Chapter 8
Engineering Disease Resistance in Rice

K. K. Kumar, E. Kokiladevi, L. Arul, S. Varanavasiappan, and D. Sudhakar

Abstract Rice diseases cause substantial yield loss in rice. Through conventional breeding, resistance genes (R-gene) were transferred into elite rice genotypes particularly against the fungal blast and bacterial blight diseases. Main drawback of this approach is that, in the long term, breakdown of resistance occurs due to evolution of new virulent pathogen strains. In the current scenario, developing rice with durable broad-spectrum resistance through genetic transformation is gaining importance. In this direction, genetic transformation of rice was being carried out for the past two decades via expressing pathogenesis-related (PR) proteins, antimicrobial peptide, and genes governing signaling pathways as well as elicitor proteins. In spite of several reports, the expression of PR proteins and antimicrobial peptides did not yield desirable disease control in rice. Better understanding of disease resistance mechanism in plants helped in identifying critical transcription factors (TFs) involved in disease resistance. Overexpression of *NPR1* encoding non-expressor of pathogenesis-related protein 1 and *OsWRKY45* transcription factors in rice showed strong disease resistance to multiple pathogens and at the same time resulted in fitness cost. Recently, transgenic rice with high level of resistance to important rice diseases was achieved by expressing *NPR1* and *WRKY45* under tissue-specific/pathogen-responsive promoter; thereby agronomic traits are not altered. Rice transformants expressing the pathogen-derived elicitor proteins particularly from rice blast pathogen, *Magnaporthe oryzae* is a promising approach for imparting broad-spectrum disease resistance without yield penalty. Host-delivered RNAi technology is the latest of the approaches toward enhancing disease resistance against sheath blight and viral disease of rice. Recently, genome-editing tools are being deployed in rice to enhance resistance against diseases of rice.

Keywords Rice transgenic · Disease resistance · Signalling pathway · Elicitor protein · RNAi · Genome editing

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8.1 Introduction

Rice (*Oryza sativa* L.), being one of the important cereal crops of the world, is affected by more than 70 diseases. The most important rice diseases are the blast caused by fungus *Magnaporthe oryzae* and the bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Sheath blight (ShB) caused by fungus *Rhizoctonia solani* is also one of the important diseases of rice along with a few viral diseases. These diseases are responsible for causing annual yield losses of up to 50% of rice productivity (Datta et al. 2002). Rice is known to possess many disease resistance genes (R-gene) associated with blast and bacterial blight diseases. More than 40 genes conferring BB resistance (Sundaram et al. 2014), 101 blast-resistant genes (Rajashekara et al. 2014), and 350 quantitative trait loci (QTLs) have been identified (Sharma et al. 2012). Deployment of disease resistance (R) genes and quantitative trait loci through backcross breeding method has contributed greatly to increasing rice resistance against diverse pathogens (Kou and Wang 2010). However, such effort is hampered by the resistance breakdown due to the variability in pathogen population or development of new strains due to mutation (Jones and Dangl 2006; Dangl et al. 2013). Unlike the BB or blast disease, no major resistant gene is known in rice germplasm for sheath blight disease. Breeding for sheath blight resistance has not been successful as the resistance is controlled by multiple loci, and there is no reliable source of rice germplasm with complete resistance to the disease (Liu et al. 2009; Zuo et al. 2014). In the absence of suitable genetic resistance for ShB, chemical method is the only option for its control. Therefore, breeding for varieties with durable and broad-spectrum disease resistance is critical to sustainable agricultural development.

More than 25 viruses are known to infect rice. Important viral diseases of rice include rice dwarf virus (RDV), rice black-streaked dwarf virus (RBSDV), rice stripe virus (RSV), rice tungro bacilliform virus (RTBV), and rice tungro spherical virus (RTSV). In Southern Vietnam during 2006–2007, more than 485,000 hectares of paddy fields were severely affected by rice grassy stunt virus (RGSV) or co-infection by RGSV and rice ragged stunt virus (RRSV), resulting in heavy loss and directly affecting millions of rice farmers (Cabauatan et al. 2009). In China, epidemic outbreaks of the rice black-streaked dwarf disease resulted in a grain yield decrease of 10–40%, resulting in a total loss of grain production in the rice planting areas of southern China (Li et al. 1999). Rice tungro is one of the important viral diseases of rice, which is caused by the joint infection of two unrelated viruses *Rice tungro bacilliform virus* (RTBV), a double-stranded DNA-containing virus, and *Rice tungro spherical virus* (RTSV), a single-stranded RNA virus. The most conspicuous symptoms of tungro are the stunting of plants and yellow-orange discoloration of leaves. In the recent times, genetic engineering has been sought as the method of choice for achieving disease resistance in rice.

8.2 Engineering Disease Resistance in Rice by Overexpressing Antimicrobial Proteins

Earlier generation of transgenic rice for disease resistance focused on expressing the antimicrobial proteins belonging to pathogenesis-related (PR) proteins or antimicrobial peptide to engineer rice disease resistance. Enhanced disease resistance was observed via expressing the PR proteins or antimicrobial peptide, but the level of resistance conferred was not sufficient enough for commercial cultivation.

8.2.1 Overexpression of Pathogenesis-Related (PR) Proteins

Pathogenesis-related (PR) proteins are a group of plant proteins that express during pathogen infection as a defense mechanism. Several classes of PR proteins are known in plants. Plant chitinase (PR-3) and β-1-3-glucanase (PR-2) are two hydrolytic enzymes produced by the plants to break down the chitin (N-acetyl-D-Glucosamine) and glucan (laminarin) polymer, respectively, which constitute the major components of the fungal cell wall.

