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Importance of proteomics approach on identifying defense protein in response to biotic stresses in rice (*Oryza sativa* L.)

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Abstract

Biotic stress in rice (*Oryza sativa* L.) is one of the major stresses limiting crop productivity and the geographical distribution of many important crops worldwide. A clear perceptive of the molecular mechanisms involved in plants response to biotic stress is of fundamental importance to plant science. Knowledge about these mechanisms is also critical for continued development of rational breeding and transgenic strategies to improve resistant into stress in cereal crops. Proteomic approach has become a powerful tool to study plant responses to biotic stress and also proteomics approaches are being applied in rice for the past several years to identify better mechanism to the biotic stresses-responsive proteins. A large number of proteins responsive to biotic stresses, including pathogens such as fungi, bacteria, viruses and herbivores have been studied. Identified proteins are belong to functional categories into defense mechanisms, metabolism, energy, and signaling. This review will briefly summarize and discuss about the proteomics based investigation of biotic stress-responsive proteins in rice and increasing importance of proteomics approach in defense mechanism and genetic engineering of rice crop plants.

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Introduction

Plant-pathogen interactions have been studied extensively over the years from both the plant and pathogen viewpoints. An understanding of how plants and pathogens recognize each other and differentiate to establish either a successful or an unsuccessful relationship is crucial in this field of investigation (Mehta *et al.,* 2008). Plants are under constant assault by biotic agents, including viral, bacterial, fungal pathogens, parasitic plants and insect herbivores, with enormous economic and ecological impact (Pimentel., 1991 and 2002). Plants responding to biotic stresses produce several protective compounds and proteins such as pathogenesis related (PR) proteins, directly disease related proteins and other proteins. Environmental stresses influence the plant growth and agriculture production, including the major food crop, rice (Agrawal and Rakwal, 2008, 2011; Jorrin *et al.*, 2007). In order to minimize the impact of environmental stresses on rice production, proteomics approaches have increasingly been applied to understand the regulatory mechanisms of rice responses to environmental caues including biotic stresses. Proteomics, or the analysis of the protein complement of the genome, provides experimental continuity between genome sequence information and the protein profile in a specific tissue, cell or cellular compartment during standard growth or different treatment conditions. Although the genome defines potential contributions to cellular function, the expressed proteome represents actual contributions. Moreover, by using proteomic approaches, differences in the abundance of proteins actually present at the time of sampling can be distinguished and different forms of the same protein can be resolved. The analysis of proteomes from organisms has been performed extensively by exploring the high resolution of two-dimensional electrophoresis (2-DE) coupled with MS. These data, when complemented by *de novo* sequencing, allow the unequivocal identification of proteins involved in different biological functions. The proteomic approach is a fundamental method by which we can obtain an understanding and identification of the functions of proteins expressed in a given condition (Mehta *et al.*, 2008). Biotic stresses are caused by the living organisms, such as fungi, bacteria, viruses, and insects. There are many proteomics-level studies in rice have been reported. Those biotic studies involve fungus (Kim *et al.*, 2003, 2004, 2009; Lee *et al.*, 2006), elicitor (Agrawal *et al.*, 2002; Chen *et al.*, 2007 a; Lin *et al.*, 2008, Li *et al.*, 2004 and Liao *et al*, 2009), bacterium (Mahmood *et al.*, 2006; Chen *et al* (2007 b) Kandasamy *et al.*, 2009 and Chi *et al.*, 2010), virus (Ventelon-Debout *et al.*, 2004), herbivore (Wei *et al.*, 2009). In this review we describe importance of proteomics studies of biotic stresses in rice plant and we highlight the proteins expressed during rice-virus, rice–bacterium, rice–fungus and rice-herbivore interactions reported in proteomic studies, and discuss these findings considering the advantages and limitations of current proteomic tools. These studies have increased our knowledge about biotic stresses-responsive proteins and regulatory pathways and mechanisms.

