SALT STRESS RESPONSIVE PROTEINS IDENTIFICATION IN WILD SUGAR BEET (*Beta maritima*) BY MASS SPECTROMETRY

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ABSTRACT

SALT STRESS RESPONSIVE PROTEINS IDENTIFICATION IN WILD SUGAR BEET (*Beta maritima*) BY MASS SPECTROMETRY

Salt stress is one of the major abiotic stresses in agriculture worldwide. Seven percent of the land's surface and five percent of cultivated lands are affected by salinity. Turkey is the fourth in the world and third in Europe in producing sugar beet. It is observed that salt stress affects the sugar beet negatively especially at germination and seedling stages, it limits the productivity of crop plants and affects the quality of plants.

In the present study, proteomic approach was used to investigate the salt-stress responsive proteins in wild salt-tolerant beet, *Beta maritima*. Sugar beet were grown approximately two months. After growing, they were treated with 250 mM NaCl for seven days. Control plants received no salt treatment during this period. Total proteins of leaves and root were extracted. The proteins were fragmented into peptides using in-solution digestion technique and liquid chromatography-tandem mass spectrometry (LC-MS/MS) used for identified the proteins. Totally 288 proteins were identified in leave samples and totally 259 proteins were identified in the root samples.

Identified protein results were shown that unique of salt leave proteins and up-regulated proteins of leave samples were the related to the antioxidant enzymes. On the other hand, active transporter protein of vacular ATP synthase subunit A was identified in the salt responsive of root samples.

ÖZET

YABANİ ŞEKER PANCARINDA (*Beta maritima*) TUZA DUYARLI PROTEİNLERİN KÜTLE SPEKTROMETRE KULLANILARAK TANIMLANMASI

Verimli tarım alanlarının tuzlanması, tüm dünyada giderek büyük bir sorun haline gelmektedir. Dünya üzerindeki arazilerinin yüzde yedisi ve sulama yapılan toprakların yüzde beşi tuzluluktan etkilenmektedir. Türkiye şeker pancarı üretiminde dünyada dördüncü avrupadada üçüncü sırada yer almaktadır. Şeker pancarının çimlenme ve fide dönemlerinde tuz stresinden olumsuz etkilendiği, üretimde önemli derecede verim kayıpları meydana getirdiği ve bitkinin kalitesini etkilediği gözlemlenmiştir.

Bu çalışmada, yabani şeker pancarının tuza dayanıklı türü olan *Beta maritima* kullanılarak tuz-stresine toleranslı proteinler proteomik yaklaşım kullanılarak incelenmiştir. Yaklaşık iki ayda yetiştirilen şeker pancarı bitkileri yedi gün boyunca 250 mM NaCl maruz bırakılmıştır. Kontrol bitkilerine bu süre içinde tuz uygulanmamıştır. Yapraktaki toplam proteinler izole edilmiştir ve solüsyon içinde parçalama tekniği kullanılarak proteinler peptitlere parçalanmıştır. Sıvı kromatografisi-ikili kütle spektrometresi (LC-MS/MS) kullanılarak proteinler tanımlanmıştır. Toplam 288 protein yaprak örneklerinde, 259 protein de kök örneklerinde tanımlanmıştır.

Tanımlanan protein sonuçlarına bakıldığında tuz uygulanan yaprak örnekleri ve fazla tanımlanan yapraktaki proteinler antioksidant enzimlerken, tuz uygulanmış kök örneklerinde vakular ATP sentez subunit A proteini tanımlanmıştır.

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CHAPTER 1

INTRODUCTION

1.1. The Biology of Sugar Beet

1.1.1. General Description

The sugar beet belongs to the *Chenopodiaceae* family. This family has approximately 1400 species such as *B. Vulgaris*, *B. maritima*, *B. Patula*, etc. and 105 genera (Watson and Dallwitz 1992). Fodder beet/mangolds, red table beet, Swiss chard/leaf beet, and spinach are the most economically important species in this family.

The sugar beet production of area is totally 6.96 million hectare in 1998 all over the world (Holtschulte 2000). It means that the sugar beet is the most important crop among the cultivated forms. The International Database for *Beta* (Frese and Hintum 1989) contains information that provided by germplasm holdings in 24 countries. And the German-Dutch Cooperation on *Beta* Genetic Resources, the Turkish genebank and the Polish Gene Bank organized collecting data to complete the world holding.

However sugar beet is an annual under certain conditions, it is normally biennial species (Smith 1987). In the first year, the sugar beet plant produces a large fresh taproot and seed stalks grow up in the second year. Sugar beet root crops are planted in the spring and harvested in the same year. In the next growing season, cold temperatures of 4- 7 °C is needed for the root to dash and for beginning the reproductive stage (Smith 1987).

During, the vegetative stage, the first growing season, sugar beet has smooth, dark green leaves. Conjunctions of the stem, a white, fleshy taproot develops (Duke 1983). During, the reproductive stage, the second growing season, planted stalk bolts from the root. This seed stalk grows approximately 1.2-1.8 metres tall. Stem with small leaves returns to the less petiolate leaf and finally sessile leaves develops. Sugar beets produce a perfect flower. Flower is surrounded by a slender green bract (Smith 1987).

The ovary forms a fruit. Each fruit contains a single seed. The ovaries are surrounded by the flower cluster (Duke 1983). If a flower occurs singly, a monogerm seed is formed. Otherwise multigerm beet seed is formed by collection of two or more flowers.

1.1.2. Content of Sugar Beet

White sugar, pulp and molasses for food are produced by sugar beet roots. A typical sugar beet root consists of 75.9% water, 2.6% non-sugar, 18.0% sugar and 5.5% pulp. 83.1% sugar fraction is obtained as crystalline sucrose, 12.5% is obtained as molasses (Bichsel 1987). Sugar is a carbohydrate that uses for many purposes such as flavour, aroma, texture, colour and body of foods. In addition to produce pure sugar, dried sugar beet pulp is produced by sugar factories. Another important by-product is sugar beet molasses that has viscous liquid containing about 48% saccharose. To produce yeast, chemicals, pharmaceuticals, sugar beet molasses are used.

1.1.3. The Centres of Origin of the Sugar Beet

Descriptions of sugar beets as plants with enlarged roots, record the earlier of the 12th century (Toxopeus 1984). To the 18th century, German scientists began to produce beets to increase the sugar content of their roots (American Sugar beet Growers Association 1998).