8.2.1.1 Overexpression of Chitinase

First attempt to engineer disease resistance in rice was done by Lin et al. (1995) by overexpressing rice chitinase gene, *chi11*, using constitutive maize ubiquitin promoter and showed enhanced resistance to *R. solani*. Subsequently, rice *chi11* gene was used to transform different genotypes of rice (Nishizawa et al. 1999; Datta et al. 2000, 2001; Kumar et al. 2003; Sridevi et al. 2003; Kalpana et al. 2006; Maruthasalam et al. 2007). Recently, a high expressing novel chitinase gene was isolated from the sheath blight-resistant QTL region (qSBR11-1 on chromosome 11) of resistant *indica* rice variety Tetep (Richa et al. 2016). Transformation of susceptible *japonica* rice line Taipei 309 (TP309) with the novel rice chitinase gene provided enhanced resistance against sheath blight pathogen, *R. Solani* (Richa et al. 2017). Li et al. (2009) transformed rice overexpressing *Momordica charantia* class I chitinase gene (McCHIT1) and showed an enhanced resistance to *R. solani* and *M. oryzae*. Compared to chitinases of plant origin, chitinases from biocontrol agents exhibit higher antifungal activity. Shah et al. (2009) transformed rice cv. PB1 with an endochitinase gene (*cht42*) from a fungus *Trichoderma virens* and recorded 62% reduction in sheath blight disease index.

Reports on co-expression of rice chitinase gene along with other PR protein showed synergistic effect for disease control in rice. The co-transformants expressing both *tlp* and *chi11* in rice showed an elevated resistance against *R. solani*

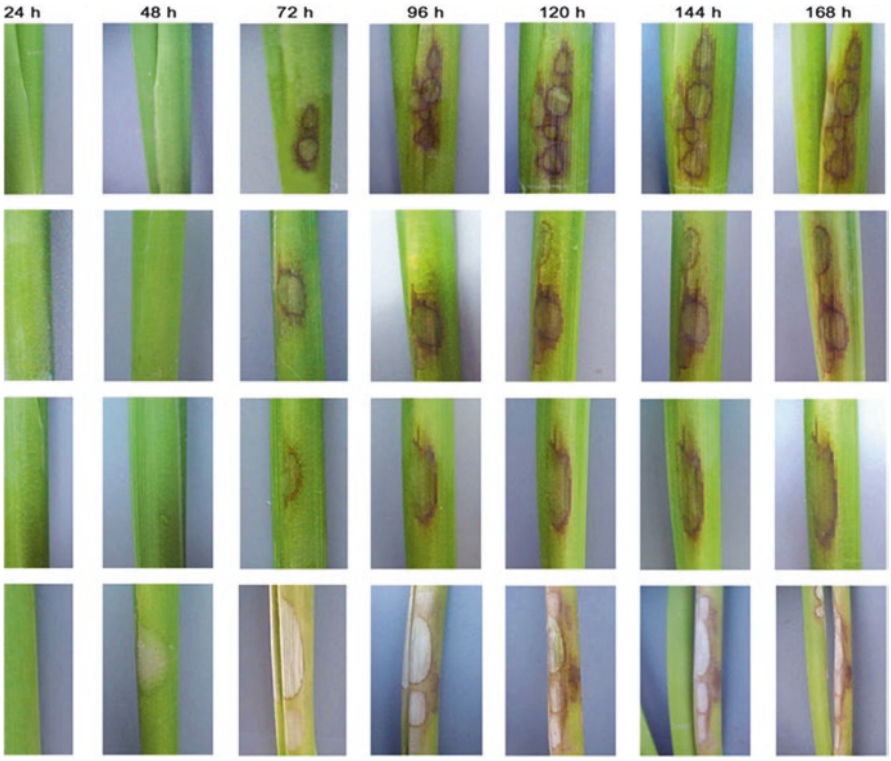

Fig. 8.1 Pyramiding of PR proteins (*chi11* and *tlp*) in transgenic rice *cv*. Pusa Basmati1 (PB1) demonstrated enhanced level of resistance to sheath blight disease. Bioassay was done in intact leaf sheaths of non-transgenic and transgenic PB1 lines using sheath blight pathogen. Reaction of SM-PB1-9 (*chi11*) (**a**) SM-PB1-5 (*tlp*) (**b**) SM-PB1-1 (*Chi11* + *tlp* + *Xa21*) (**c**) and untransformed PB1 (**d**) to sheath blight pathogen infection at 24, 48, 72, 96, 120, 144, and 168 h after infection (HAI). (Source: Maruthasalam et al. 2007)

(Fig. 8.1) and *Sarocladium oryzae* than plants expressing either *tlp* or *Chi11* (Kalpana et al. 2006; Maruthasalam et al. 2007). Transgenic rice plants transformed with *Chi11*, *tlp*, and *Xa21* and displayed resistance to both sheath blight and bacterial leaf blight (Fig. 8.2) (Maruthasalam et al. 2007). Transgenic rice constitutively co-expressing *tlp-D34* (thaumatin-like protein) gene and *chi11* showed enhancement of sheath blight resistance with disease index reduced to 39% (Shah et al. 2013). Co-expression of a rice basic chitinase gene and a ribosome-inactivating protein in rice caused a significant reduction in sheath blight development (Kim et al. 2003). Co-expression of *OsChi11* and *Osoxo4* genes in a green tissue-specific manner provided 63% resistance against sheath blight without affecting agronomically important traits (Karmakar et al. 2016). Maize phosphoenolpyruvate carboxylase (PEPC) promoter was used for *OsChi11* expression, and rice $P_{D540–544}$ promoter was used for *Osoxo4* gene expression.

