immunity, resulting in a reduction of growth of the necrotrophic fungal pathogen, Botrytis cinerea, when plants are first subjected to the 22 amino-acid flagellin peptide elicitor, flg22 (Laluk et al., 2011). Taken together, this work suggests that the activation of PTI is sufficient for broad-based immune responses to a diversity of plant pathogens.

**Inheritance of Resistance**

In the 1940s, seminal research by Harold H. Flor established the foundation for modern molecular plant pathology, formulating what is referred to as the ‘Gene-for-Gene’ hypothesis (Figure 4). Using the interaction between flax (Linum usitatissimum) and the rust pathogen Melampsora lini, Flor demonstrated that the outcome of this host–pathogen interaction could be defined through a tractable, yet simple genetic relationship. In brief, Flor demonstrated that the inheritance of resistance in the host and the ability of the pathogen to cause disease are defined by the reciprocal relationship and presence of ‘matching’ gene pairs. In the plant, this gene is defined as a ‘resistance (R) gene’, and in the pathogen, the complementary gene is called the ‘avirulence (Avr) gene’ (Flor, 1955). Subsequent work has shown that an alteration in the function, or a complete loss of either the R or Avr gene, results in the onset of disease in infected plants. This initial discovery has had a significant impact on plant breeding, and has ushered in a new era in modern agriculture; for the first time, the genetic basis of resistance was defined in a manner that gave researchers a means to track the ‘genotype × phenotype’ relationship. More importantly, this work provided a blueprint from which additional work in this area can explore the potential of conferring broad pathogen resistance in plants through the expression of dominant, conserved R genes.

In most cases, the primary tenets of the ‘Gene-for-Gene’ hypothesis are still widely used, and as noted above, provide the basis for the formulation of numerous hypotheses aimed at defining the genetic interactions between plants and microbes. While
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Classical Illustration of the genetic foundation for the ‘Gene-for-Gene’ interaction. Proposed by Flor in the 1940s, this foundational hypothesis describing the inheritance of resistance in plants and the maintenance of virulence in pathogens posits that for every gene in a plant that confers resistance, there is a corresponding, host-recognised, gene in the pathogen that confers avirulence. In the presence of both genes (i.e. $R + Avr$), plants are resistant. When either gene is lacking (i.e. $r + avr$), plants are susceptible. This basic process has been used to define a number of $R/Avr$ interactions in plants in the 70+ years since it was first discovered, and moreover, it provided a tractable mechanism to guide a number of plant breeding outcomes aimed at the generation and selection of plants with broad pathogen resistance. Image reproduced from Hammond-Kosack and Kanyuka (2007).

not broadly applicable to all host–pathogen interactions, much of our understanding of the activation of immune signalling in plants relies on the fundamentals described by Flor almost 75 years ago. As is discussed below, plant breeders often use the inheritance of dominant resistance (i.e. $R$) gene as a tool to develop and introduce new resistance traits from wild relatives into commonly grown crop species. In some cases, this results in stable, durable outcomes. Such is the case of Rxo1/AvrRxo1, which has been demonstrated to confer resistance in rice to many bacterial pathogens, further supporting the utility of genetic modification of important crop species for the improvement of disease resistance traits (Liu et al., 2014).

Plant Resistance Genes

By the early 1990s, advanced tools in molecular biology and genetics had opened new avenues of research in plant pathology, giving rise to the cloning of the first avirulence genes in plant pathogens (Staskawicz et al., 1984), the identification and cloning of the first plant resistance genes (Mindrinos et al., 1994; Bent et al., 1994) and the sequencing of the first plant genome (Arabidopsis Genome Initiative, 2000). With these new tools, researchers were armed with the resources to not only explore the mechanisms of resistance in plants and the virulence of their associated pathogens but also apply fundamental research and knowledge to a broad range of crop species. As shown in Table 1, work in this area has not only improved our understanding of the basis of plant immune signalling but also generated a wealth of knowledge, specific to disease resistance, which can be used to address additional challenges affecting crop production and food security.