The centre of origin of beet (*Beta*) may be the Middle East, near Tigris and Euphrates Rivers. It is believed that wild type of beets to be west into the Mediterranean and north along the Atlantic sea coast. Established that species of B. trigyna, B. lomatogona, and B. macrorhiza spread of north into the mountains of Turkey, Iran, and the Caucasus Mountains of Russia (Cooke and Scott 1993). So, wild types of beet disperse east through most of Eastern Asia.

1.2. Salt Toxicity

1.2.1. General Description

Plants are affected by several enviromental stresses generally attributed to abiotic stress (drought, extreme temperatures, salinity, etc.) and biotic stresses induced by pathogens (fungi, viruses, etc.). Salt stress is one of the major abiotic stresses in agriculture worldwide. The United Nations Environment Program estimates that approximately 20% of agricultural land and 50% cropland in the world is salt-stressed (Flowers and Yeo, 1995). It means that seven percent of the land's surface and five percent (77 million ha) of cultivated lands are affected by salinity (Flowers, et al. 1997). Therefore, these values evidence that the salt stress is one of the most serious environmental stress that limits the productivity of crop plants and affects the quality of plants.

Sodium chloride application has an important role for better sugar yield in sugar beet (Draycott and Bugg 1978). Plant mass is decreased due to inhibition in cell division and cell enlargement by salinity so production of protein and nucleotide is inhibited (Isla, et al. 1998). Niazi, et al. (2002, 2004) observed that concentration of chlorophyll is higher under saline conditions. Different plant species shown salt sensitivity at various growth stages. But a macronutrient in the growth medium is not affect the concentration of a micronutrient (Mn,Zn Fe and B) in different plant parts under saline conditions (Hu and Schmidhalter 1997, 2001). Under salinity conditions, different cultivars of the same plant has different behavior (Flowers and Hajibagheri 2001, Qadir, et al. 2001).

1.2.2. Effects of Salt Toxicity in Plants

Plants need to the mineral nutrients (elements) to grow and develop. These elements are important in their biological functions. But enviromental conditions can cause stress on plant and these conditions affect the plant life negatively. One of the

major stress is salinity. And known that plant growth is more effected by salt than other toxic substances (Xiong and Zhu 2002). Plants are classified as glycophytes or halophytes according to their capacity to grow on high salt medium. Most plants are glycophytes that cannot tolerate salt-stress. In contrast to glycophytes, halophytes have the capacity to accommodate extreme salinity because of very special anatomical and morphological adaptations or avoidance mechanisms (Flowers, et al. 1986). In other explains it depends on adaptations of plants physiology that include ion compartmentalization, osmolyte production, germination responses, osmotic adaptation, selective transport and uptake of ions, enzyme responses and genetic control.

High salt concentration decreases the soil water potential. Therefore, plant water potential is lowered (Flowers, et al. 1986). Plant must have a defensive system to survive under salinity condition. But the mechanism of uptake of Na^+ into plant cells is not clear. Ion transporters are considered to play an important role in salt tolerance. It has been reported that Na^+ enters the root cells through different cation channels. Voltage-independent cation (VIC) channels are thought to be the major way for Na^+ to move into plant cells (Amtmann and Sanders 1999, Schachtman and Liu 1999, Tyerman and Skerrett 1999, White 1999). Molecular mechanisms of VIC channels are not clear yet. Potassium channels are thought to be one route for Na^+ entry to root cells. Since Na^+ and K^+ have the same charge (Blumwald, et al. 2000), it is possible that these channels can also be used to move NaCl.

The aim of most researches are to improve the resistance of crop plants for salinity conditions. To develop of abiotic stress-tolerance plants, regulatory proteins and genes encoding of different structural have been used over the past 5-6 years. Usage of regulatory genes is more effective approach for developing stress-tolerance plants. So, to understand the molecular basis of regulatory genes are very important for understanding salinity tolerance mechanism.

It is known that saline environments affect plant growth in two ways: (i) salts reduce cell turgor decreasing the external water potential; and (ii) salts accumulate in leaves and become toxic (Greenway and Munns 1980; Munns and Termaat 1986). In the first “osmatic” phase, salts in the external solution cause water stress, plant growth is decrease. In the second “salt-specific” phase, accumulation of ion in the leaves induce toxic levels, reduce the photosynthetic area, and so declines in growth (Munns 1993, Munns, et al. 1995). The time between these two phases depends on the sensitivity of the plant to salt and its ability to exclude Na^+ and Cl^- from the shoot.

Evidence points out that plant salt tolerance works at a cellular level. Cellular mechanisms include ion sequestration in vacuoles or ion exclusion at plasma membranes. Plasma membrane ATPase and vacuolar ATPase are proton pumps that provide an energy source for transport of ions across the plasma membrane and tonoplast, respectively. Membrane Na^+/H^+ antiporters use of the proton gradient formed by these pumps to exchange Na^+ for H^+ across a membrane. Therefore, activity and expression of these proton pumps and Na^+/H^+ antiporters are investigated in varity plant species under salt-stress.

1.2.2.1. Homeostasis

Salt overly sensitive (*sos*) mutants of *Arabidopsis* have been screened to identify genes and cellular processes in plant salt tolerance. The *sos* genes, SOS1, SOS2 and SOS3, were cloned and characterized (Liu and Zhu 1998). The functions of these three are genetically Ca^{2+} dependent. These molecular genetic analysis of *sos* mutants give an important results that they are plasma membrane Na^+/H^+ antiporter. The SOS1 gene encodes a Na^+/H^+ antiporter and upregulated under salinity. A SOS3-SOS2 protein kinase complex controls the sodium efflux through SOS1 under salinity. A shematic representation of the SOS signaling pathway for ion homeostasis are shown in Figure 1.1.

