Engineering Disease Resistance in rice

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Abstract

More than three billion people depend on rice (Oryza sativa L) as a staple food. The filamentous rice blast fungus (Maganaporthe oryzae Couch) poses a most serious threat in rice cultivation worldwide. Rice and blast fungus combination has emerged as the model pathosystem due to intensive research carried out in these two organisms over the last several years. Previously, natural host defence mechanisms have been meticulously utilized in defending economically important crop rice from the attack of the phytopathogen. The application of more recent techniques in biotechnology and genetic engineering of agricultural crop plants has the potential to further advance the development of blast resistant rice plants. In this article, we discuss how transgenic expression glucose oxidase and over-expression of OsCDR1 in rice helped us to understand host plant resistance. We look forward toward analyzing rice innate immunity to engineer its resistance against the pathogen, leading to durable and broad-spectrum resistance.

Keywords: Rice innate immunity, blast resistance genes, blast fungus, glucose oxidase, *OsCDR1*

Introduction

Rice blast fungus causes the most serious disease and it has been reported from more than 85 countries (Kato 2001). In the absence of crop protection strategies rice yields can go down by 10-30%. Current strategies include breeding for durable resistance and use of fungicides. A rigorous phylogenetic analysis has led to redefining of rice blast fungus as *Magnaporthe oryzae* which is different from *M. grisea* based on multi-locus genealogy and mating experiments. The isolates capable of infecting crabgrass (*Digitaria* sp.) are known as *M. grisea* and those infecting rice, millets and other grasses (*Oryza*, *Setaria*, *Lolium*, *Eragrotis* and *Elusine*) are known as *M. oryzae* (Couch and Kohn 2002). *M. oryzae* is an ascomycetous fungus and its genome has been sequenced (Dean 2005). Rice with a comparatively small genome size (~400 Mb), availability of genetic resources, and its genetic relatedness to other major cereals make it an attractive model crop plant to study the biology of molecular plant-fungus interactions. Genome sequence of both subspecies of rice – *indica* (Yu et al 2002) and *japonica* (Goff et al 2002) as well as a high quality annotated version of the *japonica* genome are available from international rice genome sequencing project (IRGSP 2005).

Genetic improvement of rice through conventional breeding methods has been an effective strategy for developing high yielding varieties. This has been accomplished by transferring genes from the wild relatives to the cultivated species of rice through distant hybridization. Modern biotechnological techniques can contribute to the agronomic improvement of rice by overcoming some of the limitations in traditional breeding methods. The transgenic approach of plant genetic manipulation provides access to an unlimited gene pool for the transfer and expression of desirable genes irrespective of their evolutionary and taxonomic status. Genetic transformation of rice provides numerous opportunities for the improvement of existing cultivated varieties of rice. Rice transformation is now routine in many laboratories around the world. Gene transfer techniques such as PEG-mediated, electroporation, microprojectile bombardment, and *Agrobacterium*-mediated transformation are the most widely used methods for introducing foreign genes into rice (Peng et al 1992; Christou 1997; Hiei et al 1997; Yokoi et al 2000). In addition to efficient transformation methods, improvement of rice by genetic engineering depends on availability of agronomically important genes. Significant advances in cell biology, gene delivery techniques, and molecular biology of transgene expression have allowed the identification of desirable genes for introgression into rice

Disease resistance is a major challenge. We discuss some of the recent advances in this area under the following categories: (i) elucidation of initial

recognition mechanisms, identification of signaling molecules, and pathways to better understand plant responses to pathogen infection; (ii) introduction of genes into plants to detoxify microbial compounds that act as fungal pathogenicity factors; and (iii) expression of genes, singly and in combination, producing antifungal proteins and other antimicrobial products, in transgenic plants.

Review of recent advances in research

Innate immunity

Most plant species are resistant to most plant pathogens. Thus, pathogen isolated from one plant species in most cases cannot infect, reproduce and cause disease on other distantly related species. An important and interesting question is what determines host range of a pathogen. Plants possess an innate system that efficiently detects and limits the growth of pathogens. Many of the phytopathogenic fungi initiate infection with the germination of conidiospores on the plant leaf surface. This follows formation of an infection structure such as an appressorium from which infection hyphae develops and results in the creation of penetration peg. These hyphae breach host epidermal cell walls and the infection induces dome-shaped extensions of the inner surface of the wall known as papilliae, with the help of physical pressure and enzymatic degradation. The tips of the infection hyphae then expand to form multi-fingered feeding structures that invaginate but don't penetrate the plasma membrane of the host. In addition, symbiotic fungi and oomycetes can also invaginate feeding structures (haustoria), into the host cell membrane. Plants lack mobile immune cells or a somatic immune system, unlike animals. Rather, they rely on an innate immunity system and on the systemic signals emanating from the site of infection. Research on the innate immune system is of great interest for advancing basic understanding of this important stress response as well as for improvement of crop plants. It is now understood that there are three layers of the plant innate immune system. They are (i) PAMP (pathogen associated molecular patterns) triggered immunity (PTI), (ii) effector triggered immunity (ETI) and (iii) systemic acquired resistance (SAR).

