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REVIEW

Biotic Stress-Responsive Rice Proteome: An Overview

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Abstract Biotic stresses affect the plant growth, seed quality, and crop yield. The monocot model rice crop plant is no exception and is affected by a variety of biotic stress factors. High-throughput proteomics approaches are being applied in rice for the past several years to exploit better understanding the biotic stresses-responsive regulatory mechanisms. A large number of proteins responsive to biotic stresses, including pathogens and herbivores, have been cataloged. Cataloged proteins mainly belong to functional categories into metabolism, energy, defense mechanisms, and signaling. To date, majority of these proteins have not been functionally characterized yet, except the pathogen-related 10 protein, PBZ1. This review will briefly summarize and discuss: (1) the proteomics-based investigation of biotic stress-responsive proteins in rice and (2) increasing importance of proteomics approach in defense biology and engineering the next-generation rice/crop plants.

Keywords Biotic stress · Rice · Proteomics

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Introduction

Environmental stresses influence the plant growth and agriculture production, including the major food crop, rice (Agrawal et al. 2006, 2009; Agrawal and Rakwal 2006, 2008, 2011; Jorrín et al. 2007). Rice (*Oryza sativa*) supplies the energy requirement for more than two billion of the world's population, especially in Asian countries. In Korea, rice blast disease alone caused the losses of 8% total products in the mid-1970s (International Rice Research Institute (IRRI), http://irri.org/our-science/rice-breeding/). In the year 2010, low temperature, typhoons, heavy rainfall, and insufficient sunshine hours decreased the rice production by 11.6% (Statistics Korea, http://kostat.go.kr/portal/).

To minimize the impact of environmental stresses on rice production, high-throughput technologies have increasingly been applied to understand the regulatory mechanisms of rice responses to environmental cues including biotic stresses. Proteomics is one of the high-throughput technologies applied to plants for the past one decade (Agrawal and Rakwal 2008). There has been tremendous progress in gel-based and gel-free proteomics techniques and their applications to address relevant biological questions.

Biotic stresses are caused by the living organisms, such as fungi, bacteria, viruses, and insects. To date, a total of 16 proteomics-level studies in rice have been reported. Those biotic studies involve elicitor (Agrawal et al. 2002; Chen et

al. 2007a; Lin et al. 2008; Liao et al. 2009), fungus (Kim et al. 2003, 2004, 2009; Lee et al. 2006), bacterium (Mahmood et al. 2006; Chen et al. 2007b; Kandasamy et al. 2009; Chi et al. 2010), virus (Ventelon-Debout et al. 2004), herbivore (Wei et al. 2009), and mammalian (Fan et al. 2011). In this review, we summarize proteomics studies of biotic stresses in rice. These studies have increased our knowledge on biotic stresses-responsive proteins and regulatory pathways and mechanisms. We discuss below each research topic defined under a specific biotic stress heading.

Elicitor

Based on the gene-for-gene theory, proteins or chemicals recognized by host receptors trigger host-defense responses by activating and/or suppressing defense signaling and metabolic pathways (Agrios 2005; Allwood et al. 2008; De Wit et al. 2009). Previous studies 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 at least four proteomics studies that deal with elicitors (Table 1). 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 sulfate-polyacrylamide gel electrophoresis) and two-dimensional (2-D) 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. 2007a). A total of ten differentially expressed protein spots were detected. Of these, seven upregulated and one downregulated 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). A 2-DGE 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 were upregulated, representing 11 unique proteins. The upregulation 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 (qRT-PCR) analysis of these two corresponding genes revealed that these proteins are also regulated at transcriptional, as well as post-transcriptional levels.

CSB I, a 102-kDa glycoprotein from *Magnaporthe oryzae* (*M. 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 2-DGE-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).

Fungal Pathogens

Magnaporthe oryzae Comparative proteomics analyses of interactions between pathogen and rice were carried out by many research groups. The *M. oryzae* interaction with rice causes the rice blast disease, one of the most serious fungal diseases in rice, and has been known to cause a great loss of rice production (Talbot 1995). The first proteomics study was reported in the 2003 using the SCCs system (Kim et al. 2003). Fourteen spots encoded six different family protein were identified at 24 and 48 h after inoculation. Most of the identified proteins were related to pathogen or defense responses, including PBZ1, SalT, RLK, β-glucosidase, OsIRL, and OsPR10. In the year 2004, they extended the study on characterizing *M. oryzae*-responsive proteins from rice leaves infected with compatible (KJ301) and incompatible (KJ401) fungus (Kim et al. 2004). A total of eight spots representing seven unique proteins were identified by MALDI-TOF-MS. Those proteins were Glu1, Glu2, TLP, POX, PBZ1, RLK, and OsPR10. Among them, PBZ1, RLK, and OsPR10 were highly conserved in both SCCs and leaves in response to *M. oryzae*, suggesting that those proteins may be 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). Study of secreted proteins in the proteomics field is known as secretome, which has recently been comprehensively reviewed (Agrawal et al. 2010). Pure secreted proteins were subjected to a 2-DGE-based proteomics approach. Twenty-

