Proteomic Studies: Contribution to Understanding Plant Salinity Stress Response

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Abstract: Salinity stress significantly abridged the productivity of global crops. Developing and improving the salinity stress-tolerant species is urgent to continue the food supply in the coming decades; otherwise many individuals might die due to hunger or food insecurity. The genome of plants under saline conditions represents physiological alterations; however, it does not represent the change of protein level reflected by corresponding gene expression at the transcriptome level. While proteins are more reliable determinant since they are directly involved in shaping salinity stress-adapted novel phenotype of physiological traits. Moreover, protein profiles display greater changes then the transcript levels. Therefore, exploring the protein complement of the genome would be naturalistic to elucidate the mechanism of salt tolerance in plants. In this review, an attempt is made to present the role and implementation of proteomic studies in response to plant salinity stress and its significant contributions so far made for better understanding the complex mechanism of the plant under salinity stress. Moreover, brief characteristics of plants in saline conditions and the limitation of proteomic studies are further discussed.

Keywords: Abiotic stress, Genome, Plant proteomics, Proteome, Salinity, Tools.

1. INTRODUCTION

Producing more plants, especially crops and foods for the growing world population is a necessity to escape from foodless days in the upcoming decades for many individuals, especially in developing countries. However, the major abiotic stress factors, predominantly salinity stress lead to the major reductions of the yield of crops. About 1.125×10^7square kilometres of the world's land area is salt-affected (approximately 8.5%), and if the forest, wetlands, unsuitable, high biodiversity land area excluded the total salt-affected area reduced to 9.71×10^6 square kilometres. Among the salt-affected land, 5% highly, 10% extremely, 20% moderately and 65% slightly salt-affected lands [1, 2].

There are no continents left from the adverse effect of soil salinity and the areas salt-affected are distributed into 17 regions of the world's land area namely (descending order based on the total salt-affected land) the Middle East, Oceania, North America, Former USSR, East Asia, South America, West Africa, the USA, East Africa, South Asia, South Africa, Canada, Southeast Asia, Central America, East Europe, West Europe and Japan [1]. Moreover, due to high salinity stress, approximately 20% of irrigated land is salt-affected (4.5×10^5 out of 2.27×10^6 square kilometres) [3, 4]; yearly 1.5×10^4square kilometres of lands is becoming useless for agricultural production, and for the continuation of such increased soil salinization of arable land would have devastating worldwide effects and resulted up to 50% land loss by the year 2050 [1, 5].

Since the salinity stress severely affected the yield of crops, it has a high local impact on economic loss. An estimation by Ghassemi and his colleagues mentioned that the global income loss was about $US 11400 million and $US 1200 million every year in irrigated and non-irrigated lands, respectively because of salinity stress [6]. In fact, the salinity stress threatens the sustainability of the agricultural industry. Soil can be salinized by both natural and human factors. Primarily, natural factors including weathering of parent material, deposition of sea salt carried in wind and rain and inundation of coastal land by tidal water are the major causes of soil salinity. While human factors induced secondary soil salinization that includes the rise of the water table due to excessive irrigation using underground water, irrigation with salt-containing water and poor drainage [1].

A soil condition characterized by a high concentration of soluble salt that significantly reduces the yield of most crops is called salinity. If the electric conductivity of saturation extract (ECe) of soils is 4 dSm^{-1} or more at 25°C, the soils are considered as saline soils. Approximately 40 mM NaCl that generates approximately 0.2 MPa osmotic pressure is equivalent

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to ECe of 4 dSm^{-1} [7]. Most of the crops are saline sensitive. Some of the crops and forages are moderately tolerance or moderately sensitive and very few are tolerant species, such as (threshold ECe of dSm^{-1}) *Triticum turgidum* (5.9), *T. aestivum* (8.6), *Beta vulgare* (7.0), *Hordeum vulgare* (8.0), *Secale cereal* (11.4), *Gossypium hirsutum* (7.7), *Cyamopsistetragonoloba* (8.8) [8]. Salinity stress can cause both hyper-ionic and hyperosmotic stress which can lead to plant demise. The variation of responses of crops to salinity stress greatly differs because of their strategies either to stress escape or to stress resistance characteristics [9]. Interestingly, halophytes are well-adapted and thrive under high salinity by using two strategies, salt tolerance, and salt avoidance. Generally, halophytes follow three mechanisms of salt tolerance; reduction of the Na^+ influx, compartmentalization, and excretion of Na^+ [10]. Therefore, understanding the mechanisms of plant salinity stress is crucial in order to develop salinity stress-tolerant species to meet the food supply for the increasing population.