Rice-fungus interactions

Considerable advances have been achieved in the last 10 years in the identification of the determinants of plant-fungus interactions. When pathogenic fungi start the infection process, secreted and intracellular proteins are up- or downregulated, improving the predation ability of fungi (Murad *et al.*, 2006 ; Murad *et al.*, 2007). Rice blast disease the *Magnaporthe grisea*-rice interaction is a model system for understanding plant disease because of its great economic importance, and also because of the genetic and molecular genetic tractability of the fungus (Talbot., 2003) and also *M. grisea* is the most serious disease of cultivated rice (*Oryza sativa* L.), in most rice-growing regions of the world. There are at least four proteomics studies that deal with funi in rice (Table 1). A pioneering study on rice proteomics was performed to analyse the protein profile after *M. grisea* infection, and was conducted using infected leaf blades fertilized with various levels of nitrogen (Konishi *et al.*, 2001). Rice plants grown with high levels of nitrogen nutrient are more susceptible to infection by the blast fungus (Long *et al.*, 2000). Although this study failed to establish any correlation

between nitrogen application and disease resistance, leaf proteins revealed some minor changes when plants grown under different levels of nitrogen were compared (Rakwal and Agrawal., 2003). Twelve proteins, including the rice thaumatin-like protein (TLP) and (PR-5), were identified with accumulation changes at different levels of nitrogen. Another study of the same interaction was performed by Kim *et al* (2003). This study showed that fourteen spots encoded six different family protein were identified at 24 and 48 h after inoculation. Fourteen protein spots were induced or increased by the treatments, which including Twelve proteins from six different genes were identified. Rice pathogen-related protein class 10 (OsPR-10), isoflavone reductase-like protein (PBZ1), β-glucosidase, and putative receptor-like protein kinase (RLK) were among those induced by rice blast fungus, Six isoforms of probenazole-inducible protein (PBZ1) and two isoformsof salt-induced protein (SalT) that responded to blast fungus, elicitor, and jasmonic acid were also resolved on a 2-DE gel and identified by proteome analysis. PBZ1, OsPR-10 and SalT proteins from incompatible reactions were induced earlier and to a greater extent than those in compatible reactions. Another study in this field was performed by kim *et al* (2004), in order to analysis of pathogen-responsive proteins from rice leaves that induced by rice blast fungus. In this study rice leaf was infected with compatible (KJ 301) and incompatible (KJ 401) fungus. Matrix-assisted laser desorption/ ionization-time of flight analysis of these differentially displayed proteins, showed them to be two receptor-like protein kinases (RLK), two β-1,3-Glucanases (Glu1, Glu2), Thaumatin-like protein (TLP), Peroxidase (POX), probenazole-inducible protein (PBZ1), and rice pathogenesis-related protein 10 (OsPR-10). Among these proteins, RLK, TLP, PBZ, and OsPR 10 proteins were induced more in the incompatible interactions than in compatible ones. This results suggesting that those proteins may be very important for rice defense system. As the expressed proteins in the extracellular space (ECS) serve as the first line of defense against biotic stresses including the pathogens, the same group cataloged the proteins secreted into the ECS upon fungal elicitor or *M. oryzae* attack using the SCCs system (Kim *et al*., 2009). Another rice–fungus interaction study reported recently was that of sheath blight, caused by the fungus *Rhizoctonia solani*. Unlike *M. oryzae*, *R. solani* causes rice sheath blight disease mostly in the sheath tissue. *R solani* responsive proteins in sheath tissue were investigated against two rice strains Labelle (incompatible) and LSBR-5 (compatible). Seven protein spots were found to be upregulated in both Labelle and LSBR-5 strains, including proteasome subunit, RuBisCO large subunit (LSU), and defense-related protein β-1,3-glucanase. Fourteen protein spots were upregulated or downregulated in response to compatible strain LSBR-5 only, such as ascorbate peroxidase (APX), chitinase, chaperonin, and 14- 3-3-like protein. (Lee *et al*., 2006).

Rice-elicitor interactions

Several studies have reported that plant defense mechanisms are associated with the recognition by plant receptors of specific elicitor molecules (Leister *et al*., 1996; Dangl and Jones., 2001; Nurnberger and Brunner., 2002). Based on the gene-for-gene theory, proteins or chemicals recognized by host receptors trigger host-defense responses by activating and suppressing defense signaling and metabolic pathways (Allwood *et al*., 2008 and De Wit *et al*., 2009). Previous studiesabout elicitor have shown that plant defense responses are also elicited by a group of stimuli called elicitors, such as polysaccharides, small proteins, or chemicals (Hahn, 1996). There are four proteomics studies was done in response to elicitor in rice plant. The first proteomics analysis of rice defense by elicitor was reported in the year 2002, where 2-week-old rice seedlings were treated with chitosan, a major component of fungal cell wall (Agrawal *et al*., 2002). The one-dimensional (1-D; also referred to as SDS-PAGE, sodium dodecyl sulfatepolyacrylamidegel electrophoresis) and two-dimensional (2-DE) gel-based proteomics approach identified four strongly upregulated protein spots, encoding two unique defense related proteins, OsPR5 and OsPR10. The measured mRNA level of the genes corresponding to these two proteins using Northern