PAMP Triggered immunity (PTI)

The first layer of innate immunity is governed by host sensors known as pattern recognition receptors (PRRs) that recognise pathogen- (or microbial-) associated molecular patterns (PAMPs or MAMPs) displayed on the surface of the pathogens. This can halt further colonisation by microbial pathogens. The host sensors are typically receptor kinases (Ronald et al 2010). The PRRs detect lipo-polysaccharides (LPS), peptides, chitin, double-stranded RNA, microbial DNA and other molecules of microbial origin (Ronald et al 2010). CEBiP, a rice host sensor, belonging to class LysM recognises a microbial molecule chitin from rice blast fungus (Kaku et al 2006). Expression of chimeric receptor in rice plants exhibited necrotic lesions in response to chitin and became more resistant to *M. oryzae* (Kishimoto et al 2010). These results demonstrated that generation and expression of host sensors is a viable strategy for engineering host resistance.

Effector triggered immunity (ETI)

Successful pathogens breach the first layer of innate immunity by deploying effectors which contribute to pathogen virulence. However, a given effector may be specifically recognized by NBS-LRR proteins, directly (gene-for-gene hypothesis) or indirectly (guard hypothesis). This phenomenon results in effector triggered immunity (ETI), which is considered as accelerated and amplified PTI response (Jones and Dangl 2006). ETI is more specific and is predicted to be less durable because effectors are highly variable among different strains of the same pathogen (Chen and Roland, 2011). Some of the characterised NBS-LRR proteins that confer resistance against *M. oryzae* include the following: Pita (Bryan et al 2000; Jia et al 2000), Pib (Wang et al 1999), Piz-t (Zhou et al 2006), Pikm (Ashikawa et al 2008), Pit (Kawano et al 2010), Pid3 (Shang et al 2009), Pi2 (Zhou et al 2006), Pi5 (Lee et al 2009), Pi9 (Qu et al 2006), Pi36 (Liu et al 2007), Pi37 (Lin et al, 2007), and Pb1 (Hayashi et al, 2010) and Pia (Okuyama et al 2011). The NBS-LRR proteins are crucial for rice innate immunity system. Some of the effectors characterised from *M. oryzae* are: AvrPita (Jia et al 2000), AvrPiz-t (Shang et al 2009), Avr-Pik/km/kp (Lee et al 2009) and AvrPia (Qu et al 2006). It is remarkable that NBS-LRR-mediated disease resistance is effective against obligate pathogens and hemi-biotrophic pathogens but not against necrotropic pathogens. Pathogens may evade ETI by either shedding or diversifying the effector proteins already recognised by the host. They may also acquire additional effectors that suppress ETI. This, in turn, results in gene specificities so that ETI can be triggered again (Jones and Dangl 2006).

Systemic acquired resistance (SAR)

Systemic acquired resistance (SAR) is characterized by an activation of a broad spectrum of host defence mechanisms, locally at the site of the initial pathogen attack as well as systemically, in tissues untouched by the pathogen. SAR can provide resistance against diverse organisms such as fungi, bacteria, and viruses. Induced defense reactions associated with SAR

involve both biochemical and cytological changes, and depend on the production of a signal that is translocated to other parts of the plant, where it triggers resistance (Schneider et al 1996). SAR provides enhanced and long-lasting systemic immunity to secondary infection by a range of biotrophic, hemi-biotrophic and necrotrophic pathogens (Grant and Lamb 2006). Non-expressor of Pathogenesis-Related1 (NPR1) is a central positive regulator of SAR signalling (Fobert and Despres 2005). SAR is usually associated with the generation of reactive oxygen species, accumulation of salicyclic acid (SA), induction of pathogenesis-related genes, reinforcement of plant cell wall, etc. Identification of mobile signals that govern SAR and mechanisms by which they are perceived in distal tissues remains unclear. SA was considered as the critical signalling component in manifestation of SAR. However, evidence suggests that signal translocation probably uses lipid-derived (jasmonate-based) signals and lipid chaperones (Grant and Lamb 2006).

Natural resistance to rice blast disease

Breeders have developed numerous cultivars resistant to rice blast. The emergence of pathotype variation in *M. oryzae* populations has been proposed as the principal mechanism involved in loss of resistance (Ou 1985). Compatibility of plant–pathogen interactions is governed by the gene-for-gene model (Flor 1971) in many pathosystems (Brown et al 1995). Because emergence of new pathotypes (or races) of a pathogen can often break down host resistance that is based on a single resistance (*R*) gene, the mechanisms underpinning pathotype variation, especially the sources and dynamics of new pathotypes, have been extensively investigated in several pathosystems that follow the gene-for-gene model .including the rice blast system (Valent et al 1994).

There has been considerable progress in understanding mechanisms of blast resistance and the process of pathogenesis of the blast fungus. Availability of genome sequence of both, rice (Goff et al 2002; Yu et al 2002; IRGSP 2005) and *M. oryzae* (Dean et al 2005) have significantly enriched our understanding of the genes involved in disease resistance.

Rice belongs to the genus *Oryza* which includes more than 25 wild species including perennial and annual species, which are either diploid or tetraploid. The *indica* and *japonica* are subspecies of *Oryza sativa*, which are largely grown and consumed by 50% of world human population. Continuous selection of domesticated wild rice for the development of high yielding and well adapted varieties has led to genetic erosion, which, in turn has narrowed variability in rice. Several useful major and minor blast resistance genes have been identified. However, the potential resistance sources hidden in the wild species of rice largely remain unexplored.