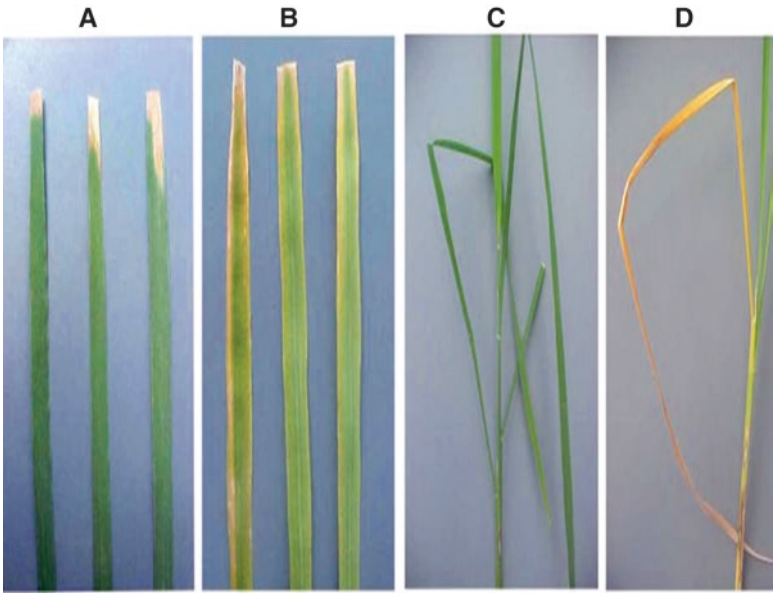

Fig. 8.2 Transgenic rice plants pyramided with *chi11+ tlp + Xa21* showed resistance to sheath blight and bacterial leaf blight. Reaction of SM-PB1-1 (**a**) and untransformed PB1 (**b**) to ShB infection at 168 HAI. Reaction of SM-PB1-1 (**c**) and non-transgenic PB1 (**d**) to *Xoo* infection at 14 days after inoculation. (Source: Maruthasalam et al. 2007)

8.2.1.2 Overexpression of other PR Proteins in Rice

Oxalic acid (OA) is a nonhost-specific toxin secreted by certain plant pathogens during infection (Dutton and Evans 1996). Plant oxalate oxidase (OxO) enzyme degrades OA into CO_2 and hydrogen peroxide (H_2O_2). OxO-generated H_2O_2 may function as a secondary messenger in the activation of phytoalexin biosynthetic pathways, hypersensitive response (HR), systemic acquired resistance (SAR), and PR gene expression in plants. Genome analysis of rice showed four tandemly duplicated *oxo* genes (*Osoxo1–Osoxo4*) in chromosome 3, with *Osoxo4* playing a role in disease resistance (Carrillo et al. 2009). Transgenic rice overexpressing the rice oxalate oxidase 4 (*Osoxo4*) gene under a green tissue-specific promoter (rice PD540–544) exhibited 50% protection against *R. solani* without any agronomic imbalance (Molla et al. 2013).

Germin-like protein (GLP) gene family is one of the important defense gene families that have been considered to play an important role in several aspects of plant development or stress tolerance (Knecht et al. 2010). One of the rice GLP genes, *OsGLP2-1*, was significantly induced by blast fungus (Liu et al. 2016). Overexpression of *OsGLP2-1* quantitatively enhanced resistance to leaf blast, panicle blast, and bacterial blight (Liu et al. 2016). *OsGLP2-1*-mediated resistance to blast and bacterial blight was involved in the activation of jasmonic acid (JA)-dependent pathway instead of salicylic acid (SA)-dependent pathway.

Osmotin and osmotin-like proteins (OLP) belong to thaumatin-like proteins (TLP) of the PR-5 family because they all contain a typical thaumatin domain. It is involved in plant permeability stress and defense responses because of its antibacterial properties in vivo against a broad range of plant pathogens (Narasimhan et al. 2005). Xue et al. (2016) found that *OsOSM1* expression is strongly induced by *R. solani* in ShB-resistant rice variety YSBR1. Overexpression of *OsOSM1* (OsOSM1ox) in susceptible variety Xudao 3 significantly increases resistance to SB in transgenic rice (Xue et al. 2016). They found that JA-responsive marker genes are induced in OsOSM1ox lines and suggest that the activation of JA signalling pathway may account for the increased resistance in transgenic OsOSM1ox lines.

8.3 Engineering Disease Resistance in Rice by Overexpressing Antimicrobial Peptides

Antimicrobial peptides (AMPs) are used by both plant and animal systems to destroy microorganism, including bacteria, fungi, mycoplasma, and viruses. AMPs are characterized to possess high anti microbial activity and be very quick in killing microbes and at the same time are nontoxic to eukaryotic cells. Defensins are small antifungal peptides (~5 KDa) of eukaryotic origin present in plant, animal, and insects. Plant defensins (PR-12) are low molecular weight cysteine-rich peptide thought to affect cell membrane of microbes and prevent its ion uptake. Transgenic rice expressing *Dahlia merckii* defensin (*DM-AMP1*) gene gave better level of protein (up to 80%) to the two important rice fungal pathogens *M. oryzae* and *R. solani* (Jha et al. 2009). The Dm-AMP1 signal peptide had successfully targeted the Dm-AMP1 to apoplast in transgenic rice. Transgenic rice expressing the antimicrobial peptide from onion (*Ace*-AMP1) improved their resistances to blast, sheath blight, and bacterial blight by 86%, 67%, and 82%, respectively (Patkar and Chattoo 2006). Rice overexpressing *Rs-AFP2* defensin gene from *Raphanus sativus* suppressed the growth of *M. oryzae* and *R. solani* by 77% and 45%, respectively (Jha and Chattoo 2010). Transgenic expression of Rs-AFP2 was not accompanied by an induction of PR gene expression, suggesting that the expression of Rs-AFP2 directly inhibits the pathogens. The antimicrobial peptide of humans, LL-37, is a 37-residue-long peptide which possesses broad-spectrum antibacterial activity and was used for rice transformation. Transgenic rice expressing the LL-37 peptide in the intercellular space showed enhanced disease resistance against bacterial leaf blight and blast (Lee et al. 2017). To avoid degradation by the plant proteases, the fusion of vicilin signal peptide at the N-terminal of LL-37 directed it to intercellular space. The *pGD1* (phosphogluconate dehydrogenase) promoter from rice was used to induce stable expression of SP-LL-37 in transgenic rice. Giant silk moth (*Hyalophora cecropia*) encodes the antimicrobial protein cecropin A and cecropin B. Transgenic rice plant expressing plant codon-optimized *cecropin A* gene exhibited resistance to rice blast without an induction of PR gene expression (Coca et al. 2006). Similarly,

