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Review on Proteomics Technologies and Its Application for Crop Improvement

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Abstract
Proteomics is the study of proteins and their interactions in a cell. Within the wide field of functional OMICS, proteomics has become a useful tool and the emphasis is shifting from genomics to the protein compliment of the human organism. Because proteome reflects more accurately on the dynamic state of a cell, tissue, or organism, much is expected from proteomics to yield better disease markers for diagnosis and therapy monitoring. Hence the present review was to review proteomics technologies and their applications for crop improvement. The advent of proteomics technologies for global detection and quantitation of proteins creates new opportunities and challenges for those seeking to gain greater understanding of diseases. High-throughput proteomics technologies combining with advanced bioinformatics are extensively used to identify molecular signatures of diseases based on protein pathways and signalling cascades. Mass spectrometry plays a vital role in proteomics and has become an indispensable tool for molecular and cellular biology. However, future developments may enable faster and more sensitive proteomics studies and Proteomics alone cannot provide all the information required for understanding cellular processes. Therefore Complementary approaches in genomics, metabolomics and bioinformatics will have to be used together with proteomics to permits a more holistic view of biological systems and their alterations in disease, so that the maximum benefit can be realized.
Keywords: Bioinformatics, mass spectrometry, proteomics, Two-dimensional electrophoresis

1. INTRODUCTION

Proteomics is a recent member of the 'omics' family that has gained rapid momentum at the turn of the century, particularly in the area of therapeutics. In 1994, around 20 years ago, is considered the year of birth for "proteomics", being the term an extension of the word "proteome" first coined by Marc Wilkins while being a Ph.D. student at Australia's Macquarie University (Agrawal *et al*., 2013). Proteomics is the large scale study of proteins particularly their composition, structures, functions, and interactions of the proteins directing the activities of cell (Wilkins *et al.,* 1995). The main theme of interest proteomics it gives a much better understanding of an organism than genomics because genomics can give a rough estimation of expression of a protein. It is much more complicated than genomics, mostly because while an organism's genome is more or less constant, the total protein expression profile always changes with time, micro and macro environmental conditions (Holman *et al*., 2013).

The main goal of proteomics is to study, know and understand "how", "where", "when", and "what for" are the several hundred thousand of individual protein forms produced in a living organism, how do they interact with one another and with other molecules to construct the cellular building, how can they be modified and work in order to fit in with programmed growth and development, and to interact with their biotic and a biotic environment (Smith *et al*.,2013).

In the last decade, there have been many improvements in protein separation a technique, including two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), liquid chromatography (LC) and much progress has also been recently achieved in the analysis of proteins from tryptic digests using mass spectrometry (MS) and database searching. The newest generation of MS combined with good separation techniques is capable of providing rapid and confident protein identifications (Buts *et al.,* 2014).

Plant proteomic projects include structural proteomics of the whole organism, organs, tissues, cells, and sub cellular compartments, as well as comparative proteomics on various processes (Holman et al., 2013). Much attention has been paid to proteomic studies on crop plants in recent years subjected to various a biotic stresses and biotic factors such as looding, drought, salinity, acidity, and nutrient limitation (Petricoin *et al*., 2011).

The global scale analysis of plant proteins is expected to yield more direct understanding of function and regulation than analysis of genes. To meet the current challenges of food insecurity, genes and proteins that control crop architecture and or stress resistance in a wide range of environments will need to be identified to facilitate the biological improvement of crop productivity (Emam *et al.,* 2014). So the objective of this paper is to review proteomics technologies and its application for crop improvements.

2. Genomics to Proteomics

With the completion of the Human Genome Project, the emphasis is shifting to the protein compliment of the human organism. This has given rise to the science of proteomics, the study of all the proteins produced by cell and organism, which involves the identification of proteins in the body and the determination of their roles in physiological and patho physiological functions. The term "proteome" refers to all the proteins expressed by a

genome. While a genome remains unchanged to a large extent, the proteins in any particular cell change dramatically as genes are turned on or off in response to the environment. Since it is proteins that are directly involved in both normal and disease-associated biochemical processes, a more complete understanding of disease may be gained by looking directly into the proteins within a diseased cell or tissue. Genomics has provided a vast amount of information linking gene activity with disease, but it does not predict PTM that most proteins undergo. Therefore, DNA sequence analysis does not predict the active form of a protein and RNA quantitation does not always reflect the corresponding protein levels. It is believed that through genomics and proteomics, new disease markers and drug targets can be identified, which will ultimately help design products to prevent, diagnose, and treat diseases.