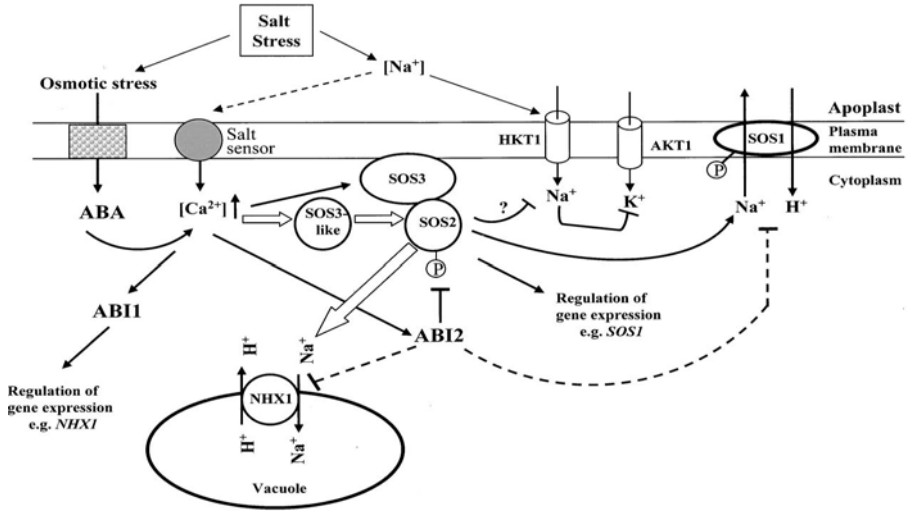

Figure 1.1. SOS signaling pathway for ion homeostasis under salt stress in *Arabidopsis*

Salt stress increased the Ca^{+2} concentration and it activates the protein kinase SOS3. Then SOS3 activates the protein kinase SOS2. Activated SOS2 phosphorylates SOS1, a plasma membrane Na^+/H^+ antiporter, which then transports Na out of the cytosol. The transcript level of SOS1 is regulated by the SOS3-SOS2 kinase complex. SOS2 also activates the tonoplast Na^+/H^+ antiporter that sequesters Na^+ into the vacuole. Na^+ entry into the cytosol through the Na^+ transporter HKT1 may also be restricted by SOS2. ABI1 regulates the gene expression of NHX1, while ABI2 interacts with SOS2 and negatively regulates ion homeostasis either by inhibiting SOS2 kinase activity or the activities of SOS2 targets. Double arrow indicates SOS3-independent and SOS2-dependent pathway (Zhu, et al. 2005).

1.2.2.2. Detoxification

Oxidative stress is an important stress that caused by salinity. Oxidative stress is produced by the formation of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and singlet oxygen ($^1O_2$). ROS is produced is a normal function of aerobic metabolism. But under normal conditions the negative effects of ROS can be eliminated. Salinity conditions increases the ROS production (Xiong and Zhu 2002). The excessive ROS can damage proteins, lipids and nucleic acids (Halliwell and Guteridge 1985). To eliminated the ROS effect, antioxidant compounds such as ascorbic acid, glutathione, thioredoxin, and ROS scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione peroxidase (GPX) are employed by plants. The first step of defense mechanism is conversion of superoxide anions to hydrogen peroxide and water. This step is produced by superoxide dismutase (SOD). If the superoxide anion is not neutralized at this step, it reacts with reduced transition metals such as Fe^{2+} to produce highly reactive hydroxyl radicals by the Fenton reaction. Therefore, SOD is the very important enzyme in the defence mechanism against oxidative stress. Then, producing hydrogen peroxide converted to oxygen and water by catalysing the several classes of peroxidases and catalases. There is no elimination mechanism for $OH^{\cdot}$. These mechanisms shown in Figure 1.2.

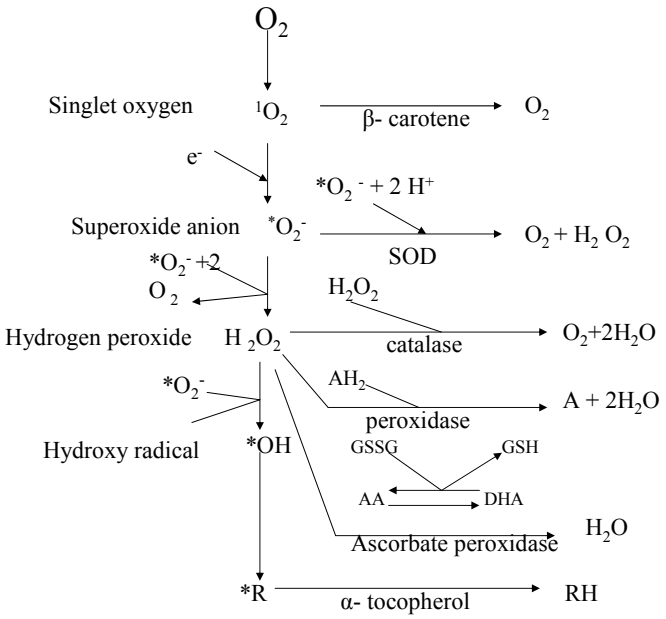

Figure 1.2. Mechanisms of reactive oxygen species formation and ROS/antioxidant signaling pathways

1.2.3. Plants That Tolerance to Salt Toxicity

To make a useful description of the molecular mechanisms active in the response of the NaCl treatment, it needs to characterize the components of these mechanisms, including proteins. Therefore, proteomic analysis based on the 2-DE-MS technology, as a large-scale one, has been widely used to investigate salinity response in plants, including NaCl-treated of pea (*Pisum sativum L.*), rice (*Oryza sativa L.*), wheat (*Triticum aestivum L.*), *Suaeda aegyptiaca*, maize, tobacco, *Synechocystis*, *Arabidopsis*.

The identification of stress tolerance genes will need a range of genetic resources and molecular tools (Leung, et al. 2001). The complete sequencing of the genomes of *Arabidopsis* (Ausubel 2000) and rice (Barry 2001) makes these species useful for understanding of salinity stress and to identify NaCl-responsive genes.

CHAPTER 2

PROTEOMICS AND MASS SPECTROMETRY

2.1. What is the Proteomic?

The term of proteomic was initially proposed by Marc Wilkins in 1994 at the *Conference on Genome and Plant Maps* (Siena, Italy) as the "*PROTEin complement expressed by a gen OME*". Proteome study is represent a comprehensive study of all proteins describe at a given time, in given condition, in a given organism (Barbier-Brygoo and Joyard 2004). And primary amino-acid sequence can be explained by a proteomic study. Moreover, other properties of proteins such as their relative amounts, specific activity, state of modification, three-dimensional structure, can be expressed. These informations are very important for the description of biological systems.

DNA ⟶ **mRNA** ⟶ **Proteins**

Genomics Genomic Expression Proteomic Expression

Genes are transcribed into mRNA. Because cells make alternative splicing, one gene can lead to different mRNA molecules. Then, these mRNA species are translated into proteins. These proteins can become very active by adding post-translational modifications (PTM) or interaction with other proteins. After all of these processes, many different protein isoforms can be form by one gene. Lastly, proteomics can be describe newly 'omics' disciplines of metabolomics. All of these processes named as 'systems biology' that will used for understanding of cellular biology.