Table 1 Summary of proteomics reports on biotic stresses in rice

	Stress type	Methods	Plant condition	Regulated protein/functional groups
Elicitor	Chitosan	1-DGE, 2-DGE (p*I* 3.5–10), *N*-terminal Edman sequencing	Seedling leaves, total protein	ROS (APX), defense (OsPR5, OsPR1)
	Chitooligosaccharide	2-DGE (p*I* 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 (p*I* 4–7, 18 cm), MALDI-QTOF-MS or LC–MS/MS	Seedling leaves, total protein	Energy (fructose-bisphosphatealdolase, NADH-ubiquinone 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 (p*I* 4–7, 18 cm), LTQ-MS/MS	Fourth-leaf stage leaves, total protein	Defense (PR10a, PR5), ROS (catalase, Mn-SOD, Cu/Zn-SOD, GST), signaling (nucleoside diphosphate kinase), stress (translationally controlled tumor protein homolog, chaperonin), protein biosynthesis (translational elongation factor Tu), metabolism (malate dehydrogenase, transketolase, fructose-bisphosphate aldolase)
Fungus	*Magnaporthe oryzae*	2-DGE (p*I* 4–7, 18 cm), MALDI-TOF-MS	SCCs, total protein	Defense (PBZ1, OsPR10, RLK), stress (SalT), ROS (OsIRL)
	M. oryzae	2-DGE (p*I* 4–7, 18 cm), MALDI-TOF-MS	Fourth- and fifth-leaf stage leaves, total protein	Defense (PBZ1, OsPR10, Glu1, Glu2, RLK, POX, TLP)
	Rhizoctonia solani	2-DGE (p*I* 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 proteasome non-ATPase regulatory) energy (G3PDH, rubisco large subunit), metabolism (ribulose-bisphosphate carboxylase)
	M. oryzae	2-DGE (p*I* 4–7, 18 cm), MALDI-TOF-MS	SCCs medium	Defense (chitinase, DUF26, α-amylase, β-expansin, putative germin A/putative oxalate oxidase, Jacalin-like lectin domain)
Bacterium	*Xanthomonas oryzae*	2-DGE (p*I* 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)
	X. oryzae	2-DGE (p*I* 4–7, 17 cm), MS/MS	*Xa21*-transgenic SCCs, plasma membrane protein	ROS (APX, quinone reductase), stress (LMW HSP), signaling (H1-ATPase, protein phosphatase)
	Pseudomonas fluorescens	2-DGE (p*I* 4–7, 17 cm), MS/MS	Seedlings sheaths, total protein	Stress (p23 co-chaperone), ROS (GSTZ5, thio-redoxin h), metabolism (ribulose-bisphosphate carboxylase, nucleotide diphosphate kinase), protein degradation (proteosome)
	Sinorhizobium meliloti	2-DGE (p*I* 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)

Table 1 (continued)

	Stress type	Methods	Plant condition	Regulated protein/functional groups
Virus	Rice yellow mottle virus (RYMV)	2-DGE (p*I* 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 (2,3-biphosphoglycerate-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)
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-beta-glucosidase, fructose-bisphosphate aldolase, sucrose synthase), transport (aquaporin)

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

one differentially expressed protein spots were identified in response to elicitor and fungal attack, including 2 highly expressed protein families, chitinase (9 spots), and domain of unknown function (DUF)26 (5 spots). All five DUF26 family proteins were upregulated. The DUF26 family proteins have been shown to be defense-related proteins in various plants (Kim et al. 2004, 2009; Wrzaczek et al. 2010). Among the other identified secreted proteins were oxalate oxidases/germin, expansin, amylase, and Jacalin-like domain protein, which were upregulated in response to fungal treatment. Their protein abundance had good agreement with their corresponding gene's mRNA levels, as judged by qRT-PCR.

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) (Lee et al. 2006). 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.

Bacteria

Pathogenic bacterium Two proteomics analyses involved in *Xanthomonas oryzae* (*Xoo*), which is the causative agent of rice leaf blight (Mahmood et al. 2006; Chen et al. 2007b). A comparative proteomic analysis of cytosolic and membrane fraction proteins from leaf blades of 3-week-old rice seedlings inoculated with compatible or incompatible *Xoo* strains (Mahmood et al. 2006). A total of 20 differentially expressed protein spots were identified out of 366 total detected spots, which were involved in energy (ATP synthase, RuBisCO LSU), metabolism (transketolase, GADPH, triosephosphate isomerase), defense (PR5, PBZ1, SOD, peroxyredoxin), and protein synthesis. Two of them, triosephosphate isomerase and unknown proteins, were specific to incompatible interaction. Ten of them were reported to be specific to the compatible interaction, such as ATP synthase, RuBisCO LSU, and peroxyredoxin. Eight protein spots were commonly induced in both incompatible and compatible interactions, including PBZ1, PR5, SOD, and one RuBisCO LSU. Another study used the SCCs system to identify Xoo-responsive proteins (Chen et al. 2007b). PM proteins were prepared from the transgenic rice SCCs harboring Xa21-GFP fusion protein using PEG precipitation. The prepared PM fractions were confirmed by GFP luminescent, and subjected to 2-DGE-based proteomics analysis. A total of 11 protein spots representing 10 unique proteins were identified, including protein phosphatase, hypersensitive-induced response protein (OsHIR1), prohibitin (OsPHB2), APX, and heat shock proteins (HSPs). Xa21is a receptor kinase protein located on PM, which senses signals from its molecular patterns from *Xanthomonas oryzae* (Song et al. 1995). The activation of Xa21 leads to the activation of rice innate immunity (Lee et al. 2009). Recently, an interactome using

yeast two hybrid was carried out to understand the intercellular signaling of Xa21 and other stress-related proteins (Seo et al. 2011). This protein–protein interaction study may give a different way to understand the biotic and abiotic signaling cross-talk in rice.

Symbiotic bacterium 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. 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 trigger different type of defense response pathways.

Virus

The rice yellow mottle virus (RYMV) is one of the most damaging viral pathogens of rice. The RYMV possesses a single-stranded RNA with four open reading frames. 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 HSP.

Herbivores

The first rice–herbivores interaction study using proteomics was carried out in the year 2009 using the brown planthopper (BPH), which is one of the most serious rice pests (Wei et al. 2009). A total number of 50 proteins were significantly modulated, which were related to biotic stimulation (9 proteins), stress response (9 proteins), protein metabolism and modification processes (10 proteins), carbohydrate metabolism (9 proteins), amino acid and derivative metabolism (4 proteins), photosynthesis (5 proteins), and transport (4 proteins). Among these biotic stimulation-related proteins, five peroxidase proteins, allene oxide cyclase, APX, and dioxygenase were detected, indicating that herbivores may cause the accumulation of ROS in the host. The upregulation of aquaporin isoforms by herbivores suggested that osmotic stress may have some role during herbivory-induced damage to the tissues.

Functional Dissection of Identified Biotic Stresses-Responsive Proteins

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), and Jacalin-like domain protein.

The pathogen-related (PR) proteins, 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, 2004; Mahmood et al. 2006; Chi et al. 2010; Ventelon-Debout et al. 2004). Meanwhile, 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, 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.

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). The OsRLK (DUF26) was detected only in response to rice blast fungus attack from SCCs, rice leaf, and SCCs

secretomes, but not other biotic stresses, suggesting the OsRLK may be important in rice blast fungus defense system (Kim et al. 2003, 2004, 2009).

The β-1,3-glucanases were regulated by environmental stresses, wounding, phytohormone, and development (Henning et al. 1993; Vogeli-Lange et al. 1994; Leubner-Metzger et al. 1998; Akiyama and Pillai 2001). 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.

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). 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). OsAPX2 protein was responsive to BPH, and an APX from SCCs plasma membrane was responsive to *Xoo* infection (Wei et al. 2009; Chen et al. 2007b). These two proteins were detected in rice seedlings after treatment with *ovine saliva* (Fan et al. 2011). These data suggest that the ROS detoxifying enzymes may function in multiple biotic stress responses.