As noted above, research over the past 20 years has ushered in significant advances in our understanding of the genetic basis of pathogen resistance signalling in plants. During this time, with parallel advances in genomics and bioinformatics, the number of resistance genes identified and characterised from a multitude of plant species has grown significantly, and in many regards, this new information reminds us that we are still in the infancy of our understanding and the deployment of this knowledge (Table 3). Indeed, since the identification and isolation of the first plant $R$ genes almost 25 years ago, additional other $R$ genes – and genes associated with their function – have been cloned from important staple crops, including barley, potato, tomato, rice and wheat (Dangl et al., 2013). Using a combination of functional genomics and bioinformatics, coupled to lab-based molecular-genetic approaches, research over the past 20 years has revealed that most of the $R$ proteins identified can be classified into just a few categories; this classification is based largely on predicted structural motifs (i.e. coiled-coil domains). For a thorough overview of the modular structures of plant $R$ proteins, refer to the review by Dangl and Jones (2001); Figure 5). Among the plant $R$ proteins described thus far, the largest group is represented by those containing a nucleotide-binding (NB) site and leucine-rich repeat (LRR) domain (i.e. NB-LRR); this family is further segregated based on the presence of a leucine-zipper (LZ) or coiled-coil (CC) sequence at the amino terminus. Interestingly, NB-LRR resistance proteins from plants possess high sequence homology to the Toll and Interleukin 1 receptor (TIR) proteins associated with...
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Race Specific R proteins

Race non-Specific R proteins

Cell wall

Plasma membrane

Cytoplasm

Kinase

Kinase

ECS

PEST

LRD

CC

NB

TIR

RRS1

N,L6, RPP5

Mla10, RPM1, RPS2

BS2

Pi-ta

Pto, PBS1

Xa27

RPG1

Pathogen Avirulence and Effector Genes

The activation of PTI in response to pathogen infection is a highly effective mechanism to restrict pathogen invasion and proliferation. So, how do pathogens survive and propagate in the face of a resistance mechanism that is, more often than not, sufficient to prevent infection and disease? In simplest terms, pathogens – whether from plants or animals – evolve strategies to overcome host defences; in short, this is accomplished by either evading preformed defence responses or by blocking the activation of host processes, such as PTI. As a tractable system and working model to define the inheritance of resistance, the ‘Gene-for-Gene’ hypothesis offers a logical framework within which numerous host–pathogen interactions can be studied. However, as a means to describe the virulence capacity of a pathogen, and moreover, to define how pathogens successfully overcome host defence signalling, the R–Avr interaction does not fully explain this process. In fact, it begs the question: Why does a virulent pathogen make and secrete Avr proteins into their host, only to be recognised by the plant and to lead to the activation of resistance?

To address this, a number of research groups have also focused on the identification and characterisation of virulence factors from a broad range of bacterial, fungal and viral plant pathogens (Table 1). Similar to virulence strategies previously identified in human pathogenic microorganisms, it was discovered that plant pathogenic bacteria (e.g. Pseudomonas syringae, Erwinia amylovora) utilise a type-three secretion system (T3SS) to deliver a suite of proteins – termed effectors – into the plant cell during infection. As noted above, the T3SS identified in plant pathogens

Figure 5 R protein classes and their cellular location. The predicted domains of R proteins which confer either race-specific or race-non-specific resistance are presented schematically: CC, coiled-coil domain; TIR, Toll and Interleukin 1 receptor-like motif; NB, nucleotide binding site; LRD, leucine-rich domain; LRR, leucine-rich repeat; NLS, nuclear localisation signal; ECS, endocytosis signal; PEST, Pro-Glu-Ser-Ther-like sequence; WRKY, motif characteristic of some plant transcription factors; 1, 2, 3, 4 – novel domains that lack significant homology to known proteins; 5, domain with homology to a B-lectin; 6, structure with a weak similarity to a PAN domain; 7, structure with homology to epidermal growth factor (EGF)-like domain; Cl-2, Cl-4 and Cl-5 confer resistance to Cladosporium fulvum races expressing, respectively, Avr2, Avr4 and Avr5; L6 flax rust resistance 6; Mla10, resistance to Blumeria graminis f. sp. hordei expressing Avra10; RPM1, resistance to P. syringae pv. maculicola expressing AvrRpm1 or AvrB; RPP5, resistance to Hyaloperonospora parasitica expressing ATR5. Image reproduced from Hammond-Kosack and Kanyuka (2007).
was homologous to that described in a number of well-studied Gram-negative bacterial pathogens of humans (e.g. Escherichia coli; Salmonella; He et al., 2004). Using this system, pathogenic bacteria deliver what are termed type-three secreted effectors (T3Es), which are protein virulence factors hypothesised to have evolved as a means to alter host physiology during the infection process. As noted above, and more relevant to their role during host–pathogen signalling, T3Es broadly function to suppress the activation of plant defence signalling, including processes associated with PTI signalling. To date, at least 28 unique T3Es have been identified as secreted proteins from the model plant pathogenic bacteria, P. syringae pv. tomato DC3000, and the collective works from a number of groups have shown that T3Es target a broad range of cellular processes – and their associated regulators – during infection (Figure 2 and Table 2; Xin and He, 2013).