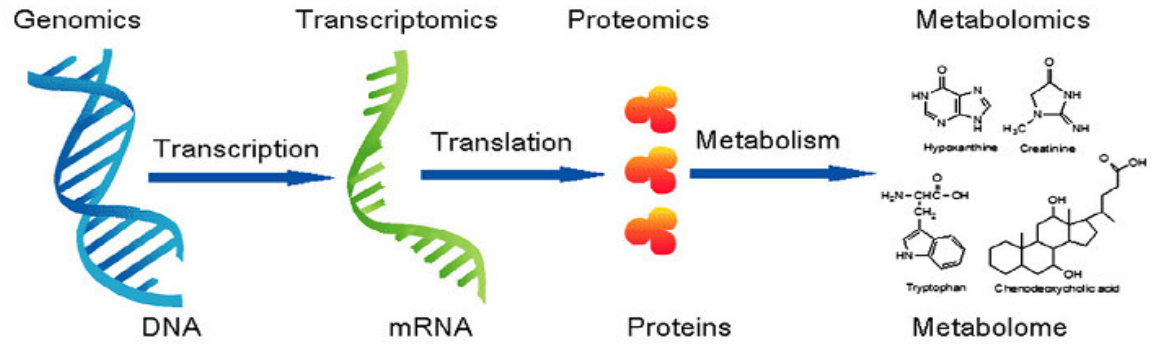

Fig1. Schematic representation of omics technologies

2.1. TYPES OF PROTEOMICS

Based on the protein response under stress conditions proteomics are classified into different groups.

2. 1.1. Expression proteomics

Expression proteomics is used to study the qualitative and quantitative expression of total proteins under two different conditions. Like the normal cell and treated or diseased cell can be compared to understand the protein that is responsible for the stress or diseased state or the protein that is expressed due to disease of a given environments.2-D gel electrophoresis, mass spectrometry technique were used to observed the protein expressional changes, which is present and absent in tumour tissue, when compared with normal tissue. Which are over expressed and under expressed can be identified and characterized protein activities multi-protein complexes, and signalling pathways (Hinsby *et al*., 2003). Identification of these proteins will give valuable information about molecular biology of tumour formation and disease-specific manner for use as diagnostic markers or therapeutic targets.

2.1. 2. Structural proteomics

Structural proteomics helps to understand three dimensional shape and structural complexities of functional proteins. Structural proteomics can give detailed information about the structure and function of protein complexes present in a specific cellular organelle. . Different technologies such as X-ray crystallography and NMR spectroscopy were mainly used for structure determination (Junjie *et al*., 2015).

2.1. 3. Functional proteomics

Functional proteomics explains understanding the protein functions as well as unrevealing molecular mechanisms within the cell then depend on the identification of the interacting protein partners. The association of an unknown protein with partners belonging to a specific protein complex involved in a particular mechanism would in fact, be strongly suggestive of its biological function. Furthermore detailed description of the cellular signalling pathways might greatly benefit from the elucidation of protein- protein interactions (Kamal *et al*., 2015).

2. 2. Proteomics Technologies

The complete characterization of a proteome is a formidable task and the degree of success achieved depends on the methods available and their amenability to automation and high throughput formats (Junjie *et al*., 2015).Parameters such as the complexity of the protein mixture, levels of expression and medication and intracellular localization all impact the choice of proteomics technology to be used (Holman *et al*., 2013). In proteomic analysis both analytical and bioinformatics tools were used to characterize protein structure and functions. Analytical techniques 2-D gel electrophoresis, MALDI-TOF-MS was used. In case of bio-informatics numbers of software tools were also used.