blot analysis showed good correlation with the accumulated protein abundance. A plasma membrane (PM) proteomics study of rice suspension cultured cells (SCCs) in response to fungal cell wall elicitor chitooligosaccharide was performed in the year 2007 (Chen *et al.*, 2007). A total of ten differentially expressed protein spots were detected. Of these, seven up regulated and one down regulated protein spots were identified by MS analysis. Most of these proteins were kinase family, involved in defense (MAR-binding protein MFP1 and calcium-dependent protein kinase) and stress response (putative zinc finger protein), suggesting that the PM proteins play an important role in defense signal transduction during pathogen attack. Probenazole (PBZ) is a chemical that elicits defense responses in plants including accumulation of salicylic acid (Iwai *et al.*, 2007). In another studies proteomic approach was employed to identify differentially regulated proteins in rice leaves in response to PBZ treatment (Lin *et al.*, 2008), Thirty-one abundant protein spots were identified by LC-MS/MS, where 9 protein spot were up regulated, representing 11 unique proteins. The up regulation of two phenylpropanoid pathway-related enzymes, putative leucine aminopeptidase (PLA), and caffeic acid 3-O-methyltransferase (COMT), suggested that PBZ may activate either flavonoid-type phytoalexin and/or lignin biosynthesis. A quantitative real-time PCR (qRTPCR) analysis of these two corresponding genes revealed that these proteins are also regulated at transcriptional, as well as post-transcriptional levels. Another study about elicitor was CSB I elicitor, a 102-kDa glycoprotein from fungi *Magnaporthe oryzae* was also reported to induce defense responses in rice (Li *et al.*, 2004). Four-leaf stage rice plant treated with CSB I was analyzed by the 2D-PAGE-based proteomics approaches (Liao *et al.*, 2009). A total of 18 protein spots were identified by LC-MS/MS and that included 11 upregulated and 7 newly synthesized protein spots. Those identified proteins were related to reactive oxygen species (ROS) detoxifying (Cu/Zn-SOD, Mn-SOD, and GST), programmed cell death (PCD), signal transduction, and defense (OsPR10a and OsPR5).

Rice-bacterium interactions

Bacteria rely on diverse secretion pathways in order to overcome plant defences and to establish successful colonization of the host plant. Getting a comprehensive insight into the interaction of bacteria with rice will be an important step toward understanding diseases and resistance mechanisms that have evolved in plants. To investigate the role of defence-responsive proteins in the rice-*Xanthomonas oryzae* pv. *oryzae* interaction, Mahmood *et al* (2006) applied a proteomic approach. Cytosolic and membrane proteins were fractionated from the rice leaf blades 3 days post inoculation with incompatible and compatible *X. oryzae* pv. *Oryzae* races. From 366 proteins analysed by 2DE, 20 were differentially expressed in response to bacterial inoculation which were involved in energy [ATP synthase, ribulose 1,5 bisphosphate carboxylase /oxygenase large subunit (RuBisCO LSU)], Metabolism [transketolase, GADPH, triosephosphate isomerase], Defense system [PR-5, probenazole-inducible protein (PBZ1), SOD and peroxyredoxin (Prx)], and Protein synthesis. Analyses clearly revealed that four defence-related proteins were induced for both compatible and incompatible *X. oryzae* pv. *Oryzae* races, where in PR-5 and PBZ1 were more rapid and showed higher induction in incompatible interactions and in the presence of jasmonic acid. Studying the same rice-*X. oryzae* pv. *oryzae* interaction, Chen *et al* (2007 b) analysed proteins from rice plasma membrane to study the early defence responses involved in XA 21-mediated resistance. XA 21 is a rice receptor kinase, predicted to perceive the *X. oryzae* pv. *oryzae* signal at the cell surface, leading to the 'gene-for-gene' resistance response. They observed a total of 20 proteins differentially regulated by pathogen challenge at 12 and 24 h post inoculation, and identified at least eight putative plasma membrane-associated and two non-plasma membrane-associated proteins (Table 1) with potential functions in rice defence.