In addition, genome sequencing, combined with functional studies using non-model systems, has revealed bacterial pathogens with a small amount of secreted effectors, as in the case of E. amylovora (Triplet et al., 2010). However, the reduced number of secreted effectors does not seem to affect the virulence of the pathogen on its host, as recent disease epidemics have been documented in apple orchards throughout the United States over the past decade (Malnoy et al., 2012). Alternatively, other plant pathogens are predicted to have an expansive secreted effector repertoire, as is the case of the oomycete pathogen Phytophthora infestans, the causal agent of the Irish Potato Famine, which has 563 predicted effector proteins, which have RNA expression levels that are induced during early stages of infection and contain an RXLR motif (Hass et al., 2009). Interestingly, many T3Es have been shown to target basic physiological processes within the host cell to promote its virulence during infection, suggesting that specifically inhibiting these basic cellular processes are important in promoting pathogen growth and reproduction.

### The Guard Hypothesis

While a number of predicted structural (i.e. domain motifs) differences exist among the various families of R proteins identified thus far in plants, many share a number of striking similarities with regard to functionality in immune signalling. With regard to specificity, it is hypothesised that the majority of R proteins function to recognise pathogen effectors – either by direct association or via the consequence of effector activity – and activate resistance in response to pathogen infection according to the basic criteria established in the ‘Gene-for-Gene’ hypothesis. At a fundamental level, we now know that recognition of pathogens by plants is mediated by the R–Avr interaction; we also know that this recognition can occur through direct association of the R–Avr pair (Jones and Dangl, 2006; Liu et al., 2014; e.g. Pita-AvrPita, or via the indirect association of these paired genetic determinants (Chisholm et al., 2006). In the case of the latter – the indirect recognition and activation of resistance by R–Avr pairs – research has shown that the association of plants and pathogens can be described and conceptually defined through an elegant series of evolutionary steps; these include recognition of the invading pathogen, adaptation by the pathogen and the evolution of new resistances. The identification of the T3SS and their associated T3Es opened a number of new directions in the field of molecular plant pathology. In the late 1990s, several hypotheses were proposed to define how R and Avr proteins function, and moreover, how each specifies the activation of resistance or promotes the elicitation of susceptibility. During this time, a number of important