transgenic rice plants expressing *cecropin B* exhibited reduction in lesion due to BB pathogen infection (Sharma et al. 2000; Coca et al. 2004). Overall, the overexpression of antimicrobial protein in transgenic rice confers enhanced level of resistance to all important diseases of rice.

8.4 Engineering Broad-Spectrum Disease Resistance in Rice

Plants defend against microbial pathogen attack by activating a variety of defense systems that are mediated through multiple signalling pathways. Plant defense signalling is mainly mediated through the plant hormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). In general, plants upon exposure to pathogen induce two well-known forms of immune responses: SA-mediated systemic acquired resistance (SAR) and JA/ET-mediated inducible systemic resistance (ISR). The induced immune response often confers durable, broad-spectrum, and systemic resistance against different pathogens at distal tissue from the infection or treatment site. Many transcription factors are successfully used for engineering disease resistance in rice (Table 8.1). Transcription factors NPR1 and WRKY45 act as key positive regulator of SA-mediated pathway in plants. The SA pathway in rice appears to branch into OsNPR1/NH1 and WRKY45-mediated sub-pathway. Plant-inducible immune response can also be triggered by exogenous application of a number of elicitors or elicitor transgene expression.

8.4.1 Rice Transgenic Plants Expressing NPR1 Gene

8.4.1.1 AtNPR1

Arabidopsis thaliana NPR1 (*AtNPR1*) is a key positive regulator, which acts downstream of the signal molecule SA in regulating gene expression of SAR pathway (Cao et al. 1994). Transgenic rice constitutively expressing the *AtNPR1* gene results in disease resistance to bacterial pathogen *Xoo* but had a negative impact on growth and agronomic traits due to triggering lesion-mimic/cell death (LMD) phenotype (Fitzgerald et al. 2004). In another study, transgenic rice plants constitutively expressing *At*NPR1 have been reported to exhibit negative physiological consequences in the form of growth retardation, height reduction, and decreased seed production (Quilis et al. 2008). Green tissue-specific expression of *AtNPR1* using the $P_{D540–544}$ promoter in rice confers resistance to the sheath blight pathogen, with no concomitant abnormalities in plant growth and yield parameters (Molla et al. 2016). They demonstrated that an increase in the *AtNPR1* transcript levels in the transgenic rice plants resulted in the activation of many defense-related PR genes, and the elevated induction of PR genes appeared to translate into enhanced resistance

Table 8.1 Genetic transformation of rice with transcription factor associated with disease resistance

Sl. No.	Gene	Promoter	Type of expression	Transgenic *indica*/*japonica* variety	Disease resistance	Alteration in phenotype	Reference
1.	*OsWRKY6* NCBI GenBank: BK005009.1	Constitutive; CaMV35S	Overexpression	*Japonica* cv Nipponbare	Strong resistance to BLB	Growth retardation	Choi et al. (2015)
2.	*OsWRKY11* RGAP[a]: LOC_Os01g43650	Constitutive; CaMV35S	Overexpression	*Japonica* cv. Nipponbare	Enhanced resistance to BLB	Enhanced tolerance to drought stress, reduction in height	Lee et al. (2018)
3.	*Os*WRKY13 NCBI GenBank: EF143611	Constitutive; maize *ubiquitin*	Overexpression	*Japonica* cv. Mudanjiang 8	Resistance to BLB and blast	Decreases tolerance to cold and salt stresses as well as to retarded growth and development	Qiu et al. (2007, 2008)
4.	*OsWRKY30* NCB GenBank: DQ298180	Constitutive; maize *ubiquitin*	Overexpression	*Japonica* cv. Xiushui 11	Enhanced resistance to rice sheath blight and blast	Increased drought stress, exhibited no obvious morphological changes	Peng et al. (2012)
5.	*OsWRKY45-1* NCBI GenBank: AK066255 RAP-DB[b]: Os05g0322900	Constitutive; maize *ubiquitin*	Overexpression	*Japonica* cv. Nipponbare	Strong resistance to blast but reduces resistance to BLB	Transgenic rice plants varied with growth conditions, adverse effects on agronomic traits	Shimono et al. (2007)
6.	Os*WRKY45-2* *indica* cv. Minghui 63 *NCBI GenBank:* GQ331927 *OsWRKY45-1* *japonica cv.* Nipponbare NCBI GenBank: GQ331932	Constitutive; maize *ubiquitin*	Downregulation of OsWRKY45-1 and overexpression of OsWRKY45-2	*Indica* cv. Minghui 63 or *Japonica* cv. Mudanjiang 8	Enhanced resistance to Xoo and Xoc (*Xanthomonas oryzae pv oryzicola*). However, overexpression of both allele of WRKY confers enhanced resistance to blast	Reduces adaptation to salt, cold, and drought stresses	Tao et al. (2009)

