Plant innate immunity in rice: a defense against pathogen infection

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ABSTRACT

A large number of pathogenic microorganisms cause rice diseases that lead to enormous yield losses worldwide. Such losses are important because rice is a staple food for more than half of the world’s population. Over the past two decades, the extensive study of the molecular interactions between rice and the fungal pathogen Magnaporthe oryzae and between rice and the bacterial pathogen Xanthomonas oryzae pv. oryzae has made rice a model for investigating plant–microbe interactions of monocotyledons. Impressive progress has been recently achieved in understanding the molecular basis of rice pathogen-associated molecular pattern-immunity and effector-triggered immunity. Here, we briefly summarize these recent advances, emphasizing the diverse functions of the structurally conserved fungal effectors, the regulatory mechanisms of the immune receptor complexes, and the novel strategies for breeding disease resistance. We also discuss future research challenges.

Keywords: plant immunity, pathogen effectors, rice, diseases, Magnaporthe oryzae, Xanthomonas oryzae pv. oryzae

INTRODUCTION

Many microbial pathogens attack crop plants and cause huge yield losses that threaten global food security. Although application of chemicals has significantly reduced plant diseases, planting of resistant cultivars remains the most effective and environmentally friendly strategy to control crop diseases. Rice (Oryza sativa) is an important crop that is grown in Asia, Africa, and South and Central America. Over half of the global population consumes rice as the main food source. Throughout the growing season, a variety of pathogens, including fungi, bacteria, viruses, and nematodes, infect different parts of rice plants and greatly reduce yields. In the last two decades, considerable knowledge has been obtained regarding the recognition of pathogens by rice plants and the signaling events in rice innate immunity. Here, we summarize the advances in understanding rice innate immunity and the application of that understanding to the breeding of disease-resistant varieties. We also discuss the major challenges for future research.

PLANT INNATE IMMUNITY

Over the last two decades, extensive genetic and molecular studies of plant–microbe interactions in several model systems have revealed that plants have evolved a two-branched innate immune system that detects and wards off various pathogens, resulting in disease resistance [1]. According to the standard zigzag model to illustrate the plant two-branched immune system in response to pathogens, the first branch uses transmembrane pattern-recognition receptors (PRRs) that recognize conserved pathogen-associated molecular patterns (PAMPs) that recognize conserved pathogen-associated molecular patterns (PAMPs), leading to an immune response called PAMP-triggered immunity (PTI). To circumvent PTI, fungal, bacterial, viral, and nematode pathogens evolve effector proteins that suppress host defenses leading to effector-triggered susceptibility (ETS). The second branch, which mostly acts within the cell, uses highly polymorphic resistance (R) proteins that respond to pathogen effectors, leading to a rapid and robust effector-triggered immunity (ETI). However, this zigzag
model does not fully apply to some unique aspects in plant–virus interactions \[11\]. Although there are limited comparative studies between antiviral and antibacterial/antifungal immune responses, some reviewers proposed that RNA silencing (RNAi) evolved by plant that recognize viral double-stranded RNA (dsRNA, corresponds to PAMP from fungi and bacteria) may have similar functions as PTI in blocking viral infection \[11, 12\]. As the result of the plant–virus coevolution, viral suppressors of RNAi (VSRs) are regarded as effectors to overcome host RNAi (regarding as ETS) \[11, 12\]. Plant R proteins that recognize VSRs as avirulence proteins can mediate a strong defense as ETI \[11\].

**MAJOR DISEASES IN RICE**

Seventeen rice diseases caused by fungi, bacteria, nematodes, and viruses are listed in Table 1. Based on scientific and economic importance, the most important of these are rice blast caused by the fungus *Magnaporthe oryzae*, bacterial blight caused by the bacterium *Xanthomonas oryzae pv. oryzae* (*Xoo*), root knot caused by the nematode *Meloidogyne graminicola*, white tip caused by the nematode *Aphelenchoides besseyi* and rice stem nematode disease caused by the nematode *Ditylenchus angustus*. These pathogens were selected as the top 10 plant pathogenic fungi, bacteria, and nematodes, respectively; by the review articles published in the cited references.

### Table 1. Major fungal, bacterial, nematode, and viral diseases of rice.

| Diseases of rice | Pathogen | Rice yield loss | References |
|------------------|----------|-----------------|------------|
| **Fungal disease** | Magnaporthe oryzae | Up to 100% | \[2\] |
| Rice blast | Rhizoctonia solani | Up to 50% | \[3\] |
| False smut | Ustilaginoidea virens (Cooke) Takah | Up to 44% | http://www.apsnet.org/publications/imageresources/Pages/FI00163.aspx |
| Sheath rot | Sarocladium oryzae (Sawada) W. Gams & D. Hawksworth | Up to 85% | http://www.knowledgebank.irri.org/rice.htm |
| Brown spot | Cochliobolus miyabeanus | Up to 45%, caused ‘Great Bengal Famine’ in 1942 | \[4\] |
| Bakanae | Fusarium fujikuroi | Yield reductions and mycotoxin contamination | \[5\] |
| **Bacterial disease** | Xanthomonas oryzae pv. oryzae | 10–50% | \[6\] |
| Bacterial leaf streak | Xanthomonas oryzae pv. oryzicola | 8–32% | \[7\] |
| Bacterial panicle blight | Burkholderia glumae | Up to 85% | \[8\] |
| **Nematode disease** | Meloidogyne graminicola | Up to 87% | \[9\] |
| Rice root-knot nematode | Aphehelenchoides besseyi | Up to 50% | http://pest.ceris.purdue.edu/pest.php?code=NEABABB |
| Rice white tip nematode | Ditylenchus angustus | 20–90% | http://www.cabi.org/isc/datasheet/19285 |
| Rice stem nematode | Heteroderda elachista | Unknown | \[10\] |
| Rice cyst nematode* | Heteroderda oryzae | Up to 42% | \[10\] |
| Rice cyst nematode* | Heteroderda sacchari | Similar with *H. oryzae* | \[10\] |
| **Viral disease** | Rice stripe virus | 30%–40% | http://en.jaas.ac.cn/zbs/highlights.asp |
| Rice black streaked dwarf | Rice black streaked dwarf virus | ~60% | http://www.cabi.org/isc/abstract/19881671518 |
| Southern rice black streaked dwarf | Southern rice black streaked dwarf virus | Up to 100% | http://en.jaas.ac.cn/zbs/highlights.asp |
| Rice yellow mottle | Rice yellow mottle virus | 10%–100% | http://www.knowledgebank.irri.org/training/fact-sheets/pest-management/diseases/item/rice-yellow-mottle-virus-fact-sheet |

*Total of four species of *Heteroderda* genus have been discriminated that cause rice cyst nematode disease.
Molecular Plant Pathology [13–15] (Fig. 1). In recent years, the following re-emerging diseases have become increasingly important: rice sheath blight caused by Rhizoctonia solani, rice false smut caused by Ustilaginoidea virens (Cook) Takah, rice bacterial panicle blight caused by Burkholderia glumae, and rice stripe disease caused by rice stripe virus (RSV) [3,16–18]. Over the past two decades, the rice/M. oryzae and rice/Xoo pathosystems in particular have been the focus of intensive studies and have become molecular models for research on plant-microbe interactions. The major advances in the understanding of rice innate immunity against bacterial and fungal pathogens were recently reviewed by Liu et al. [19]. In this review, we consider the new progress in the two model pathosystems and novel insights into rice innate immunity against nematode and viral pathogens.