Rice blast resistance genes

A list of 26 genes blast resistance genes was published in Rice Genetics Newsletter (RGN) in 1995. Since then, several other blast resistance genes have been identified Molecular markers have been used extensively to identify and map genes for blast resistance. (Jia and Martin, 2008; Lin et al 2007; Gowda et al 2006; Barman et al 2004; Monosi et al 2004; Sallaud et al 2006; Naqvi et al 1995). . Several major blast resistance genes have been cloned; *Pib* (Wang et al 1999), *Pita* (Bryan et al 2000), *Pikh* (Sharma et al 2005), *Pi9* (Ou et al 2006), *Pi2*/*Pizt* (Zhou et al 2006), *Pi36* (Liu et al 2007), and *Pi37* (Liu et al 2007) and shown to belong to NBS- LRR family of resistance genes.

Utilisation of resistance genes and broad spectrum resistance

Rapid evolution and emergence of new aggressive *M. oryzae* isolates can be managed using cultivar mixtures and multilines. Crop cultivar heterogeneity might make the pathogen adapt more slowly to the resistance genes deployed. By planting mixtures of hybrid, blast resistant and susceptible cultivars intraspecific crop diversification has been achieved (Zhu et al 2000). Multilines are seed mixtures comprising of several near isogenic lines (NILs) each carrying different *R* genes (Ishazaki et al 2005; Takeuchi et al 2006). Sasanishiki and Koshihikari are examples of multilines (Koizumi et al 2004).

A lineage exclusion hypothesis was proposed to design approaches to disease management (Zeigler et al 2005). This hypothesis relies on a couple of assumptions: (i) rice blast populations consist of sets of flow amongst them and (ii) resistance deployed is associated with a fungal fitness cost. Thus, it makes sense to stack more than a single resistance gene in a cultivar and this approach of gene pyramiding is an effective strategy to prevent breakdown of blast disease resistance. When this strategy is followed selection pressure on a single blast isolate is reduced (Bonman et al 1992; Hittalmani et al 2000). Pyramided lines with resistance gene combinations *Pi*1 and *Piz*-5, and *Pi*1, *Piz*-5 and *Pi-ta* exhibit a broadened resistance spectrum in India and Philippines (Hittalmani et al 2000). Rice breeding line Jefferson carrying *Pik* and *Piz* genes has retained its resistance since 1997 (Fjellstrom et al 2004; McClung et al 1997).

Another strategy is the deployment of a broad spectrum of *R* genes which confer resistance against different

strains of the fungus (Bonman et al 1992; Kawata et al 2003). *R* genes are tagged with tightly-linked molecular markers which are utilised in MAS to achieve accurate introgression of combinations of these genes (Babujee and Gnanamanickam 2000; Fjellstrom et al 2004). Molecular markers (RAPD, RFLP, AFLP, SCAR, STS, CAPS etc.) offer great scope for improving efficiency of conventional plant breeding by using molecular markers linked to the trait. This is especially advantageous to use in breeding for a pathogen.

Innovative studies underway

Understanding host resistance by expressing glucose oxidase

Oxidative burst, mediated by hydrogen peroxide (H_2O_2), has been recognized as a key component of plant defense response during an incompatible interaction. To determine if elevated levels of H_2O_2 lead to cell death, activation of defense genes and enhanced resistance to diverse pathogens, transgenic rice plants expressing a fungal glucose oxidase gene (*GOX*) were generated using both constitutive and inducible expression systems. Both constitutive and wound or pathogen-induced expression of GOX led to increases in the endogenous levels of H_2O_2, which in turn caused cell death. Elevated levels of H_2O_2 also activated the expression of several defense genes and these transgenic plants showed enhanced resistance to both bacterial and fungal pathogens (Kachroo et al 2003). In comparison to inducible expression, constitutive expression of GOX resulted in 3–10-fold higher levels of the *GOX* transcript and the corresponding enzymatic activity. Such increased levels of GOX, which would result in elevated levels of H_2O_2 caused improper seed set and decreased seed viability in transgenic plants constitutively expressing *GOX*. These results have suggested that pathogen inducible expression of heterologous genes may be a practical and robust way of generating broad spectrum disease resistance.

A basal level of GOX activity was observed in transgenic plants expressing *GOX* under the control of the *PAL* promoter. Both wounding and pathogen infection led to up-regulation of GOX activity and a corresponding increase in the levels of H_2O_2, which resulted in cell death. Since a pathogen or wound inducible promoter was used to control the expression of *GOX* gene, enhanced resistance in these transgenic plants could be triggered by either of these responses and was equally effective. Wounding of transgenic plants prior to challenge with a pathogen did not enhance the resistance status of such plants further, suggesting that pathogen infection alone was sufficient to confer enhanced resistance in these plants. It has been shown that plants produce $O2-$ and H_2O_2 through a mechanism similar to the mammalian neutrophil NADPH oxidase system (Auh and Murphy 1995; Pugin et al 1997; Kawasaki et al 1999). Rapid generation of H_2O_2 during the oxidative burst is a key component of the plant defense response to pathogen challenge (Levine et al 1994; Alvarez et al 1998).