7.	*OsWRKY45* NCBI GenBank: AK066255 RAP-DB[b]: Os05g0322900	Constitutive; *OsUbi7*	Overexpression	*Japonica* cv. Nipponbare	Resistance to BLB and blast	Minor negative effects on agronomic traits	Goto et al. (2015)
8.	*OsWRKY45* NCBI GenBank: AK066255 RAP-DB[b]: Os05g0322900	*Pathogen inducible; PR1b with ADH* 5′-UTR	Inducible expression	*Japonica* cv. Nipponbare	Resistance to blast and blast	Agronomic traits comparable to control untransformed rice	Goto et al. (2016)
9.	*OsWRKY62* NCBI GenBank: AK067834 RAP-DB[b]: Os09g0417800	Constitutive; maize *ubiquitin*	Downregulation (RNAi)	*Japonica* cv. Nipponbare	Susceptible to blast and BLB, thus act as positive regulator	Plays a negative role in pathogen defense under hypoxia stress	Fukushima et al. (2016)
10.	*OsWRKY67* RAP-DB[b]: Os05g0183100	Constitutive; CaMV35S	Overexpression	*Japonica* cv. Dongjin (DJ)	Enhanced resistance to BLB and blast	Plant growth retardation	Vo et al. (2018)
11.	*Os*WRKY76 NCBI GenBank: AK068337	Constitutive; maize *ubiquitin*	Overexpression	*Japonica* cv. Nipponbare	Enhances susceptibility to *M. oryzae* and *Xoo*	Increases cold tolerance	Yokotani et al. (2013)
12.	*OsEREBP1* RGAP[a]: LOC_Os02g54160	Constitutive; maize *ubiquitin*	Overexpression	*Japonica* cv. Kitaake	Resistance to BLB	Confers drought and submergence tolerance	Jisha et al. (2015)
13.	*OsMYC2* RGAP[a]: Os10g42430 or RAP-DB[b]: Os10g0575000	Constitutive; maize *ubiquitin*	Downregulation (RNAi)	*Japonica* cv. TP309	Showed enhanced resistance against BLB	Suppressed seedling growth compared to wild-type plants under blue light and showed little effect under white light	Giri et al. (2017)

(continued)

Table 8.1 (continued)

Sl. No.	Gene	Promoter	Type of expression	Transgenic *indica*/*japonica* variety	Disease resistance	Alteration in phenotype	Reference
14.	*OxDC* gene from *Bacillus subtilis* named as *Bacisubin* NCBI GenBank: HQ452341	Constitutive; CaMV35S	Overexpression	*Japonica* cv. Nipponbare	Resistance to rice blast and sheath blight	Normal growth, development, and grain production in rice	Qi et al. (2017)
15.	*OsXrn4* gene NCBI GenBank: AK070933	Constitutive; CaMV 35S	Overexpression	*Japonica* cv. Nipponbare	Conferred resistance to rice stripe virus	–	Jiang et al. (2018)
16.	MAPK kinase 10.2 RGAP[a]: Loc_Os03g12390	Constitutive; maize *ubiquitin*	Overexpression	*Japonica* cv. Nipponbare	Enhances resistance to *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*)	Increases rice tolerance to drought stress	Ma et al. (2017)
17.	*OsCIPK30* (calcineurin B-like proteins) interaction protein kinase protein. RAP-DB[b]: Os01g0759200	Constitutive; maize *ubiquitin*	Overexpression	*Japonica* cv. Nipponbare	Delay the RSV symptoms and show milder RSV symptoms	Transgenic plants showed normal growth	Liu et al. (2017)
18.	*OsMADS26* RAP-DB[b]: Os08g0112700	Constitutive; maize *ubiquitin*	RNAi lines	*Japonica* cv. Nipponbare	Resistance to blast and BLB	Displayed enhanced tolerance to water deficit. Moderate impact on plant development	Khong et al. (2015)

19.	Os*ACS2* NCBI GenBank:AK064250	Pathogen-inducible; *PBZ1* promoter	Inducible expression	*Japonica* cv. Kitaake	Broad-spectrum disease resistance to necrotrophic and hemibiotrophic fungal. Resistance to a field isolate of *R. solani*, as well as different races of *M. oryzae*	Enhance resistance to fungal pathogens without negatively impacting crop productivity	Helliwell et al. (2013)
20.	*AtNPR1* *NCBI GenBank:* ATU76707	Constitutive; maize *ubiquitin*	Overexpression	*Japonica* cv. Taipei 309 (TP309)	Resistance to the bacterial pathogen *Xoo*	Detrimental side effects under controlled environment to develop lesion-mimic/cell death (LMD) phenotype	Fitzgerald et al. (2004)
21.	*AtNPR1* *NCBI GenBank:* ATU76707	Constitutive; maize *ubiquitin*	Overexpression	*Japonica* cv. Senia	Confers resistance against blast, bakanae diseases (caused by *Fusarium verticillioides)* and foot root disease (caused by *Erwinia chrysanthemi*) of rice. Higher susceptibility to infection by the *Rice yellow mottle virus* (*RYMV*)	Growth retardation and reduced height, observed spontaneous lesions when growing under suboptimal conditions (growth chamber). Susceptible to salt and drought stress	Quilis et al. (2008)

(continued)

Table 8.1 (continued)