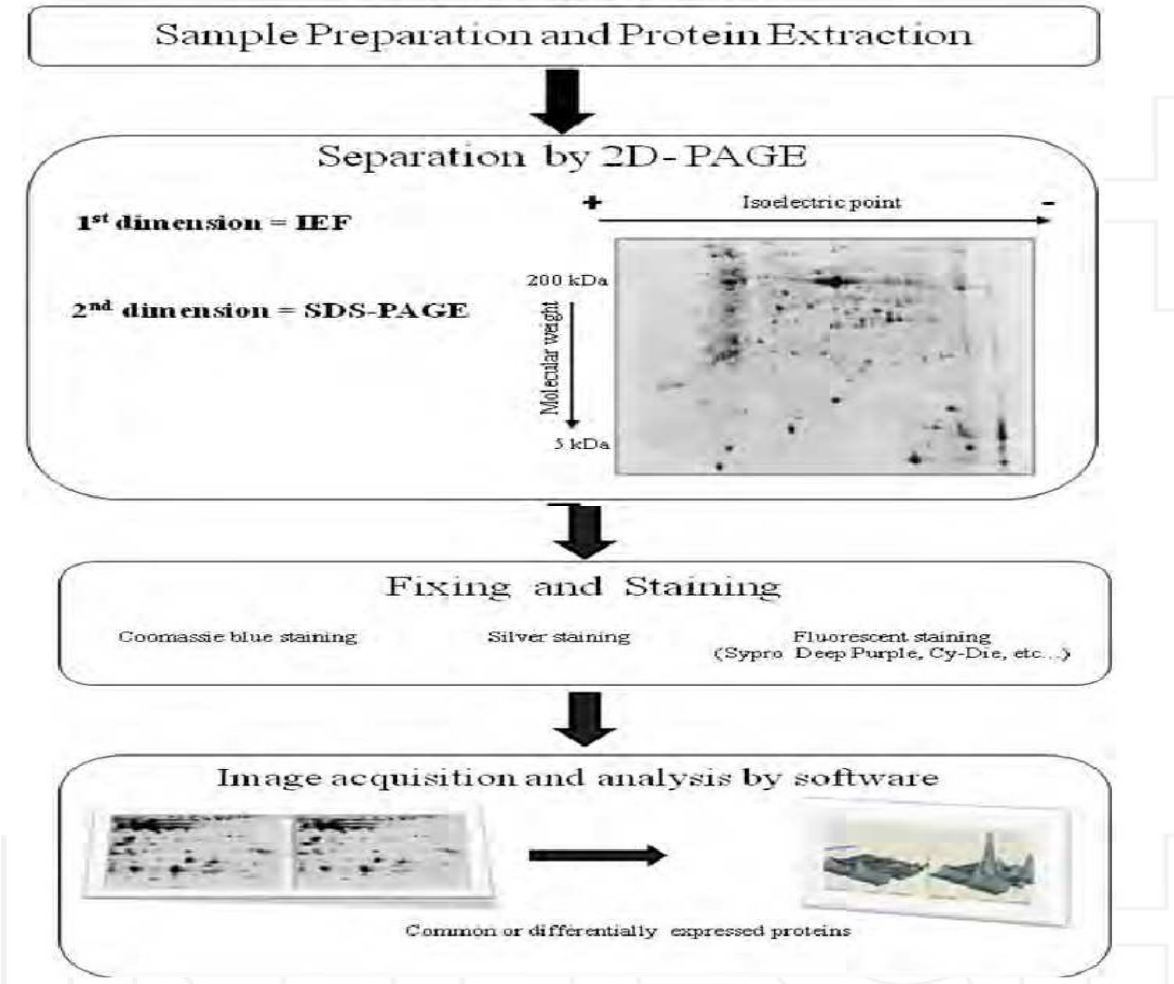

Fig 2. Work flow schemes in proteomics

2.2.1. Two-dimensional polyacrylamide gel electrophoresis

Two Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE) is used to separate proteins from a mixture providing information such as: molecular weight, isoelectric point, presence or absence of proteins in a sample and PTMs (Lodha, *et al*., 2013). It can achieve the separation of several thousand different proteins in one gel. High-resolution 2-D PAGE can resolve up to 10,000 protein spots per gel. Stains such as Coomassie blue, silver, SYPRO Ruby and Deep Purple can be employed to visualize the proteins (Wu *et al.,* 2014b).The proteins are separated by charge (isoelectric point) in the first dimension and by mass in the second dimension. In isoelectric focusing, the proteins migrate in a pH gradient to the pH at which they have no net charge. The most common proteins are separated by isoelectric point in the horizontal direction and by size in the vertical direction and this map of protein spots can be considered as the "protein finger print" of that sample.