2.1.1. Identify a Protein in Proteomics

If gen expression is analyzed at the protein level, there is a huge increase in complexity. Point out that human genome has approximately 40,000 genes. And estimated that encoded proteins by these genes is two to three orders of magnitude higher than the genes number. It is mean that encoded protein number vary from 200,000 to 2 million, due to splicing, post-translational modifications (PTMs) of proteins, such as phosphorylation, methylation, and protein degradation (Barbier-Brygoo and Joyard 2004) . To identify the proteins, firstly, their functional properties must be known. Proteins that based on the same gene can be mostly identical. There might have only small difference in their functionally details. But simple listing of the proteins is not enough. All the interactions between proteins must be describe as much as possible and quantitative outline of proteins is necessary. So, to identify a large number of proteins and distinguish between close relatives, protein identification tools are used. Mass spectrometry is used to identify proteins by partial analysis of their digestion-derived peptides. Databases fills in the missing sequence information. Because sequence databases and experimental data are limited.

It has become clear that organism complexity is produced by a complex proteome than by a complex genome. Proteome is explained as the time-specific protein and cell-specific protein complement of the genome, that all proteins expressed in a cell at one time, containing isoforms and protein modifications (Juri Rappsilber and Matthias Mann 2002). The proteome is much more dynamic than the genome. Because the genome is fix for one cell, mostly identical for all cells of an organism, and not change in a species. On the other hand, the proteome is very dynamic with time, effects the external factors, and change considerably between cell types.

2.1.2. Types of proteomics

Proteomic applications have three types: expression proteomics, structural proteomics and functional proteomics.

Expression proteomics purposes to identify all the protein species in a cell, tissue or organism at a certain time. Structural proteomics aims to identify the molecular structure of the protein in a given process and to relate this information to the database of identified genes. Functional proteomics is dealed with the identification of functions, activities, and interactions of all the proteins in proteome.

2.2. Plant Proteomics

Improvement in structural and functional genomics has been accelerated by discoveries of genes in microorganism, animals and plants (Pandey and Mann 2000). Plant proteomics has accessed after completing sequence of the genomes of *Arabidopsis thaliana* (Ausubel 2000) and rice (*Oryza sativa*) (Barry 2001). And so unprecedented numbers of plant genes has added the databases.

Although plant proteomics is in its early years, it will likely to become an active field in plant biology with increasing the databases, gen annotation, the use of expressed sequence tag (EST). Since resolution of protein spots on a 2D (two-dimensional) gel is limited, researchers have focused on protein isolation from cellular compartments of any cell or tissue instead of contend with total protein complement. Different analytical levels for proteomic studies have been demonstrated such as; from protein fractionated on the consist of their post-translational modifications (phosphoproteome, by Laugesen et al.), to protein complexes (respiratory chains supercomplexes of plant mitochondria, by Eubel et al.), organelles (chloroplast proteomics by Van Wijk), subcellular compartments (plant membrane proteomics, by Ephritikhine et al.; and cell wall proteomics, by Rose), plant cell (Chlamydomonas proteomics by Staubler and Hippler). Then, Riccardi et al. discussed functional proteomics on tissues, organ or plants, Schneider et al. discussed Swiss-Prot protein knowledgebase and lastly Schwacke et al. discussed plant membrane proteome database (Barbier-Brygoo and Jacques Joyard, et al. 2004). Also a group of European scientists formed a European Union-supported consortium, around 1996, to study the proteome of the plasma membrane of tobacco and Arabidopsis and 2-DE reference maps produced (Rouquie, et al. 1997, Santoni, et al. 1999). Maize roots analyzed during hypoxic acclimation (Chang, et al. 2000). Moreover, there have been several studies to experiment different plant species with

abiotic and biotic stress conditions, example of abiotic stresses, heat (Waters, et al. 1996), cold (Singh and Laroche 1990), salt (Serrano and Gaxiola 1994), drought (Bray 1993), heavy metals (Rauser 1990) and some studies include biotic stresses; soil nutrient problems (Kang, et al. 2004) whereas bacterial (Jorrin, et al. 2006), fungal (Campo, et al. 2004) and viral diseases (Ventelon- Debout, et al. 2003). These biotic and abiotic stresses effect the plants leaf's and root's physiological and morphological. So plants develop defense mechanisms to protect themselves against biotic and abiotic stresses. Proteomic approach used to identify the proteins that plants produce under these extreme conditions. For this purpose two-dimensional (2-DE) gel electrophoresis use to detect the spots and then these spots analyse by mass spectrometry. Soft ionization methods (MALDI and ESI) use for mass spectrometry.

2.3. Two-Dimensional Gel Electrophoresis

One-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (1-D SDS-PAGE or 1-DE) separates total protein extracts according to protein's molecular weight (size) difference and it separates only 80-100 different protein components. It is not enough value since cell proteomes are extremely complex having several thousand of proteins. On the other hand, 2-DE can separate thousands of proteins simultaneously. Therefore, two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (2-D SDS-PAGE or simply 2-DE), introduced by O'Farrell (1975), is still the most accepted method in proteomics studies.

2-DE separates protein mixture including to two dimension. The first dimension (isoelectric focusing, IEF) sepearates proteins according to isoelectric point (pI), whereas the second dimension (sodium dodecyl sulfate polyacrylamide gel electrophoresis, SDS-PAGE) separates proteins according to molecular weight. Today's modern 2-DE systems has a capacity to separate up to 10,000 protein spots on one gel. 2-DE systems can analyzed more than 5000 proteins simultaneously, having nearly 2000 proteins routinely, and can detect and quantify nearly 1 ng amount of protein per spot.

The second dimension of 2-DE separates proteins according to their molecular weight, in other words their mobility in polyacrylamide porous gel. SDS-PAGE can be

performed on horizontal or vertical systems (Görg, et al. 1995). Vertical systems are refered when multiple runs in parallel are required. Pore size of the polyacryamide gel can be controlled by varying the total acrylamide content of the gel and cross-linker content of the total acrylamide. IPG strips having low polyacrylamide content can acts as a stacking gel due to concentrated, ready and nonrestictive protein zones within. Therefore there is no need to use stacking gel with vertical 2-DE systems.