Table 1. Proteins identified on biotic stresses in rice using proteomic approaches.

Biotic factor	Stress type	Methods	Plant condition	Regulated protein/functional
Fungus	*Magnaporthe oryzae*	2-DE (pI 4-7, 18cm), MALDI-TOF-MS	SCCs medium total protein	Defense (PBZ1, OsPR10, RLK), stress (SalT) and ROS (OsIRL)
	M. oryzae	2-DE (pI 4-7, 18cm), MALDI-TOF-MS	Fourth- and fifth-leaf stage leaves, total	Defense (PBZ1, OsPR10, Glu1, Glu2, RLK, POX, TLP)
	Rhizoctonia solani	2-DGE (pI 3–10, 17 cm), ESI-Q-TOF-MS	6-week-old sheaths, total protein	Defense (β-1,3-glucanase, chitinase, 14-3-3-like protein), stress (chaperonin 60), ROS (stromal APX), protein degradation (26S proteasomenon-ATPase regulatory), energy (G3PDH,), metabolism (ribulose bisphosphate carboxylase)

Table 1 (continued)

Elicitor	Chitosan	1-DGE, 2-DGE (pI 3.5-10), N-terminal Edman sequencing	Seedling leaves, total protein	ROS (APX), defense (OsPR5,OsPR1)
	Chitooligosaccharide	2-DGE (pI 4–7, 17 cm), MS	Xa21-transgenic SCCs, plasma membrane protein	Signaling (PKN/PRK1 protein kinase-like, Putative MAR-binding protein MFP1, Calcium-dependent protein kinase)
	Probenazole	2-DGE (pI 4-7, 18 cm), MALDI-QTOF-MS or LC–MS/MS	Seedling leaves, total protein	Energy (fructose bisphos phatealdolase, NADHubiquinone oxidoreductase, G3PDH), metabolism (glycosyl hydrolase, glucose-1- phosphate adenyltransferase, glutamine synthetase), secondary metabolism (phenylalanine ammonialyase, caffeic acid 3-omethyltransferase), protein destination and storage (leucine aminopeptidase), ROS (GSTU17), stress (chaperonin 60), protein synthesis (chloroplast translational elongation factor Tu), cell structure (germin-like protein)
	CSB I	2-DGE (pI 4–7, 18 cm), LTQ-MS/MS	Fourth-leaf stage leaves, total protein	Defense (PR10, PR5), ROS (catalase, Mn-SOD, Cu/Zn-SOD, GST), signaling (nucleoside diphosphate kinase), stress (chaperonin), protein biosynthesis (translational elongation factor Tu), metabolism (malate dehydrogenase, transketolase, fructose-bisphosphate aldolase)
Bacterium	*Xanthomonas oryzae*	2-DGE (pI 3.5-10, 11 cm), N-terminal Edman sequencing and MALDI-TOF-MS	3-week-old leaves,cytosolic, and membrane protein fractions	Defense (PBZ1, PR5), ROS (SOD, peroxiredoxin), energy (RuBisCO LSU, ATP synthase), metabolism (transketolase, GADPH, ribose-5-phosphate isomerase), protein synthesis (50S ribosomal protein)
Table1(continued)	*Pseudomonas fluorescens*	2-DGE (pI 4-7, 17 cm), MS/MS	Seedlings sheaths, total protein	Stress (p23 co-chaperone), ROS (GSTZ5, thioredoxinh), metabolism (ribulose-bisphosphate carboxylase, nucleotide diphosphate kinase), protein degradation (proteosome)
	Sinorhizobium Meliloti	2-DGE (pI 3.5-10, 13 cm), MALDI-TOF-MS	Seedlings roots/sheaths/ leaves, total protein	Defense (exoglucanase), stress (DnaK-type molecular chaperone, chaperonin 60), ROS (catalase, POX), energy (G3PDH, ATP synthase, RuBisCO LSU), protein degradation (subtilisin-like proteinase, aminopeptidase N, 20S proteasome), metabolism (glutamine synthetase), signaling (CRT, peroxisomal targeting signal protein-like)
Herbivore	Brown planthopper (BPH)	iTRAQ, nESI-LC-QqTOF-MS	Fourth-leaf stage leaf sheaths, total protein	Stress (DREPP2 protein, 70 kDa HSP protein), ROS (OsAPX2, GSTF2, catalase, peroxidase, DOX), protein synthesis and degradation (40S ribosomal protein, peptidyl-prolyl cis–trans isomerase) metabolism (glucan endo-1,3-betaglucosidase, fructose bisphosphate aldolase, sucrose synthase), transport (aquaporin)
Virus	Rice yellow mottle virus (RYMV)	2-DGE (pI 4–7, 24 cm), MALDI-TOF-MS or MS/MS	SCCs, total protein	Defense (PR-10a, α-amylase), stress (RAB25, chaperonin CPN60-2, HSP 70, LMW HSP, SalT), ROS (Mn-SOD, Cu/Zn-SOD), metabolism (2,3biphosphoglycerate-independent

