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Review article

A decade of plant proteomics in South Korea: the International plant proteomics organization (INPPO) perspective and involvement

Su-ji Lee[1], Kyu Young Kang[2,3], Nam-Soo Jwa[4], Dea-Wook Kim[5], Ganesh Kumar Agrawal[6], Abhijit Sarkar[6,7], Renu Deswal[8], Jenny Renaut[9], Dominique Job[10], Randeep Rakwal[6,11,12*], and Sun Tae Kim[1*]

[1]Department of Plant Bioscience, Pusan National University, Miryang, 627-706, South Korea
[2]Division of Applied Life Science (BK21 program), Gyeongsang National University, Jinju 660-701, South Korea
[3]Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 660-701, South Korea
[4]Department of Molecular Biology, Sejong University, Gunja-dong, Seoul 143-747, South Korea
[5]National Institute of Crop Science, Rural Developmental Administration, Suwon 441-857, South Korea
[6]Research Laboratory for Biotechnology and Biochemistry (RLABB), GPO Box 13265, Kathmandu, Nepal
[7]Department of Botany, Banaras Hindu University, Varanasi 221005, India
[8]Department of Botany, University of Delhi, Delhi-7, India
[9]Centre de Recherche Public-Gabriel Lippmann, Department of Environment and Agrobiotechnologies, Belvaux, GD, Luxembourg
[10]CNRS/UCBL/INSA/Bayer CropScience Joint Laboratory, UMR 5240, Bayer CropScience, 14-20 rue Pierre BAIZET, F-69263, Lyon cedex, France
[11]Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba Ibaraki 305-8572, Japan
[12]Department of Anatomy I, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa, Tokyo 142-8555, Japan

***Corresponding author: Sun Tae Kim, stkim71@pusan.ac.kr; Randeep Rakwal, plantproteomics@gmail.com**

Abstract

In this review, we discuss the contribution of proteomics focusing on plant responses to various environmental stimuli in South Korea. Due to improvements in proteomics methods and applications in various research fields in South Korea, plant proteomics have and will continue to provide systematic approaches to address biological questions and understanding of the network between plants and environmental stimuli. We also introduce the International Plant Proteomics Organization (INPPO; www.inppo.com), a global initiative to advance plant proteomics research worldwide that is also designed to highlight national plant proteomics topics via a common global platform. Thus, we expect INPPO to generate further interest and activity among South Korean plant proteomics researchers and within the plant biology scientific community. Finally, to help network the plant proteomics community in South Korea, the creation of an INPPO-South Korea chapter is proposed.

Keywords: Abiotic stress, Biotic stress, Environment, INPPO, Plant Proteomics.
Abbreviations: HSPs: heat shock proteins; MS: mass spectrometry; LCM: liquid culture medium; PEG: polyethylene glycol; PCD: programmed cell death; PVDF: polyvinylidene difluoride; RuBisCO: ribulose-1,5-bisphosphate carboxylase/oxygenase; 2-DGE: two-dimensional gel electrophoresis; SCCs: suspension cultured cells.

Introduction

Crop plants and the start of proteomics and technological advances

Crops and plants of economical importance are the primary targets of proteomics research in South Korea, which is generally conducted to improve seed quality and yield. Rice, being a major crop, has always been a major focus of research. South Korea has large quantities of natural and cultivated rice germplasms and researchers that have been thoroughly trained in investigation of these resources. In the past decade, proteomics has increasingly been applied as a high-throughput technology to complete our understanding of plant systems under diverse environments (Thiellement et al., 2007, Agrawal and Rakwal 2008a). Plant proteomics in South Korea is believed to have started in the late 1990s, when a research group led by Professor Kyu Young Kang initiated proteomics investigations of rice plants at Gyeongsang National University. In their first published study, they described a novel method for depletion of the most abundant leaf protein in rice, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), via a simple polyethylene glycol (PEG) fractionation technique (Kim et al., 2001). This pioneering method demonstrated how a simple extraction method combined with two-dimensional gel electrophoresis (2-DGE) could increase the detection of rare or low-abundant proteins, and hence tremendously increase coverage of the leaf proteome. The same group also developed a 1-DGE (SDS-PAGE; sodium dodecyl sulfate polyacrylamide gel electrophoresis)-based methodology to fractionate and enrich high-/low-molecular mass proteins of the rice leaf (Kim et al., 2003a). In that study, the ability to combine 1-DGE with