Elevation of H_2O_2 levels can affect plant defense in several ways, presumably by stimulating cross-linking of proline-rich proteins of the cell wall (Bradley et al 1992; Brisson et al 1994), and inducing several plant genes involved in cellular protection and defense (Chen et al 1993; Mehdy 1994; Lamb and Dixon 1997). H_2O_2 is also required for initiating programmed cell death which leads to SAR (Dangl et al 1996; Alvarez et al 1998). We observed that programmed-like cell death was initiated by wounding and pathogen infection and defense related genes were induced stronger in 583 *PAL::GOX* rice plants. These results suggest that elevated H_2O_2 levels in transgenic rice, conferred disease resistance through an oxidative burst-mediated defense response.

A surprising finding was that the peroxidase gene *PIR3* was found to be suppressed at the RNA level in *GOX*-expressing plants, suggesting that peroxidase might play an important role in H_2O_2-mediated defense response. It was shown that pathogen inducible H_2O_2-manipulation, led to programmed- like cell death and PR gene activation in transgenic rice. Furthermore, it was also shown that pathogen inducible expression of heterologous genes is an effective way to confer broad spectrum disease resistance in crop plants and is less likely to cause any developmental abnormalities or metabolic changes.

The suppression of peroxidase is likely to reduce the capability of cells to scavenge H_2O_2, which in turn stimulates the accumulation of H_2O_2 and acceleration of plant cell death (PCD) (Mittler et al 1998). It will be worth studying, whether severely reduced peroxidase levels will result in enhanced disease resistance in peroxidase antisense transgenic plants like in the case of reduced catalase levels (Takahashi et al 1997; Chamnongpol et al 1998).

Understanding host resistance by over-expressing *OsCDR1*

Plant aspartic proteases (AP) play key roles in the regulation of biological processes, such as the recognition of pathogens and pests and the induction of effective defense responses. A large number of AP (>400) have been identified *in silico* in the rice genome. None have previously been isolated and functionally characterized for their involvement in disease

resistance. A gene (*OsCDR1*) from rice which encodes a predicted aspartate protease was isolated and characterised. Expression of *OsCDR1* was induced upon treatments with benzothiadiazole (BTH) and salicylic acid, which are signal molecules in plant disease resistance responses. Ectopic expression of *OsCDR1* in *Arabidopsis* and rice conferred enhanced resistance against bacterial and fungal pathogens. Enhanced disease resistance was observed in transgenic plants which was correlated with induction of pathogenesis-related gene expression and was shown by mutational analysis to be dependent on AP activity of the transgene-encoded product. OsCDR1 accumulates in intercellular fluids (IF) in transgenic plants. Infiltration of IF from transgenic *Arabidopsis* plants into leaves of wild-type (WT) *Arabidopsis* resulted in induced the systemic defense response. These results demonstrated the conservation of CDR1 function between rice and *Arabidopsis* during the disease resistance response (Prasad et al 2009).

Studies showed that *OsCDR1* was transcriptionally activated in response to treatments with BTH or SA but not with jasmonic acid (JA). Previous studies have demonstrated that BTH is effective in inducing disease resistance and in activating defense responses in rice (Gorlach et al 1996; Xiong et al 2001). Unlike most dicot plants, rice plants normally contain relatively high constitutive levels of free SA (Silverman et al 1995). However, in spite of high endogenous levels, rice leaves have an SA-inducible glucosyl transferase activity (SA-GTase), an enzyme that conjugates SA (Silverman et al 1995), suggesting that rice leaves can respond to exogenous SA. Whether SA is a signal molecule in rice disease resistance response has not been well established, although exogenous application of SA to rice plants was found to induce various genes that play important roles in different stress responses (Xu et al 2006; Li et al 2008). Functional validation of *OsCDR1* following identification through bioinformatics was achieved in transgenic *Arabidopsis* lines that ectopically expressed *OsCDR1*.

Interestingly, *OsCDR1:Arabidopsis* accumulated high levels of SA and showed several-fold induction of the defense-related genes *PR1* and *PR2* but not of *PDF1*.2, a marker of JA-mediated responses. The accumulation of SA is an important step in the signal transduction pathway leading to SAR, disease resistance, and susceptibility (Delaney et al 1994). It has been proposed that at least one of the mechanisms of SA effect is through up-regulation of levels of active oxygen species such as H_2O_2. Leaves treated with SA have been shown to accumulate H_2O_2 in *Arabidopsis* and rice (Ganesan and Thomas 2001). Interestingly, the transgenic plants also exhibited oxidative bursts leading to generation of H_2O_2 which have been previously shown to be associated with *PR1* gene expression at a step in signal transduction upstream of SA (Bi et al 1995).

The high level of *PR* gene expression in *OsCDR1*-transgenic plants suggests a role for *OsCDR1* in SA-mediated disease resistance signaling pathways. This is further supported by the observation that over-expression of *OsCDR1* in transgenic *Arabidopsis* plants enhanced resistance against infection by *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis* but not against *Alternaria brassicicola.* Both *P. syringae* and *H. arabidopsidis* have biotrophic phases of pathogenicity whereas *A. brassicicola* is a necrotrophic pathogen. Expression of *PR* genes and the SA-dependent signaling pathway are involved in the regulation of defense responses against biotrophic pathogens, while the *PDF1*.2 gene and the JA and ethylene signalling pathway are involved in modulating defense responses against necrotrophic pathogens (Gupta et al 2000; Kunkel and Brooks 2002; Spoel et al 2003).