Sl. No.	Gene	Promoter	Type of expression	Transgenic *indica*/*japonica* variety	Disease resistance	Alteration in phenotype	Reference
22.	*AtNPR1* NCBI GenBank:NM_105102	Green tissue-specific expression; $P_{D540-544}$ promoter NCBI GenBank: KJ857554	Tissue-specific expression	*Indica* cv. Pusa SugandhII II (PSII)	Confers resistance to the sheath blight pathogen	Transgenic plants are phenotypically similar to the non-transgenic wild type under greenhouse conditions	Molla et al. (2016)
23.	*AtNPR1*	*Pathogen inducible; TFB1* promoter plus 5′ leader sequence TAIR[c]: AT4G36988	Inducible expression	*Japonica* cv. Zhonghua 11 (*ZH11*)	Broad-spectrum disease resistance. Resistance to BLB, fungal blast, and bacterial leaf streak	No compromise on rice plant fitness	Xu et al. (2017b)
24.	*BjNPR1* *NCBI GenBank:* AY667498	Constitutive; CaMV35S	Overexpression	*Indica* cv. Chaitanya and Samba Mahsuri	Enhanced resistance to rice blast, sheath blight, and bacterial leaf blight diseases	Improvement in certain agronomic traits such as increases in plant height, panicle length, flag-leaf length, number of seeds/ panicle, and seed yield/ plant	Sadumpati et al. (2013)

25.	*OsERF922* *RAP-DB*[b]*:* *Os01g0752500*	Expression of sgRNA under rice U6 promoter	Gene knockout using CRISPR/Cas9	*Japonica* cv. Kuiku131	Enhanced resistance to blast	Main agronomic traits are not altered	Wang et al. (2016a)
26.	*Bsr-d1* RGAP[a]: *LOC_Os03g32230*	Constitutive; maize *ubiquitin for RNAi*	Downregulation (RNAi) and gene knockout using CRISPR/Cas9	*Japonica* cv. TP309 rice	Broad-spectrum blast disease resistance	Agronomic character not altered	Li et al. (2017)

[a]RGAP Rice Genome Annotation Project
[b]RAP-DB Rice Annotation Project Database
[c]TAIR the Arabidopsis Information Resource

of transgenic rice to *R. solani*. Expression of *AtNPR1* under pathogen-inducible promoter can overcome fitness cost in rice. Earlier plant defense gene expression was thought to be regulated at transcriptional level by the pathogen-inducible promoter. However recently, Xu et al. (2017a) did global transcriptome profiling on *Arabidopsis* plant exposed to elf18 elicitor and discovered that fundamental layer of regulation also occurs at translation level during defense response. In this study, they identified a pathogen-inducible TFB1 gene in *Arabidopsis* that is rapidly and transiently induced upon pathogen challenge. *TFB1* promoter with 5′ leader sequence (before the start codon for *TFB1*) contains two untranslated ORF (uORFs) in it. Translation of TBF1 is normally suppressed by these two uORFs within the 5′ leader sequence (Pajerowska-Mukhtar et al. 2012). Xu et al. (2017b) transformed rice with a construct that expresses *AtNPR1* under *TFB1* promoter cassette (*TFB1* promoter plus 5′ leader sequence with two pathogen-responsive upstream open reading frames, $uORFs_{TFB1}$). Thus, they engineered broad-spectrum disease resistance in rice without compromising on rice plant fitness. The rice plants displayed resistance to BLB, fungal blast, and bacterial leaf streak. Thus using *TFB1* cassette, it is possible to develop transgenic plants with enhanced broad-spectrum disease resistance with minimal adverse effects on growth and development. In an another study, rice co-expressing *AtNPR1* and *OsCHI11* under green tissue-specific promoter showed enhanced sheath blight tolerance as compared to single-gene transformants (Karmakar et al. 2017).

8.4.1.2 OsNPR1/NH1

Overexpression of *OsNPR1/NH1* was shown to confer strong resistance to both *Xoo* and *M. oryzae* (Chern et al. 2005; Sugano et al. 2010). Overexpression of *OsNPR1*/NH1 in rice induced constitutive activation of *PR* gene expression, accompanied by lesion-mimic symptoms and light hypersensitivity (Chern et al. 2005). Overexpression of *OsNPR1 c*onferred disease resistance to bacterial blight but also enhanced herbivore susceptibility in transgenic plants (Yuan et al. 2007). Sugano et al. (2010) conducted experiments to determine the function of *OsNPR1* and found that overexpression of *OsNPR1* led to increased activity in defense mechanisms against pathogens but reduced cellular activity with regard to photosynthesis and protein synthesis that leave the plant more vulnerable to herbivore predation.

8.4.1.3 BjNPR1

Transgenic *indica* rice expressing *Brassica juncea NPR1* (*BjNPR1*) exhibits enhanced resistance to rice blast, sheath blight, and bacterial leaf blight diseases (Sadumpati et al. 2013). Rice transformants with higher levels of *BjNPR1* revealed improvement in certain agronomic traits such as increases in plant height, panicle

length, flag-leaf length, number of seeds/panicle, and seed yield/plant as compared to the untransformed plants.

8.4.2 Rice Transgenic Plants Expressing OsWRKY45

Although discovered recently, WRKY transcription factors are becoming one of the best characterized classes of plant transcription factors and are at the forefront of research on plant defense responses. More recent studies have provided direct evidence for the involvement of specific WRKY proteins in plant defense responses. Interaction between rice and *Xoo* is a classical example of host-pathogen interaction and serves as an ideal model system for investigation. WRKYs, a class of plant-specific transcription factors, act as a key regulator of plant immune response (Ulker and Somssich 2004). The "WRKY" domain of ~60 amino acids binds to the cognate *cis*-acting "W" box motif (C/T)TGAC(C/T) in the promoter of several downstream target genes. Rice overexpressing *OsWRKY45* under strong constitutive promoter (maize ubiquitin promoter, *PZmUbi*) showed extremely strong disease resistance to both rice blast and leaf blight but at significant costs on rice growth and yield (Shimono et al. 2007; Shimono et al. 2012). The WRKY45-OX rice plants cultivated in a growth chamber showed restricted growth, and those cultivated in a greenhouse showed only minor growth retardation (Shimono et al. 2007).To reduce the negative effect of *WRKY45* overexpression in rice, Goto et al. (2015) optimized expression of *WRKY45* gene in rice using a moderate-strength constitutive rice ubiquitin promoter (POsUbi7). Transgenic rice plants expressing WRKY gene at moderate level showed strong resistance to both blast and BLB diseases in a greenhouse, although the degree of resistance was a little weaker than that of the representative PZmUbi line (Goto et al. 2015). At the same time, adverse effects of environmental factors on WRKY45-ox lines are alleviated in POsUbi7 lines, whereas most of the PZmUbi plants died after the low-temperature treatment, indicating that a high level of WRKY45 expression rendered rice plants cold sensitive.