Typically, 2DE is used in expression proteomics studies where the focus is on studying alterations in protein expression profiles due to the appearance of a new protein spot, or the disappearance of a protein spot, or changes in the intensity of an existing protein spot. While the resolution of complex protein mixtures obtained with 2DE is far superior to that with conventional one-dimensional protein electrophoresis. Nowadays, 2-D PAGE analysis is often coupled with the MS technology and it has provided with higher resolution, improved reproducibility, and higher loading capacity for preparative purposes with the intent of subsequent spot identifications (Kim *et al*., 2013).

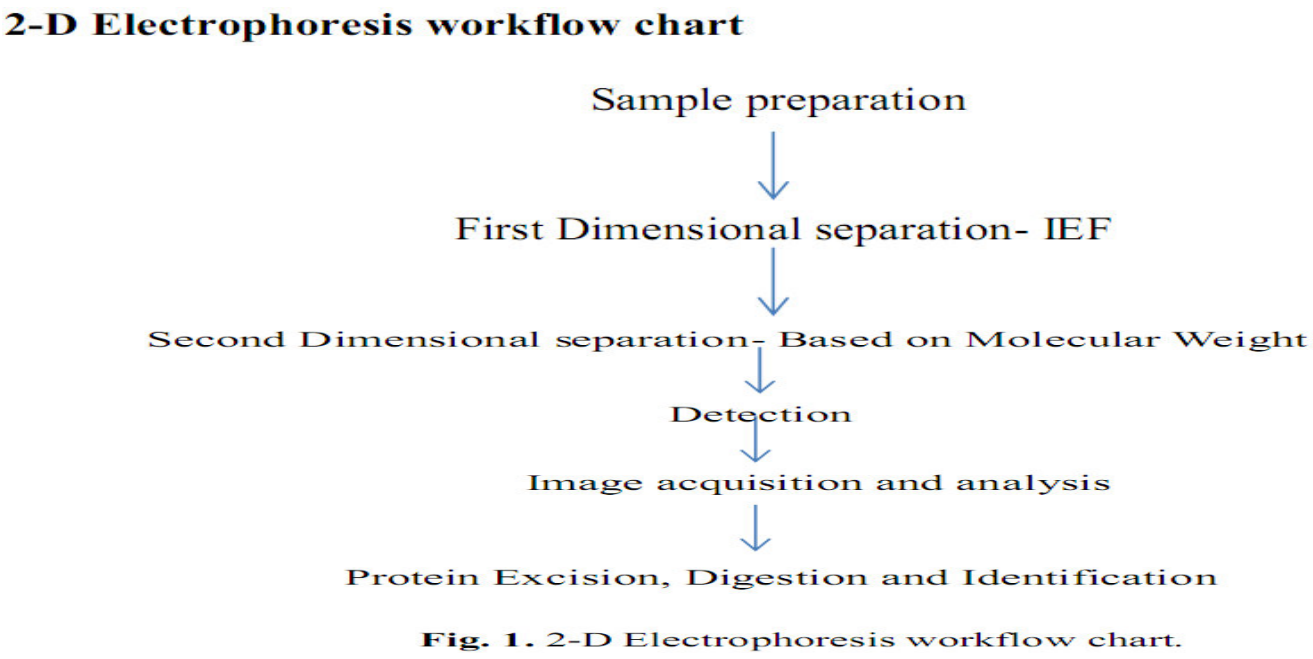

Fig. 1. 2-D Electrophoresis workflow chart.

2. 2.2. Mass Spectrometry for Protein Characterization

A relatively new and rapidly evolving development in proteomics research has been the application of mass spectrometry (MS) which, in conjunction with the development of comprehensive protein databases (Kamal *et al*.,2015) and advances in computational methods (Lahm and Langen,2000), is being used for high-throughput characterization and identification of proteins.MS can be used to determine the molecular weight as well as the amino acid composition of proteins at low concentrations (attomole to femtomoles and it is also easily adaptable to high-throughput formats, which has made it the method of choice for protein identification and characterization (Wu *et al*., 2014b).