	phosphoglycerate mutase, phosphoglycerate dehydrogenase, aldolase) energy (G3PDH), protein degradation (ubiquitin-like protein, ribosomal 40S), protein synthesis (elongation factor 1-b′), signaling (translation initiation factor 5A)

*Abbreviations: pI (isoelectric point), LC-MS/MS (liquid chromatography tandem mass spectrometry), LMW(low molecular weight), MALDI-TOF-MS (matrix-assisted laser desorption-time-of-flight mass spectrometry), MS (mass spectrometry), nESI (nanoelectrospray ionization), 1-DGE (one-dimensional gel electrophoresis), Os (Oryza sativa), PR (pathogenesis-related), qRT-PCR (quantitative reverse transcriptase-polymerase chain reaction), SCCs (suspension-cultured cells), 2-DGE two-dimensional gel electrophoresis.

Symbiotic bacterium and rice

The symbiotic bacterium grows together with its host plant, and could promote host growth and improve yield. Two studies investigated the changes in protein profile upon interaction between rice and two symbiotic bacteria, *Pseudomonas fluorescens* and *Sinorhizobium meliloti* (Kandasamy *et al.*, 2009; Chi *et al.*, 2010). The culture of *P. fluorescens* promoted rice growth in seedling stages (Kandasamy *et al.*, 2009). Six protein spots out of 23 differentially expressed protein spots were identified from rice sheath infected with *P. fluorescens*. The *S. meliloti*-infected rice tissues revealed new evidence on how symbiotic bacterium contributes to host growth (Chi *et al.*, 2010). A total of 21, 20, and 12 differential protein spots were identified from root, sheath, and leaf, respectively, which were involved in Energy, protein destination/storage, and defense-related proteins were expressed in all tissues, indicating that those proteins were common for the pathogen interactions. Metabolism and signal transduction-related proteins were detected in root and sheath, while cell growth/division-related proteins were found only in leaf. Furthermore, the symbiotic bacterium-regulated proteins were significantly different from those of proteins induced by pathogenic bacterium, such as PBZ1. This study suggested that symbiotic/pathogenic bacterium can trigger different type of defense response pathways in rice.

Rice-virus interactions

For the success of plant infection, viruses must first be transmitted either mechanically or by a vector (transmission), replicate in plant cells (replication), subsequently move through plasmodesmata to neighbouring cells (cell-to-cell movement) and, finally, attain the vascular tissue to circulate systemically through the phloem to the sink tissues of the host (vascular movement). After being unloaded from the phloem, viruses establish systemic infection through new cycles of replication and cell-to-cell/vascular movement. In both compatible (susceptible host) and incompatible (resistant host) interactions, viruses use plant host proteins to complete the steps of the infection process and suffer the influences of plant host proteins as a counteraction against the infection. The genes that encode these proteins have been studied extensively in numerous host-virus systems, mainly using transcriptional analysis (Whitham., 2006). The rice yellow mottle virus (RYMV) is one of the most damaging viral pathogens of rice. RYMV is endemic to Africa (Pinel *et al.*, 2000; Abubakar *et al.*, 2003), and is considered as very destructive for rice production. Proteomics approach was also used to understand the effect of interaction between RYMV and susceptible (IR64; *O. sativa indica*) or partial resistant (Azucena; *O. sativa japonica*) types rice on protein changes (Ventelon-Debout *et al.*, 2004). A total of 19 and 13 differential protein spots were identified due to susceptible and resistance interactions. Those proteins were related to metabolism, stress, and translation. A group of commonly proteins were also identified in both types of interactions, such as Superoxide dismutase, α-amylase, Ubiquitin-like protein, Chaperonin, and Heat shock proteins.