In *Arabidopsis*, over-expression of *AtCDR1* causes dwarfing (Xia et al 2004) whereas, out of four transgenic lines studied, only one line (At17) showed growth retardation and an early leaf senescence phenotype. The leaf senescence phenotype observed in the At17 transgenic line might be due to higher expression of *OsCDR1* or the nature of the T-DNA integration site. *OsCDR1* activation is likely to lead to the generation of an endogenous extracellular peptide elicitor, as has been proposed for *Arabidopsis* CDR1 (Xia et al 2004). Indeed, local infiltration of IF from OsCDR1: *Arabidopsis* plants into WT *Arabidopsis* leaves induced the systemic defense response, and an elicitor activity was detected in the IF. The hypothesized elicitor released by OsCDR1 can rapidly activate basal defense responses in local and systemic leaves, suggesting a high capacity for mobility. Further, evaluation and extension of this approach to the rice system is of high agronomical importance because rice is susceptible to a broad spectrum of diseases. Hence, transgenic rice plants overexpressing *OsCDR1* were generated and analysed for the expression of various defense-related genes and for their resistance to *M. oryzae* and *X. oryzae.*

Expression of the different PR proteins is commonly used as a marker for SAR. Although activation of PR protein- encoding genes by compatible pathogens evidently does not lead to resistance in the plant against the invading pathogen, these genes may be effective if activated before the challenge inoculation occurs. In some cases, constitutive overexpression of *PR* genes could further enhance resistance to certain pathogens

(Jach et al 1995; Epple et al 1997). Hence, *OsCDR1* was overexpressed under a constitutive promoter which led to activation of defense-related genes, including *PBZ1/PR10* and *PR1*. Although variable levels of *OsCDR1* and defense-related genes were observed among the transgenic lines, there was a correlation between the levels of *OsCDR1* and the degree of induction of defense-related genes. In rice, *OsPR1* genes are induced through the coordinated action of SA-, JA-, and ABA signaling pathways and underlie the SAR response (Mitsuhara et al 2008). Expression characteristics of the 12 *OsPR1* genes indicate that the resistance obtained in *OsCDR1*: transgenic rice might be due to SA- but not JA- or ABA-mediated disease resistance signaling (Prasad et al 2009). Overexpression of *OsCDR1* in *Arabidopsis* and rice and previously reported over-expression of *CDR1* in *Arabidopsis* showed elevated *PR* gene expression and acquired enhanced resistance against phytopathogens; these results suggest for the presence of similar defense pathways in *Arabidopsis* and rice (Prasad et al 2009; Xia et al 2004). It is important to note that overexpression of *OsCDR1* in rice had no deleterious effects in our experimental conditions. This observation also satisfies the criteria of agricultural genetic engineering which include creating crops that are durable to stresses, require fewer chemical inputs, and do not show unwanted agronomic effects. These findings should greatly facilitate study on disease-resistance pathways in rice and other monocots, and may have a profound impact on controlling diseases in economically important crops such as cereals.

Prospects of achieving breeding goal

Achieving durable resistance

Recently we have witnessed paradigm-shifting advances in the field of plant-microbe interactions. However, most of the observations are based on the study of the *Arabidopsis-Pseudomonas syringae* system. Currently, *O. sativa – M. oryzae* has emerged as an important model to study plant-fungus interactions for crop plants. It is important to further anlayse the biology of rice innate immunity to engineer pathogen tolerance leading to durable and broad- spectrum resistance.

REFERENCES

Alvarez ME, Pennell RI, Meijer PJ, Ishikawa A, Dixon RA and Lamb C. 1998. Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. *Cell* 92: 773–784.

Ashikawa I, Hayashi N, Yamane H, Kanamori H, Wu J, Matsumoto T, Ono K and Yano M. 2008. Two adjacent nucleotide-binding site-leucine-rich repeat class genes are required to confer *Pikm*-specific rice blast resistance. *Genetics* 2267-2276.

Auh C-K and Murphy TM. 1995. Plasma Membrane redox enzyme is involved in the synthesis of O_2- and H_2O_2 by *Phytophthora* elicitor-stimulated rose cells. *Plant Physiol* 107**:** 1241–1247.

Babujee L and Gnanamanickam SS. 2000. Molecular tools for characterization of rice blast pathogen (*Magnaporthe grisea*) population and molecular marker-assisted breeding for disease resistance. *Curr Sci* 78:248-257.

Barman SR, Gowda M, Venu RC and Chattoo BB. 2004. Identification of a major blast resistance gene in rice cultivar Tetep. *Plant Breed* 123: 300-302.

Bi Y, Kenton P, Mur L, Darby R and Draper J. 1995. Hydrogen peroxide does not function downstream of salicylic acid in the induction of PR protein expression. *Plant J* 8:235-245.

Bonman JM, Vergel D, Dios TI, Bandong JM and Lee EJ. 1992. Breeding rice for resistance to pests. *Ann Rev Phytopathol* 30:507-528.

Bradley DJ, Kjellbom P and Lamb CJ. 1992. Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response. *Cell* 70: 21–30.

Brisson LF, Tenhaken R and Lamb C. 1994. Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. *Plant Cell* 6: 1703–1712.

Brown JKM. 1995. Pathogens' responses to the management of disease resistance genes. In: *Advances in plant pathology*. Andrews JH, and Tommerup IC, eds. Academic Press, New York, pp 75-102.

Bryan GT, Wu K, Farrall L, Jia Y, Hershey HP, McAdams SA, Faulk KN, Donaldson G K, Tarchini R and Valent B. 2000. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. *Plant Cell* 12:2033-2045.