2-DGE was also demonstrated. This method revealed the usefulness of a classical SDS-PAGE in the proteomics field for in-depth investigation of a proteome. In later years, successive development of several methodologies in rice proteomics allowed Kyu Young Kang's group to conduct thorough proteomics investigations of rice and develop comprehensive maps of the proteome responses of rice to multiple biotic and abiotic stresses (reviewed in Agrawal and Rakwal 2006, 2008b, 2011, Agrawal et al., 2009, 2010). These and other technical advancements, along with the confidence in proteomics technology for addressing biological questions (Bradshaw and Burlingame 2005) have led to its widespread utilization by a number of research groups at Universities and Institutions. Most, if not all of these groups are highlighted onto on the map of South Korea in Fig. 1.

Plant proteomics database in South Korea

Increases in the number of research groups and publications reflect the rapid progress in plant proteomics that has occurred in South Korea in the past decade. Many scientists are now working intensively on numerous biological questions pertaining to agriculture crops and plants of their interest (Fig. 2). The plant systems that are being used for such investigations range from the dicot model *Arabidopsis thaliana* to the monocot cereal model crop rice (*Oryza sativa* L.), and include numerous other important crop plants, such as wheat, maize, soybean, rapeseed (canola), buckwheat, and banana. Investigations of plant responses to biotic (i.e., bacterial, fungus, and viral pathogens) and abiotic (i.e., agrochemicals, cold, drought, heat, heavy metals, light, ozone, salt, UV-B, volatile organic compounds, and water logging) stresses through proteomics techniques have been the major focus of South Korean plant proteomers since the technique was first introduced. In fact, this tremendous progress in plant proteomics was highlighted in a special issue of the Journal of Plant Biotechnology (a peer reviewed South Korean Scientific Journal published by the Korean Society of Plant Biotechnology) (Lee et al., 2011). This special issue in a national journal, and increased numbers of research publications to keep up with leading international journals, advocated the achievements made in South Korean plant proteomics and increased the awareness of the potential for the use of proteomics to study plants among plant biologists. Such attention and increased funding are required to induce the next green revolution to feed and meet the demands of the ever-growing human population under global climate changes (Swaminathan 2000). In the following sections, we look at two critical aspects of plant proteomics research in South Korea, abiotic and biotic stress proteomics in plants, particularly crops such as rice.

Abiotic stress proteomics

Rice proteomics has achieved remarkable progress in the use of high-throughput techniques, as well as generation of meaningful biological data pertaining to developmental stages, tissues and organs, and against biotic and abiotic stresses. Agrawal and Rakwal have systematically and comprehensively reviewed the progress in rice proteomics during 2000 to 2010 (Agrawal and Rakwal 2006, 2008b, 2011, Agrawal et al., 2009, 2010, Rakwal and Agrawal 2003). In Korea, a total of 29 papers on plant proteome responses to abiotic stresses including agrochemicals, heavy metals, cold, heat, drought, water logging, and salt streses were published from 2005 to 2010. These papers are summarized in Fig. 3, along with an illustration of the proteomics approaches. Most experiments to