Although it has deficiency, such as a poor ability to separate proteins with high molecular weight (above 200 kDa), poor solubility of hydrophobic proteins, difficulties in resolving and identifying very acidic or basic proteins and low-abundance proteins and limited dynamic range, it is a powerful method for the analysis of complex protein mixtures extracted from cells, tissues or other biological samples.

2.4. Detection of Protein Spots and Image Analysis

The last procedure of 2-DE experiment is to detect the protein spots on gels either by universal or by specific staining methods. Universal staining methods detect protein spots with Coomassie blue dye, silver staining, negative staining with metal cations (e.g. zinc imidazole), staining or labeling with organic or fluorescent dyes, detection by radioactive isotopes, and by immunological detection. On the other hand, specific staining methods are used for detecting PTMs (e.g. phosphorylation, methylation, etc.). For ideal protein detection on two-dimensional gel, some properties are very important such as it should be sensitive (low detection limit), reproducible, well-matched with mass spectrometry and it should also have linear and wide dynamic range. Unfortunately, there is no method that matches with these properties exactly. Detection methods of coomassie blue staining and silver staining are the most used methods for proteomic studies.

The most chosen detection method is the coomassie brilliant blue (CBB) staining. Because it is low cost and to use it easy. It colors the proteins on a gel with dark blue. CBB R-250, CBB R-350 and CBB G-250 dyes are commercially available. It is a simple procedure for removing of dye from gel. So it is suited for mass spectrometry. Its detection range is approximately 50 ng to 1000 ng. On the other hand,

CBB staining has a problem during destaining, spots are partially destained. Quantification cannot be accurate because steady state between dye and protein is not established totally.

After staining the gel, computer programs such as PD Quest, Bio-Rad and Delta2D, Decodon are used to convert the gel images into digital data using a scanner or camera. These programs have a ability for spot detection, spot editing, background correction, gel matching, quantification, etc.

In conclusion, to get exact identification of protein spots (newly expressed and up- or down-regulated) in polyacrylamide gel, these spots are cut out from gel and digested (in-gel digestion) into peptide fragments with specific enzyme (generally trypsin). And then identified using mass spectrometry and database searches.

2.5. In Solution Digestion

In solution digestion is very important method for producing peptides from proteins in proteomic studies. Because whole proteins extracted and recovery of peptides with this technique.

After preparing the protein solution, it treated with a protease (most common trypsin). Completion of the analysis is generally taken two days. During the first day, samples are prepared for overnight digestion and the second day samples are lyophilized and then reconstituted in a solution for MS analysis. This method provides reduction and alkylation of the cysteine-containing peptides.

Although there are several enzymes to be used for in solution digestion, the most commonly used one is trypsin for sequencing experiments with tandem mass spectrometry (MS/MS). Because trypsin cleaves amide bonds in proteins at the C-terminal side of lysine (K) and arginine (R) residues, if either of these are not followed by a proline residue in the C-terminal direction. Other important point is the it produces small peptides, generally in the mass range of 600-2500 Da.

2.6. Mass Spectrometry

Mass spectrometry (MS) is used to identify unknown compounds or to characterize the structure of a molecules. MS is the study of gas-phase ions. MS became an important tool in the field of biochemistry by the development of fast atom bombardment (FAB) in 1981 (Barber, et al. 1981). It has been used for the analysis of protein and peptides since 1989, when two new "soft" techniques for gas phase ionization of large, polar, and highly charged molecules were established. The introduction of new soft ionization techniques are named as electrospray ionization by Fenn and co-workers (ESI) (Fenn, et al. 1989) and matrix-assisted laser desorption/ionization by Karas and Hillenkamp (MALDI) (Karas and Hillenkamp 1988).

Mass spectrometers have mainly three essential parts, namely the ionization source, the mass analyzer, and the detector.

The ionization source (e.g. electrospray, matrix-assisted laser desorption) is the first component of the MS. It produces ions from the analyzed (liquid or solid) samples. The second component is the mass analyzer (e.g. quadrupole, time-of-flight) which determines the mass-to-charge ratio (m/z) of ions derived from the analyte. The third and last component is the detector (photomultiplier, microchannel plate, electron multiplier) which detects the ions resolved by the mass analyzer and it transforms the ion beam into a usable signal. Each of these three parts of mass spectrometer is under vacuum-pump systems which is required for their function.

2.6.1. Ion Sources

The analyzed samples are ionized by ion sources before to analysis in the MS. A variety of ionization techniques are used for this aim. Some ionization techniques are very energetic and produce extensive fragmentation. Other techniques are softer, called a "soft" ionization techniques, and produce molecular species. The two most common "soft" ionization techniques are electrospray ionization (ESI) and matrix-assisted laser

desorption/ionization (MALDI), due to ionization without fragmentation which allow the formation of ions. So they give molecular weight information.

2.6.1.1. Electrospray Ionization (ESI)

Electrospray ion sources used in mass spectrometers are firstly designed and its working principles are firstly described by Fenn and co-workers in 1985 (Whitehouse, et al. 1985). ESI ionize the sample at atmospheric pressure then transfer the ions into the mass spectrometer. To combine an atmospheric pressure source compartment with an analyzer compartment cause problem that this combination must be kept at a very low pressure (10^{-5} Torr). This problem is solved by introducing focusing lenses with very small openings between both compartments. An electrospray is produced by appliying a strong electric field, under atmospheric pressure, to liquid passes through a capillary tube with a weak flux. This electric field cause a charge accumulation at the liquid surface, which break to form highly charged droplets. Then these droplets pass through a curtain of heated inert gas, most often nitrogen. The drop appears spherical at low voltages, then elongates in the stronge electric field; when the surface tension is broken, the shape of the drop changes to a 'Taylor cone' and the spray appears.

