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Plant innate immunity in rice: a defense against pathogen infection

Wende Liu[1,*] and Guo-Liang Wang[1,2,*]

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*

[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China and [2]Department of Plant Pathology, Ohio State University, Columbus, OH 43210, USA

*Corresponding authors. E-mails: wang.620@osu.edu; liuwende@caas.cn

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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 environmental-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 immunity 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), 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

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

Diseases of rice	Pathogen	Rice yield loss	References
Fungal disease			
Rice blast	*Magnaporthe oryzae*	Up to 100%	[2]
Rice sheath blight	*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			
Bacterial blight	*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			
Rice root-knot nematode	*Meloidogyne graminicola*	Up to 87%	[9]
Rice white tip nematode	*Aphelenchoides besseyi*	Up to 50%	http://pest.ceris.purdue.edu/pest.php?code=NEABABB
Rice stem nematode	*Ditylenchus angustus*	20–90%	http://www.cabi.org/isc/datasheet/19285
Rice cyst nematode[a]	*Heterodera elachista*	Unknown	[10]
	Heterodera oryzicola	Up to 42%	[10]
	Heterodera oryzae	Unknown	[10]
	Heterodera sacchari	Similar with *H. oryzicola*	[10]
Viral disease			
Rice stripe	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

[a]Total of four species of *Heterodera* genus have been discriminated that cause rice cyst nematode disease.

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

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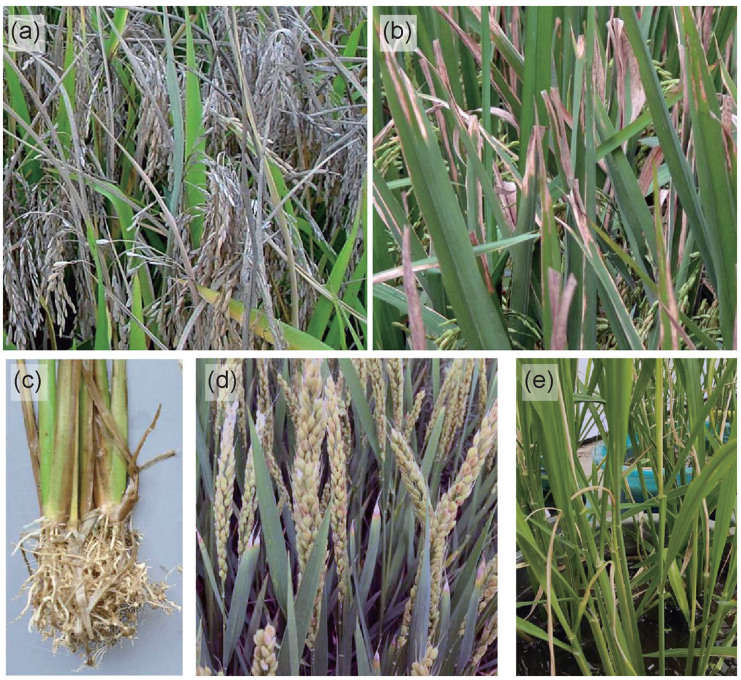

Figure 1. The five most important diseases of rice according to the journal *Molecular Plant Pathology*. (a) Rice blast (neck/panicle blast) caused by the fungus *M. oryzae*, a potent pathogen that infects all parts of rice but causes the greatest losses when it incites neck/panicle blast. The fungus initiates neck/panicle blast by infecting nodes on the rice stem; this is followed by massive hyphal growth, resulting in the rotting of the neck and the failure of grain filling. Image provided by Wende Liu. (b) Rice bacterial blight caused by *X. oryzae* pv. *oryzae*. After *X. oryzae* pv. *oryzae* invades the plant through hydathodes or wounds, it multiplies in xylem vessels. Image courtesy of Yongfeng Liu, Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, China. (c) Root knot caused by the nematode *M. graminicola*. *Meloidogyne graminicola* larvae infect rice roots, causing characteristic terminal swellings/galls on the roots and draining plant photosynthates and nutrients. Infection of young plants may be lethal, whereas infection of mature plants decreases yield. (d) Rice white tip caused by the nematode *A. besseyi*. The nematode enters rice florets, proliferates, and causes characteristic whitening of the leaf tips; the leaf tips then die, and grain yield is reduced. Images in panels c and d are from Wenkun Huang, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, China. (e) Rice stem nematode disease caused by *D. angustus*. This nematode is located in rice stubble and glumes during its dormant stage. During the growing season, it penetrates the leaf sheaths and stalks, where it develops, reproduces, and causes leaf distortion and seed abortion. Image courtesy of Jichun Wang, Institute of Plant Protection, Jilin Academy of Agricultural Sciences, China.

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* (Cooke) 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 repertoire 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, OsFLS2, 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 (*r*equired for *a*ctivation of *Xa21*) genes that activate the XA21-mediated immune response [28]. These genes are loca ted in a single operon (*raxSTAB*) that includes a tyrosine sulfotransferase (RaxST) and three

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Table 2. PRR genes and co-receptors that are important for rice immunity.