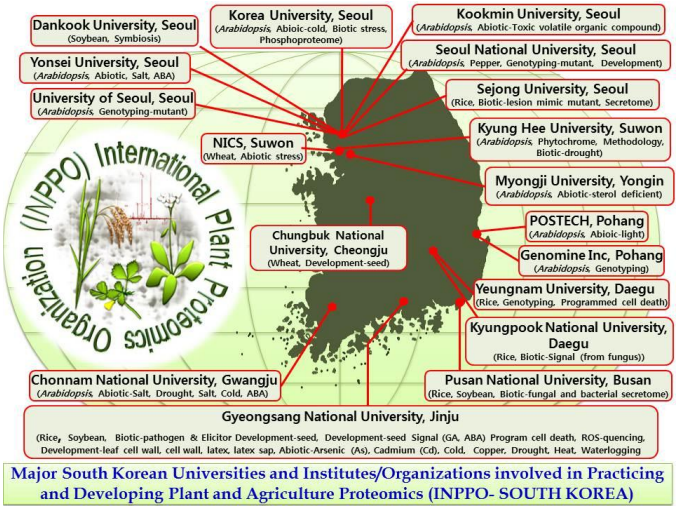


Fig 1. Plant proteomics research in South Korea and a potential INPPO-South Korea platform.

investigate abiotic stresses responses were carried out using leaf and root tissues of rice and *Arabidopsis*. Below, we briefly summarize proteomics studies of abiotic stresses in rice as a model crop system. These studies have increased our knowledge of abiotic stress-responsive proteins and regulatory pathways and mechanisms in rice.

Agrochemical and heavy metal stress

A total of 11 papers published between 2005 and 2010 have analyzed changes in the proteome to investigate the effects of two agrochemicals, glyphosate and glufosinate, and of heavy metals such as arsenic (As), cadmium (Cd), and copper (Cu). Proteome-wide changes in response to heavy metals comprise the largest set of papers (five papers), with As in leaf (Lee et al., 2008, Ahsan et al., 2010) and root (Ahsan et al., 2008a), Cd in leaf/root (Lee et al., 2010) and seedling (Ahsan et al., 2007a), and Cu in seedling (Ahsan et al., 2007b). Proteomics analysis revealed that most of the proteins related to energy production and metabolism, such as the RuBisCO large subunit (LSU) and detoxification, were increased. Conversely, proteomics analysis of rice leaves treated with glyphosate (an herbicide used to inhibit aromatic amino acid synthesis) and glufosinate (an herbicide inhibiting glutamine synthetase to catalyze ammonia assimilation) revealed that antioxidant enzymes and photosynthetic/metabolic-related proteins such as RuBisCO LSU were deceased (Ahsan et al., 2008b, Lee et al., 2008), suggesting that plant cells maintain a homeostasis in photosynthetic and antioxidative machineries corresponding to the environmental stresses imposed on plants.

Temperature stress

In addition, Lee et al. (2007a, 2009) investigated cold-responsive proteins in leaves and roots of rice exposed to chilling stress conditions (5 or 10ºC) in a time-course experiment. Proteins such as cysteine proteinase, thioredoxin peroxidase, a RING zinc finger protein-like, drought-inducible late embryogenesis abundant protein, and a fibrilin-like protein were identified. Another study of the leaf proteome of rice exposed to 42ºC for 12 and 24 h was also conducted (Lee et al., 2007b). Based on increased biochemical changes such as ion leakage and lipid peroxidation, it was suggested that high temperature treatment induced oxidative stress in rice leaves.