### Table 2 Major devastating plant pathogens

<table>
<thead>
<tr>
<th>Species</th>
<th>Kingdom</th>
<th>Biological characteristics</th>
<th>Predicted effectors</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnaporthe oryzae (Rice Blast)</td>
<td>Fungus</td>
<td>Filamentous ascomycete fungus; causes 10–30% yield losses</td>
<td>&gt;40 cloned and mapped</td>
<td>Dean et al. (2012); Valent and Khang (2010)</td>
</tr>
<tr>
<td>Xanthomonas oryzae pv. oryzae</td>
<td>Bacteria</td>
<td>Rod shaped gram-negative; 10–50% yield losses</td>
<td>~16 effectors are translocated into the host cell</td>
<td>Furutani et al. (2009)</td>
</tr>
<tr>
<td>Pseudomonas syringae pv. tomato</td>
<td>Bacteria</td>
<td>Yield losses of 5–75% in the field</td>
<td>~28 effectors have been shown to be translocated to the host cell</td>
<td>Xin and He (2013)</td>
</tr>
<tr>
<td>Botrytis cinerea (Grey Mold)</td>
<td>Fungus</td>
<td>Necrotrophic fungus, infects more than 200 plant species</td>
<td>~880 predicted by genome sequencing</td>
<td>Amselem et al. (2011)</td>
</tr>
<tr>
<td>Pseudoperonospora cubensis (Cucurbit Downy Mildew)</td>
<td>Oomycete</td>
<td>Obligate biotroph; causes yield losses up to 100%</td>
<td>125 predicted RXLR effectors from genome sequencing</td>
<td>Burkhardt et al. (2015)</td>
</tr>
<tr>
<td>Erwinia amylovora (Fire Blight)</td>
<td>Bacteria</td>
<td>Gram-negative; outbreaks cause devastating losses up to 100%</td>
<td>~5 secreted</td>
<td>Triplet et al. (2010)</td>
</tr>
<tr>
<td>Phytophthora infestans</td>
<td>Oomycete</td>
<td>Hemibiotroph; causal agent of the Irish Potato Famine</td>
<td>563 predicted RXLR effectors from genome sequencing</td>
<td>Hass et al. (2009)</td>
</tr>
<tr>
<td>Common name</td>
<td>Genome size</td>
<td>Major pathogens</td>
<td>Number of NB-LRR genes</td>
<td>Year of sequence</td>
</tr>
<tr>
<td>---------------------</td>
<td>-------------</td>
<td>-----------------------------------------------------</td>
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<td>------------------</td>
</tr>
<tr>
<td>Human</td>
<td>2.91 Gb</td>
<td><em>Mycobacterium tuberculosis</em>, <em>Escherichia coli</em> O157:H7</td>
<td>Not applicable</td>
<td>2001</td>
</tr>
<tr>
<td>Rice</td>
<td>389 Mb</td>
<td><em>Xanthomonas oryzae</em>, <em>Magnaporthe oryzae</em></td>
<td>600</td>
<td>2002 and 2005</td>
</tr>
<tr>
<td>Papaya</td>
<td>372 Mb</td>
<td><em>Papaya ringspot virus</em> (PRSV)</td>
<td>55</td>
<td>2008</td>
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<tr>
<td>Cucumber</td>
<td>367 Mb</td>
<td><em>Pseudoperonospora cubensis</em>, <em>Phytophthora capsici</em></td>
<td>61</td>
<td>2009 and 2011</td>
</tr>
<tr>
<td>Sorghum</td>
<td>730 Mb</td>
<td><em>Fusarium moniliforme</em>, <em>Puccinia purpurea</em></td>
<td>211</td>
<td>2009</td>
</tr>
<tr>
<td>Apple</td>
<td>742 Mb</td>
<td><em>Erwinia amylovora</em></td>
<td>799</td>
<td>2010</td>
</tr>
<tr>
<td>Soybean</td>
<td>1.1 Gb</td>
<td><em>Fusarium virguliforme</em>, <em>Pseudomonas syringae pv. glycinea</em></td>
<td>Not analysed</td>
<td>2010</td>
</tr>
<tr>
<td>Coffee</td>
<td>710 Mb</td>
<td><em>Hemileia vastatrix</em> (Coffee Rust)</td>
<td>561</td>
<td>2014</td>
</tr>
<tr>
<td>Loblolly Pine</td>
<td>22 Gb</td>
<td><em>Fusarium circinatum</em></td>
<td>Not analysed</td>
<td>2014</td>
</tr>
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discoveries were made, including the formulation of the basis for what has now become the leading paradigm in the field of molecular plant pathology: the ‘Guard Hypothesis’. During the early stages of research aimed at defining the molecular and biochemical basis of gene-for-gene-mediated resistance, the hypothesis that the interaction of R and Avr proteins functioned as a ‘lock and key’ mechanism remained the most logical explanation to define how plants and pathogens communicate (Staskawicz et al., 1984). At the same time, it did little to explain how plants and pathogens evolve to overcome the seemingly ‘back-and-forth’ evolution of resistance and susceptibility. In 1998, a review by Van Der Biezen and Jones proposed what has now known as the ‘Guard Hypothesis’:

Conceivably, one particular Avr product could correspond to one specific pathogenicity target, which, in turn, could be safeguarded by one matching R protein. In order to adapt rapidly to pathogen Avr modification or loss, novel recognition specificities in R proteins are created through the generation of sequence variation in the β-sheet of the LRR domain.