Chamnongpol S, Willekens H, Moeder W, Langebartels C, Sandermann H Jr, Van Montagu M, Inzé D and Van Camp W.1998. Defense activation and enhanced pathogen tolerance induced by H_2O_2 in transgenic tobacco. *Proc Natl Acad Sci USA* 95: 5818–5823.

Christou P. 1997. Rice transformation: bombardment. *Plant Mol Biol* 35:197-203.

Couch BC and Kohn LM. 2002. A multilocus gene genealogy concordant with host preference indicates segregation of a new species *Magnaporthe oryzae* from *M. grisea. Mycologia* 94:683–693.

Dangl JL, Dietrich RA and Richberg MH. 1996. Death don't have no mercy: Cell death programs in plant-microbe interactions. *Plant Cell* 8: 1793–1807.

Dean RA, Talbot NJ, Ebbole DJ, Farman ML, Mitchell TK, Orbach MJ, Thon M, Kulkarni R, Xu JR, Pan H, Read ND, Lee YH, Carbone I, Brown D, Oh YY, Donofrio N,Jeong JS, Soanes DM, Djonovic S, Kolomiets E, Rehmeyer C, Li W, Harding M, Kim S, Lebrun MH, Bohnert H, Coughlan S, Butler J, Calvo S, Ma LJ, Nicol R, Purcell S, Nusbaum C, Galagan JE and Birren BW. 2005. The genome sequence of the rice blast fungus *Magnaporthe grisea.* 2005. *Nature* 434:980-986.

Delaney TP, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, Gaffney T, Gut-Rella M, Kessmann H, Ward E and Ryals J. 1994. A central role of salicylic acid in plant disease resistance. *Science* 266:1247-1250.

Epple P, Apel K and Bohlmann H. 1997. Overexpression of an endogenous thionin enhances resistance of *Arabidopsis* against *Fusarium oxysporum*. *Plant Cell* 9:509-520.

Flor HH. 1971. Current status of the gene-for-gene concept. *Ann Rev Phytopath* 9:275-296.

Fobert PR and Despres C. 2005. Redox control of systemic acquired resistance. *Curr Opin Plant Biol* 8, 378-382.

Ganesan, V., and Thomas, G. 2001**.** Salicylic acid response in rice: influence of salicylic acid on H_2O_2 accumulation and oxidative stress. *Plant Sci* 160:1095-1106.

Gnanamanickam SS, Babujee L, Priyadarisini VB, Dayakar BV, Leena Kumari D, Sivaraj R and Leong SA. 2000 Lineage-exclusion resistance breeding: pyramiding of blast resistance genes for management of rice blast in India. *Adv Rice Blast Res* 15:172–179.

Goff S A, Ricke D, Lan T, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H, Hadley D, Hutchison D, Martin C, Katagiri F, Lange BM, Moughamer T, Xia Y, Budworth P, Zhong, Miguel T, Paszkowski U, Zhang S, Colbert M, Sun W, Chen L, Cooper B, Park S, Wood T C, Mao L, Quail P, Wing R, Dean R, Yu Y, Zharkikh A, Shen R, Sahasrabudhe S, Thomas A, Cannings R, Gutin A, Pruss D, Read J, Tavtigian S, Mitchell J, Eldredge G, Scholl T, Miller R M, Bhatnagar S, Adey N, Rubano T, Tusneem N, Robinson R, Feldhaus J, Macalma T, Oliphant A and Briggs S. 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). *Science* 296:92 – 100.

Gorlach J, Volrath S, Knauf-Beiter G, Hengy G, Beckhove U, Kogel KH, Ostendorp M, Staub T, Ward E, Kessman H and Ryals J. 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *Plant Cell* 4:629-643.

Gowda M, Roy-Barman S and Chattoo BB. 2006. Molecular mapping of a novel blast resistance gene *Pi38* in rice using SSLP and AFLP markers. *Plant Breed* 125: 596-599.

Grant M. and Lamb C. 2006. Systemic immunity. *Curr Opin Plant Biol* 9: 414-420.

Gupta V, Willits MG and Glazebrook J. 2000. *Arabidopsis thaliana* EDS4 contributes to salicylic acid (SA)-dependent expression of defense responses: evidence for inhibition of jasmonic acid signaling by SA. *Plant Microbe Interact* 13:503-511.

Hayashi N, Inoue H, Kato T, Funao T, Shirota M, Shimizu T, Kanamori H, Yamane H, Hayano-Saito Y, Matsumoto T, Yano M and Takatsuji H. 2010. Durable panicle blast-resistance gene Pb1 encodes an atypical CC-NBS-LRR protein and was generated by acquiring a promoter through local genome duplication. *Plant J* 64: 498-510.

Hiei Y, Komari T and Kubo T. 1997. Transformation of rice mediated by *Agrobacterium tumefaciens. Plant Mol Biol* 35: 205-218

Hittalmani S, Parco A, Mew TV, Zeigler RS and Huang N. 2000. Fine mapping and DNA marker- assisted pyramiding of the three major genes for blast resistance in rice. *Theor Appl Genet* 100:1121-1128.