Rice-herbivores interactions

Brown planthopper (BPH), which is one of the most serious rice pests. The first rice–herbivores interaction study using proteomics approaches was carried out in the year 2009 using the (Wei *et al.*, 2009). A total number of 50 proteins spot were significantly modulated, which were related to Biotic

stimulation (9 proteins), Stress response (9 proteins), Metabolism and modification processing proteins (10 proteins), Carbohydrate metabolism (9 proteins), Amino acid and derivative metabolism (4 proteins), Photosynthesis (5 proteins), and Transport (4 proteins). Among these 9 protein that related to biotic stimulation, five peroxidase proteins, allene oxide cyclase, APX, and dioxygenase were detected that indicating herbivores may cause the accumulation of ROS in the host. The up regulation of aquaporin isoforms by herbivores suggested that osmotic stress may have some role during herbivory-induced damage to the tissues.

Understanding the importace proteoimic on biotic stresses interactions in rice

In this review, we have presented the recent proteomic studies performed to better understand rice interactions to fungus, elicitor, bacterium, virus and herbivores. Taken together, the data available reveal that several proteins are commonly expressed in diverse pathosystems (Fig. 1). Based on the proteomics analysis, a group of proteins were targeted for functional dissection and to understand the underlying regulatory mechanisms of rice response to biotic stresses. Those proteins include the defense-related proteins (PBZ1, OsPR10, RLK, β-1,3-glucanase, and chitinase), ROS-related proteins (SOD, APX, and OsIRL), Stress related proteins (HSP and chaperonin). The pathogen-related proteins (PR), especially the PR10 family, were found to be highly response to biotic stresses in several plants. One of the PR10 family proteins is the PBZ1, which was differentially regulated in response to probenazole, fungal elicitor, *M. oryzae, X. oryzae, S. meliloti*, and RYMV (Lin *et al.*, 2008; Kim *et al.*, 2003 and 2004; Mahmood *et al.*, 2006; Chi *et al.*, 2010; Ventelon- Debout *et al.*, 2004).

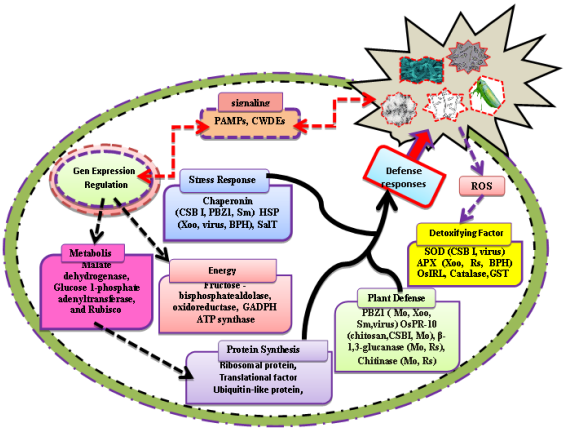

Fig. 1. Overview of plant–pathogen interactions and insights into proteomic studies of the proteins involved in these processes. This model illustrating biotic stress-responsive proteins in rice. Proteins of various biological functions are expressed in response to biotic stresses. Those proteins are mainly involved in biotic stress response, including ROS detoxification, plant defense, metabolism and energy. Abreviation: BPH brown planthopper, Mo (*Magnaporthe oryzae*), Sm (*Sinorhizobium meliloti*), ROS (reactive oxygen species) , Rs (*Rhizoctonia solani)*, Xoo *(Xanthomonas oryzae)*. GADPH (GAyceraldehyde-3-phosphate dehydrogenase.