Since this time, overwhelming evidence from a number of plant–pathogen interactions, including those from plants interacting with bacteria, fungi and oomycetes, have dissected the molecular and biochemical mechanisms underpinning pathogen recognition and immune signalling in plants. Of these, one of the best-characterised examples is that from the A. thaliana–P. syringae interaction, whereby the activation of multiple R proteins is ‘guarded’ by the plant protein RIN4. As illustrated in Figure 3, RIN4 association with the R proteins RPS2 and RPM1 functions to negatively regulate resistance activation. Upon delivery of the T3Es AvrRpt2, AvrRpm1 and/or AvrB into the host cell, RIN4 is targeted by the activity of the effectors, and this process leads to release of the negative regulation of these R proteins and immune signalling is activated. Thus, through this indirect mechanism, plants have evolved a means to recognise pathogens by ‘guarding’ the activation of resistance through the utilisation of a conserved pathogen target (Chisholm et al., 2006).

Interplay of Plant Growth and Development in Defence

Above, we described how resistance is activated by specific pathogen-triggered responses, which in turn leads to the amplification of host signalling pathways required for robust immune signalling. As an illustration of the complexity of defence activation and attenuation, recent work has shown that many of the basal immune signalling pathways in plants are associated with processes that regulate host physiology (e.g. growth and development and circadian response), as well as response to environmental change and abiotic stress (Hua, 2013; Huot et al., 2014). From these collective works, a complex, often-interlinked regulatory network has been deciphered.

During its life cycle, any given plant may come into contact with a multitude of biotic and abiotic pressures; during this, plants must balance signalling between growth, development and survival. As an illustration of the convergence between plant growth and immune signalling, recent work has shown that the basal immune signalling responses – that require the function of PTI-associated receptor complexes – also play essential roles in host signalling processes associated with environment (Huot et al., 2014). As detailed above, the identification and characterisation of FLS2 and EFR opened the door to a multitude of studies aimed at defining the primary immune signalling processes required for pathogen perception and the activation of plant immunity. From this work, a complex web of interactions was uncovered, leading to the identification of additional signalling molecules required for the activation – and regulation – of immunity in response to the basal recognition of pathogens. Among these, the identification of BIK1 and BAK1 has led to an increased understanding of the balancing relationships between immunity, host physiology and environmental response. BIK1 was identified as being required for resistance activation in Arabidopsis in response to the necrotrophic fungal pathogens B. cinerea and Alternaria brassicicola; interestingly, however, BIK1 is not required, but instead blocks immune signalling in response to infection by the bacterial pathogen P. syringae (Macho and Zipfel, 2014). As an immune regulator, BIK1 is required for the activation of PTI-associated processes, a process mediated in part by its physical association with key RLKs (e.g. FLS2). As a key regulator of host physiology, and in the absence of pathogens, BIK1 is known to be required for plant response(s) associated with ethylene perception and signalling, processes that function antagonistically to the key immune-associated hormone (e.g. jasmonic acid and salicylic acid) response pathways (Macho and Zipfel, 2014).

Similar to BIK1, another signalling component associated with PTI receptor activation and signalling has been identified that potentially links PAMP signalling with growth and hormone response. In brief, the LRR receptor kinase BAK1, described above, was shown to bind the hormone brassinolide, a member of the brassinosteroid family of hormones that promotes cell division and elongation. Upon brassinolide binding, BRI1 activates BAK1 through a series of phosphorylation cascades, from which it is hypothesised that the recruitment and subsequent activation of BAK1 to membrane-specific signalling systems mediates the differential signalling of defence and growth (Macho and Zipfel, 2014). At present, the function of BAK1 in innate immune signalling is hypothesised to be independent of its function in hormone signalling.

Plant–Pathogen Interactions and the Influence of the Environment

As a function of immune activation and signalling (i.e. ETI), the impact of environment and temperature has also been demonstrated to impact the performance of plant R and pathogen Avr genes. Indeed, substantial research in this area has shown that changes in temperature affect a wide range of biotic and abiotic factors, including response to light, growth and development (Hua, 2013). For example, temperature fluctuations have been
found to have significant impacts on pathogen virulence; in mammals, many pathogen virulence genes are induced at 37 °C and attenuated at temperatures at or below 30 °C (Steen et al., 2002; Kimes et al., 2012). Similarly, in plant–pathogen interactions, numerous virulence factors are induced at low (i.e. c. 20 °C) temperatures and repressed at temperatures above 30 °C (Hua, 2013). From this work, it has been hypothesised that a complex trade-off exists, in part as a direct function of pathogen physiology: at high temperatures, the activation of ETI is reduced in part due to a block in effector secretion, while at the same time, PTI signalling is enhanced as a consequence of enhanced bacterial proliferation (Hua, 2013).