RICE PRR REPERTOIRE AND PTI

In a long-term evolutionary arms race with pathogenic microorganisms, plants have evolved a repertoiue of PRR genes that recognize the conserved microbial PAMPs, leading to the inhibition of pathogen infection [1]. Plant PRRs are cell-surface receptors that perceive PAMPs released from the infecting pathogens in the extracellular environment; the perception of PAMPs by PRRs results in PTI responses. Plant PRRs are represented by transmembrane receptor-like kinases (RLKs), which typically contain extracellular leucine-rich repeats and an intracellular kinase domain, and receptor-like proteins (RLPs), which lack a kinase domain [20]. Because RLPs lack a cytoplasmic kinase domain, they recruit proteins containing kinase domains for the activation of the downstream signaling pathways. More than 1131 RLK genes have been identified in the rice genome; this is nearly two times the number in Arabidopsis and probably results from duplication events in the RLK genes of rice [21]. RLPs form a second major class of cell-surface receptors in plants, and the rice genome encodes 90 RLP genes [22]. Together, these receptor classes respond to a wide variety of activating ligands (lipid, protein, nucleic acids, carbohydrate, etc.) from various exogenous sources, such as pathogens and host-derived endogenous danger signals. Studies have increasingly shown that conserved PAMPs such as bacterial flagellin, peptidoglycan, lipopolysaccharide, and fungal chitin can be sensed by rice cells and trigger innate immunity [23–26].

Several rice PRR proteins including XA21, Os-FLS2, CEBiP, OsCERK1, LYP4, and LYP6 have been well characterized (Table 2). The rice RLK gene Xa21 was one of the first innate immune receptor genes to be isolated and confers resistance to a wide range of Xoo strains [27]. The XA21-mediated signaling network has been intensively studied through genetic and biochemical approaches [19,23]. A number of previous studies have identified several Xoo Rax (required for activation of Xa21) genes that activate the XA21-mediated immune response [28]. These genes are local to a single operon (raxSTAB) that includes a tyrosine sulfotransferase (RaxST) and three
components (RaxA, RaxB, and RaxC) of a predicted type 1 secretion system [28]. Based on these findings, researchers hypothesized that a tyrosine-sulfated, type 1-secreted protein activates XA21-mediated immunity. Consistent with this hypothesis, a sulfated, 21-amino acid (AA) synthetic peptide (RaxX21-sY) derived from RaxX protein secreted by Xoo was proved to be essential for triggering XA21-mediated resistance [36]. Interestingly, RaxX residues between 40 to 55 share remarkable similarity with Arabidopsis signaling factor PSY1 (sulfated, secreted 18-AA peptide) and four predicted rice PSY1 orthologs [36]. The high similarities suggest that when a rice plant lacks XA21, Xoo and other Xanthomonads might use sulfated RaxX to mimic PSY1-like peptides in order to suppress host defense responses and facilitate infection [36].

OsFLS2 is the rice ortholog of Arabidopsis FLS2, and heterologous expression of OsFLS2 in the fls2 mutant can restore the fls2 mutant defects in Arabidopsis [29]. Like FLS2, OsFLS2 can directly recognize flg22 and trigger an immune response in rice [30]. These results indicate that the flg22 signaling pathway is conserved between Arabidopsis and rice and that OsFLS2 may also provide PTI-mediated defense in rice. Researchers have characterized several chitin immune receptors (CEBiP, OsCERK1, LYP4, and LYP6) that directly or indirectly recognize chitin fragments and trigger defense responses in rice [24,25,31]. Intriguingly, OsCERK1, LYP4, and LYP6 are also important for triggering immune responses to bacterial PGN in rice [24]. Furthermore, recent evidence indicates that the receptor-like cytoplasmic kinases OsRLCK185 and OsRLCK176 function downstream of OsCERK1 in the chitin and PGN signaling pathways, suggesting that chitin and PGN share intracellular signaling components [33]. Therefore, OsCERK1 functions as an adaptor in conjunction with OsLYP4 and OsLYP6 and plays dual roles in PGN and chitin signaling in rice innate immunity. These results demonstrate that multiple PRR proteins may work together to respond to PAMPs in rice.

### RICE R GENE REPERTOIRE AND ETI

It is well known that nucleotide-binding and leucine-rich repeat domain (NLR) proteins function as immune receptors in both animals and plants [37]. However, plant genomes contain many more NLRs than animal genomes, indicating differences in the two immune systems. The rice genome, for example, contains about 480 NLR genes while the human genome has only about 10 [38]. Interestingly, the majority of the cloned R genes encode NLR proteins (Table 3), although several atypical R proteins containing a variety of conserved protein domains/motifs are also identified (Fig. 2). Details concerning the structure and function of the cloned R genes have been reviewed and discussed in Liu et al. [19].

In the last 2 years, five new R genes (Pi50, Pi64, Xa10, Xa23, and STV11) have been cloned. Among them, Pi50 and Pi64 encode typical NLR proteins [39,40]. NLR genes are usually located in clusters in plant genomes; of the 480 NLR genes in rice, for example, 263 reside in 44 clusters [38]. Rice R genes Pi2, Pi9, and Piz-t are located in one of these NLR gene clusters on chromosome 6, and at least eight R genes are located at this locus in both wild and cultivated rice [41]. The newly cloned Pi50 gene is located at the Pi2/9 locus and confers broad-spectrum resistance to M. oryzae [42].

| PRR gene | Protein structure | Function | Reference |
|----------|-------------------|----------|-----------|
| CEBiP    | LysM RLP          | Chitin receptor | [25] |
| LYP4     | LysM RLP          | Chitin and PGN receptor | [24] |
| LYP6     | LysM RLP          | Chitin and PGN receptor | [24] |
| OsFLS2   | LRR RLK          | Recognizes flg22 and triggers immunity | [29,30] |
| XA21     | LRR RLK          | Recognizes RaxX21-sY and triggers immunity | [27] |
| OsCERK1  | LysM RLK         | Co-receptor of CEBiP, LYP4 and LYP6 | [31] |
| OsRLCK185| Receptor-like cytoplasmic kinases | Interacts with OsCERK1 and important for chitin- and PGN-induced immunity | [32] |
| OsRLCK176| Receptor-like cytoplasmic kinases | Interacts with OsCERK1 and important for chitin- and PGN-induced immunity | [33] |
| OsSERK1  | LRR RLK          | Regulates BR-mediated development signaling | [34] |
| OsSERK2  | LRR RLK          | Co-receptor kinases of XA21 and regulates BR-mediated development signaling | [35] |

**Table 2. PRR genes and co-receptors that are important for rice immunity.**
Table 3. The cloned rice resistance genes and *M. oryzae* and *X. oryzae* pv. *oryzae* avirulence genes.

| Resistant genes | Avirulence genes | References |
|-----------------|------------------|------------|
| R gene          | Encoding protein | Avr gene   | Encoding protein | Pathogen                  | |
| **Pib**         | NB-LRR           | AvrPib     | 75 AA secreted protein | *Magnaporthe oryzae* |
| **Pi-1a**       | NB-LRR           | AvrPi-1a   | 224 AA secreted protein |
| **P9**          | NB-LRR           | AvrP9      | 91 AA secreted protein |
| **Pi2**         | NB-LRR           | ND         | –                    |
| **Piz-t**       | NB-LRR           | AvrPiz-t   | 108 AA secreted protein |
| **Pi-d2**       | Lectin RLK       | ND         | –                    |
| **Pi33**        | –                | ACE1       | Polyketide synthase  |
| **Pi c**        | –                | AvrPi c    | 70 AA secreted protein |
| **Pi36**        | NB-LRR           | ND         | –                    |
| **Pi37**        | NB-LRR           | ND         | –                    |
| **Pi50**        | NB-LRR           | ND         | –                    |
| **Pi64**        | NB-LRR           | ND         | –                    |
| **Pikm**        | NB-LRR           | Avr-Pik/km/kp | 113 AA secreted protein, five alleles (A–E) |
| **Pit**         | NB-LRR           | ND         | –                    |
| **Pi5s**        | NB-LRR           | ND         | –                    |
| **Pi3**         | NB-LRR           | ND         | –                    |
| **Pi13-A4**     | NB-LRR           | ND         | –                    |
| **Pi54**        | NB-LRR           | ND         | –                    |
| **Pi5h**        | NB-LRR           | ND         | –                    |
| **Pik**         | NB-LRR           | Avr-Pik/km/kp | 113 AA secreted protein, five alleles (A–E) |
| **Pikp**        | NB-LRR           | Avr-Pik/km/kp | 113 AA secreted protein, five alleles (A–E) |
| **Pi14**        | NB-LRR           | Avr-Pia    | 85 AA secreted protein |
| **Pi-CO39**     | NB-LRR           | AvrI-CO39  | 89 AA secreted protein |
| **Pi25**        | NB-LRR           | ND         | –                    |
| **Pi1**         | NB-LRR           | ND         | –                    |
| **pi21**        | Proline-containing protein |
| **Pb1**         | NB-LRR           | ND         | –                    |
| **ND**          | –                | PWL2       | 145 AA secreted protein |
| **xa5**         | TFIIA transcription factor | Avrxa5/PthXo7 | Xanthomonas oryzae pv. oryzae |
| **xa13**        | MtN3/saliva domain protein | Avrxa13/PthXo1 | TALE |
| **Xa25**        | MtN3/saliva domain protein | ND | |
| **Xa3/Xa26**    | LRR-RLK          | AvrXa3     | TALE                 |
| **Xa27**        | Rice unique gene | AvrXa27    | TALE                 |
| **Xa1**         | NB-LRR           | ND         | –                    |
| **Os11N3**      | Homolog of nodulin MtN3 | AvrXa7 | TALE |
| **(OsSWEET14)** |                   |            |                      |
Table 3 (Continued.)