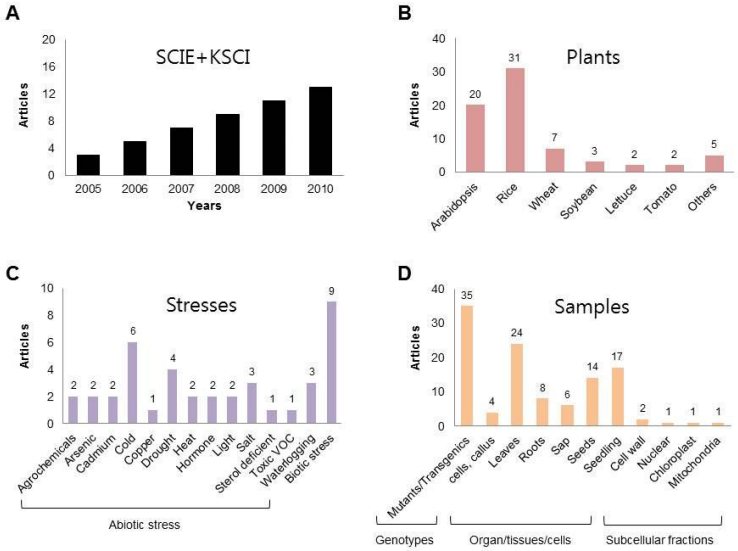
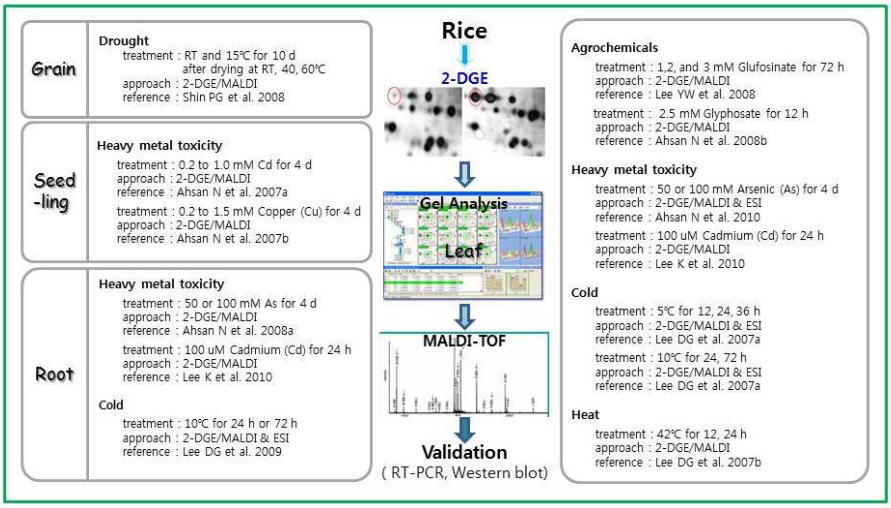


Fig 2. Studies, objectives, and contributions of the plant proteomics in Korea (adopted from Lee et al., 2011).



Fige 3. A summary of published papers on rice abiotic stress proteomics (adopted from Lee et al., 2011).

Seventy-three out of 1000 proteins separated by 2-DGE were identified by mass spectrometry (MS), 48 of which were identified as heat shock proteins (HSPs), energy and metabolism-, redox homeostasis-, and regulation-related proteins after heat treatment. One important finding was that many small HSPs (sHSPs) are newly up-regulated. The sHSPs play a central role in the complex cellular network (Wehmeyer et al., 1996), and it has been suggested that plants protect themselves from heat stress using a complex mechanism, of which sHSPs are a part (Lee et al., 2007b).

Biotic stress proteomics

Biotic stressors such as bacteria, viruses, fungi, insects, and weeds not only have the potential to cause damage, but also are the direct cause of vast economic losses of crops. Proteomics studies against biotic stress factors have been conducted in *Arabidopsis*, pepper, rice, and wheat (Fig. 4). Many of these studies were conducted using the blast lesion mimic (*blm*) mutant. This mutant is associated with biotic stress response proteins coupled to pathogens or defense-related signals. As a major fungal pathogen, an interaction between *Magnaporthe oryzae* (*M. oryzae*) causing rice blast disease and rice plants was reported by Jung et al., (2005, 2006), Kim et al., (2009), and Ryu et al., (2009). We discuss these investigations in the following sections while focusing on studies dealing with the interaction of *M. oryzae* and rice.