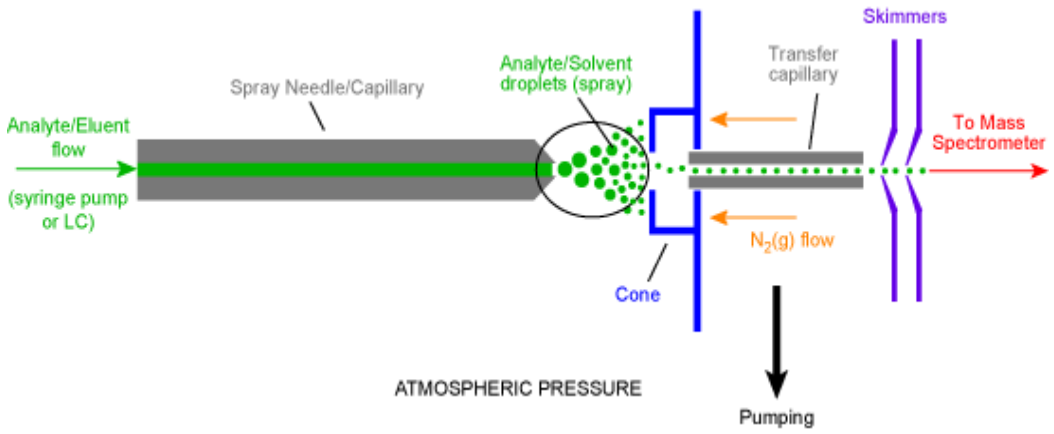

Figure 2.1. Schematic Representation of an ESI source
(Source The University of Bristol, School of Chemistry 2008)

Electrospray ionization is very efficient in ionization process and, as a result, sensitivity of electrospray-based experiments. The other important characteristic of electrospray ionization is its general compatibility with high-performance liquid chromatography (HPLC) solvent system and this property makes ESI useful equipment in proteomic studies.

2.6.1.2. Matrix-Assisted Laser Desorption/Ionization (MALDI)

Matrix-assisted laser desorption/ionization (MALDI) was first described by Karas and Hillenkamp in 1988 (Karas and Hillenkamp 1988). MALDI is obtained in two steps. In the first step, the compound to be analyzed is mixed in a solvent. This solvent contains small organic molecules in solution, called a matrix that has a strong absorption at the laser wavelength. The compound is usually mixed with the matrix solution in a ratio 1:1000, respectively. This mixture is dried before analysis, any liquid solvents are removed. And so ‘solid solution’ deposits of analyte-doped matrix crystals.

In the second step, bulk portions of this solid solution is removal by intense pulses of laser for a short time. Laser generates rapid heating of the crystals by the accumulation of a large amount of energy in the condensed phase. Matrix molucules are excitated during the rapid heating by the laser. And this rapid heating causes sublimation of the matrix crystals and increasing the matrix into the gas phase. Remaining little internal energy is transferred to the analyte molecules. And lastly, ionization reactions ocur at any time during this process.

The MALDI process is very sensitive ionization method. The matrix minimizes sample damage from the laser pulse by absorbing most of the energy. And the matrix also increases the efficiency of energy transfer from laser to the analyte. Therefore, the sensitivity is highly increased. It is also more common than other laser ionization techniques. Lastly, because the absorption properties and size of the compuond to be analyzed do not affected the process, MALDI permits to the desorption and ionization of analytes with very high molecular mass in excees of 100 000 Da. The most common matrix-assisted laser desorption/ionization experiment is applied to proteome experiments is the direct analysis of protein digests.

2.6.2. Mass Analyzers

After the ions are produced by ion sources, they are seperated according to their mass-to-charge (m/z) ratios by mass analyzers. There are several mass analyzers. The most common and useful ones for biomolecules are quadrupole mass analyzer, time-of-flight (TOF), quadrupole ion traps, and Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry. Different ion sources are connected to these different of mass analyzers. TOF mass analyzer requires the ions to be produced in package. So it is well suited for pulse laser sources and connected to MALDI. Triple quadrupole-TOF or ion traps coupled to ESI for analyzing large biomolecules. MALDI-TOF instruments and ESI-tandem MS instruments are used in most proteomic studies. MALDI-TOF MS is used for identification of proteins by peptide masses (peptide mass fingerprints; PMF) and ESI MS/MS expresses peptide fragmentation.

There are three main properties of an analyzer are; the upper mass limit, the transmission and the resolution. The upper mass limit determines the measured highest value of the m/z ratio. The transmission is the ratio between the number of ions reaching the detector and the number of ions produced in the sources. The resolution is the ability of mass analyzer to separate ions of similar m/z.

2.6.2.1. Time-of-Flight Mass Analyzer (TOF)

The linear time-of-flight mass analyzer was firstly described by Stephens in 1946. It became the commercial instrument after published the design of a linear TOF mass spectrometer by Wiley and McLaren in 1955.

The working principles of time-of-flight mass analyzers are simple. Firstly, all the ions are given the same amount of kinetic energy by acceleration in an electric field. High voltage causes the electric field. After acceleration, the ion enters a field-free region. In this region, it travels at a velocity that is inversely proportional to its m/z. It means that, ions with low m/z travel more rapidly than ions with high m/z. And then,

required time is measured for the travel of ion through the length of the field-free region.

The commercial TOF instrument can get resolution of 10,000 or greater (Chernushevich, et al. 2001). Resolution in a TOF mass analyzer is effected by the dimensions the instrument, the length of the flight tube, and the accelerating the voltage. For improving sensitivity, reflectron mode can be placed at the end of the drift zone. It can be used by refocusing of ions with the same m/z on the reflectron detector. So, the reflectron is increase the length of the flight tube. To increase the flight time by lowering the acceleration voltage is also increase the resolution. On the other hand, lowering the voltage reduces the sensitivity. The only way to have both high resolution and high sensitivity is to use a long flight tube for a higher resolution and acceleration voltage at least 20 kV for higher sensitivity.

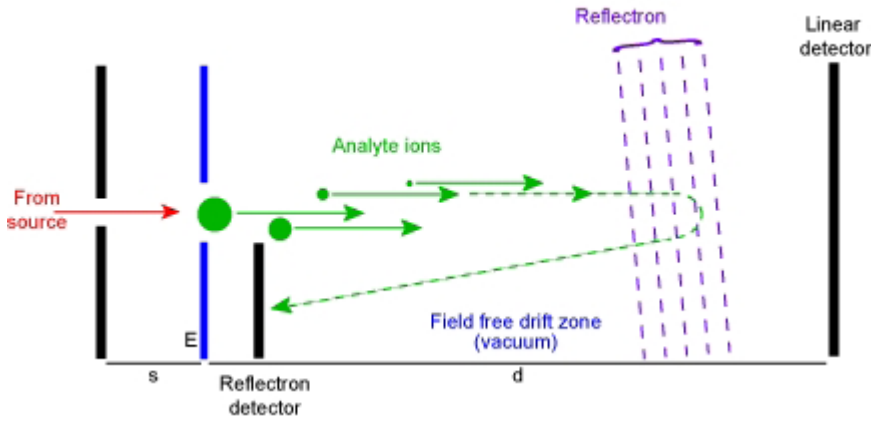

Figure 2.2. Schematic Representation of a Time-of-Flight Mass Spectrometer (Source The University of Bristol 2007)

The TOF is well suited to ionization techniques like MALDI. Because MALDI produce ions in short, and well-defined pulses as the TOF analysis required. So the MALDI-TOF system is a very sensitive method. It detects low quantities (10^{-15} to 10^{-18} mole) of sample.