| Resistant genes | Avirulence genes | Pathogen | References |
|-----------------|------------------|----------|------------|
| R gene          | Encoding protein | Avr gene | Encoding protein | |
| Xa10            | Executor R protein, encodes 126 AA, with four potential transmembrane helices | AvrXa10 | TALE | [79] |
| Xa23            | Executor R protein, encodes 113 AA, with four potential transmembrane helices | AvrXa23 | TALE | [80] |
| Rxo1d           | NB-LRR           | AvrRxo1  | –            | Xanthomonas oryzae pv. oryzicola | [81,82] |
| STV11           | Sulfotransferase | ND       | –            | RSV | [18] |

*The function of these three R genes requires two NB-LRR members.
These two R genes share the same NB-LRR gene locus.
The gene has not been cloned yet.
This gene was cloned from maize.
ND = not determined.

Figure 2. A diagram showing the domain diversity of rice atypical R proteins. Eleven rice R proteins with different domains are illustrated, including the bulb-type mannos-specific lectin (B lectin) domain, the protein tyrosine kinase (Pkinase_Tyr) domain, the heavy metal-associated (HMA) domain, the proline-rich motifs (PRMs), the transmembrane helices (TM), the transcription initiation factor IIa, gamma subunit (TFIIA_gama_N) domain, the transcription initiation factor IIa, gamma subunit (TFIIA_gama_C), the sugar efflux transporter for intercellular exchange (Min3_slv) domain, the PQ loop repeat (PQ-loop) domain, the leucine rich repeat (LRR) domain, and the sulfotransferase family (Sulfotransferase_3) domain. Protein domains/motifs were predicted by the SMART program (http://smart.embl-heidelberg.de/) with a normal mode. Figures are not drawn to scale.

The Pi50 cluster contains four duplicated genes (Pi50_NBS4_1/2 and Pi50_NBS4_3/4) that differ in only four AAs [39]. Complementation tests revealed that Pi50_NBS4_1/2 but not Pi50_NBS4_3/4 confer Pi50-mediated blast resistance in rice [39]. Pi50 shares more than 96% AA sequence identity with Pi2, Pi9, and Piz-t, suggesting that Pi50 is derived from the functional divergence of duplicated genes [39]. The allelic gene Pi64 encodes a 1288-AA protein and is localized in both the cytoplasm and nucleus [40]. Pi64 is constitutively expressed in all tissues and at all development stages, and confers a high level of resistance to both leaf and neck blast in rice [40].

Both Xa10 and Xa23 are executor R proteins that confer the transcription activator-like effector (TALE)-dependent resistance to bacterial blight in rice [79,80]. The XA10 protein localizes as hexamers in the endoplasmic reticulum (ER) and such localization coincides with the ER Ca$^{2+}$ depletion and XA10-induced cell death in plants [79]. These results suggest that XA10 is an inducible protein that triggers programmed cell death by a conserved mechanism involving disruption of the ER and of cellular Ca$^{2+}$ homeostasis. The Xa23 protein shares 50% identity with XA10, and these two executor R proteins also have a similar predicted transmembrane helices structure [80]. Xa23 transcription is specifically activated by the TALE AvrXa23, and XA23 can trigger a strong immune response in rice, tobacco, and tomato [80]. The promotors of both Xa10 and Xa23 contain a TALE-binding element that is essential for cognate TALE-induced resistance [79,80]. These results suggest that the rice genome has evolved an executor R gene family, the members of which function in disease resistance by recognizing the cognate TALEs in Xoo.
STV11, which confers durable resistance to RSV, was recently cloned by a map-based cloning strategy [18]. The gene encodes a sulfotransferase that can catalyze the conversion of salicylic acid (SA) into sulfonated salicylic acid (SSA) in RSV-infected plants, and SSA is more effective than SA in triggering RSV resistance and in inhibiting viral replication [18]. Moreover, SSA may also serve as a signal to enhance SA biosynthesis through a positive feedback mechanism after RSV infection; SA may contribute to the inhibition of viral replication in the RSV-infected plants [18]. STV11-R is prevalent in cultivated indica rice cultivars, whereas the susceptible allele STV11-S is prevalent in japonica cultivars. The cloning of STV11 will facilitate the breeding of RSV-resistant rice through molecular marker-assisted selection; such resistance will greatly improve RSV management in rice production.

Our understanding of rice resistance to nematodes has lagged behind the soybean-nematode pathosystem. For instances, two soybean cyst nematode (SCN) resistance genes (Rhg1 and Rhg4) have been cloned through a map-based cloning strategy [83,84]. The Rhg1 gene encodes three proteins [an AA transporter (Glyma18g02580), an a-SNAP protein (Glyma18g02590), and a WI12 (wound-inducible domain protein), (Glyma18g02610)], all of which are essential for the resistance to SCN [83]. A physical structure study revealed that the rhg1 locus that encodes these three proteins is present in multiple copies (10 tandem copies) in SCN resistant lines, whereas only one copy is present in susceptible cultivars [83]. Overexpression of the individual genes is ineffective, but overexpression of the three genes together enhances SCN resistance [83]. These results suggest that variation in the copy number of multiple genes at Rhg1 mediates SCN resistance in soybean. Rhg4 encodes a ubiquitous enzyme (serine hydroxymethyltransferase) that is responsible for interconversion of serine and glycine and that is important for cellular one-carbon metabolism [84]. Two genetic polymorphisms (R130P and Y358N) were detected in the Rhg4 alleles of resistant versus susceptible cultivars, suggesting that these two AAs are important for the regulatory function of this enzyme [84]. A linkage mapping study revealed a major resistance gene (Has-1\(_{19}\)) against rice cyst nematode caused by Heterodera sacchari and it was delimited to a 8.2 cM interval between the markers RM254 and RM206 on chromosome 11 in rice [85]. However, the gene encodes Has-1\(_{19}\) have not been cloned. Because another three species of cyst nematodes (H. oryzae, H. elachista, and H. oryzae) also frequently infect rice and cause significant annual yield lost, additional identification and cloning of genes responsible for resistance to the cyst nematodes that attacks rice is urgently needed.