Rice plant-pathogen interactions and programmed cell death

***M. oryzae* and rice**

The rice blast fungus *M. oryzae* is a causal agent of rice blast disease, one of the most serious pathogens of the rice plant in most rice-growing regions of the world. Kyu Young Kang and colleagues have meticulously carried out comparative proteomics analyses of the interactions between *M. oryzae* and rice (Fig. 5). The first proteomics analysis was conducted in 2003 using a rice suspension cultured cells (SCCs) system (Kim et al., 2003b) inoculated with rice blast fungus or treated with elicitors to identify pathogen-responsive proteins. In 2004, these authors extended the study to characterization of *M. oryzae*-responsive proteins from rice leaves infected with

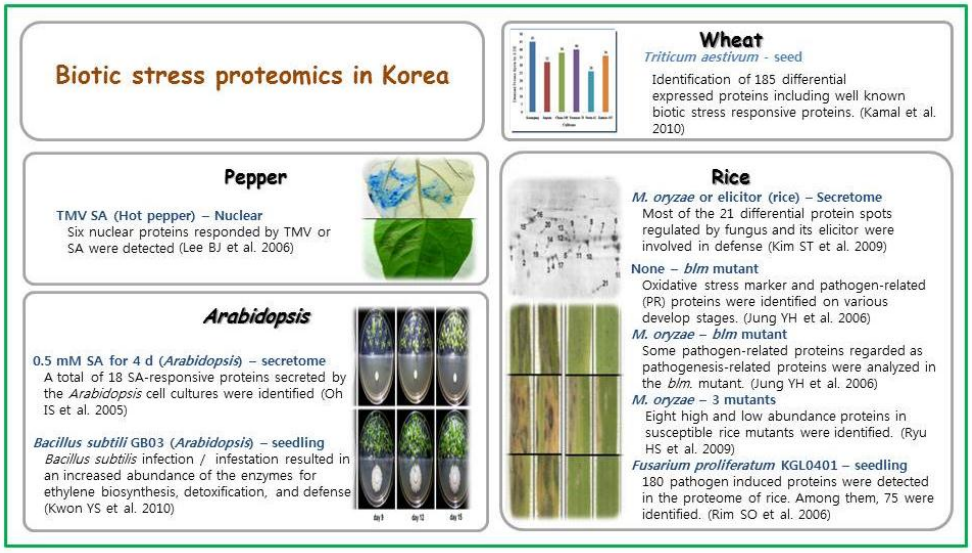


Fig 4. A summary of published papers on rice biotic stress proteomics (adopted from Lee et al., 2011).

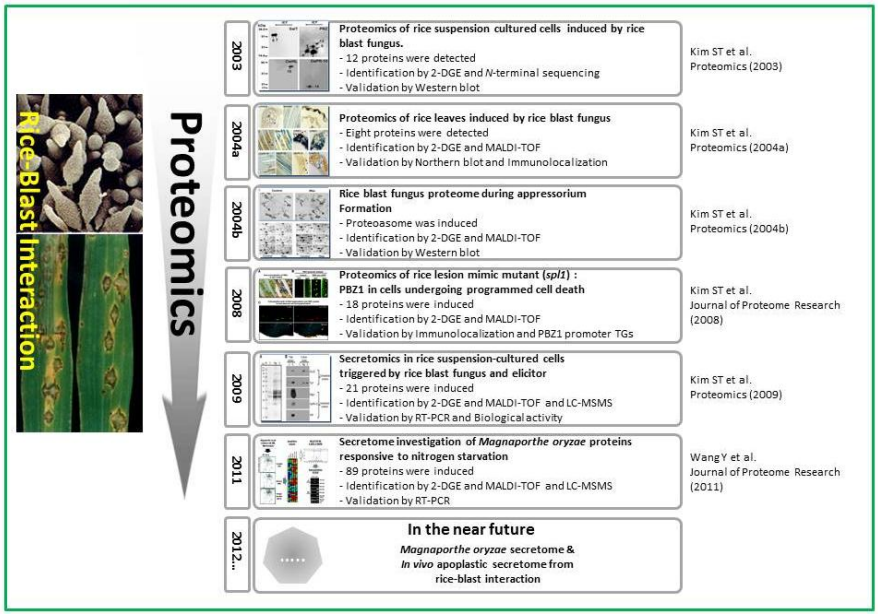


Fig 5. History of rice proteomics during rice-blast pathogen interaction.