The MALDI Q-TOF MS gives both peptide mass fingerprints and amino acid sequence. This system identifies a sample with amino acid sequence. There is no need to be use a different mass spectrometer when the peptide mass fingerprinting is failed.

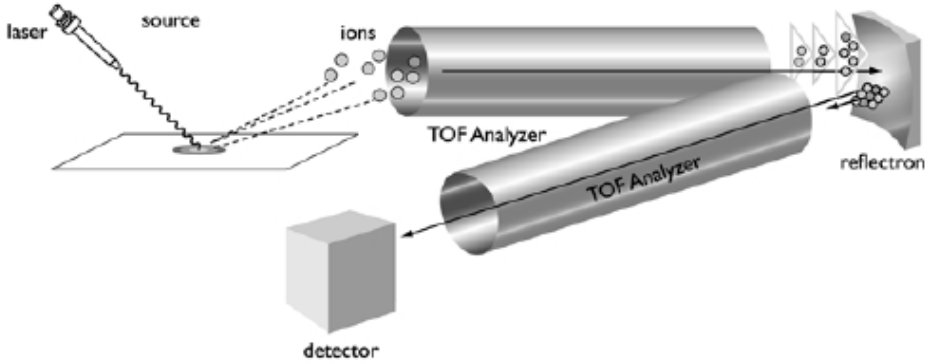

Figure 2.3. A MALDI-TOF Instrument
(Source Liebler 2002)

2.6.2.2. Quadrupole Ion Traps

The quadrupole analyzer was firstly described as 'ion trap' by Paul and Steinwedel in 1960. And Stafford was modified it to a mass spectrometer. Ion trap mass analyzer is made up of a circular electrode, with two ellipsoid caps on the top and the bottom. The formed ions are directed into the ion trap. And applying the RF and DC voltages, ions are trapped in that place. As the ions repel each other in the trap, their trajectories expand as a function of time. To prevent ion losses by this expansion, helium gas is used to take excess energy from the ions by collision.

Ion trap mass analyzers are high sensitivity. Because transmission of ions from ion sources to detector and use of the ions produced by the ion source are very efficient. The main reason behind the high sensitivity of ion trap mass analyzer is the ability to allow ions to be "stored" and then selectively ejected from the ion trap (Yates 1998).

2.6.3. Detectors

The ion beam passes through the mass analyzer and then is detected and transformed into a usable signal by a detector. Different type of detectors exist. They

are classified in two categories. First category of detectors are; the photographic plate and the Faraday cage. They measure of the charges that reach the detector directly. Second category of detectors are; the photomultiplier, electron multiplier and microchannel detectors that they are increase the intensity of signals. Microchannel plate detectors are commonly used in modern commercial instruments (Dubois, et al. 1999).

2.6.4 Tandem Mass Spectrometry (MS/MS)

Tandem mass spectrometry, MS/MS, is a general method involving at least two stages of mass analysis. In the most MS/MS experiment, a first analyzer is used to isolate a precursor ion. A second spectrometer analyzes the product ions. The simply principle of MS/MS is an ion is selected by the first spectrometer MS_1, fragmented through collision and the fragments are analyzed by the second spectrometer MS_2. Thus ions with a selected m/z value, observed in a standard source spectrum, can be chosen and fragmented to obtain their product ion spectrum.

To analyze a complex mixture, e.g. natural products, a separation technique, such as liquid chromatography (LC), gas chromatography (GC), is coupled with mass spectrometry.

The coupling of liquid chromatography is more delicate because gas-phase ions must be produced for mass spectrometry. Liquid chromatography normally is used for nonvolatile compounds. And in LC/MS, the sample is first separated with high-performance liquid chromatography (HPLC), and then mass spectrometry is used for detection.

2.7. LC-ESI-MS/MS

Ion mobility (IM) coupled with mass spectrometry (MS) has evolved a powerful analytical technique. This combination provides investigating of the structural and conformational properties of bio-molecules in the gas-phase.

The working principle of ion mobility spectrometry (IMS) is to separate ions according to mobility differences. For this aim, ions flow through an inert gas by applying a weak electric field. A uniform and static electric field uses for flowing ions through the background gas in a classical ion mobility devices, or drift tubes. Because sensitivity issues associated with classical drift tubes and providing the faster mass spectral gaining, extensive development has produced in IM–MS techniques in the last decade.

Drift tube devices needs to high sensitivity due to duty cycle related to gating packets of ions into the device. The issue of duty cycle can be discontinuous ion sources such as MALDI, where each laser shot can provide the packet for mobility separation. A periodic focussing dc drift tube uses to lowered the effect of ion loss due to radial diffusion, or radio frequency (RF) uses to place ions with axial fields as the mobility separator.

Time of Flight (TOF) is used for an analyser and inparticular orthogonal acceleration (oa) TOF technology provides full mass spectra in a short time such as millisecond and produced wide ion mobility peaks. The other mass analysers such as quadrupoles or ion traps do not includes these properties. This hybrid quadrupole/IM separator/oa-TOF instrument also namedly as The Synapt High Definition Mass Spectrometry (HDMS). And a schematic diagram of the hybrid quadrupole/IM separator/oa-ToF instrument (the Synapt HDMS system) shown in Figure 2.4.