The ‘Disease Triangle’ predicts that the onset and incidence of disease is coincident with an interaction of factors unique to the host plant, the pathogen and the environment. In short, high disease pressure is observed when pathogen populations increase, which can be influenced by any number of factors, including environmental temperatures, host performance and intensive monoculture. When faced with these pressures, pathogens adapt – through mutation – to overcome resistance. Higher temperatures typically favour increased pathogen proliferation; this is true in both mammals and plants. In addition, in plants, higher temperatures generally mean additional abiotic stresses placed on the plant, such as drought and heat stress (Nakashima et al., 2014). As a function of host performance, higher temperatures are also hypothesised to accelerate the breakdown (or loss in performance) of R genes; this is predicted to be a result of increased pathogen pressure on the host. There are, however, examples of R genes whose performance is directly influenced by temperature. For example, in wheat, the R gene Yr59 has been shown to perform ‘better’ at higher temperatures, as revealed by increased resistance to the rust pathogen Puccinia striiformis f. sp. tritici. At low temperatures, pathogen virulence is favoured, and wheat is susceptible to infection and disease (Bryant et al., 2014). There are also described examples where the converse is true: the inhibition of R gene-mediated resistance at higher temperatures has also been described.

Genome-Enabled Approaches in Plant Biology and Agriculture

Over the past two decades, the elucidation of the genetic architecture of numerous plant species has provided a treasure-trove of valuable resources to not only define the molecular-genetic and biochemical basis of numerous plant processes but also provide a foundation to further describe the evolution of these processes. This work achieved a milestone in the early 2000s with the completion of the Arabidopsis genome, and since this time, the further development and use of new technologies have led to a substantial increase in the number of resources and tools at our disposal (Table 3). For example, in recent years, significant advances in genome sequencing (e.g. Illumina) and their application to host–pathogen interactions have resulted in high-throughput cost-effective methods for plant genotyping and sequencing transcriptional changes during infection (Hamilton and Buell, 2012). In total, these technologies have facilitated countless advances in transcriptomic-based studies, facilitating not only a better understanding of the function and consequence of transcriptional reprogramming during infection but also the impact of environmental stress on the ultimate outcome of the host–pathogen interaction (Wang et al., 2014b). Since the release of the Arabidopsis genome sequence in 2000, the number of plant genome sequences elucidated continues to increase at a seemingly limitless pace (Table 3). In total, comparative analyses of these genomes has led to a greater understanding of the complexity and evolution of plants, as well as has identified a number of shared commonalities related to the function of cellular processes, including the mechanisms that underpin plant–pathogen interactions. Indeed, the enabling power of the Arabidopsis genome sequence has allowed scientists to identify important protein homologues in non-model species to further investigate and define the conservation and function of immune signalling across species. In many cases, sequence-similar homologues have been shown to have similar functions in model and non-model plant species. For example, the citrus orthologue of the Arabidopsis protein NDR1 (NON-SPECIFIC DISEASE RESISTANCE1) has been shown to functionally complement the Arabidopsis ndr1 mutant plants (Century et al., 1995; Knepper et al., 2011) to restore resistance to bacterial pathogens (Lu et al., 2013). Future comparative analyses between model and non-model systems may similarly prove pivotal in defining key mechanisms required for durable, sustainable resistance in important crop species.

Plant Pathogens and Genome Editing

As described above, recent advances in sequencing technologies have resulted in the generation of a wealth of information useful in guiding plant breeding to address abiotic and biotic stress. In addition, previous work on fundamental questions in plant–pathogen interactions has led to the discovery and creation of enabling technologies for genome editing technology and crop improvement. One such example is the identification of transcription activator-like (TAL) effectors, a novel class of pathogen-derived proteins – secreted effectors – that are produced and delivered by Gram-negative bacterial pathogens and delivered into the host during infection. Once delivered into the host, TAL effectors translocate into the host nucleus where they bind specific DNA sequence targets; this binding results in transcriptional changes, and ultimately, regulates the expression of host susceptibility genes, which results in cellular changes that benefit the pathogen to cause disease (Bogdanove et al., 2010). From the fundamental work in this area, numerous advances in our understanding of TAL effector-targeted gene function have been made, including the identification of specific host genes that are activated by TAL effectors, such as the activation of Os8N3 by the Xanthomonas oryzae pv. oryzae (Xoo) effector, PthXo1 (Bogdanove et al., 2010). In short, this work found that constitutive overexpression of Os8N3, a gene required for pollen development in rice, resulted in plants that showed enhanced susceptibility to mutant Xoo strains lacking PthXo1, whereas silencing of
OsSN3 resulted in rice plants that showed enhanced resistance. Through this work, as well as numerous additional work from other groups, methods have been developed to create targeted mutations of specific host gene sequences by fusing TAL effectors with an endonuclease resulting in transcription activator-like effector nucleases (TALENs; Bogdanove et al., 2010). As a tool for improving agriculture, this method shows promise as a useful tool to modify plant genomes for trait selection. Lastly, in addition to the use of TALEN-based methods, a quickly developing alternative genome editing system, CRISPER-Cas, has shown substantial promise as a tool that uses single guide RNA methods to make targeted deletions within the genome; in plant research, this is being used to generate plants that are ‘immune’ to the impact of pathogen manipulation and ultimate elicitation of disease. This technology has been demonstrated to function at extremely high specificity, thus showing promise as a rapid means to generate agricultural plant lines with improved environmental (abiotic) and biotic stress response (Kumar and Jain, 2014; Wang et al., 2014a).

Enhancing Crop Performance and Food Production

To say that the acceptance of GM crops still has many hurdles to overcome before being universally accepted and deployed is an understatement. However, one cannot ignore the potential for genome-assisted selection and breeding of elite traits to address many of the numerous challenges facing agriculture. For example, a report by Jonathan Jones outlines many of the success stories since the first release of key GM crops. As noted by Jones (2011), of the four major crops that are grown worldwide (corn, canola, soybean and cotton), a collective yield increase of approximately 30 million metric tons has been recorded as a direct result of the utilisation of GM-based technologies. Indeed, one such example of fundamental knowledge generated through the study of plant resistance is the deployment of Bt (Bacillus thuringiensis)-toxincrops, a GM technology that has demonstrable success in controlling insect damage to crop plants. In addition, Bt corn plants were found to have reduced levels of fungal (e.g. Fusarium) growth, which in turn led to a reduced accumulation of harmful mycotoxins in harvested corn (Wu, 2006). Finally, genetic modification technologies have also been effective in combating plant diseases that, if left unchecked, will decimate entire agricultural industries. Such is the case of papaya production in Hawaii, whereby through the use of GM-enabled approaches, the plant is protected from papaya ringspot virus (PRV; Gonsalves, 1998). Although there are many success stories associated with the generation and deployment of GM-based technologies, broad acceptance is far from guaranteed.

Conclusion

Over the past decade, numerous advances in plant breeding and genome technologies have enabled significant improvements in the performance and yields of staple crops around the globe. For example, since 1950, rice yields around the world have more than doubled. Similarly, the production of both wheat and corn has seen significant increases in production (Tester and Langridge, 2010). In parallel to increased food yields of each of these crops, traits have also been incorporated into lines to address a number of biotic and abiotic stresses most commonly associated with performance and yield. These include traits associated with improvements in disease resistance (Dangl et al., 2013), environmental stress response (Nakashima et al., 2014) as well as a number of instances of traits associated with improvements in nutritional qualities (e.g. ‘Golden Rice’; Paine et al., 2005). To date, many of these yield increases have been realised through the utilisation of a hybrid approach, coupling traditional breeding to the implementation of elite lines selected based on genotype. As a result of combining the power of genomics with traditional methods in breeding, the time needed to identify, characterise and incorporate important traits associated with crop performance has been significantly reduced. Thus, while these advances can be seen as a step in the right direction in terms of meeting the demands that population and climate place on agriculture, we have only begun to scratch the surface in terms of our utilisation of research and technology to feed the growing population. As outlined throughout this article, current research aimed at defining the fundamental processes and mechanisms associated with plant resistance to biotic stress has led to significant advances in our understanding of the broader role that environment and human influence play on crop performance and durability (Figure 1). As population growth and global temperatures continue to rise, current research objectives must maintain a focus on addressing the multitude of genetic and environmental interactions that drive plant performance under pathogen challenge. To combat this problem, multidisciplinary approaches, including classical plant breeding, genomics, molecular biology, ecology and plant pathology, are needed now more than ever to meet the demands of population growth and the impact of human and environmental stress on agricultural production.

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References

Pathogen Resistance Signalling in Plants


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Further Reading