Understanding the responses of plants to salinity stress is arduous. Although the gene expression of plants under saline conditions can delineate the responses of plants, the alterations of gene expression at the transcript level do not reflect the changes at the protein level [11, 12]. While proteins are more reliable determinant since they are directly involved in shaping novel phenotype by adjustment of physiological traits to the altered environment. Moreover, protein profiles display greater changes than the transcript levels. Both structural proteins and functional proteins involved in the regulation of plant epigenome, transcriptome, and metabolome directly response to both plant developmental and health stage as well as ambient environment to shape phenotype of plants. Additionally, protein function is not dependent only on its molecular structure, but also on its cellular localization, post-translational modifications and interacting partners [13-16]. Therefore, exploring the protein complement of the genome would be naturalistic to elucidate the mechanism of salt tolerance in plants.

Proteomic studies revealed the whole proteome of an organism and potential protein markers whose changes in abundance determine the quantitative changes in physiological traits that can be used for a description of genotype's level of stress tolerance. In this review, studies dealing with plant proteome changes in response to salinity stress are focused to describe the role of proteomics to the understanding mechanism of plant salinity stress. Additionally, the limitation of proteomic studies further discussed.

2. BRIEF MAJOR CHARACTERISTICS OF PLANT SALINITY STRESS

Elevated levels of salinity deteriorate soil structure and impede desirable air-water balance that is essential for normal biological processes of the plants. Salinity stress severely affects morphological and agronomical traits involving plant growth, development and productivity that include leaf colour and area, number of leaves, plant height, root length and shape, fresh and dry mass, and moisture contents [17, 18], number of flowers, number of branches and head diameter, and peduncle length of plants [19]. Dehydration, necrosis, wilting, chlorosis, and abscission of leaves as well as reduced plant height, root length, leave length and width were observed in the higher concentration of NaCl [17]. Salinity stress also elicited the production of metabolites [20].

Salinity stress contributes a high concentration of salt ions-mainly Na^+ and Cl^-, as well as some other salt ions, such as Ca^{2+}, K^+, CO_3^{2-}, NO_3^-, SO_4^{2-}— in soil-water solution, resulting decreased soil-water potential reveals. Hence, a decrease in water uptake by roots due to the osmotic pressure induced by high salinity on plant cells. Habitually, the concentration of some ions, particularly Na^+ is actively maintained at the low level in the plant cell cytoplasm through ATP-dependent ion pumps, such as Na+/H+-ATPases, V-ATPases, and inorganic pyrophosphatases (iPPases). In contrast, at the high concentration of salt ions induced osmotic stress and a disbalance in intracellular ion homeostasis that lead to an accumulation of several osmolytes, for example, raffinose-derived oligosaccharides, glycine betaine, proline as well as high molecular hydrophilic proteins from late embryogenesis-abundant (LEA) super family in the osmotic adjustment of cell cytoplasm. The osmotic adjustment also leads to enhanced sequestration of inorganic ions in the central vacuole. Furthermore, the osmotic effect is rapid, usually first few hours after introducing to the stress environment. It is common to all type of dehydration stresses, while the severity of the ionic effect is increased with time and specific to salinity stress (for review, see [4, 21-23]).

3. TOOLS AND TECHNOLOGIES OF PROTEOMIC STUDIES

In general, there are two approaches performed in the proteomic analysis of samples. The first one is the most commonly used method (1) gel-based proteomic analysis and (2) the second one is the comparatively less common method- gel-free analysis in the field of plant science. The overall workflow of proteomic

analysis is shown in Figure **1**. Gel-based analysis solely depends on the two-dimensional polyacrylamide gel electrophoresis (2-DE). Nowadays differential in-gel electrophoresis (DIGE) is used to avoid the problems associated with 2-DE. Gel-free technologies include multidimensional protein identification technology (MudPIT) for peptide separation, isotope-coded affinity tagging, isobaric tagging for relative and absolute quantification, stable isotope labelling of amino acids in cell culture, isotope-coded protein labelling for peptide quantification, and label-free methods (peak integration or spectral counting) [24].

4. PROTEOMIC STUDIES IN RESPONSE TO PLANT SALT STRESS

At the first Siena meeting in 1994, Marc Wilkins coined the term *'proteome'* for the first time, a scientific buzzword that is now associated with 133,606 publications in the field of proteome/proteomics as currently listed at the National Center for Biotechnology Information (NCBI) website, of which 15,642 publications are associated with proteome/proteomics with stress studies, and only 543 publications represent the proteome/proteomics studies associated with plant salinity stress [28]. It refers to a large-scale comprehensive study of a specific proteome (the

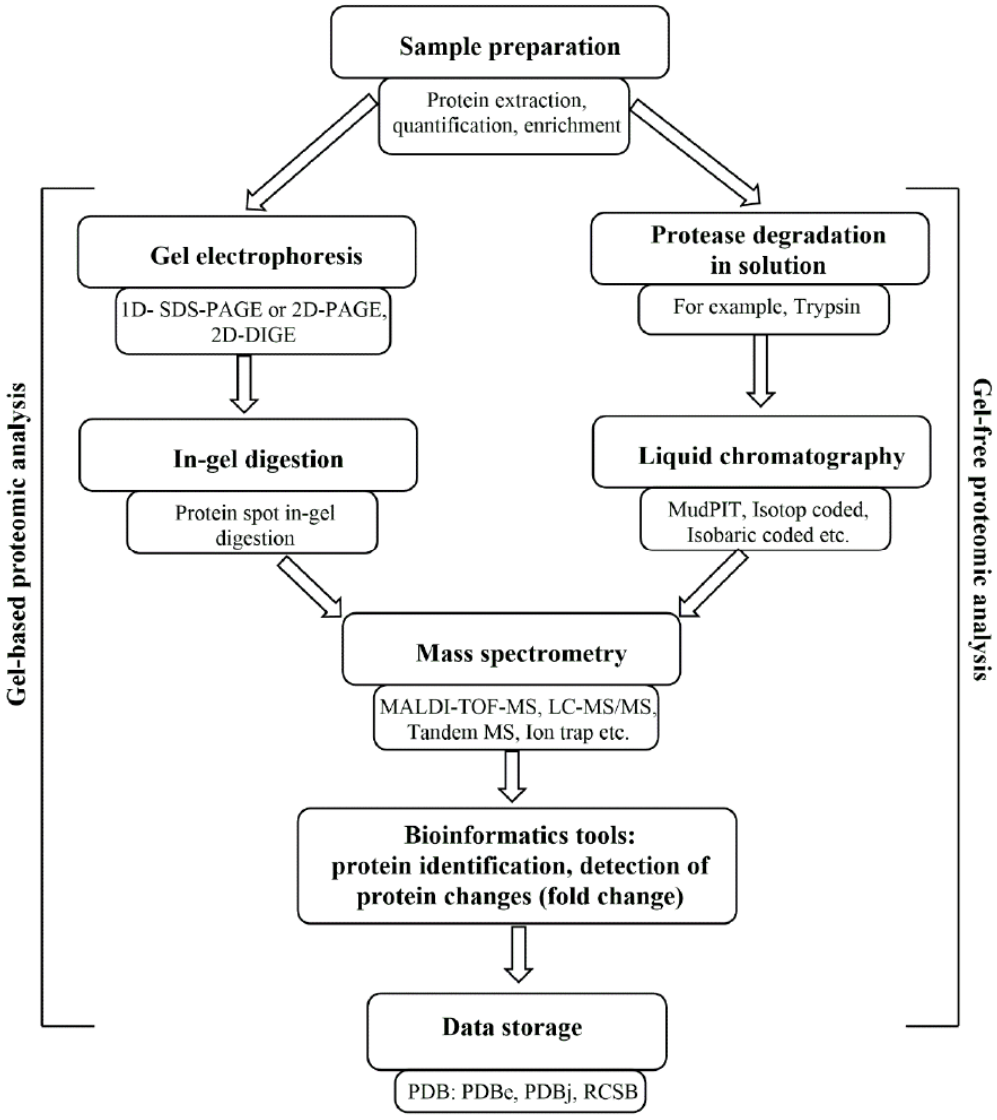

Figure 1: The overall workflow of proteomic analysis. SDS-PAGE: Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis; 2D-PAGE: Two Dimensional Gel Electrophoresis; DIGE: Differential Gel Electrophoresis; MALDI-TOF-MS: Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight-Mass spectrometry; LC-MS/MS: Liquid Chromatography with tandem mass spectrometry; MudPIT: multi-dimensional protein identification technology; PDB: Protein Data Bank; PDBe: Protein Data Bank in Europe; PDBj: Protein Data Bank in Japan; RCSB: Research Collaboratory for Structural Bioinformatics. The information adapted from [25-27].

protein complements of the genome) to understand the cellular processes [29]. The systematic analysis of proteome includes information on abundances of protein, variations, and modifications of proteins as well as interacting partners and networks of proteins [30].

Plant proteome is differentially expressed upon exposure to salinity stress. Proteomics has a broad spectrum use in protein profiling study of plants under stress conditions. It has direct contribution to find genes and proteins involved in plant salinity stress response and tolerance acquisition process [16, 26, 31]. Genes encoding proteins for osmolyte synthesis, ion channels, receptors, and salt-responsive signalling factors or enzymes to salt-sensitive plants can confer salinity-tolerant phenotypes [32]. Therefore, identification and characterization of salt-responsive proteins through the high-throughput proteomics studies are the fundamental roots to develop salt-tolerant plant varieties.

In the field of plant abiotic stress research, comparative analysis of differential expression of proteomes between control (non-stressed) plants and stressed plants is the most common study. To a lesser extent, the comparison of proteomes isolated from two different genotypes or plant species with contrasting levels of salt stress is also studied. The sets of proteomes are distinguished focusing on the both protein quality and quantity by differential-expression of proteomics analysis, which is aimed at the protein identification and relative quantitation [26]. Several studies related to the comparative analysis of proteome between plants subjected to salt stress and control treatments have been studied in economic crops: rice [33-37], wheat [38-42], barley [43-48], tomato [49-51], soybean [52-55], and model plant *Arabidopsis thaliana* [56-58] and medicinal plants: *Andrographis paniculata* [59, 60], *Brassica napus* [61], *Bruguiera gymnorhiza* [62, 63], *Cucumis sativus* [64], *Kandelia candel* [31, 65, 66], *Suaeda aegyptiaca* [67], *Suaeda salsa* [68] and *Vitis vinifera* [69]. The proteomics approach has long been dominated by the techniques of two-dimensional gel electrophoresis (2DGE) followed by protein identification using mass spectrometry (MS) analysis such as matrix-assisted laser desorption ionization-time-of-flight/time-of-flight mass spectrometry (MALDI-TOF/TOF-MS).

5. ROLE OF PROTEOMICS IN ENHANCING BIOLOGICAL DISCOVERIES

Proteomic technologies are becoming widespread in their use in various fields of biological sciences including identification of stress-responsive proteins to develop the stress-tolerant plant, the discovery of cell-surface markers/biomarkers and drug development [70, 71]. The goal of proteomics is to provide complementary and critical information by revealing the regulation, quantities, activities and interaction of proteins that are present in complex biological systems— whole organism, specific tissue or cellular compartment— under certain conditions in the given time [72].

In recent years, proteomics is active in plant genomics area, where proteomic tools are used to identify proteins derived from mixtures extracted from tissues/cells in response to developmental and certain environmental conditions and to quantify expression levels of those identified proteins [25]. Using these techniques, researchers can simultaneously display and determine thousands of proteins per experiment. Moreover, it is possible to detect and estimate reliably the relative expressions of those differentially expressed proteins in different conditions and measure absolute proteins expression of single proteins in a complex mixture [73].

Proteins as the cellular building blocks directly assert the potential function of genes via enzymatic catalysis, molecular signalling, and physical interactions. However, structure, functions, abundance, and the number of proteins in an organism cannot be predicted although mRNA levels accurately measured using DNA chip array [74, 75]. mRNA is not always a good reflection of the level of proteins present in the cell. Expression of many proteins may be regulated at the levels of translation and the rates of degradation of mRNA and proteins differ significantly between genes. The change of expressions could be due to protein modifications, proteolysis, subcellular localization, or interaction with other proteins [74]. Additionally, most of the post-translational modifications cannot be predicted from genomic or mRNA data. It is important to know how proteins interact with each other in a cell and how these interactions response to internal and external signals. These notable events in the lifetime of proteins can only be determined through a proteomics approach [74, 76].

Protein profile or mapping of a cell, tissue, organ, or organism is crucial for functional identification of each protein and their metabolic pathways in stress conditions. It is a valuable natural genetic resource, which could be helpful to discover genes and gene products conferring tolerance to various stresses [47].

Some of these proteins might be differentially expressed upon exposure to adverse environmental conditions such as drought, salinity, and temperature with a different time course. Therefore, plant proteomic studies concentrated on profiling the proteins of whole plants or various parts of plants from model plants *Arabidopsis thaliana* and crops [47]. Particularly, this technique allowed finding several up and down-regulated proteins under environmental stresses through mass spectrometry analysis.

6. PLANT PROTEOME RESPONSES TO SALINITY STRESS

Plants under salinity stress alter their gene expressions profoundly to acclimatize themselves in adverse conditions, which changes the composition of plant transcriptome, proteome and metabolome. Several studies confirmed that protein accumulation changed significantly under stress conditions [31, 36, 62, 77]. Effects of salinity stress on the proteome compositions are studied on main crops and few trees including rice [33-37], wheat [38-42], barley [43-48], tomato [49-51], soybean [52-55], and model plant *Arabidopsis thaliana* [56-58] and medicinal plants- *Andrographis paniculata* [59, 60], rapeseed- *Brassica napus* [61], Mangrove trees- *Bruguiera gymnorhiza* [62, 63], cucumber [64], Mangrove tree- *Kandelia candel* [31, 65, 66], *Suaeda aegyptiaca* [67], halophytes-seepweed [68] and grapes [69]. Zhang *et al.* (2012) reported that more than 2171 salt-responsive proteins have been found in shoots, leaves, roots, seedlings, radicles, hypocotyls, grains, gametophytes, and unicells from different plant species.

The specific salt-responsive proteins have been identified with the help of gel electrophoresis along with mass spectrometry as well as bioinformatics tools that are applied in identifying gel spot patterns and physiological states [78, 79]. The recent advances of proteomic techniques have provided a profound perception of stress-responsive proteins and corresponding mechanisms involved in stress response. A cellular defence mechanism is mainly involved in the expression of associated genes and the corresponding proteins. Three major groups of genes, involved in the stress response, are includes [80]: genes- (i) that play role in the signalling cascades and are associated with transcriptional regulation; (ii) that have a role in the protection of membranes and proteins; (iii) that are involved in uptake and transport of ions and water molecules. Without the help of the proteomics, it was almost impossible to determine those proteins involved precisely in stress response.

7. IMPORTANCE OF PROTEOMIC ANALYSIS OF SALT-RESPONSIVE PROTEINS

Proteins, unlike transcripts, are direct effectors of the plant stress response. It covers enzymes catalysing changes in metabolite levels as well as components of transcription and translation machinery [26]. Proteins also play a direct role in the stress-acclimation process by changing their level in the tissue of stressed plants. These changes of protein accumulation under stress are closely interrelated to plant phenotypic response to stress determining plant tolerance to stress [26, 81, 82]. In contrast, changes in gene expressions at transcriptome levels cannot always show exactly the changes at protein levels due to the effects of post-transcriptional regulatory mechanism such as nuclear export and mRNA localization, transcript stability, translational regulation, and protein degradation [83-87]. Therefore, an investigation of plant proteome under stress at the protein level is highly important that can significantly contribute to our understanding of physiological mechanisms, plant phenotypes, and cellular and molecular processes underlying plant stress response and tolerance, including photosynthesis, energy metabolism, ROS scavenging, ion/osmotic homeostasis, signalling transduction, transcription and translational regulation, and cytoskeleton dynamics.

In the proteomic analysis of salinity stress plants, the main purpose is the identification of potential stress proteins that can be used as protein markers. These markers could be used to know the quantitative data of some physiological parameters to the salinity stress conditions [26]. The physiological parameters are used to measure stress tolerance capacity of a plant species [88]. Proteomic studies are also useful for supplying clues about proteins of unknown function. Identification of salt stress-responsive proteins, especially defence proteins and determination of their expression patterns in response to stress are crucial to understanding their role in salt tolerance mechanism. Understanding these protein functions to stress adaptation through proteomics analysis would be the basis for effective strategies to improve the salt tolerance capacity of plants using biotechnological tools [89]. Furthermore, low abundant proteins and novel regulatory mechanisms in salt stress signalling and metabolism pathways could be identified.

8. LIMITATIONS OF PROTEOMICS ANALYSIS

The successful implementation of proteomics has already been made for a farm animal to establish the

nutraceutical properties of the milk proteome [90] or to monitor the *in vivo* performance of livestock animals, such as cows, goats, sheep and buffalo [91, 92] as well as disease control in human [93-96]. Similarly, the proteomics approach is increasingly used in the crops plants as well as other plants to understand the molecular insight of plants in response to stresses [24, 33, 34, 38, 39, 43, 44, 49, 52, 65, 97, 98]. However, even though a large scale identification of crops proteins has been done with the most advanced MS technologies and state-of-the-art proteome databases, a full proteome coverage has not been achieved yet, even in model plants *A. thaliana or Oryza* species [99]. Nearly 300,000 non-redundant peptides matching to about 25,000 unique proteins are currently available via MASCP Gator, representing 70% of the expected Arabidopsis proteome [100, 101]. The current limitations of plant proteomics [100] are stated below:

- A complete proteome yet not established even for model plant *A. thaliana* although large scale crops protein identified.
- Inefficiency to detect low abundance proteins in response to biotic and abiotic stress even with the most advanced MS instruments. Most cases these proteins are the key regulatory proteins to the stress conditions.
- Pre-fractionation of protein extracts combined with Ribulose-1,5-bisphosphate carboxylase/-oxygenase (RuBisCO) depletion can facilitate the detection of Leucyl aminopeptidases (LAPs); however, protein fractionation and enrichment generate additional variability in biological samples. Moreover, it is not compatible with high-throughput quantitative proteomic methods.
- Crop breeding purposes, revealing the broader relevance of the post-translational modifications (PTMs) is crucial which is remains to be established.
- Characterization of the phosphoproteomics has a great role in the crop breeding and improvement strategies which remains challenging because protein phosphorylation is generally rapid and transient.
- Large-scale analyses of the plant acetylome have not been reported yet which is an interesting post-translational regulatory mechanism and can be an interesting breeding marker.
- More economical molecular and functional markers are needed that can be discovered through proteomic studies; however, the potential of crop proteomics for the development of novel markers for plant breeding has not been fully realized yet.

CONCLUDING REMARKS

Proteins play a crucial role in determining the stress-adapted novel phenotype of plants that developed in response to stress. Therefore, proteomic studies could contribute to a better understanding of physiological and molecular mechanisms underlying in the plant stress response. Salinity stress can modify the protein structure and changes the gene expressions involved in signalling cascades, transcriptional regulation, uptake, and transport of water and ion. Proteomics could determine the proteins involved in those regulatory pathways. Although there are few limitations available in proteomic studies, the advances of proteomics would enhance the development of salinity stress tolerance plant in near future as well as it will depict the way of improving plant breeding strategies in response to stress environment.

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