Proteomic Analysis of Salinity Stress-responsive Proteins in Plants

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Abstract: Salinity is one of the most severe environmental factors that may impair crop productivity. Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years and up to 50% by the year 2050. Therefore, breeding for salinity stress tolerance in crop plants (for food supply) and in forest trees (a central component of the global ecosystem) should be given high research priority in plant biotechnology programs. Molecular control mechanisms for abiotic stress tolerance are based on the activation and regulation of specific stress related genes. Abiotic stresses usually cause protein dysfunction. Maintaining proteins in their functional conformations and preventing the aggregation of non-native proteins are particularly important for cell survival under stress. Cells subjected to salt stress showed a protective response which enabled them to survive. In the last few years, considerable progress has been made in the analysis of the transcriptome to study salt stress either alone or in combination with other abiotic stresses. However, there is no review that highlights the studies conducted to-date on proteomic analysis of salinity stress-responsive proteins in plants. The present review summarizes the effect of salinity in plants and the current initiatives in proteomic research for the analysis of plant salt tolerance. The importance of this review is to improve the salt tolerability of plants by understanding the alterations of proteins in plants.

Key words: Salinity, stress proteins, plants, salt stress, heat shock proteins

INTRODUCTION

Abiotic pressures like salt stress and chemical insulance can impose limitations on crop productivity and also limit land available for farming, often in regions that can ill afford such constraints, thus highlighting a greater need for understanding how plants respond to adverse conditions with the hope of improving tolerance of plants to environmental stress (Joseph et al., 2010). Living systems respond to changing environment by changing gene expression. In addition to changes in the level of expression of existing proteins, some new proteins may be synthesized. Such proteins not only can serve as markers, but may also play important role in adjusting to that specific environment. Targeting of these proteins to specific location within or outside the cell may also yield important clues to their function (Naqvi et al., 2009). Several workers have detected a number of proteins induced by salt, reflecting the complexity of biochemical and physiological responses. There are many reports showing that these protein changes are accompanied with the biological changes of the adaptation process, which make the organism more fit in the altered environment (Singh et al., 1985; Hurkmans et al., 1988).

A wide range of environmental stresses (such as high and low temperature, drought, alkalinity, salinity, UV stress and pathogen infection) are potentially harmful to the plants (Breusegem et al., 2001). Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Wang et al., 2003). Salt stress in soil or water is one of the major stresses especially in arid and semi-arid regions and can severely limit plant growth and productivity (Allakhverdiev et al., 2000; Koca et al., 2007). It is estimated that about 20% of the agricultural land in the world is affected by salinity (Flowers and Yeo, 1995) and salinity is a crucial constraint to realizing sustainable development of agriculture in future. Therefore, it is of great significance to investigate the molecular mechanism of salinity tolerance in plants and improve stress tolerance of crops.

Soil salinity: Earth is a salty planet, with most of its water containing about 30 g of sodium chloride L⁻¹. This is continually affecting the land on which crops will be grown. Excessive soil salinity is an important constraint limiting the distribution of plants in natural habitats and is an increasingly severe agricultural problem in arid and
Semi-arid regions (Lauchli and Epstein, 1990). This problem has been observed to be getting further aggravated (Munns et al., 2002). This has led to research into the physiological and biochemical mechanisms underpinning salt tolerance, with the aim of improving crop plants. It affects osmotic stress, decreasing water availability, ionic stress, changes in the cellular ionic balance (Kirst, 1989).

Global scarcity of water resources and the increase in salinization of soil and water are having a high impact on agricultural productivity (Silva et al., 2008). Roots play a number of important roles during plant growth and development and typically are the first part of the plant to encounter soil salinity (Aydi et al., 2008; Meot-Duros and Magné, 2008). When growing in saline soils, roots have to cope with two types of stresses, osmotic and ionic (Lin and Kao, 2001b). These stresses, in turn, cause reduced water uptake and inhibition of root growth (Munns, 1993).