Recently, many new resistance genes have been mapped via genome-wide association studies (GWASs) of large collections of rice germplasm. Wang et al., for example, investigated 366 diverse indica rice accessions using 0.8 million single-nucleotide polymorphisms (SNPs) and identified 30 loci that are significantly related to resistance to M. oryzae [86]. In that study, a new R gene locus was identified on chromosome 3 where no blast R gene had been previously reported [86]. Using 372 diverse rice cultivars collected from 82 countries and 700 000-SNP arrays, Kang et al. identified 97 loci associated with blast resistance (LABRs) against five diverse isolates [87]. Among these loci, 82 are new regions, and 15 are co-localized with known blast resistance loci [87]. Further functional analysis of the candidate genes in the LABR_64 region via RNAi technology identified two new R alleles at the Pi5 locus [87]. These results suggest that GWAS is an efficient strategy for rapid allele discovery and that GWAS, when coupled with RNAi technology, will help researchers dissect complex disease resistance in rice. Another recent study investigated the function of 332 NLR genes that were cloned from five blast-resistant rice cultivars [88]. Strikingly, 98 of them confer resistance to one of the tested blast isolates, demonstrating that a systemic approach can increase the efficiency of R gene cloning in rice.

**PATHOGEN EFFECTORS AND THEIR HOST TARGETS**

In a broad sense, effectors are pathogen proteins and small molecules that can alter host cell structure and function [89]. Avr effectors are those molecules that are recognized by the cognate host R proteins directly or indirectly in plant cells; the recognition triggers a rapid and robust hypersensitive reaction. To date, a total of 21 Avr effector genes have been cloned in rice pathogens, and these include 13 from M. oryzae, 7 from Xoo, and 1 from Xoc (Table 3). The identification of these Avr genes has greatly facilitated the investigation of the molecular basis of the interaction between Avr effectors and R proteins. The examples of direct and indirect interactions between two types of proteins and host targets of the Avr effectors have recently been reviewed [19,90].

**AvrPib** and **AvrPi9** were recently cloned in M. oryzae. **AvrPib**, the cognate Avr gene of the R gene Pib, was cloned using a map-based cloning strategy. It encodes a 75-AA protein with no homology
to any protein in the database [43]. Phenotyping and genotyping of 60 *M. oryzae* isolates collected from five geographically distinct areas suggested that *AvrPib* has undergone host-driven selection [43]. Resequencing of the *AvrPib* allele of 108 diverse isolates revealed that transposable element (TE) insertion (frequency 81.7%) is the prevalent mechanism that leads to the loss of its avirulence function [43]. *AvrPi9*, the Avr gene of the R gene *Pi9*, was cloned using a comparative genomic approach with virulent mutant strains derived from a sequential planting method [47]. The *AvrPi9* protein is highly expressed at early stages of *M. oryzae* infection [47]. Moreover, the *AvrPi9* protein localizes in the biotrophic interfacial complex and appears to be translocated into rice cells during infection [47]. Like *AvrPib*, TEs also play an important role in acquisition of virulence in the *AvrPi9* alleles in *M. oryzae*.

*Magnaporthe oryzae* secretes various effectors that enter infected rice cells and then move to neighboring cells, presumably targeting host proteins to prepare for infection [91]. Several host targets of Avr effectors have been recently characterized. For instance, the *AvrPiz*-t effector targets the rice RING E3 ligase APIP6 and suppresses PTI [92]. Interestingly, the interaction between *AvrPiz*-t and APIP6 leads to their mutual degradation [92]. Transgenic rice plants expressing the *APIP6* RNAi construct have reduced PTI responses and reduced basal resistance to *M. oryzae* [92], suggesting that *APIP6* positively regulates rice innate immunity. A recent study showed that *APIP6* interacts with and degrades OsELF3-2 (ortholog of *Arabidopsis* flowering and circadian regulator ELF3) [93]. The *oself3-2* T-DNA mutant and RNAi plant exhibit enhanced resistance to *M. oryzae* [93], indicating that OsELF3-2 negatively regulates rice innate immunity against *M. oryzae*.

The exocyst is an octameric protein complex that functions in vesicle trafficking. Its subunits *Exo70B2* and *Exo70H1* in *Arabidopsis* are involved in the response to pathogens, with *Exo70B2* having a more important role in cell wall apposition formation related to plant defense [94]. The Avr-Pii effector targets two rice *Exo70* proteins (*OsExo70-F2* and *OsExo70-F3*) to form a protein complex in rice cells [95]. Functional assays showed that *OsExo70-F3* but not *OsExo70-F2* is specifically involved in Pii-dependent resistance [95]. Moreover, overexpression of *Avr-Pii* or silencing of *OsExo70-F2* and -F3 genes in rice did not affect the virulence to compatible *M. oryzae* strains [95]. These results suggest that the Avr-Pii targets *OsExo70-F3* and the rice exocytosis pathway are important for ETI and that *OsExo70* functions as a decoy or helper in Pii/Avr-Pii interactions.

## HORMONE-MEDIATED IMMUNITY IN RICE

Rice hormones such as SA (salicylic acid), JA (jasmonate acid), and ET (ethylene) are important regulators of immune responses [96–98]. Two excellent reviews summarized the advances in understanding the functions of various hormones in rice immunity in 2013 [99,100]. Here, we provide the recent progress on hormone-mediated immunity in rice during the past few years.

SA, JA, and ET are three main hormones that play important roles in plant immunity. SA is usually considered to regulate immunity against biotrophic pathogens, whereas JA and ET are believed to be involved in resistance to necrotrophic and insect pests [101]. However, this dichotomy does not fully fit into the monocotyledonous plant rice [10]. Different from the dicot plant *Arabidopsis*, rice plants challenged by fungal and bacterial pathogens do not show SA accumulation [102]. However, rice plants indeed respond to exogenous SA treatment [102]. These results suggest that rather than the endogenous SA level, the involvement of SA in rice defense responses is more dependent on the SA signaling [99].

Accumulating evidence reveals that extensive crosstalk between different hormones exists in rice plants in response to pathogen infections. For instance, the rice DELLA protein SLR1 (slender rice1) represses the transcription of gibberellic acid (GA)-responsive genes and functions as a key regulator of GA signaling [103]. Vleesschauwer et al. recently found that SLR1 functions in resistance to hemibiotrophic but not necrotrophic pathogens [104]. Moreover, they demonstrated that SLR1 mediates resistance through integrating and amplifying both SA- and JA-dependent defense signaling pathways in rice [104]. A recent transcriptome study of root-knot nematode-infected rice plants reveals that a number of well-identified marker genes involved in the SA/JA/ET pathways show significantly differential expression patterns between susceptible and resistant interactions [105]. These results indicate that various plant hormones are involved in the rice–nematode interaction and further in-depth studies are needed to decipher the underlying mechanism of hormone-mediated resistance in this pathosystem.

Plant hormone pathways are often targeted by pathogen effectors for suppression of hormone-mediated immunity. For example, *M. oryzae* encodes an antibiotic biosynthesis monoxygenase (Abm) that converts endogenous free JA into hydroxylated JA (12OH-JA) to attenuate rice innate immunity during fungal colonization [106]. The wild-type strain of *M. oryzae* secretes 12OH-JA during host
penetration to avoid the defense response, whereas the Abm mutant of M. oryzae accumulates methyl JA (MeJA), which induces rice defense [106]. Notably, M. oryzae also secretes Abm after invasion, and the secreted Abm appears to convert plant JA into 12OH-JA to facilitate host colonization [106], indicating that Abm is an effector protein that is important for M. oryzae pathogenicity. The host target of Abm remains to be identified. In addition to inducing or manipulating host hormone biosynthesis, most plant pathogens are producing hormones as virulence factors [107]. For example, rice bakanae disease pathogen Fusarium fujikuroi produces chemically similar GA that probably functions as a suppressor of host defense responses through modulating hormonal balance in plants [107]. Many gall-forming bacteria and biotrophic fungi produce cytokinins (CKs) that are required for the establishment of diseases [107]. However, the underlying mechanism of CKs produced by plant pathogens during infection remains largely unknown. Recently, Chanclud et al. identified the gene CKS1 (cytokinin synthesis 1) that is required for CK synthesis and full virulence in M. oryzae [108]. Moreover, they showed that the CKs produced by M. oryzae are important for dampening host defense and affecting plant nutrients (sugar and AAs) distribution that facilitate for fungal growth in and around the infection site [108], indicating that this fungal-secreted CKs are key effectors that are similar with the TALE from bacteria. Interestingly, Bockhaven et al. recently found that rice plants treated with 2 mM silicon (Si) significantly increase resistance to the brown spot fungus Cochliobolus miyabeanus [109]. Rather than suppressing rice ET signaling, Si application increases resistance to rice brown spot probably through interfering with the production and/or action of ET in C. miyabeanus [109]. These results suggest that impairment of hormone production in pathogens is an efficient strategy to control plant diseases resistance.