Mass spectrometry is an analytical technique that produces spectra of the masses of the atoms or molecules comprising a sample of material. The spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules, such as peptides and other chemical compounds. It works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass to charge ratios (Lodha *et al.,* 2013).

Frequently used ionization methods include electro spray ionization (ESI), matrix-assisted laser desorption/ionization (MALDI), and surface-enhanced laser desorption/ionization (SELDI). Matrix-assisted laser desorption-ionization (MALDI) and electro spray ionization (ESI) are two technologies that are commonly used for protein ionization (Yang, *et al.,* 2015).

2.2.3. MALDI-TOF-MS

The MALDI process has as its energy source the laser pulse, as opposed to the electrostatic potential in ESI, to ionize peptides. Protein sample have been characterized by SDS PAGE by generating peptide maps. These peptide maps have been used as fingerprints of protein or as a tool to know the purity of a known protein in a known sample. Mass spectrometry gives a peptide map when proteins are digested with proteolytic enzymes like trypsin. This peptide map can be used to search a sequence database to find a good match from the existing database (Jacoby *et al*., 2013b).

Time-of-flight (TOF) and quadrupole mass analyzers have been developed for use in mass spectrometers (Wu *et al.,* 2014b), and of these, TOF analyzers are more common because of their ease of operation. TOFs are commonly used with a MALDI ion source, whereas quadrupole analyzers are combined with an ESI source. The accurate determination of protein molecular weights is mainly achieved using a MALDI-TOF instrument. These peptide mass finger prints are compared with a Data base of virtual peptide mass finger prints generated by the theoretical digestion of known proteins by specific proteases (Kamal *et al*., 2015).

2.2.4. Electro spray ionization

This technology involves the production of gaseous ions by application of a potential to a flowing liquid, resulting in the formation of a spray of small droplets with solvent-containing analyte. Solvent is removed from the droplet by heat or another form of energy such as collision with a gas, and multiply charged ions are formed. Finally, electrostatic repulsion is sufficiently high to cause desorption of the analyte ions, which are then passed to the mass spectrometer. In ESI mass spectrometry the protein sample is in solution, and a potential is applied to create a fine mist of charged droplets that are subsequently dried and introduced into the mass analyzer (Lahm and Langen,

2000**)**. In contrast to MALDI, ESI produces highly charged ions without fragmentation of the ions in the gas phase **(**Mann *et al*., 2001**)**.

2.2.5. Chromatographic techniques

Chromatography comprises different group of methods which are utilized for the separation of closely related components of mixtures. Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (the stationary phase), while the other (the mobile phase). The phases are chosen such that components of the sample have differing affinities for each phase. The most commonly employed separation technique for Bio analysis is high performance liquid chromatography (HPLC), also known simply as LC.

There are increasing applications in proteomics research due to its ability to analyze large, fragile bio molecules. With advancements in ionization methods and instrumentation, LC combined with MS has become a powerful technology for the characterization and identification of peptides and proteins in a complex mixture. This technology shows advantages over gel-based techniques in terms of speed, sensitivity, scope of analysis and dynamic range, since it can incorporate a wide choice of detection methods (Chandana *et al*., 2013).

2.2.6. Protein Microarrays

Protein chips, protein biochips or protein microarrays are micro proteomic technologies for studying protein interaction and function. This technology uses only a very small amount of crude sample (e.g. cell lysates, urine or serum) from the patient (Geho *et al*., 2010).Protein chips are viewed as the most promising tools for proteome-wide analysis. This technology enables thousands of proteins to be analysed (a more rapid profiling approach compared with 2DGE) and it enables screening for specific types of post-translational modification.

Based on the comparisons of protein mass profiles from any two samples from different biological and pathological conditions, potential bio markers or disease-related protein targets could be identified. It is used for rapid profiling chip array system for a variety of studies, each exploiting the key strengths and advantages of this technology:(i) it being amendable to analyse small and usually limited quantities of biological samples (0.5–500 mol); (ii) the ability to detect and evaluate proteins without the need for tagging, labelling or processing; (iii) the sensitivity of the system with the ability to detect fmol concentrations of proteins; and (iv) the rapidity in obtaining results, thus making it amendable for analysis even in large epidemiological settings.