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]

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].

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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*	[43,44]
Pi-ta	NB-LRR	*AvrPi-ta*	224 AA secreted protein		[45,46]
Pi9	NB-LRR	*AvrPi9*	91 AA secreted protein		[47,48]
Pi2	NB-LRR	ND	–		[49]
Piz-t	NB-LRR	*AvrPiz-t*	108 AA secreted protein		[49,50]
Pi-d2	B lectin RLK	ND	–		[51]
Pi33[c]	–	*ACE1*	Polyketide synthase		[52]
Pii [c]	–	*AvrPii*	70 AA secreted protein		[53]
Pi36	NB-LRR	ND	–		[54]
Pi37	NB-LRR	ND	–		[55]
PiS0[a]	NB-LRR	ND	–		[39]
Pi64	NB-LRR	ND	–		[40]
Pikm[a]	NB-LRR	*Avr-Pik/km/kp*	113 AA secreted protein, five alleles (A–E)		[53,56]
Pit	NB-LRR	ND	–		[57]
Pi5[a]	NB-LRR	ND	–		[58]
Pid3	NB-LRR	ND	–		[59]
Pid3-A4	NB-LRR	ND	–		[60]
Pi54	NB-LRR	ND	–		[59]
Pish	NB-LRR	ND	–		[61]
Pik	NB-LRR	*Avr-Pik/km/kp*	113 AA secreted protein, five alleles (A–E)		[53,62]
Pikp	NB-LRR	*Avr-Pik/km/kp*	113 AA secreted protein, five alleles (A–E)		[53,63]
Pia[a,b]	NB-LRR	*Avr-Pia*	85 AA secreted protein		[53,64]
Pi-CO39[a,b]	NB-LRR	*Avr1-CO39*	89 AA secreted protein		[65]
Pi25	NB-LRR	ND	–		[66]
Pi1	NB-LRR	ND	–		[67]
pi21	Proline-containing protein	ND	–		[68]
Pb1	NB-LRR	ND	–		[69]
ND	–	*PWL2*	145 AA secreted protein		[70]
xa5	TFIIA transcription factor	*Avrxa5/PthXo7*		*Xanthomonas oryzae* pv. *oryzae*	[71]
xa13	MtN3/saliva domain protein	*Avrxa13/PthXo1*	TALE		[72]
Xa25	MtN3/saliva domain protein	ND			[73]
Xa3/Xa26	LRR-RLK	*AvrXa3*	TALE		[74,75]
Xa27	Rice unique gene	*AvrXa27*	TALE		[76]
Xa1	NB-LRR	ND			[77]
Os11N3 (*OsSWEET14*)	Homolog of nodulin MtN3	*AvrXa7*	TALE		[78]

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Table 3 (Continued.)

Resistant genes		Avirulence genes			References
R gene	Encoding protein	Avr gene	Encoding protein	Pathogen	
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]
Rxo1[d]	NB-LRR	*AvrRxo1*	–	*Xanthomonas oryzae* pv. *oryzicola*	[81,82]
STV11	Sulfotransferase	ND	–	RSV	[18]

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

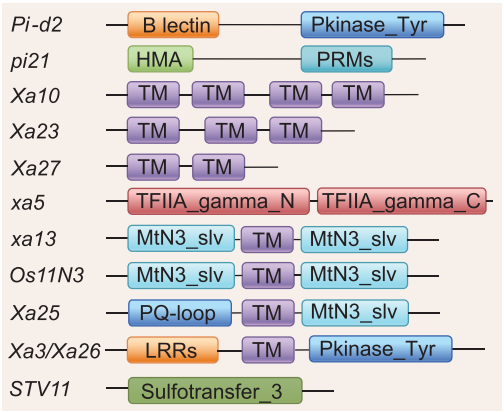

Figure 2. A diagram showing the domain diversity of rice atypical R proteins. Eleven rice R proteins with different domains are illustrated, including the bulb-type mannose-specific lectin (B lectin) domain, the protein tyrosine kinase (Pkinase_Tyr) domain, the heavy metal-associated (HMA) domain, the proline-rich motifs (PRMs), the transmembrane helices (TM), the transcription initiation factor IIA, gamma subunit, helical (TFIIA_gamma_N) domain, the transcription initiation factor IIA, gamma subunit (TFIIA_gamma_C), the sugar efflux transporter for intercellular exchange (MtN3_slv) domain, the PQ loop repeat (PQ-loop) domain, the leucine rich repeat (LRR) domain, and the sulfotransferase family (Sulfotransfer_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 promoters 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*.

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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 sulphonated 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*^{Og}) 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*^{Og} have not been cloned. Because another three species of cyst nematodes (*H. oryzicola*, *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

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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 monooxygenase (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

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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 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 disulphide 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 tritici-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 Pikp-HMA and Avr-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 by150% 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].

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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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