**STRUCTURAL INSIGHT INTO RICE/PATHOGEN SYSTEMS**

Advances in X-ray crystallography promise to deepen our understanding of the recognition between plant NLRs and pathogen effectors at the molecular level. The technique has been recently used to analyze the interaction between rice NLRs and M. oryzae effectors. According to X-ray crystallography, the Avr effector AvrPiz-t adopts a six-stranded β-sandwich-fold structure, and Cys62 forms a disulfide bond with Cys75 [110]. de Guillen et al. recently used NMR spectroscopy to determine the 3D structures of the M. oryzae effectors Avr1-CO39, Avr-Pia, and AvrPiz-t and of the Pyrenophora triticum-repentis (wheat tan spot pathogen) effector ToxB [111]. The analysis showed that these effectors have very similar six β-sandwich structures that are stabilized by a disulfide bridge between two conserved cysteins located in similar positions of the proteins. These sequence unrelated but structurally similar fungal effectors were termed MAX effectors. Most M. oryzae MAX effectors are highly expressed early during infection. Determining whether the MAX effectors have similar functions in pathogenesis and whether they can target conserved host proteins will require further investigation.

Maqbool et al. recently used biochemical, structural, and activity-based assays to study how the rice NLR protein Pik directly interacts with the M. oryzae effector Avr-Pik [112]. Coexpression of Pik-PikD and the analysis of the 3D crystal structure of their complex revealed that Avr-PikD has high affinity binding to the so-called integrated HMA domain in Pikp [112]; this binding initiates immunity responses. Furthermore, mutated Avr-PikD compromises the interaction with the Pikp-HMA domain and therefore abolishes the Avr-PikD-Pikp-triggered defense response in rice [112].

Finally, a recent copurification and crystal structure study revealed that the Xanthomonas type III effector AvrRox1-ORF1 binds to a molecular chaperone AvrRox1-ORF2 to form a tetramer complex with a distinct fold containing a novel kinase-binding domain [113]; the AvrRox1-ORF2 chaperone is structurally different from typical effector-binding chaperones. This tetramer complex is structurally homologous to zeta toxin:epsilon antitoxin [113]. AvrRox1-ORF1 encodes a T4 polynucleotide kinase-like domain that might directly phosphorylate a host target [113].

**BREEDING OF DISEASE-RESISTANT RICE**

Researchers have estimated that crop yields must be increased by 150% before 2030 to meet the global food demand [114]. This increase in yield will be difficult to achieve because of many limiting factors including pathogens. During the past decades, the breeding of disease-resistant rice cultivars has greatly increased yield in China and several Asian countries. For example, many R genes against M. oryzae, Xoo, and RSV have been integrated into new rice cultivars through marker-assisted selection and genetic engineering breeding strategies in China [114]. Readers are referred to a recent comprehensive review on the progress of rice molecular breeding in China [114].
In addition to conventional approaches, novel strategies based on host-induced gene silencing (HIGS), *Xanthomonas* spp. transcription activator-like effector nucleases (TALENs), and a bacterial monomeric DNA endonuclease CRISPR-associated protein 9 (CRISPR/Cas9) have been successfully used to increase resistance against pathogens in plants. The first successful application of HIGS in disease control was the expression of papaya ringspot virus (PSRV) coat protein in transgenic papaya plants to inhibit PSRV infection [115]. Growing evidence suggests that the expression of dsRNA molecules that target important genes in nematodes, fungi, and even insects might also generate resistant plants [116]. For instance, transgenic plants expressing fungal virulence gene constructs can specifically silence host targets in the case of the pathogenic fungi *Blumeria graminis*, *Fusarium* species, and *Puccinia striiformis* F. sp. *tritici* [117–119]. The use of HIGS to control rice blast and sheath blight is being studied in several laboratories and may generate transgenic lines with resistance to multiple pathogens if the target pathogen DNA sequence is highly conserved.

Genome-editing technology has great potential for the engineering of plants that have a broad spectrum of resistance but are free of antibiotic markers. TALENs encode artificial bipartite enzymes that consist of a modular DNA-binding domain and the FokI nuclease domain [120]. The DNA-binding domain has been engineered to recognize a specific DNA sequence. The ability to precisely edit a specific host gene, such as the target of a bacterial virulence gene, can result in the development of transgenic crops that thwart the virulence strategy of *Xanthomonas* spp. For example, resistant and hygromycin-free rice plants have been generated with TALEN technology; the resistance of these plants is based on the targeting of the bacterial blight susceptibility gene *Os11N3* (also called *OsSWEET14*) [121]. The CRISPR/Cas9-based gene-editing tool is becoming increasingly important. This technology simply uses engineered 20 base pair (bp) RNA guide sequence that binds to its DNA target site of interests to cause DNA cleavage and mismatching repairing or homologous replacement [122]. To date, CRISPR/Cas9-based gene editing has been used for many organisms, including the model crop plants rice, maize, and wheat [123]. Simultaneous editing of three mildew resistance locus o (Mlo) genes in hexaploid bread wheat led to the generation of heritable resistance to the powdery mildew fungus *Blumeria graminis* f. sp. *tritici* (Bgt) [124]. A new method to edit plant genomes without introducing foreign DNA into cells was recently reported; this may alleviate regulatory concerns related to genetically modified plants [125]. With this new method, transgenic plants were generated from the protoplasts of *Arabidopsis thaliana*, tobacco, lettuce, and rice transfected with purified Cas9 protein and guide RNA. These plants contain only small insertions or deletions that are indistinguishable from naturally occurring genetic variations. In the future, improvements in the application of CRISPR/Cas9 technology will likely lead to novel and broad-spectrum disease resistance in crops.

**CONCLUSION AND PERSPECTIVES**

During the last two decades, tremendous progress has been made in understanding the innate immune receptor complex in rice. More than 40 rice PRR and R genes have been identified and functionally characterized. These genes help regulate the defense responses to bacterial, fungal, and viral pathogens. Breakthroughs have included the determination of rice immune receptors and how such receptors recognize fungal and bacterial ligands, the understanding of the structure of the rice immune receptor complex, and the development of novel strategies for rice diseases management. Research is needed in the following areas: (1) the connections and interactions between the signaling components of rice PRR and NLR-mediated resistance for defense activation, (2) the function of transcriptional factors that receive signals from PRRs and NLRS and that control the downstream defense gene activation in the nucleus, (3) the role of epigenetic regulations in rice immunity, and (4) the application of our increasing understanding of rice innate immunity to achieve disease control in rice fields.

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**REFERENCES**

1. Boller T and He SY. Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science* 2009, 324: 742–4.
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2. Dean RA, Talbot NJ and Ebbole DJ et al. The genome sequence of the rice blast fungus Magnaporthe grisea. Nature 2005; 434: 980–6.

3. Zheng A, Lin R and Zhang D et al. The evolution and pathogenic mechanisms of the rice sheath blight pathogen. Nat Commun 2013; 4: 1424.

4. Condon BJ, Leng Y and Wu D et al. Comparative genome structure, secondary metabolite, and effector coding capacity across Cochliobolus pathogens. PLoS Genet 2013; 9: e1003233.

5. Wiemann P, Sieber CM and von Bargen KW et al. Deciphering the cryptic genome: genome-wide analyses of the rice pathogen Fusarium fujikuroireovirus complex regulation of secondary metabolism and novel metabolites. PLoS Pathog 2013; 9: e1003475.

6. Lee BM, Park YJ and Park DS et al. The genome sequence of Xanthomonas oryzae pathovar oryzae KACC10331, the bacterial blight pathogen of rice. Nucleic Acids Res 2005; 33: 577–86.