Another PR10 family protein OsPR10 was also highly activated in response to multiple biotic stresses, including elicitor chitosan, CSB I, and fungal infection in SCCs and leaves (Agrawal *et al.*, 2002; Liao *et al.*, 2009; Kim *et al.*, 2003 and 2004). Moreover, the biochemical analyses of PBZ1 and OsPR10 revealed RNase activity (Kim *et al.*, 2008a, 2011). By using transgenic rice harboring GFP reporter under PBZ1 promoter, the PBZ1 was shown to be closely associated with fungal infection and programmed cell death (Kim *et al.*, 2008b). These data suggested that the PR10 protein family may be critical for host biotic stress defense mechanism. The b-1,3 GIu have been reported to be induced in many other plants in response to various pathogens, environmental stresses, wounding, phytohormone, and development (Henning *et al.*, 1993; Vogeli-Lange *et al.*, 1994; Leubner- Metzger *et al.*, 1998; Akiyama and Pillai., 2001). Members of these families are induced when exposed to pathogens and have antifungal activity

(Van Loon and Van Strien., 1999). A comprehensive analysis of β-1,3-glucanase family genes was performed. Among 27 analyzed rice β-1,3-glucanases, 22 are highly regulated by *M. oryzae* infection in rice leaves, suggesting the β-1,3-glucanase association with *M. oryzae* defense mechanism (Hwang *et al.* 2007). Among those, two β-1,3-glucanase proteins, OsGlu1 and OsGlu2, were identified from leaves in response to *M. oryzae* attack, and OsGlu1 also by *R. solani*, suggesting that β-1,3-glucanase protein family might be important line of defense against antifungal attack. Thus Nishizawa *et al* (2003), it was reported that a transgenic plant harboring the rice b-1,3 GIu gene (*Gns1*) showed a lesion mimic phenotype and exhibited enhanced resistance against the rice blast fungus. One of the most rapid plant responses occurring after pathogen recognition is the oxidative burst, which involves ROS production, primarily superoxide (O_2^-) and hydrogen peroxide (H_2O_2) at the site of the attempted invasion (Lamb and Dixon, 1997). It has been suggested that the oxidative burst and cognate redox signaling may play a central role in integration and coordination of the multitude of plant defense (Lamb and Dixon, 1997). A final result of the induced oxidative burst may be its participation in the HR and PCD (Huckelhoven and Kogel, 2003; Levine *et al*, 1994). responses ROS detoxifying proteins were closely associated to the plant defense system, such as superoxide dismutase (SOD), APX (Yoshioka *et al*, 2009; Nanda *et al*, 2010). SOD, GST and CAT belong to an important group of enzymes that remove active oxygen species in plants. SOD acts as the first line of defense response against ROS, dismutating superoxide to H_2O_2 (Alscher *et al*, 2002). observed that Mn-SOD intensity increased after rice leaves were treated with O_3. Following treatment with crude extracts of rice blast fungus, the SOD gene was dramatically induced in suspension-cultured rice cells (Matsumura *et al.*, 2003). The Cu/Zn- SOD and Mn-SOD proteins were upregulated in leaves in response to elicitor CSB I (Liao *et al.*, 2009). Interestingly, the same SOD proteins were identified in rice infected with RYMV (Ventelon-Debout *et al.*, 2004). GST is the enzyme responsible for detoxifying xenobiotics by catalyzing their conjugation with tripeptide glutathione (Edwards *et al.*, 2000). OsAPX2 protein was responsive to BPH, and an APX from SCCs plasma membrane was responsive to Xoo infection (Wei *et al.*, 2009). These data suggest that the ROS detoxifying enzymes may function in multiple biotic stress responses. OsRLK is a DUF26 domain (cysteine-rich repeat domain) containing protein and is differentially regulated by pathogen infection, JA treatment, root development, and salt stress in rice (Jiang *et al.*, 2007; Zhang *et al.*, 2009).

Concluding remarks

The proteomic analysis is a very useful tool for providing complex information about differences in the plant proteome during abiotic and biotic stresses. This information can show us the complexity of the plant response to various environmental stress factors and can enable us to find the biomarkers of plant tolerance to stresses which would be usable by breeders. Moreover, it is becoming possible to identify unknown pathogens, quantify the biomarkers in different cultivars or evaluate the quality of plant products using modern proteomic techniques. The examples reviewed here demonstrate the complex cellular network that exists in different plant-pathogen interactions. Overall, the use of proteomic studies, allied to functional validation analyses, can provide fascinating contributions to the understanding of complex mechanisms, such as plant–pathogen interactions. The first step in the understanding of disease resistance is currently being met with the identification of the proteins expressed during plant-pathogen interactions. The next step will be to determine which proteins confer pathogenicity and disease resistance, and the mechanisms by which they do so. We believe that future proteomic studies, coupled with functional validation analysis, may provide new insights into disease resistance and pathogenicity.

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