Blast pathogen, *M. oryzae* hyphae, invades rice cells within 24 h post-inoculation. However, WRKY45 is induced after the *M. oryzae* invasion in rice (Shimono et al. 2007). Due to the time lag in WRKY45 protein induction, it is unable to exert its full defense potential against blast pathogen (Shimono et al. 2007, 2012). To address this issue, Goto et al. (2016) developed rice lines in which WRKY45 induction occurs soon after pathogen challenge using an early pathogen-responsive promoter. Goto et al. (2016) developed transgenic rice with strong disease resistance to blast and BB by expressing WRKY45 under the control of pathogen-responsive promoters in combination with a translational enhancer derived from a 5′-untranslated region (UTR) of rice alcohol dehydrogenase (ADH). Although pathogen-responsive promoters alone failed to confer effective disease resistance, the use of the ADH 5'-UTR in combination with them, in particular the PR1b and GST promoters, enhanced disease resistance. The 2-kb upstream sequence of PR1b showed a very early pathogen response with high level of WRKY45 expression confined to infec-

tion site. This early and strong local induction of WRKY45 may be critical for the strong disease resistance in WRKY45-expressing lines. Field trials showed that overall PR1b promoter-driven (with ADH 5'-UTR) lines performed the best without any negative effects on agronomic traits, which is comparable to control untransformed rice.

Recently, *OsWRKY67* was found to be upregulated against pathogen challenges. Activation of *OsWRKY67* by T-DNA tagging significantly improved the resistance against two rice pathogens, blast and BB. Subsequently, overexpression of *OsWRKY67* in rice confirmed enhanced disease resistance but led to a restricted plant growth of the transgenic plants with high levels of *OsWRKY67* protein. OsWRKY67 RNAi lines significantly reduced resistance to *M. oryzae* and *Xoo* isolates tested and abolished XA21-mediated resistance, implying the possibility of broad-spectrum resistance from *OsWRKY67* (Vo et al. 2018). On the other side, *OsWRKY62* was reported to act as negative regulator of innate and *Xa21*-mediated resistance against bacterial blight (Peng et al. 2008). Further in the study, transgenic rice lines overexpressing *OsWRKY62* challenged with *Xoo* were found to show significantly longer lesions than the wild-type controls. Thus, the negative role played by *OsWRKY62* was evident and suggests suppression of such a kind of negative players could be employed towards enhancing the innate defense system in rice. Similarly, overexpression of *OsWRKY72* was found to be negatively influencing BB resistance in rice (Seo et al. 2011).

8.4.3 Engineering Disease Resistance in Rice by Enhancing Ethylene Biosynthesis

Recent evidence indicates that ethylene (ET) pathway also plays a major role in mediating plant disease resistance. Six rice *ACS* genes (*OsACS1-6*) are reported to exist in the rice genome. During the rice-*M. oryzae* interaction, endogenous ET levels increased within 48 h after inoculation with a significantly higher production of ET in the incompatible Pii R-gene-mediated interaction (Iwai et al. 2006). OsACS1 and OsACS2 were significantly induced upon *M. oryzae* infection, along with the induction of an ACC oxidase (ACO) gene, *OsACO*7. Silencing of *OsACS2* and *OsACO7* by RNA interference (RNAi) resulted in increased susceptibility to rice blast (Seo et al. 2011), suggesting that OsACS2 and ET production play a positive role in rice resistance to *M. oryzae* infection. Helliwell et al. (2013) genetically manipulated the endogenous ET level in transgenic rice by expressing *OsACS2* (1-aminocyclopropane-1-carboxylic acid synthase) transgene under control of a strong rice pathogen-inducible promoter *PBZ1*. Rice plants generated exhibited increased resistance to *R. solani* and different races of *M. oryzae*. These results suggest that pathogen-inducible production of ET in transgenic rice can enhance broad-spectrum disease resistance to necrotrophic and hemibiotrophic fungal pathogens without negatively impacting crop productivity.

8.4.4 *Engineering Rice Disease Resistance by Expressing Pathogen Protein Elicitor Gene*

One promising approach to the achievement of broad-spectrum resistance is to incorporate genes that elicit general defense responses in plants. Several microbial protein elicitors have been shown to induce systemic acquired resistance in plants by activation of both SA- and ET/JA- mediated signalling pathway. The bacterial harpin and flagellin protein acts as elicitor in plants. Shao et al. (2008) introduced a harpin-encoding gene, *hrf1*, derived from *Xoo* into rice. Transgenic rice expressing *Xoo* harpin gene was highly resistant to all major races of *M. oryzae*. Bacterial flagellin expression induces disease resistance in transgenic rice (Takakura et al. 2008). Expression of the *PemG1* gene from *M. oryzae* in transgenic rice results in enhanced resistance to the rice blast fungus (Qiu et al. 2009). By characterizing the protein in the culture filtrate of rice blast fungus, two novel protein elicitors, MoHrip1 and MoHrip2, were identified, and subsequently their gene was isolated from the *M. oryzae* (Chen et al. 2012, 2014). The MoHrip1- and MoHrip2-expressing transgenic rice plants displayed higher resistance to rice blast and stronger tolerance to drought stress than wild-type rice (Wang et al. 2017). The MoHrip1 and MoHrip2 transgenic rice also exhibited enhanced agronomic traits such as increased plant height, tiller number, thousand-kernel weight, and ear number. Rice transformants overexpressing *MoSM1* protein elicitor gene from *M. oryzae* confers broad-spectrum resistance to both *Xoo and BLB* but at the same time had no effect on drought, salinity, or grain yield (Hong et al. 2017). The MoSM1-OE plants contained elevated levels of salicylic acid (SA) and jasmonic acid (JA) and constitutively activated the expression of SA and JA signaling-related regulatory and defense genes. However, no alteration in resistance to sheath blight disease was observed in MoSM1-OE lines.