IRGSP 2005. International Rice Genome Sequencing Project. 2005. The map-based sequence of the rice genome. *Nature* 436:793-800

Jach G, Görnhardt B, Mundy J, Logemann J, Pinsdorf E, Leah R, Schell J and Maas C. 1995. Enhanced quantitative resistance against fungal disease by combinatorial expression of different barley antifungal proteins in transgenic tobacco. *Plant J* 8:97-109.

Jia Y and Martin R. 2008. Identification of a new locus, *Ptr(t)*, required for rice blast resistance gene *Pi-ta*-mediated resistance. *Mol Plant Microb Interact* 21: 396-403.

Jia Y, McAdams SA, Bryan GT, Hershey HP and Valent B. 2000 Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. *EMBO J* 19:4004–4014.

Jones JDG and Dangl JL. 2006. The plant immune system. *Nature* 444: 323-329.

Kachroo A, He Z, Patkar R, Zhu Q, Zhong J, Li D, Ronald P, Lamb C and Chattoo BB. 2003. Induction of H_2O_2 in transgenic rice leads to cell death and enhanced resistance to both bacterial and fungal pathogens. *Trans Res* 12:577-586.

Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiyama C, Dohmae N, Takio K, Minami E and Shibuya N. 2006. Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. *Proc Natl Acad Sci USA 103:* 11086-11091.

Kato H. 2001. Rice blast disease. *Pestic Outlook* 12:23–25.

Kawano Y, Akamatsu A, Hayashi K, Housen Y, Okuda J, Yao A, Nakashima A, Takahashi H, Yoshida H, Wong HL, Kawasaki T and Shimamoto K. 2010. Activation of a Rac GTPase by the NLR family disease resistance protein Pit plays a critical role in rice innate immunity. *Cell Host Microbe* 7: 362-375.

Koizumi S, Ashizawa T and Zenbayashi KS. 2004. Durable control of rice blast disease with multilines. In: *Rice Blast: Interaction with rice and control.* Kawasaki S, eds. Kluwer, pp191–199.

Kunkel BN and Brooks DM. 2002. Cross talk between signaling pathways in pathogen defense. *Curr Opin Biotechnol* 5:325-331.

Lee SK, Song MY, Seo YS, Kim HK, Ko S, Cao PJ, Suh JP, Yi G, Roh JH, Lee S, An G, Hahn TR, Wang GL, Ronald P and Jeon JS. 2009. Rice Pi5-mediated resistance to *Magnaporthe oryzae* requires the presence of two coiled-coil-nucleotide-binding-leucine-rich repeat genes. *Genetics* 181: 1627-1638.

Levine A, Tenhaken R, Dixon R and Lamb C. 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* 79: 583–593.

Li D, Liu H, Zhang H, Wang X and Song F. 2008. *OsBIRH1*, a DEAD-box RNA helicase with functions in modulating defence responses against pathogen infection and oxidative stress. *J Exp Bot* 59:2133-2146.

Lin F, Chen S, Que Z, Wang L, Liu X and Pan Q. 2007. The blast resistance gene *Pi*37 encodes a nucleotide binding site–leucine-rich repeat protein and is a member of a resistance gene cluster on rice chromosome 1. *Genetics* 177:1871-1880.

McClung MA, Marchetti MA, Webb BD and Bollich CN. 1997. Registration of 'Jefferson' rice. *Crop Sci* 37:629–630.

Mehdy MC. 1994. Active oxygen species in plant defense against pathogens. *Plant Physiol* 105: 467–472.

Mitsuhara I, Iwai T, Seo S, Yanagawa Y, Kawahigasi H, Hirose S, Ohkawa Y and Ohashi Y. 2008. Characteristic expression of twelve rice PR1 family genes in response to pathogen infection, wounding, and defense-related signal compounds (121/180). *Mol Genet Genomics* 279:415-427.

Mittler R, Feng X and Cohen M. 1998. Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco. *Plant Cell* 10**:** 461–473**.**

Monosi B, Wisser RJ, Pennill L and Hulbert SH. 2004. Full-genome analysis of resistance gene homologues in rice. *Theor Appl Genet* 109: 1434-1447

Naqvi NI, Bonman JM, Mackill DJ, Nelson RJ and Chattoo BB. 1995. Identification of RAPD markers linked to a major blast resistance gene in rice. *Mol Breed* 1: 341-348.

Okuyama Y, Kanzaki H, Abe A, Yoshida K, Tamiru M, Saitoh H, Fujibe T, Matsumura H, Shenton M, Galam DC, Undan J, Ito A, Sone T and Terauchi R. 2011. A multifaceted genomics approach allows the isolation of the rice *Pia*-blast resistance gene consisting of two adjacent NBS-LRR protein genes. *Plant J* 66: 467-479.

Ou SH. 1980. Pathogen variability and host resistance in rice blast disease. *Ann Rev Phytopathol* 18:167-187.

Ou SH. 1985. *Rice diseases*. Commonwealth Mycological Institute, Kew, UK, pp 109–201.

Peng J, Kononowicz H and Hodges TK. 1992. Transgenic *indica* rice plants. *Theor Appl Genet* 83: 855-863.

Prasad BD, Creissen G, Lamb C and Chattoo BB. 2009. Overexpression of rice (*Oryza sativa* L.) *OsCDR1* leads to constitutive activation of defense responses in rice and *Arabidopsis*. *Mol Plant microb Interact* 22:1635-1644.