compatible (KJ301) and incompatible (KJ401) fungus (Kim et al., 2004a). The use of the PEG (15% w/v) fractionation technique previously developed to remove RuBisCO into the pellet fraction (Kim et al., 2001) enabled a comprehensive analysis of the *M. oryzae*-responsive proteins from rice (cv. Jinheung) leaves. Among proteins identified by *N*-terminal sequencing and MALDI-TOF-MS were the probenazole-induced protein (PBZ1), OsRLK, and OsPR10, which were highly upregulated in both SCCs and leaves in response to *M. oryzae* attack. These results also revealed that the expression of PBZ1, OsRLK and OsPR10 was faster and higher in the incompatible interactions than compatible interactions. These results imply that rapid and high-level expression of these genes may provide host plants with a self-defense mechanism against pathogen attack. Accordingly, these proteins might also serve as potential defense-related biomarkers in rice.

PBZ1 and programmed cell death

PBZ1, which is a PR 10 family protein, was found to be induced in both rice SCCs and leaves inoculated with blast fungus (Kim et al., 2003b, 2004a). Despite the above research linking PBZ1 with defense/stress response, its biological function remained unknown. Interestingly, Kim and coworkers (Kim et al., 2008) explored the role of PBZ1 as a putative cell death marker in rice. Based on these studies, PBZ1was found to be highly accumulated in tissues undergoing programmed cell death (PCD). The correlation between the accumulation of PBZ1 and PCD was demonstrated during leaf senescence, root aerenchyma formation, coleoptiles senescence, and root cap and seed aleurone layer formation (Kim et al., 2008) by monitoring PBZ1 expression level using Western blotting, immunohistochemical analysis, and promoter analysis. The results showed that the expression levels and localizations of

PBZ1 were tightly correlated with plant cell death. These results strongly suggest that PBZ1 is either a molecular marker in rice defense responses or can serve as a novel potential biomarker for cell death/PCD in rice.

M. oryzae and rice, and their secretomes

Secretomes are an emerging area for understanding interactomes between hosts and pathogens in the extracellular space within the rice leaf, which serves as a front line of defense against invading blast fungus (see review, Agrawal et al., 2010). To the best of our knowledge, there have been no reports of the in vitro and in vivo secretomes of rice leaves infected with compatible and incompatible races of *M. oryzae*. Previously, secretome analysis of rice SCCs treated with *M. oryzae* or its elicitors resulted in the identification of 21 differential protein spots (Kim et al., 2009). Mass spectrometry analysis resulted in the identification of nine chitinases, expansin, five 33 kDa secretory proteins (DUF 26), and two germin/oxalate oxidases protein. One of the identified chitinase proteins revealed strong enzymatic activity in an in-gel assay. Corresponding gene expression patterns were also validated by RT-PCR, which revealed a much earlier induction of chitinase, DUF26, and expansin in incompatible rather than compatible interactions. This secretome analysis by Kim et al. was the first to provide information about the mechanism of rice defense response against *M. oryzae* and its elicitor. However, the *in vivo* secretome of any plant-pathogen interaction has yet to be systematically investigated. In the near future, the established *in vivo* rice apoplastic proteome will provide important information enabling better understanding of the rice-*M. oryzae* interaction. While studying the blast-fungus interaction proteome, it is also desirable to examine the pathogen portion of the secretome to obtain better insight into the rice self-defense mechanism. During the host infection process, *M. oryzae* differentiates into a special structure, known as an appressorium, to penetrate the leaf surface, followed by invasive hyphae in an early stage of the biotrophic infection phase. Kim et al., (2004b) conducted a study to identify the proteins induced in the rice blast fungus *M. oryzae* during appressorium formation using 2-DGE. Protein spots differentially expressed during appressorium formation were confirmed from gels after 2-DE analysis of proteins labeled with ^{35}S methionine and stained with silver nitrate. This allowed isolation and identification of three proteins (20S proteasome a subunits, scytalone dehydratase, and serine carboxypeptidase Y) that were differentially accumulated during appressorium formation. In particular, the expression levels of the 20S proteasome proteins identified by Western blot analysis during appressorium formation and nutrient starvation increased greatly. Thus, the function of the 20S proteasome proteins might provide important information pertaining to the cellular mechanism involved in appressorium formation. Wang et al. (2011) recently presented a comparative 2-DGE-based proteomics approach that revealed the secretome of *M. oryzae* responsive to N starvation using cultured liquid complete medium, minimal medium, and N starvation minimal medium. A total of 85 protein spots differentially responsive to N starvation were identified by MALDI-TOF-MS and μLC-ESI-MS/MS. The identified proteins were mainly cell wall hydrolase enzymes (22.4%), protein and lipid hydrolyses (24.7%), ROS detoxifying proteins (22.4%), and proteins with unknown function (14.1%). The results showed that several of the identified secreted proteins had previously been found to be associated with fungal growth and pathogenicity.