**Salt tolerance in plants**: Salt tolerance in plants is a complex trait, which varies widely among closely related species and between different varieties (Sreenivasulu et al., 2000; Ashraf, 2002). Differences between closely related plants are particularly interesting to identify a small number of factors responsible for salt tolerance (Gehlot et al., 2005). Increased tolerance to salinity stress in crop plants is necessary in order to increase productivity with limited water supplies and high salinity. Tolerant genotypes respond to salinity stress with complex changes in their physiological and molecular status (Morsiy et al., 2006).

During the course of salinity stress, active solute accumulation of osmotic solutes such as soluble carbohydrates, proteins and free amino acids is claimed to be an effective stress tolerance mechanism. The adaptability of plant species to high salt concentrations in soil by lowering tissue osmotic potential was accompanied by accumulation of these osmotic solutes (Zhu, 2002; Jaleel et al., 2008). Studies of Heikal et al. (2000) and Ismail and Azoopl (2002) revealed that salt tolerance of *Vicia faba* L. was correlated with higher accumulation of ionic and osmotic solutes in salt-tolerant than that of salt-sensitive plants.

**The role of Reactive Oxygen Species (ROS) in salinity stress**: Abiotic stress conditions such as salinity and drought favor the accumulation of Reactive Oxygen Species (ROS) such as superoxide radicals (O$_2^-$), hydroxyl radicals (OH$^-$), hydrogen peroxide (H$_2$O$_2$) and cause oxidative stress (Parida and Das, 2005; Silva et al., 2008; Sudhakar et al., 2001). Salinity stress is known to trigger oxidative stress in plant tissues through the increase in reactive oxygen species (Apel and Hirt, 2004; Athar et al., 2008; Ashraf, 2009). Several reports demonstrated that salinity stress results in an excessive generation of ROS (Dionisioso-Sese and Tobita, 1998; Lin and Kao, 2001a; Hernandez et al., 2001; Lee et al., 2001; Sudhakar et al., 2001; Hernandez and Almansa, 2002; Tsai et al., 2004). Production of ROS is detoxified by both nonenzymatic and enzymatic antioxidant systems which play an important role in salt tolerance (Abdul-Jaleel et al., 2006; Valderrama et al., 2007).

**Antioxidant defense system for salt tolerance**: Salt tolerance has been found to be positively associated with a more efficient antioxidant system (Mittler, 2002; Noreen and Ashraf, 2008). A correlation between the antioxidant enzyme activities and salinity tolerance was demonstrated by comparison of tolerant cultivars with sensitive cultivars in several plant cultivars. These activities were reported to increase under salinity stress and closely related to salt tolerance of many plants (Athar et al., 2008).

Antioxidant defense systems in plants include both enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD) and glutathione reductase (GR) and non-enzymatic antioxidants such as ascorbate, glutathione and carotenoids (Parida and Das, 2005; Silva et al., 2008; Sudhakar et al., 2001). The alleviation of oxidative damage and increase resistance to environmental stresses are often correlated with an efficient antioxidative system. Such systems may be induced or enhanced by the application of chemicals such as Salicylic Acid (SA), or its derivates. Acetyl Salicylic Acid or aspirin (ASA), an artificial analogue of salicylic acid, undergoes spontaneous hydrolysis to SA in aqueous solution (Raskin, 1992).

**Proteomic approaches**: Improvements in proteomic technology regarding protein separation and detection, as well as mass spectrometry-based protein identification, have an increasing impact on the study of plant responses to salinity stress (Parker et al., 2006; Qureshi et al., 2007; Caruso et al., 2008). New insights have been obtained into salinity stress responses by comparative proteome studies of salt-stressed roots from Arabidopsis and rice (Oryza sativa). The identification of novel protein candidates associated with salinity stress (Yan et al., 2005; Jiang et al., 2007), detection of alterations in protein phosphorylation patterns (Chittetti and Peng, 2007) and, recently, the location of a salinity stress-responsive protein to the rice root apoplast with a putative function in stress signalling (Zhang et al., 2009) indicate the importance of ion uptake and transport and
regulation of water status and signal transduction processes in the root.