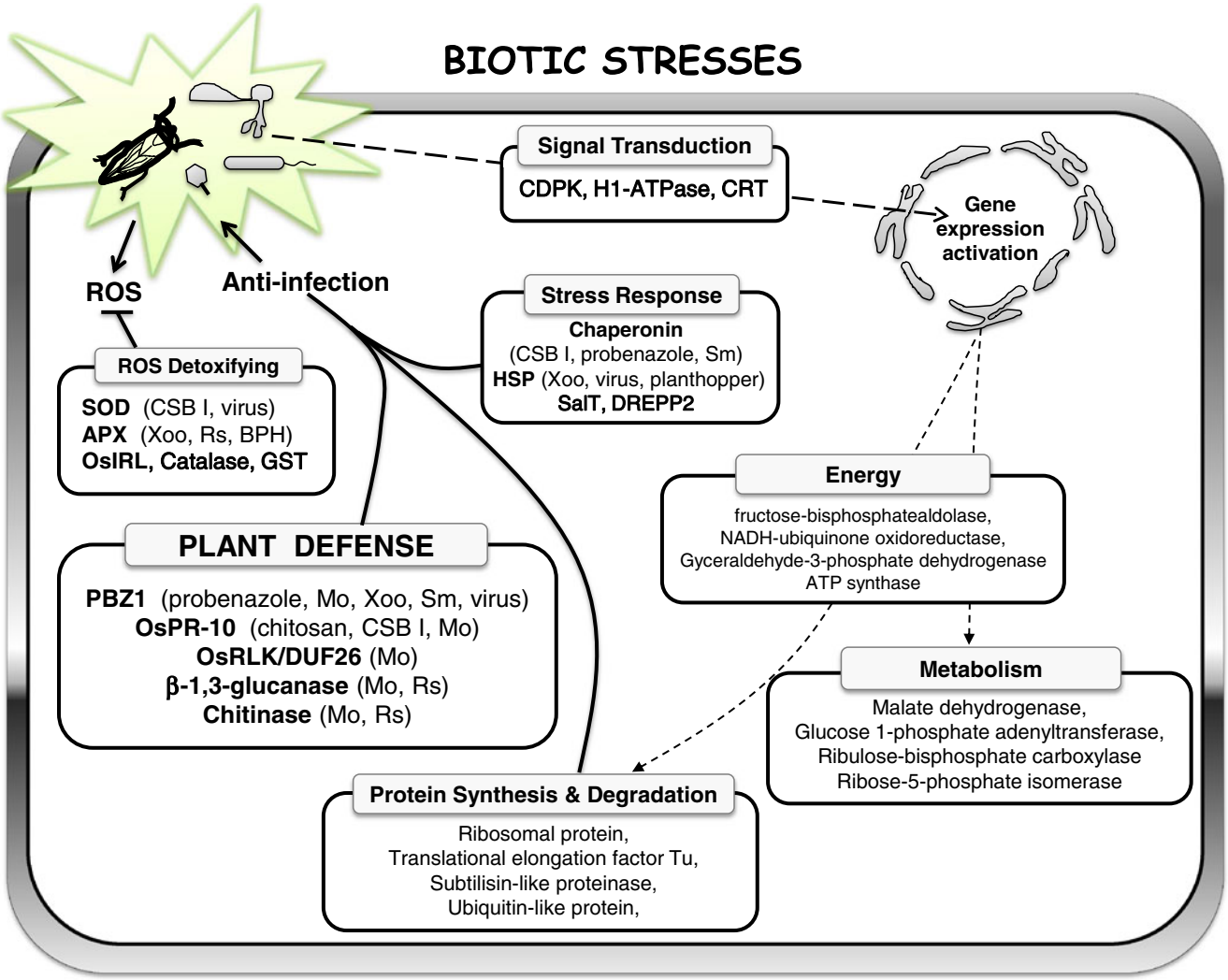

Fig. 1 An emerging model illustrating biotic stress-responsive proteins in rice. Proteins of various biological functions are expressed in response to biotic stresses. Those proteins are mainly involved in stress response, ROS detoxification, plant defense, and metabolism and energy. *BPH* brown planthopper, *Mo Magnaporthe oryzae*, *Sm Sinorhizobium meliloti*, *ROS* reactive oxygen species, *Rs Rhizoctonia solani*, *Xoo Xanthomonas oryzae*

Concluding Remarks

With the rapid progress in protein extraction, separation, and identification techniques, proteomics technology is playing a key role in developing inventory of proteins responsive to biotic stresses. The protein inventory has proved helpful in identifying common/specific proteins to biotic stresses and dissecting the rice defense pathways (Fig. 1). Nevertheless, despite the large number of cataloged proteins, the first challenge remains to saturate the proteome responsive to various biotic stresses. This saturation will reveal the potential biomarkers common or unique to each stress, and that will be used for generating the next-generation crop plants using molecular breeding and/or coupled with genetic engineering with enhanced crop yield and seed quality.

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