Pugin A, Frachisse JM, Tavernier E, Bligny R, Gout E, Douce R and Guern J. 1997. Early events induced by the elicitor cryptogein in tobacco cells: involvement of a plasma membrane NADPH oxidase and activation of glycolysis and the pentose phosphate pathway. *Plant Cell* 9: 2077–2091.

Qu S, Liu G, Zhou B, Bellizzi M, Zeng L, Dai L, Han B and Wang GL. 2006. The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site-leucine-rich repeat protein and is a

member of a multigene family in rice. *Genetics* 172:1901-1914.

Sallaud C, Lorieux M, Roumen E, Tharreau D, Berruyer R, Svestasrani P, Garsmeur O, Ghesquiere A and Notteghem JL. 2003. Identification of five new blast resistance genes in the highly blast-resistant rice variety IR64 using a QTL mapping strategy. *Theor Appl Genet* 106:794-803.

Schneider M, Schweizer P, Meuwly P and Métraux J P. 1996. Systemic acquired resistance in plants. *Int J Cytol* 168: 303-340

Shang J, Tao Y, Chen X, Zou Y, Lei C, Wang J, Li X, Zhao X, Zhang M, Lu Z, Xu J, Cheng Z, Wan J and Zhu L. 2009. 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* 182: 1303-1311.

Sharma TR, Shanker P, Singh BK, Jana TK, Madhav MS, Gaikwad K, Singh NK, Plaha, P and Rathour R. 2005. Molecular mapping of rice blast resistance gene *Pi-kh* in the rice variety Tetep. *J. Plant Biochem Biotechnol* 14:127-133.

Silverman P, Seskar M, Kanter D, Schweizer P, Metraux JP and Raskin I. 1995. Salicylic acid in rice (biosynthesis, conjugation, and possible role). *Plant Physiol* 108:633-639.

Spoel SH, Koornneef A, Claessens SM, Korzelius JP, Van Pelt JA, Mueller MJ, Buchala AJ, Métraux JP, Brown R, Kazan K, Van Loon LC, Dong X and Pieterse CM. 2003. NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. *Plant Cell* 15:760-770.

Takeuchi Y, Ebitani T, Yamamoto T, Sato H, Ohta H, Hirabayashi H, Kato H, Ando I, Nemoto H, Imbe T and Yano M. 2006. Development of isogenic lines of rice cultivar Koshihikari with early and late heading by marker-assisted selection. *Breed Sci* 56: 405–413.

Wang ZX, Yano M, Yamanouchi U, Iwamoto M, Monna L, Hayasaka H, Katayose Y and Sasaki T. 1999. The *Pib* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. *Plant J* 19: 55-64.

Xia Y, Suzuki H, Borevitz J, Blount J, Guo Z, Patel K, Dixon RA and Lamb C. 2004. An extracellular aspartic protease functions in Arabidopsis disease resistance signaling. *EMBO J* 23:980-988.

Xiong L, Lee MW, Qi M and Yang Y. 2001. Identification of defense-related rice genes by suppression subtractive hybridization and differential screening. *Mol Plant Microbe Interact* 14:685-692.

Xu R, Song F and Zheng Z. 2006. OsBISAMT1, a gene encoding S-adenosyl-L-methionine: salicylic acid carboxyl methyl-transferase, is differentially expressed in rice defense responses. *Mol Biol Rep* 33:223-231.

Yu J, Hu S, Wang J, Wong GK, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X, Cao M, Liu J, Sun J, Tang J, Chen Y, Huang X, Lin W, Ye C, Tong W, Cong L, Geng J,Han Y, Li L, Li W, Hu G, Huang X, Li W, Li J, Liu Z, Li L, Liu J, Qi Q, Liu J, Li L, Li T, Wang X, Lu H, Wu T, Zhu M, Ni P, Han H, Dong W, Ren X, Feng X, Cui P, Li X,Wang H, Xu X, Zhai W, Xu Z, Zhang J, He S, Zhang J, Xu J, Zhang K, Zheng X, Dong J, Zeng W, Tao L, Ye J, Tan J, Ren X, Chen X, He J, Liu D, Tian W, Tian C,Xia H, Bao Q, Li G, Gao H, Cao T, Wang J, Zhao W, Li P, Chen W, Wang X, Zhang Y, Hu J, Wang J, Liu S, Yang J, Zhang G, Xiong Y, Li Z, Mao L, Zhou C, Zhu Z,Chen R, Hao B, Zheng W, Chen S, Guo W, Li G, Liu S, Tao M, Wang J, Zhu L, Yuan L and Yang H. 2002, A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). *Science* 296:79 – 92.

Zeigler RS, Cuoc LX, Scott RP, Bernardo MA, Chen DH, Valent B and Nelson RJ. 1995. The relationship between lineage and virulence in *Pyricularia grisea* in the Philippines. *Phytopathology* 85:443-451.

Zhou B, Qu SH, Liu GF, Dolan M, Sakai H, Lu GD, Bellizzi M and Wang G. 2006. The eight amino-acid differences within three leucine-rich repeats between *Pi2* and *Piz-t* resistance proteins determine the resistance specificity to *Magnaporthe grisea*. *Mol Plant Microb Interact* 19:1216-1228.

Zhu Y, Chen H, Fan J, Wang Y, Li Y, Chen J, Fan J, Yang S, Hu L, Leung H, Mew TW, Teng PS, Wang Z and Mundt CC. 2000. Genetic diversity and disease control in rice. *Nature* 406: 718-722.

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