7. Bogdanove AJ, Koebnik R and Lu H et al. Two new complete genome sequences offer insight into host and tissue specificity of plant pathogenic Xanthomonas spp. J Bacteriol 2011; 193: 5450–64.

8. Lim J, Lee TH and Nahm BH et al. Complete genome sequence of Burkholderia glumae BGR1. J Bacteriol 2009; 191: 3758–9.

9. Dutta TK, Ganguly AK and Gaur HS. Global status of rice root-knot nematode, Meloidogyne graminicola. Afr J Microbiol Res 2012; 6: 8016–21.

10. Kyndt T, Fernandez D and Gheysen G. Specific adaptation of Ustilaginoidea pathogens. in molecular plant pathology. Proc Natl Acad Sci U S A 2006; 103: 11086–91.

11. Nakahara KS and Masuta C. Interaction between viral RNA silencing suppressors and host factors in plant immunity. Curr Opin Plant Biol 2014; 20: 88–95.

12. Zvereva AS and Pooggin MM. Silencing and innate immunity in plant defense against viral and non-viral pathogens. Viruses 2012; 4: 2578–97.

13. Dean R, Van Kan JA and Pretorius ZA et al. The Top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol 2012; 13: 414–30.

14. Mansfield J, Genin S and Magori S et al. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol 2012; 13: 614–29.

15. Jones JT, Haegeman A and Danchin EGJ et al. Analysis of flagellin perception mediated by flag22 receptor OsFLS2 in rice. Mol Plant-Microbe Interact 2008; 21: 1635–42.

16. Zhang Y, Zhang K and Fang AF et al. An XA21-Associated Kinase (OsSERK2) regulates immunity mediated by the XA21 and XA3 immune receptor. Cell Host Microbe 2013; 13: 347–57.

17. Ham JH, Melanson RA and Rush MC. Burkholderia glumae BGR1. Proc Natl Acad Sci U S A 2009; 106: 1424–8.

18. Wang Q, Liu YQ and He J et al. STV11 encodes a sulphotransferase and confers durable resistance to rice stripe virus. Nat Commun 2014; 5: 4768.

19. Liu WD, Liu J and Triplett L et al. Novel insights into rice innate immunity against bacterial and fungal pathogens. Annu Rev Phytopathol 2014; 52: 213–41.

20. Zipfel C. Pattern-recognition receptors in plant innate immunity. Curr Opin Immunol 2008; 20: 10–16.

21. Shiu SH, Kar lou ski VM and Pan R et al. Comparative analysis of the receptor-like kinase family in Arabidopsis and rice. Plant Cell 2004; 16: 1220–34.

22. Fritz-Laylin LK, Krishnamurthy N and Tor M et al. Phylogenomic analysis of the receptor-like-proteins of rice and arabidopsis. Plant Physiol 2008; 146: 611–23.

23. Chen X and Ronald PC. Innate immunity in rice. Trends Plant Sci 2011; 16: 451–9.

24. Liu B, Li JF and Ao Y et al. Lysin motif-containing proteins LYP4 and LYP5 play dual roles in peptidoglycan and chitin perception in rice innate immunity. Plant Cell 2012; 24: 3406–19.

25. Kaku H, Nishizawa Y and Ishii-Minami N et al. Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. Proc Natl Acad Sci U S A 2006; 103: 11086–91.

26. Desaki Y, Miya A and Venkatesh B et al. Bacterial lipopolysaccharides induce defense responses associated with programmed cell death in rice cells. Plant Cell Physiol 2006; 47: 1530–40.

27. Song WY, Wang GL and Chen LL et al. A receptor kinase-like protein encoded by the rice disease resistance gene, Xa21. Science 1995; 270: 1804–6.

28. da Silva FG, Shen YW and Dandick C et al. Bacterial genes involved in type I secretion and sulfation are required to elicit the rice Xa21-mediated innate immune response. Mol Plant Microbe Interact 2004; 17: 583–601.

29. Takai R, Isogai A and Takayama S et al. Analysis of flagellin perception mediated by flag22 receptor OsFLS2 in rice. Mol Plant-Microbe Interact 2008; 21: 62–70.

30. Shimizu T, Nakano T and Takamizawa D et al. Two LysM receptor molecules, CEBIP and OsSerk1, cooperatively regulate chitin elicitor signaling in rice. Plant J 2010; 64: 204–14.

31. Yamaguchi K,亚马达 K and Ishikawa K et al. A receptor-like cytoplasmic kinase targeted by a plant pathogen effector is directly phosphorylated by the chitin receptor and mediates rice immunity. Cell Host Microbe 2013; 13: 946–61.

32. Lu X, Xu X et al. OsSerk1 and OsSerk2 play important roles in peptidoglycan and chitin signaling in rice innate immunity. Plant Physiol 2014; 80: 1072–84.

33. Zuo SM, Zhou XG and Chen MS et al. OsSerk1 regulates rice development but not immunity to Xanthomonas oryzae pv. oryzae or Magnaporthe oryzae. J Integr Plant Biol 2014; 56: 1179–92.

34. Chen X, Zuo S and Schwessinger B et al. An XA21-Associated Kinase (OsSerk2) regulates immunity mediated by the XA21 and XA3 immune receptors. Mol Plant 2014; 7: 874–52.

35. Prutt RN, Schwessinger B and Joe A et al. The rice immune receptor XA21 recognizes a tyrosine-sulfated protein from a Gram-negative bacterium. Sci Adv 2015; 1: e1400245.

36. Li X, Kapos P and Zhang YL. NRs in plants. Curr Opin Immunol 2015; 32: 114–21.

37. Zhou T, Wang Y and Chen JQ et al. Genome-wide identification of NBS genes in japonica rice reveals significant expansion of divergent non-TIR NBS-LRR genes. Mol Genet Genomics 2004; 271: 402–15.

38. Su J, Wang W and Han J et al. Functional divergence of duplicated genes results in a novel blast resistance gene Pi50 at the Pi2/9 locus. Theor Appl Genet 2015; 128: 2213–25.

39. Ma J, Lei C and Xu X et al. Pi64, encoding a Novel CC-NBS-LRR protein, confers resistance to leaf and neck blast in rice. Mol Plant Microbe Interact 2015; 28: 558–68.

40. Jiang N, Li ZQ and Wu J et al. Molecular mapping of the Pi2/9 allelic gene Pi2-2 conferring broad-spectrum resistance to Magnaporthe oryzae in the rice cultivar Jefferson. Rice 2012; 5: 29.

41. Zhu XF, Chen S and Yang JY et al. The identification of Pi50(t), a new member of the rice blast resistance Pi2/Pi9 multigene family. Theor Appl Genet 2012; 124: 1296–304.
43. Zhang S, Wang L and Wu W et al. Function and evolution of Magnaporthe oryzae avirulence gene AvrPib responding to the rice blast resistance gene Pit. *Sci Rep* 2015; 5: 11642.

44. Wang ZK, Yano M and Yamanouchi U et al. The Pit gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. *Plant J* 1999; 19: 55–64.

45. Bryan GT, Wu KS and Farrall L et al. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene Pi-ta. *Plant Cell* 2000; 12: 2033–46.

46. Orbach MJ, Farrall L and Sweigard JA et al. A telomeric avirulence gene determines efficacy for the rice blast resistance gene Pi-ta. *Plant Cell* 2000; 12: 2019–32.

47. Wu J, Kou Y and Bao J et al. Comparative genomics identifies the Magnaporthe oryzae avirulence effector AvrPib9 that triggers Pib-mediated blast resistance in rice. *New Phytol* 2015; 206: 1463–75.

48. Xu S, Liu G and Zhou B et al. The broad-spectrum blast resistance gene Pib encodes a nucleotide-binding site-leucine-rich repeat protein and is a member of a multigene family in rice. *Genetics* 2006; 172: 1901–14.

49. Zhou B, Qu S and Liu G et al. The eight amino-acid differences within three leucine-rich repeats between Pib2 and Piz-t resistance proteins determine the resistance specificity to Magnaporthe grisea. *Mol Plant Microbe Interact* 2006; 19: 1216–28.

50. Li W, Wang B and Wu J et al. The Magnaporthe oryzae avirulence gene AvrPiz-t encodes a predicted secreted protein that triggers the immunity in rice mediated by the blast resistance gene Piz-t. *Mol Plant Microbe Interact* 2009; 22: 411–20.

51. Chen X, Shang J and Chen D et al. A B-lectin receptor kinase gene conferring blast resistance blast. *Plant J* 2006; 46: 794–804.

52. Bohnert HU, Fudal I and Diop W et al. A putative polyketide synthase/peptide synthetase from Magnaporthe grisea signals pathogen attack to resistant rice. *Plant Cell* 2004; 16: 2499–513.

53. Yoshida K, Saitoh H and Fujisawa S et al. Association genetics reveals three novel avirulence genes from the rice blast fungal pathogen Magnaporthe oryzae. *Plant Cell* 2009; 21: 1573–91.

54. Liu X, Lin F and Wang L et al. The in silico map-based cloning of Pib3, a rice coiled-coil-nucleotide-binding site leucine-rich-repeat gene that confers race-specific resistance to the blast fungus. *Genetics* 2007; 176: 2541–9.

55. Lin F, Chen S and Que Z et al. The blast resistance gene Pib3 encodes a nucleotide binding site leucine-rich repeat protein and is a member of a resistance gene cluster on rice chromosome 1. *Genetics* 2007; 177: 1871–80.

56. Ashikawa I, Hayashi N and Yamane H et al. Two adjacent nucleotide-binding site-leucine-rich-repeat class genes are required to confer Pikm-specific rice blast resistance. *Genetics* 2008; 180: 2267–76.

57. Hayashi K and Yoshida H. Repulsion of the ancient rice blast disease resistance gene Pit by the recruitment of a retrotransposon as a promoter. *Plant J* 2009; 57: 413–25.

58. Lee SK, Song MY and Seo YS et al. Rice Pi5-mediated resistance to Magnaporthe oryzae requires the presence of two coiled-coil-nucleotide-binding-leucine-rich repeat genes. *Genetics* 2009; 181: 1627–38.

59. Shang J, Tao Y and Chen X et al. Identification of a new rice blast resistance gene, Pid3, by genomewide comparison of paired nucleotide-binding-site–leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. *Genetics* 2009; 182: 1303–11.

60. Lv Q, Xu X and Shang J et al. Functional analysis of Pid3-A4, an ortholog of rice blast resistance gene Pid3 revealed by allele mining in common wild rice. *Phytopathology* 2013; 103: 594–9.

61. Takahashi A, Hayashi N and Miyao A et al. Unique features of the rice blast resistance Pish locus revealed by large scale retrotransposon-Tagging. *BMC Plant Biol* 2010; 10: 175.

62. Zhai C, Lin F and Dong Z et al. The isolation and characterization of Pik, a rice blast resistance gene which emerged after rice domestication. *New Phytol* 2011; 189: 321–34.

63. Yuan B, Zhai C and Wang W et al. The Pik-p resistance to Magnaporthe oryzae in rice is mediated by a pair of closely linked CC-NBS-LRR genes. *Theor Appl Genet* 2011; 122: 1017–28.

64. Okeyama Y, Kanazaki H and Abe A et al. A multifaceted genetics approach allows the isolation of the rice Pi-a blast resistance gene consisting of two adjacent NBS-LRR protein genes. *Plant J* 2011; 66: 467–73.

65. Ribot C, Cesari S and Abidi I et al. The *Magnaporthe oryzae* effector AVR1-C039 is translocated into rice cells independently of a fungal-derived machinery. *Plant J* 2013; 74: 1–12.

66. Chen J, Shi Y and Liu W et al. A Pid3 allele from rice cultivar Gumei2 confers resistance to *Magnaporthe oryzae*. *J Genet Genomics* 2011; 38: 209–16.

67. Hua L, Wu J and Chen C et al. The isolation of Pi11, an allele at the Pi locus which confers broad spectrum resistance to rice blast. *Theor Appl Genet* 2012; 125: 1047–55.

68. Fukunaka S, Saka N and Koga H et al. Loss of function of a proline-containing protein confers durable disease resistance in rice. *Science* 2009; 325: 998–1001.

69. Hayashi N, Inoue H and Kato T et al. Durable panicle blast-resistance gene Pbl encodes an atypical CC-NBS-LRR protein and was generated by acquiring a promoter through local genome duplication. *Plant J* 2010; 64: 498–510.

70. Sweigard JA, Carroll AM and Kang S et al. Identification, cloning, and characterization of PWL2, a gene for host species specificity in the rice blast fungus. *Plant Cell* 1995; 7: 1221–33.

71. Lyer-Pascuzzi AS, Jiang H and Huang L et al. Genetic and functional characterization of the rice bacterial blight disease resistance gene xa56. *Phytopathology* 2008; 98: 289–95.

72. Yuan M, Chu Z and Li X et al. Pathogen-induced expression loss of function is the key factor in race-specific bacterial resistance conferred by a recessive R gene xa13 in rice. *Plant Cell Physiol* 2009; 50: 947–55.

73. Liu Q, Yuan M and Zhou Y et al. A paralog of the MtN3/saliva family recessively confers race-specific resistance to Xanthomonas oryzae in rice. *Plant Cell Environ* 2011; 34: 1958–69.

74. Sun X, Cao Y and Yang Z et al. Xa26, a gene conferring resistance to *Xanthomonas oryzae pv. oryzae* in rice, encodes an LRR receptor kinase-like protein. *Plant J* 2004; 37: 517–27.

75. Xiang Y, Cao Y and Xu C et al. Xa3, conferring resistance for rice bacterial blight and encoding a receptor kinase-like protein, is the same as Xa26. *Theor Appl Genet* 2006; 113: 1347–55.

76. Gu K, Yang B and Tian D et al. R gene expression induced by a type-III effector triggers disease resistance in rice. *Nature* 2005; 435: 1122–5.

77. Yoshimura S, Yamanouchi U and Katayose Y et al. Expression of Xa1, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. *Proc Natl Acad Sci USA* 1998; 95: 1663–8.

78. Streubel J, Pesce C and Hütin M et al. Five phylogenetically close rice SWEET genes confer TAL effector-mediated susceptibility to *Xanthomonas oryzae pv. oryzae*. *New Phytol* 2013; 200: 808–19.

79. Tian DS, Wang JX and Zeng X et al. The rice TAL effector-dependent resistance protein XA10 triggers cell death and calcium depletion in the endoplasmic reticulum. *Plant Cell* 2014; 26: 497–515.
80. Wang CL, Zhang XP and Fan YL et al. XA23 is an executor R protein and confers broad-spectrum disease resistance in rice. Mol Plant 2015; 8: 290–302.

81. Zhao B, Ardales EY and Raymundo A et al. TheavrRxo1 gene from the rice pathogen Xanthomonas oryzae pv. oryzaicolarfons nohost defense reaction on maize with resistance gene Rxo1. Mol Plant Microbe Interact 2004; 17: 771–9.

82. Zhao B, Lin X and Poland J et al. A maize resistance gene functions against bacterial streak disease in rice. Proc Natl Acad Sci USA 2005; 102: 15383–8.

83. Cook DE, Lee TG and Guo XL et al. Copy number variation of multiple genes at Rhg1 mediates nematode resistance in soybean. Science 2012; 338: 1206–9.

84. Liu SM, Kandoth PK and Warren SD et al. A soybean cyst nematode resistance gene points to a new mechanism of plant resistance to pathogens. Nature 2012; 492: 256–60.

85. Lorieux M, Reverst G and Garcia Diaz SX et al. Linkage mapping of Hsa-1(Og), a resistance gene of African rice to the cyst nematode, Heterodera sacchari. Theor Appl Genet 2003; 107: 691–6.

86. Wang C, Yang Y and Yuan X et al. Genome-wide association study of blast resistance in indica. BMC Plant Biol 2014; 14: 311.

87. Kang H, Wang Y and Peng S et al. Dissection of the genetic architecture of rice resistance to the blast fungus Magnaporthe oryzae. Mol Plant Pathol 2016; 17: 959–72.

88. Zhang X, Yang S and Wang J et al. A genome-wide survey reveals abundant rice blast R-genes in resistant cultivars. Plant J 2015; 84: 20–8.

89. Hogenhout SA, Van der Hoorn RA and Terauchi R et al. A genome-wide survey reveals abundant rice blast R-genes in resistant cultivars. Plant J 2015; 84: 20–8.

90. Liu W, Liu J and Ning Y et al. Recent progress in understanding PAMP-and effector-triggered immunity against the rice blast fungus Magnaporthe oryzae. Mol Plant 2013; 6: 605–20.

91. Kiang CH, Berruyer R and Giraldo MC et al. Translocation of Magnaporthe oryzae effectors into rice cells and their subsequent cell-to-cell movement. Plant Cell 2010; 22: 1389–403.

92. Park CH, Chen S and Shirekara G et al. The Magnaporthe oryzae effector AvrPiz-t targets the RING E3 ubiquitin ligase APIP6 to suppress pathogen-associated molecular pattern-triggered immunity in rice. Plant Cell 2012; 24: 4748–62.

93. Ning Y, Shi X and Wang R et al. OsELF3-2, an ortholog of Arabidopsis ELF3, interacts with the E3 ligase APIP6 and negatively regulates immunity against Magnaporthe oryzae in rice. Mol Plant 2015; 8: 1679–82.

94. Recenova T, Hala M and Kulich I et al. The role for the exocyst complex subunits Exo70B2 and Exo70H1 in the plant-pathogen interaction. J Exp Bot 2011; 62: 2107–16.

95. Fujisaki K, Abe Y and Ito A et al. Rice Exo7 interacts with a fungal effector, AVR-Pii, and is required for AVR-Pii-triggered immunity. Plant J 2015; 83: 875–87.

96. De Vleesschauwer D, Yang Y and Cruz CV et al. Abscisic acid-induced resistance against the brown spot pathogen Cochliobolus miyabeanus in rice involves MAP kinase-mediated repression of ethylene signaling. Plant Physiol 2010; 152: 2036–52.

97. Jiang CJ, Shimojo M and Sugano S et al. Abscisic acid interacts antagonistically with salicylic acid signaling pathway in rice-Magnaporthe grisea interaction. Mol Plant Microbe Interact 2010; 23: 791–8.

98. Mei C, Qi M and Sheng G et al. Inducible overexpression of a rice allene oxide synthase gene increases the endogenous jasmonic acid level, PR gene expression, and host resistance to fungal infection. Mol Plant Microbe Interact 2006; 19: 1127–37.

99. Yang DL, Yang Y and He Z. Roles of plant hormones and their interplay in rice immunity. Mol Plant 2013; 6: 675–85.

100. De Vleesschauwer D, Gheyysen G and Hofte M. Hormone defense networking in rice: tales from a different world. Trends Plant Sci 2013; 18: 555–65.

101. Piet erse CM, Van der Does D and Zamioudis C et al. Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol 2012; 28: 489–521.

102. Silverman P, S ekk M and Canter D et al. Salicylic acid in rice (biosynthesis, conjugation, and possible role). Plant Physiol 1995; 108: 833–9.

103. Feng S, Martinez C and Gusmaroli G et al. Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. Nature 2008; 451: 475–9.

104. De Vleesschauwer D, Seifi HS and Filipe O et al. The DELLA protein SLR1 integrates and amplifies salicylic acid- and jasmonic acid-dependent innate immunity in rice. Plant Physiol 2016; 170: 1831–47.

105. Kumari C, Dutta TK and Banakar P et al. Comparing the defence-related gene expression changes upon root-knot nematode attack in susceptible versus resistant cultivars of rice. Sci Rep 2016; 6: 22846.

106. Patkar RN, Benke P and Qu Z et al. A fungal monoxygenase-derived jasmonic acid attenuates host innate immunity. Nat Chem Biol 2015; 11: 733–40.

107. Robert-Seilaniantz A, Navarro L and Bari R et al. Pathological hormone imbalance. Curr Opin Plant Biol 2007; 10: 372–9.

108. Chuancul E, Kisiala A and Emery NR et al. Cytokinrin production by the rice blast fungus is a pivotal requirement for full virulence. PLoS Pathog 2016; 12: e1005457.

109. Van Boekhoven J, Spichal L and Novak O et al. Silicon induces resistance to the brown spot fungus Cochliobolus miyabeanus by preventing the pathogen from hijacking the rice ethylene pathway. New Phytol 2015; 206: 761–73.

110. Zhang ZM, Zhang X and Zhou ZR et al. Solution structure of the Magnaporthe oryzae avirulence protein AvrPiz-t. 1 Biomol NMR 2013; 55: 219–23.

111. de Guillen K, Ortiz-Vallejo D and Gracy J et al. Structure analysis uncovers a highly diverse but structurally conserved effector family in phytopathogenic fungi. PLoS Pathog 2015; 11: e1005228.

112. Majboob A, Saitoh H and Franceschetti M et al. Structural basis of pathogen recognition by an integrated HMA domain in a plant NLR immune receptor. Elife 2015; 4: e07809.

113. Han Q, Zhou C and Wu S et al. Crystal structure of Xanthomonas AvrRxo1-ORF1, a type III effector with a polynucleotide kinase domain, and its effector AvrRxo1-ORF2. Structure 2015; 23: 1900–9.

114. Liu W, Liu J and Ning Y et al. Recent progress in understanding PAMP-and effector-triggered immunity against the rice blast fungus Magnaporthe oryzae. Mol Plant 2013; 6: 605–20.

115. Slagci T, Santin M and Albrecht K et al. Plant pathogen recognition by an integrated HMA domain in a plant NLR immune receptor. Elife 2015; 4: e07809.

116. Nunez CC and Dean RA. Host-induced gene silencing: a tool for understanding fungal host interaction and for developing novel disease control strategies. Mol Plant Pathol 2012; 13: 519–29.

117. Nowara D, Gay A and Lacomme C et al. HIGS: host-induced gene silencing in the obligate biotrophic fungal pathogen Blumeria graminis. Plant Cell 2010; 22: 3130–41.
118. Koch A, Kumar N and Weber L et al. Host-induced gene silencing of cytochrome P450 lanosterol C14alpha-demethylase-encoding genes confers strong resistance to Fusarium species. Proc Natl Acad Sci USA 2013; 110: 19324–9.

119. Panwar V, McCallum B and Bakkeren G. Host-induced gene silencing of wheat leaf rust fungus Puccinia triticina pathogenicity genes mediated by the Barley stripe mosaic virus. Plant Mol Biol 2013; 81: 595–608.

120. Podevin N, Davies H V and Hartung F et al. Site-directed nucleases: a paradigm shift in predictable, knowledge-based plant breeding. Trends Biotechnol 2013; 31: 375–83.

121. Li T, Liu B and Spalding MH et al. High-efficiency TALEN-based gene editing produces disease-resistant rice. Nat Biotechnol 2012; 30: 390–2.

122. Jinek M, Chylinski K and Forfara I et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 2012; 337: 816–21.

123. Belhaj K, Chaparro-Garcia A and Kamoun S et al. Editing plant genomes with CRISPR/Cas9. Curr Opin Biotechnol 2015; 32: 76–84.

124. Wang Y, Cheng X and Shan Q et al. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol 2014; 32: 947–51.

125. Woo JW, Kim J and Kwon SI et al. DNA-free genome editing in plants with preassembled CRISPR-Cas9 ribonucleoproteins. Nat Biotechnol 2015; 33: 1162–4.