2.2.7. Bioinformatics in proteomics

MS generally generates a large amount of numerical data and bioinformatics tools therefore are essential to match these MS data to protein, EST, and genome sequence databases. Thus, the role of bioinformatics is fundamental in order to reduce the analysis time and to provide statistically significant results. To process data efficiently, new software packages and algorithms are continuously being developed to improve protein identification, characterization and quantification in terms of high-throughput and statistical accuracy. Most search engines have been developed in academic laboratories and some of those have now been commercialized. Examples of useful Web sites and their search engines are www. proteometrics.com,http://prospector.ucsf.edu/,http://195.41.108.38/PepSeaIntro.html, www.mann.embl-heidelberg.de/Services/PeptideSearch/Pep-tideSearchIntro.html,and www.matrix-science.com/;for a complete listing.

2.3. Proteomic Techniques Offer New Tools for Plant Biotechnology

The knowledge of key proteins that play crucial roles in the proper growth and development of a plant are critical to propel the biotechnological improvement of crop plants (Hossain and Komatsu, 2013). These proteins maintain cellular homeostasis under a given environment by controlling physiological and biochemical pathways. A search of the published research literature revealed that genomics and proteomics are the two major wheels that keep the discovery of novel genes rolling, which can eventually be placed into the pipeline for crop improvement programs. To increase crop productivity, genes and proteins that are responsible for stress tolerance and disease resistance have to be identified continuously. Advancements in MS-based proteomics platforms have been considered to be "New Genomics" because MS has become an indispensable tool for the investigation of the PTMs to proteins, and protein interactions. A specific advantage of proteomics over other "Omics" techniques is the capacity to reveal post-translational modifications (PTMs), which is a prerequisite to determine the functional impact of protein modification on crop plant productivity. Finally, crop proteomics is expected to become an essential part of integrated "Omics" approaches. Furthermore, the development of various advanced tools for bioinformatics and computational science are connecting proteomics to other "-omics," and the physiological data are further opening up new methods for crop improvement studies via the signalling, regulatory, and metabolic networks underlying plant phenotypes (Froehlich et al., 2013).

2.3.1. Two-Dimensional Maps of Different Plant Tissues

Jacoby *et al.* (2013) published several articles in which an attempt was made to map proteomes of different plant tissues from rice and Arabidopsis. The proteins of the different tissues were separated by 2-DE. In line with this consensus, several plant proteomics studies have been published recently that have focused on specific sub cellular

proteomes or protein complexes such as the plasma membrane, roots, mitochondria, and chloroplasts. In addition, a few interesting studies appeared concerning the symbiosis between roots of legumes and nitrogen fixing bacteria (Jacoby *et al*., 2013).

2.3.1.1. Organ Specific Proteome Analysis of a Biotic Stress Response in Crop Plants

2.3.1.1.1. Proteomics of Leaf Photosynthesis and Senescence to Understand Crop Productivity

Leaf photosynthesis is the main source of plant biomass influencing potential crop yield. Recently, Chu *et al.* (2015) analyzed changes in protein profiles upon the development of chlorophyll deficiency in Brassica napus leaves and provided new insights into the regulation of chlorophyll biosynthesis and photosynthesis in crops. Several studies have focused on the proteomics of leaf senescence, mainly on the investigation of nitrogen mobilization from leaves during leaf senescence (Avice and Etienne, 2014). Chloroplast contains up to 75% of leaf nitrogen in the form of Rubisco enzyme components in the stroma and complex of photo system II in the thylakoid membrane (Roberts *et al*., 2012). Advances in organelle proteomic studies integrated with large scale genomic approaches and determination of enzymes with proteolytic activity have addressed the complexity of chloroplast proteolytic machinery during leaf senescence and investigated different classes of senescence-associated proteases with unique physiological roles according to their expression profiles along the senescence progress (Roberts *et al*., 2012). Glycolytic enzymes involved in sucrose synthesis are of particular interest with respect to crop yield, and have been identified by sub cellular proteomic studies of senescence, photosynthesis, and stress-responding processes in rice leaves (Zhang *et al.,* 2011a).