Furthermore, semi-quantitative RT-PCR analysis of N-responsive secreted proteins revealed a good correlation between RNA and protein levels, suggesting that they might also provide insight into *M. oryzae* pathogenicity and development of methods to control infection during the early stages. Most recently, Jwa et al. conducted secretome analysis of *M. oryzae* by mimicking the early stages of infection in vitro using a glass plate (GP), polyvinylidene difluoride (PVDF) membrane, and liquid culture medium (LCM) (Jung et al., 2012). Specifically, they used 2-DGE and tandem mass spectrometry analyses to identify 53 non-redundant proteins including previously known and novel secreted proteins. Most of the proteins were found to be involved in cell wall modification, ROS detoxification, lipid modification, metabolism, and protein modification categories. This unique study employing both hydrophobic (GP and PVDF) and hydrophilic (LCM) conditions provided a survey of the *M. oryzae* secretome that can be used to further our understanding of the early interactions between *M. oryzae* and rice leaves. In conclusion, many proteins have been found to be differentially expressed in either the plant or pathogens during their interactions. However, it is likely that information providing a clear understanding of biological processes occurring during rice and *M. oryzae* interaction will require further investigations. More in-depth analyses of the special and temporal distribution of responding proteins (e.g., *in-planta* apoplastic secretome changes) will help elucidate details pertaining to pathogen invasion strategies. In the technical aspect for analysis of proteomics, the 2-DGE approach has its own limitations in identifying extreme proteins such as highly basic, acidic, or membrane proteins as well as proteins with low abundance. Use of complementary proteomics approaches such as shotgun coupled with 1-DGE using MudPIT and iTRAQ (Weckwerth 2011) will be helpful in expanding and saturating the defense responsive proteins during plant and microbe interactions.

Future challenges for plant proteomics research in South Korea

Despite considerable progress in plant proteomics research in South Korea, there are numerous challenges ahead that must be met before the potential of proteomics technology in plant science is fully realized. We would like to bring out several points for discussion among various research groups. These are to: i) have an open sharing of information, such as proteomics techniques (i.e., extraction protocols, 1-DGE, 2-DGE, peptide identification, and protein assignment), without overlapping the objectives of each working group; ii) address major ground level problems in the country; and iii) help translate the outcome of the research to the field to produce next-generation crops with actively involving agronomists and breeders. With regard to the production of stress-resistant crops (point iii) it is without doubt that proteomics data pertaining to the complexity of plant responses to biotic and abiotic environmental factors can facilitate selection of potential biomarkers of plant tolerance to these deleterious stresses. Such biomarkers could be useful for plant biologists and breeders in marker-assisted crop and plant breeding. However, it is still necessary to determine a method of translating this information for the breeders. The National Institute of Crop Science (NICS) under the Rural Developmental Administration (RDA) in South Korea faces a similar problem. Breeders at NICS ask how the information accumulated from proteomics studies will facilitate sustainable crop production under unfavourable environmental