Many research groups had used proteomic approach for the identification of salt-responsive proteins in several plants. Salekdeh et al. (2002) identified several Salt-responsive proteins in root proteome of salt-tolerant and salt-sensitive rice varieties, including ABA- and stress-responsive proteins, ascorbate peroxidase and many others. Several proteins were found to be modulated in expression by salt concentration in a coordinated manner (Nohzadeh et al., 2007). These proteins were involved in photosynthesis, photorespiration, signal transduction, metabolism regulation, oxidative stress defense, control of ion channels and protein folding.

**Salt shock proteins:** The proteins, which accumulate in response to salt stress, are referred to as Salt Shock Protein (SSP), which may have some relevance in stress tolerance. The salinity promotes synthesis of SSP, causes either increase or decrease in the level and total and soluble protein, depending on the plant parts studied and leads to increased activity of many enzymes (Igarashi et al., 1997). The SSP-23 protein, which disappeared in B. parviflora under salinity stress, reappeared when these salinized seedlings were desalinated. These observations suggest the possible involvement of these polypeptides for osmotic adjustment under salt stress (Parida et al., 2004). Gomathi and Vasantha (2006) revealed that changes in RNA and DNA content under salinity stress might be responsible for specific expression of Salt Shock Proteins (SSP) with MW of 15, 28 and 72 kDa in tolerant genotypes, while there were completely absent in susceptible. However, supplementing GA$_1$ under salt stress condition enhanced nucleic acid content (RNA) and thus induced expression of SSP in susceptible genotypes with low intensity.

**Heat shock proteins by salt stress:** Salt, drought and heat stresses are often combined in nature. Much research is aimed toward the breeding of crop cultivars with improved salt and heat tolerances. Organisms that thrive in harsh environments must possess specific mechanisms for adapting to stressful environments. One such mechanism would be the induction of molecular chaperones, Heat Shock Proteins (HSPs), which comprise several evolutionarily conserved protein families such as Hsp100 (ChpB), Hsp90, Hsp70 (DnaK), Hsp60 (GroEL) and small Hsp (Boston et al., 1996; Harti, 1996).

Regarding HSPs many authors (Flom et al., 2006; Chan et al., 2006; Guyomarch et al., 2004; Izhaki et al., 2001; Kurek et al., 2002) confirmed that protein stil appeared to be up-regulated in response to salt stress; this protein contains two heat shock chaperonin binding motif (STI1), three Tetraprto Peptide Repeat (TPR) and two Stil domains. The up-regulation of this regulatory protein may decrease the sterility of pollen during another development. It is believed that Hsp90 interacts with TPR-containing proteins to modulate diverse cellular processes through protein-protein interaction.

Hsp 70 is confirmed as biomarker of stress produced by NaCl in marine macro algae and fresh water plant species, emphasized its role in protecting plants against stress (Ireland et al., 2004). Dual channel imaging and warping of 2-DE protein gels were also used to visualize global changes in the protein synthesis pattern of cells in response to osmotic stress (6% NaCl). Extensive studies suggest that different chaperones follow distinct strategies to prevent protein misfolding and aggregation in the highly crowded cellular environment. For example, the monomeric Hsp70 recognizes short hydrophobic peptide segments and binds at an early stage of folding (Harti, 1996; Bukau et al., 2000).

**Salt stress proteins as molecular markers:** Pareek et al. (1997) suggested that stress proteins could be used as important molecular markers for the improvement of salt tolerance using genetic engineering techniques. However, proteins produced under salt stress are not always associated with salt tolerance; consequently, using proteins as a salt tolerance indicator depends on the nature of the plant species or cultivar.

Rani and Reddy (1994) observed the presence of additional 26 kDa protein band is associated with salt tolerance in rice. High, as well as low, NaCl treatment of maize led to an unexpected high number of differentially regulated proteins in roots and shoots. Moderate salt stress (25 mM NaCl) already led to a differential regulation of 31% of shoot proteins and 45% of root proteins, without an effect on the morphology and the Na$^+$ and Cl$^-$ concentrations of the plants. High stress (100 mM NaCl) led to an uncontrolled change of more than 80% of the separated proteins. Fourteen proteins which were increased by salt stress were identified by in-gel digestion and peptide mass fingerprinting using matrix-assisted lasers desorption/ionization time-of-flight mass spectrometer (MALDI-TOF) (Zorb et al., 2004).

**Plant adaptation to salt stress by protein changes:** There are many reports showing that the protein pattern changes are accompanied by the biological changes in the adaptation process, which makes the organism more fit in the altered environment (Hunkman et al., 1989; Singh et al., 1985). However, protein changes do not always confer tolerance to salinity (De Souza Filho et al.,
Several salt-induced proteins have been identified in plant species to survive under saline conditions (Ashraf and Harris, 2004). Although plants in nature have evolved several adaptive mechanisms to cope with the presence of salts in their environment, the understanding of these mechanisms still remains incomplete. The osmotic effect involves limited water absorption due to salinity in the rhizosphere and the ionic effect consists of intracellular toxicity or imbalance due to excess ions (Silva et al., 2008).

**Biotechnological strategies for salt stress tolerance:** The basic resources for biotechnology are genetic determinants of salt tolerance and yield stability. Implementation of biotechnology strategies to achieve this goal requires that substantial research effort be focused on identifying salt tolerance effectors and the regulatory components that control these during the stress episode (Hasegawa et al., 2000). Further knowledge obtained about these stress tolerance determinants will be additional resource information for the dissection of the plant response to salinity, which will reveal how plants sense salt stress, transduce signals to mediate a defensive response and define the signal pathway outputs or effectors that accomplish the processes required for stress survival and alleviation and steady-state growth in the saline environment.

Molecular genetic and plant transformation advances have made it feasible to assess biotechnological strategies based on activated signal cascades, engineered biosynthetic pathways, targeted gene or protein expression or alteration of the natural stress responsiveness of genes for development of salt tolerant crops (Hasegawa et al., 2000; Zhu, 2001). The molecular identities of key ion transport systems that are fundamental to plant salt tolerance are now known (Hasegawa et al., 2000). More recently, the SOS salt stress signaling pathway was determined to have a pivotal regulatory function in salt tolerance, fundamental of which is the control of ion homeostasis (Hasegawa et al., 2000; Sanders, 2000; Zhu, 2000).

**CONCLUSIONS**

Plant adaptation to stress under natural conditions has some ecological advantages, the metabolic and energy costs may sometimes mask and limit its benefit to agriculture and result in yield penalty. Therefore, the improvement of abiotic stress tolerance of agricultural plants can only be achieved, practically, by combining traditional and molecular breeding. The response of plants to salt and other environmental stresses have been extensively investigated by proteomic approach for many decades, still we have not been able to understand fully the mechanism which imparts tolerance to some plants and sensitivity to others due to the complexity of the mechanism.

The discovery and use of new stress-tolerance-associated genes, as well as heterologous genes, to confer plant stress tolerance (including those unique to extreme-growth-environment organisms e.g., halophytes, thermophilic organisms), has been the subject of ongoing efforts to obtain tolerant plants. The overall progress in proteomics research is encouraging, considering the fact that plant proteomics is still in its infancy and is becoming an active and evolving field with a large impact on plant biology. In particular, there are a number of issues that need to be addressed to improve and develop proteomic analysis of plant salt stress tolerance to its full potential, which may lead to the application in breeding for enhanced tolerance in crop plants.

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**REFERENCES**