2.3.1.1.2. Xylem and Phloem Proteomics of Root-to-Leaf Signalling Pathways During Stress

Maximizing crop yields also depends on the leaves receiving an optimal supply of nutrients from the root system via the xylem vessels.). Xylem proteomic and secretomic studies have recently become one of the major areas of interest in understanding plant development and responses to environmental perturbations, and illustrated several types of xylem sap containing proteins that participate in cell wall development and repair process (Zhang *et al*., 2014a), leaf senescence (Wang *et al*., 2012), a biotic stress responses (Alvarez et al., 2008), biotic stress defense mechanisms (Gonzalez *et al*., 2012), and intercellular and intracellular communication (Agrawal *et al*., 2010). Additional studies of protein and metabolite composition of xylem sap and apoplast in soybean (Glycine max) provide further investigation of expression profiles and signaling roles of corresponding proteomes, and ultimately reveal more root contributions to pathogenic and symbiotic microbe interactions, and root-to-shoot communication (Krishnan *et al.,*2011). Other proteomic studies were predominantly focused on the analysis of phloem sap exudates from agriculturally important plants oilseed rape (Froehlich *et al*., 2012), identifying several hundred physiologically relevant proteins and ribo nucleoprotein complexes. The phloem sap proteomes showed enhanced presence of proteins involved in redox regulation, defense and stress responses, calcium regulation, RNA metabolism and G-protein signalling. Some of the important insights into the operation of the sieve tube system were revealed through proteomics studies.

2.3.1.1.3. Root Proteomics of Symbiotic Systems to Improve Legume Productivity

The symbiosis between N-fixing bacteria and legumes results in formation of root nodules and is very important in agriculture. A number of proteomic studies have attempted to investigate mutual impacts between symbiotic or pathogenic bacteria and the root of host plant in the rhizosphere under a multitude of biotic and a biotic stresses from the soil (Knief et al., 2011). Differential plant and bacteroid responses to drought stress have been revealed by proteomic analysis two different groups reported protein expression studies in nitrogen fixing root nodules of soybean and white sweet clover, respectively (Reid *et al.,* 2012). 2DE was also used to identify differentially expressed proteins during the symbiotic interaction between the bacterium Sinorhizobium meliloti strain 1021 and white sweet clover. Over 250 proteins were induced or up-regulated in the nodule, compared with the root, and over 350 proteins were down regulated in the bacteroid form of the rhizobia, compared with cultured cells. Bacteroid cells showed down-regulation of several proteins involved in nitrogen acquisition, indicating that the bacteroid were nitrogen proficient. Both studies are excellent examples of the potential of proteomics in plant symbiotic interactions (Molesini et al., 2013).

2.3.1.1.4. Progress in Crop Proteomics for Stress Responses

Stressful conditions often lead to delayed seed germination, reduced plant growth, and decreased crop yield. Komatsu and Hossain (2013) highlighted the need for organ-specific proteomic analyses to identify proteins that are commonly accumulated in organs under a wide range of a biotic stresses (Komatsu and Hossain, 2013). Jacoby *et al*. (2013) described the application of the emerging proteomic technology of multiplexed selective-reaction monitoring MS, which has increased accuracy and throughput, for enhancing these approaches and providing a clear method to rank the relative importance of the growing cohort of stress-responsive proteins. In addition to crops, proteomic techniques have been applied to the study of some plants that serve as model systems in plant science and several agriculturally important fruits (Chan, 2013) under a biotic and biotic stresses.

Takahashi *et al.* (2013) examined responses to freezing stress, which causes serious problems for agricultural management, and found that the plasma membrane plays significant roles in signal perception and cellular homeostasis, indicating that plasma membrane proteins are the most important factors in determining the

environmental stress tolerance of plants. Salt stress severely decreases crop production and growth; however, certain crop cultivars show significant tolerance against the negative effects of salinity. Many salt-responsive proteins have been detected in major crops and are thought to increase resistance to salt stress. Hossain and Komatsu (2013) described the recent contributions of proteomic studies toward the understanding of heavy metal stress responses in plants, particularly the use of redox proteomic approaches for studying heavy metal-induced protein oxidation