conditions or provide tolerance or resistance to crops under biotic and abiotic stresses. Accordingly, it is important to convey that, although plant genomes contain about 25-50,000 genes that are estimated to encode more than 1,000,000 protein isoforms because of the existence of extensive post-translational modifications of proteins, it is anticipated that such modifications (e.g., phosphorylations, methylations, acetylations, prenylations....) would constitute biomarkers of plant stress response, which cannot be identified by classical genetic approaches. Therefore these protein markers will comprise the next generation of genetic markers traditionally used in breeding, allowing further progress in plant improvement. With regards to the concerns of breeders, it is essential to tap the resources pertaining to crop varieties that are being generated in institutes such as the NICS to help bridge the gap and expectations of plant breeders by making direct links with the proteomics specialists in universities in South Korea.

The INPPO initiative and its association with plant proteomics

The International Plant Proteomics Organization (INPPO; www.inppo.com) has been founded with a major goal of the global sharing of knowledge databases gathered by plant proteomics studies worldwide. One of the major objectives of INPPO is to bring national plant proteomics issues out in the open via a common global platform. A global platform has always helped promote science and technology, and their application in human health and food security. INPPO has been established as a global platform for plant proteomics that includes ten initiatives (for details, see INPPO viewpoint paper, Agrawal et al., 2011; and INPPO Highlights, Agrawal et al., 2012b). The objectives of INPPO are to intensify cooperation in the field of plant proteomics, establish complete plant proteomes, carry out comparative and translational proteomics (Agrawal et al., 2012a), establish centralized databases, organize conferences and workshops, integrate proteomics-related activities and disseminate them through the INPPO website, provide education and training, develop interactions with other proteomic initiatives such as HUPO (the Human Proteome Organization) and MASCP (the Multinational *Arabidopsis* Steering Committee for Proteomics), and help address biological and societal questions.

To continue in line with these INPPO initiatives, we envision a national Plant Proteomics Chapter in South Korea under the INPPO umbrella, which we propose as the 'INPPO – South Korean Plant Proteomics Chapter (INPPO – South Korea)'. We sincerely hope that such a forum at the national level will bring together all plant proteomers, biologists, and breeders at a common platform to discuss the pressing problems faced, in particular by the plant and agricultural scientific communities. We look forward to the active participation of the plant proteomics community in this endeavor. Moreover, to help the younger plant proteomers progress to the next level in proteomics research, high-level training under the guidance of experts in national and international laboratories with specific expertise will be necessary. Initially, available educational infrastructure in the Universities will have to include basic proteomics facilities to generate interest in and train undergraduates and graduates in the latest proteomics skills. In the long-term, creation of more state-of-the-art high-throughput proteomics facilities dedicated to plant proteomics will be required. One good example is the facility for proteomics at Yonsei University in Seoul, which is a hub for Korea HUPO that primarily focuses on mammalian systems, but also includes plant proteomers. However, to accomplish such a large jump in plant proteomics research, one or two more similar centers will have to be created, depending on the collective will and use of the plant sciences community. Only then can the potential of the younger generation of Korean plant proteomers be fully realized. Finally, in keeping with the INPPO spirit of cohesion, coordination, and synergy, we look forward to support from our colleagues in South Korea in taking plant proteomics research to the next level.

Conclusion

In this review, we highlight proteomics research cases and trends focusing on plant responses to various stimuli including abiotic and biotic stresses in Korea. Furthermore, this review provides advanced researchers with information about the "who and who" of Korean plant proteomics dealing with plant response and networking to its environment. With the opening of the INPPO-South Korea plant proteomics chapter, we not only hope to see plant proteomics gain prominence nationally but also set an example for other countries to network plant proteomers.

Acknowledgements

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