8.5 RNAi-Mediated Gene Silencing in Rice to Engineer Disease Resistance

RNA interference, an evolutionarily conserved process that is active in a wide variety of eukaryotic organisms, is a sequence-specific gene-silencing mechanism that is induced by dsRNA (Baulcombe 2004). The dsRNA is diced into small interfering RNAs (siRNAs) of 21–24 nucleotides by an endonuclease called dicer. These siRNAs are then incorporated into the RNA- induced silencing complex to guide degradation or translational repression in a sequence-specific manner. Host-delivered RNAi (HD-RNAi) is a method which involves the production of double-stranded RNA (dsRNA) molecules targeting pathogen genes in the host plant, which will be processed further into small interfering RNA molecules (siRNAs). HD-RNAi has been successful in engineering resistance against plant virus (Duan et al. 2012),

insects (Huvenne and Smagghe, 2010), nematode (Fairbairn et al. 2007), and fungi (Nunes and Dean, 2012). Recently, Tiwari et al. (2017) demonstrated that host-delivered RNAi method can be used for the control of sheath blight in rice. They transformed rice with the hairpin RNAi construct containing fusion of two pathogenicity Map Kinase 1 (*PKM1*) genes, *RPMK1-1* and *RPKM1-2* of *R. solani.* Due to host-delivered siRNA-mediated silencing of the target genes, the expression level of *RPMK1-1* and *RPMK1-2* was significantly lower in *R. solani* infecting transgenic rice, thereby enhancing sheath blight resistance in rice.

Ding et al. (2006) has developed a Brome Mosaic Virus (BMV)-based VIGS (virus-induced gene silencing) system to produce the siRNA of the target gene in rice. BMV-based system was employed to target the three predicted pathogenicity genes, *MoABC1*, *MoMAC1*, and *MoPMK1*. Zhu et al. (2017) studied the effectiveness of BMV-mediated HIGS (host-induced gene silencing) in silencing three predicted pathogenicity genes of *M. oryzae*. Inoculation of BMV viral vectors in rice resulted in systemically generating fungal gene-specific small interfering RNA(siRNA) molecules, which inhibited disease development and reduced the transcription of targeted fungal genes after subsequent *M. oryzae* inoculation (Zhu et al. 2017).

Virus resistance mediated by natural resistance genes and RNA silencing-mediated virus resistance are currently two major research focuses (Sasaya et al. 2014). Plant uses RNA silencing as a natural defense mechanism against plant viruses. Thus RNA silencing has been successfully exploited for engineering virus resistance in plants including rice. So far no natural resistance gene discovered for RBSDV in rice germplasm (Nicaise 2014). Rice black-streaked dwarf virus (RBSDV) is a dsRNA virus that causes severe yield loss in rice grown in Asia. Wang et al. (2016b) transformed rice with hairpin RNAi (hpRNAi) construct targeting four RBSDV genes, *S1*, *S2*, *S6*, and *S10*, encoding the RNA-dependent RNA polymerase, the putative core protein, the RNA silencing suppressor, and the outer capsid protein, respectively. Transgenic rice plants expressing the RBSDV hpRNA showed strong virus resistance in both the field and artificial assay system. Wang et al. (2016b) showed that long hpRNA targeting multiple viral genes can be used to generate stable and durable virus resistance in rice. They did small RNA deep sequencing on the RBSDV-resistant transgenic lines and detected siRNAs from all four viral gene sequences in the hpRNA transgene, indicating that the whole chimeric fusion sequence can be efficiently processed by dicer into siRNAs. Earlier to this report, transgenic rice plants containing an hpRNA transgene targeting the *P9-1*-encoding gene were almost immune to RBSDV infection (Shimizu et al. 2011).

8.6 Genome Engineering in Rice for Disease Resistance

Genome-editing technologies offer possibility of genome modification in a site-directed manner. Three popular genome-editing methods are zinc finger nucleases (ZNFs), transcription activator-like effector nucleases (TALENs), and CRISPER/

Cas9 system. Among the three methods, CRISPER/Cas9 system is an effective system for introducing mutation in the gene of interest in crop plants. Gene editing was successfully used for engineering disease resistance in rice. The rice bacterial blight susceptibility gene *Os11N3* (also called *OsSweet14*) was disrupted using TALEN genome-editing tool to provide *Xoo* resistance (Li et al. 2012). The *SWEET* gene encodes sucrose efflux transporter family and is hijacked by *Xoo*, using its endogenous TAL effectors AvrXa7 or PthXo3, to activate the gene and thus divert sugars from the plant cell so as to satisfy the pathogen's nutritional needs and enhance its persistence. Recently, Wang et al. (2016a) mutated (loss-of-function) the *OsERF922* gene by CRISPR/Cas9 method. Mutated rice lines thus created showed enhanced rice blast resistance without affecting the main agronomic traits. A natural allele of a C2H2-domain transcription factor gene, *bsr-d1*, confers broad-spectrum resistance to rice blast in Digu rice variety (Li et al. 2017). CRISPR/Cas9-mediated knockout of Bsr-d1 enhanced blast resistance without alteration in agronomic character (Li et al. 2017).

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