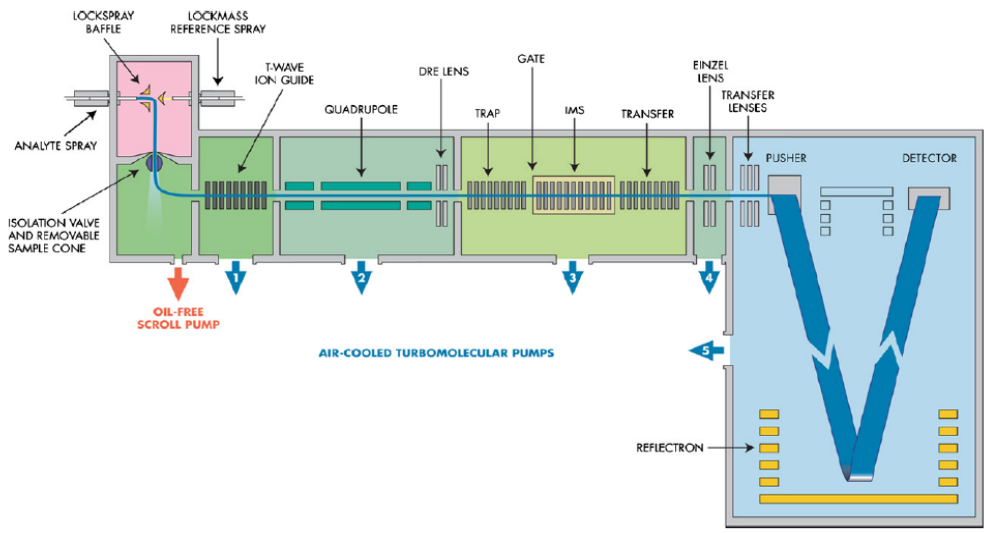

Figure 2.4. Schematic Diagram of the Synapt HDMS System
(Source Waters Corporation, Milford, USA 2008)

The IM section includes three parts; trap, IMS and transfer parts. The part of ion mobility spectrometry (IMS) provides extra dimension of fast, gas phase and ion separation. Therefore it provides higher ion definition. IMS has been described as "Plasma Chromatography" or "Ion Chromatography". The mobility of an ionised molecule is dependant on its size (shape) and charge state. The other parts of IM are trap and transfer parts that they consist of 33 electrode pairs with RF applied. But the final electrode on the trap is dc-only for gating periodically ions into the IMS. Both cells are enclosed; they share a common gas. Typically these cells are worked at 10^{-2} mbar pressure range. Although no travelling wave is used in the trap cell, the transfer cell has a continually running wave to ensure the mobility separation is maintained on transit to the oa-ToF. And fragmentation can be induced in both the trap and transfer region.

Time Aligned Parallel Fragmentation (TAP) is a unique capability of the Synapt HDMS System. Time Aligned Parallel (TAP) fragmentation enables the acquisition of first and second generation product ions. The second generation product ions are associated to the first generation product ions by their drift time.

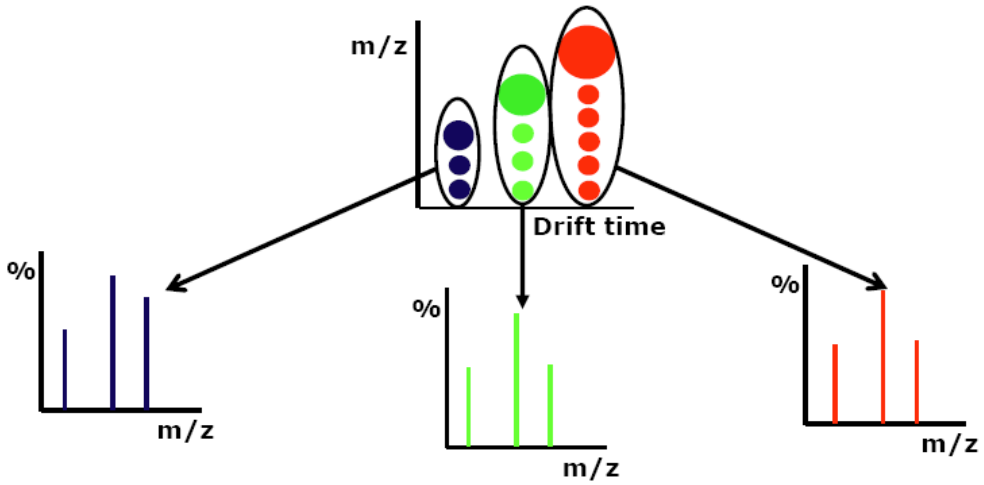

Figure 2.5. Schematic Diagram of Time Aligned Parallel Fragmentation (TAP)
(Source Waters Corporation, Milford, USA 2008)

2.8. Protein Identification by Mass Spectrometry

The identification of peptides and proteins from biological sources is central to proteomic experimentation. Variety of methods has been used for this purpose historically, but mass spectrometry, in particular tandem applications (MS/MS) has become the centre of this experiments because of its efficiency, accuracy, and sensitivity. To analyzed the proteins by mass spectrometry, a 'top-down' or a 'bottom up' approaches are used. These approaches can be explained as the form of whole-protein analysis (known as the 'top-down' approach) or analysis of enzymatically produced peptides (known as the 'bottom up' approach).

In the 'top-down' approach, whole proteins are purified and fragmented in the mass spectrometer. This approach typically deliver 100% sequence coverage for proteins that less than 70 kDa. A mixture of protein is seperated in the gas phase firstly and then known mass of specific protein ions are isolated and fragmented. And the more powerful Fourier transform (FT) mass spectrometers are used in this approach because it has the capacity to fragment whole proteins with very high mass accuracy.

In the 'bottom-up' approach, after whole proteins are purified, they are fragmented with a highly specific enzyme such as trypsin, and the resulting mixtures of 1-3 kDa peptides are analyzed using various combination of chromatography followed by tandem MS analysis.

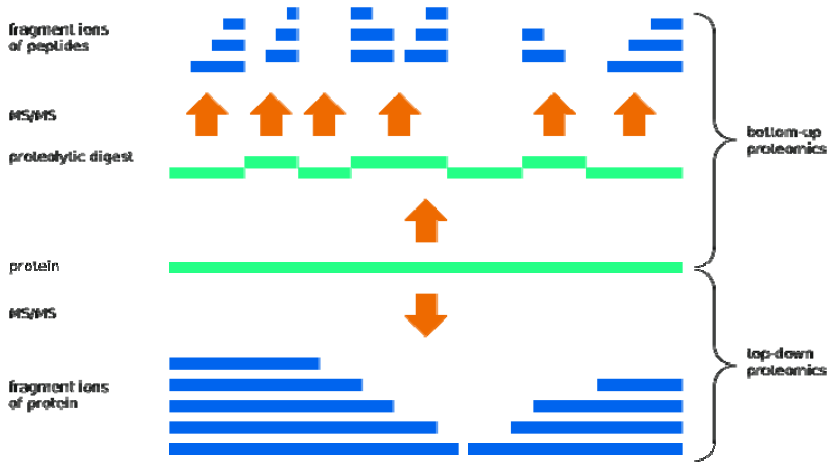

Figure 2.6. Schematic Representation of a 'top-down' or a 'bottom-up' