

Rice Proteomics and Beyond

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Editorial

Rice is the most widely consumed staple food for both developed as well as developing world, more so for Asia. According to the data of FAOSTAT (2012), rice has the third-highest worldwide production after sugarcane and maize, among all agricultural crops [1]. Developing countries account for 95% of the total rice production, with China and India contributing for nearly half of the world output [1].

The main focus of rice research has been on crop improvement to increase productivity and adaptation to adverse climatic conditions. While rice genome sequence has been available for years now, high quality and uniform annotation is a necessity for genome sequence data to be fully utilized by researchers. Towards this, the completion of Rice Annotation Project (RAP) database (<http://rapdb.dna.affrc.go.jp/>), based on the new chromosome pseudomolecule Os-Nipponbare-Reference-IRGSP-1.0 (a joint version of IRGSP and MSU pseudomolecules) [2] is important. The existence of a common gene set and uniform annotation allows rice research community to work from a common resource so that their results can be more easily interpreted by other laboratories.

Proteomics has been a forte for rice research community in recent years and is likely to become an active area with large impact on rice research. Mass spectrometry-based proteomics can provide translation level expression evidence for the predicted protein-coding genes, a proteogenomics approach, where large-scale proteome data is used in genome annotation refinement [3]. The past decade has seen a significant change in rice proteomics. Proteomes have been established for most rice tissues and organs during its normal growth and development under variety of abiotic and biotic stresses [4-6]. Climatic variations, the world over, have resulted in increased stress to rice, limiting their growth and development as well as the productivity. The studies on stress-responsive proteomes have increased our understanding about such stresses in rice. Functional dissection of protein complexes in rice has been accomplished in a low-throughput manner (i.e. targeted proteomics) to uncover their biology. Besides basic research, proteomics applications have been explored to screen transgenic plants and to evaluate the safety of rice as food. Recent years have also seen the emergence of new proteomics resources and tools for functional analysis. PhosphoRice: a meta-predictor of rice-specific phosphorylation sites (<http://bioinformatics.fafu.edu.cn/PhosphoRice>) [7] has been widely used for predicting unidentified phosphorylation sites. OryzaPG-DB: rice proteome database based on shotgun proteogenomics (<http://oryzapg.iab.keio.ac.jp/>) [3], and PRIN: a predicted rice interactome network (<http://bis.zju.edu.cn/prin/>) [8] are also important resources. This ongoing progress of bioinformatics resources for proteomics analysis is helpful not only in elucidating the function of individual proteins, but also provides an insight into their dynamic interaction network in rice as well as other crop plants.

Proteomics studies in rice can be categorized into gel-based (1-DE, 2-DE, and 2-DIGE), gel-free (LC-MS/MS, MudPIT, iTRAQ), and a combination of these two approaches [9,10]. Recently, gel-free quantitative approaches have provided for large-scale and high throughput protein identification in rice. However, a gel-based approach employing 2-DE is still the method of choice in laboratories all over the world. For the study of differentially expressed proteins, 2-DE allows simultaneously to detect, quantify and compare up to thousand protein spots isoforms, including post-translational modifications, in the same gel in a wide range of biological systems. Nevertheless, it is equally true that 2-DE based proteomics has its limitations as it is biased against certain classes of proteins including low abundance and hydrophobic proteins. Contrarily, the presence of high-abundance proteins also interferes with reproducible separation of proteins and/or MS detection. For example, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) accounts for up to 65% of the total soluble leaf protein [11]. Therefore, better approaches for sample fractionation or enrichment is still needed in order to achieve highly resolved 2-D gels. The work on depletion of RuBisCO in rice using PEG fractionation technique [12] was a major step in this direction. The employment of protamine sulfate precipitation (PSP) method has been a successful development for the RuBisCO depletion, where low abundance proteins are highly enriched [13]. Among the protein extraction methods, trichloroacetic acid (TCA)/acetone precipitation and phenol remain the most popular for total protein suitable for gel-based and gel-free proteomics approaches.

Even though rice proteomics research is progressing at a breakneck speed, organelle specific analysis still remains underexplored [14-16]. In the year 2014, there are more than 25 publications on rice proteomics in a variety of avenues, highlighting the tremendous headway that has been made in generating large-scale data sets of tissue composition, protein function and protein expression profiles. Han et al. [17,18] used both gel-based and gel-free proteomics methods to study the rice embryo during germination. Another study used iTRAQ to analyze the proteome of rice spikelets during grain filling [19]. One particular area of interest is the investigation of stress-responsive proteome. Many studies focusing on the rice-Magnaporthe oryzae interaction were undertaken with the aim of analyzing the mechanisms in regulation of rice resistance against such diseases [20-22]. Dehydration, hypersalinity, high temperature, and metal stress proteomes were also explored in this crop using both gel-free and gel-based techniques [23-27]. Yang et al. [28] carried out a global proteomic analysis of indica and japonica rice varieties using 2-DE and MALDI-TOF MS. The study identified many important proteins involved in indica-japonica differentiation. However, it has been widely accepted that proteomics data alone does not lead to a comprehensive understanding of the mechanisms behind any of the processes. Thus, the study by He et al. is significant as it used an integrated approach to compare the transcriptome, proteome and

metabolome of the rhizome to other tissues of red rice [29]. This species is characterized by a number of traits, both quantitative and qualitative, which can be used for improvement of rice cultivars. The study led to the discovery of a number of genes and proteins that are specifically expressed in the rhizome tissues of red rice, which likely play roles in regulating rhizome differentiation and growth. A large number of gene transcripts from *Magnaporthe oryzae*, the fungus that causes rice blast disease in cultivated rice, were also identified even though there was no evidence of the disease itself. This suggests that red rice is resistant to this pathogen, and may have genes which when transferred to cultivated rice will bequeath it with resistance to rice blast disease. These examples highlight the progress of proteomic research in evaluation of rice plant growth and development and productivity caused by climatic changes and patho-stresses.

Rice, due to its genome availability and existence of numerous cultivars [30], is considered as a model crop. The recent progress in rice proteomics research is set to continue considering the value of this crop as food, fodder and research material. This large-scale generation of useful information would in future be used to improve not only rice but also other crops of agricultural importance.

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