2.3.1.1.5. Post-translational modification

Proteomics has the significant advantage of being able to discern not only changes in expression levels but also in PTMs. Analysis of a protein for PTM such as phosphorlylation and glycosylation are very important for understanding issues such as activity, stability, and turnover. 2-DE and MS is especially useful for the analysis of expressed proteins with PTM, such as alkylation, glycosylation, and phosphorlylation, which may be the most important regulatory proteins in a biological cell. Protein ubiquitination is a key regulatory mechanism that controls protein abundance, localization and activity. Several large-scale analyses of protein ubiquitination in plants have been reported (Mitsui *et al*., 2013). For example, in Arabidopsis, affinity purification using an anti-ubiquity antibody and the subsequent use of MS/MS analysis has been performed to identify ubiquitinated proteins (Mitsui *et al*., 2013).

2.3.1.1.6. Analyses of Food Quality, Safety and Nutritional Values

The field of proteomics has been used to analyze the differences between the nutritional values of food crops through the analysis of their proteomes. Mitsui *et al.* (2013) reported that heat stress increased the expression of invertases in tomato fruits, thus increasing their sucrose content and producing sweeter tomatoes. Proteomics have investigated the reason that heat treatment for peach fruits will improve the peach fruit quality and shelf-life, and the reason was the differentially expressed proteins that were involved in fruit development and ripening (Zhang *et al.,*2011a). A combination of 2-DE and IgE reactive proteins using an allergic patient's sera has been applied as an approach to characterize the allergencity of food proteins (Chan, 2013). Proteomic analysis of rice leaf, root, and seed showed the presence of many allergenic proteins in the seeds, which implicate the uses of proteomic analysis of foods for the presence of allergens (Aghaei and Komatsu, 2013).

2.4. Challenges in Utilization of Proteomics Studies

Certain disadvantages are limiting the use of proteomics. Proteins are dynamic and interacting molecules, and their changeability can make proteomic snapshots difficult. There is the need for a more sensitive analytical system and the absence of an effective method for large-scale data comparison (Petricoin, 2011). Unlike DNA sequencing, protein sequencing is a relatively costly and laborious process. The other challenge is that there are a few protein sequences available and if available they are either too short or highly conserved thus difficult to study variation (Seung *et al*., 2006). Within the proteome, the many observed layers of complexity begin with an RNA processing mechanism called alternative splicing in which a single gene can produce multiple versions of a protein (Roberts *et al*., 2012). Post translational modifications are also another source of protein variation.

3. Summary and Conclusion

Proteomics has emerged as an indispensable methodology and will remain to be one of the fastest growing areas in research for large scale protein analysis in functional genomics. Proteomics is a useful and powerful tool for investigating protein changes induced by various conditions. The global scale analysis of proteins is expected to yield more direct understanding of function and regulation than analysis of genes. Gel-based 2-DE proteomic approaches combined with gel-free MS-based quantitative proteomic techniques have been widely used for crop proteome analysis. The complex mixtures of proteins with the dynamic range of protein concentrations in plant cells have been analyzed more in-depth using a combination of separation techniques based on sub cellular proteomics in different stress responding organs and tissues. The recent proteomic studies have contributed to elucidation of complex relationship between stress tolerance and crop productivity, which would enable the development of novel breeding strategies resulting in an increase in crop productivity and environmental performance.

4. Future line of work

The present technological achievements are well suited for high throughput screening of proteomic states. Yet, automation of the various steps of proteomic procedures, e.g. cell disruption, 2DE-separation, peptide generation, MS identification and data interpretation are far beyond reality. However, future developments may enable faster and more sensitive proteomics studies especially on chips or microarray techniques. To meet the current challenges of food insecurity, genes and proteins that control crop architecture and/or stress resistance in a wide range of environments will need to be identified to facilitate the biological improvement of crop productivity; however, proteomics alone cannot provide all the information required for understanding cellular processes. Therefore, complementary approaches in genomics, metabolomics and bioinformatics will have to be used together with

proteomics to permits a more holistic view of biological systems and their alterations in disease, so that the maximum benefit can be realized. As a result, systems biology approaches will continue to detect connections between broad cellular functions and pathways of biochemical and genetic analysis of the biological system in questions.

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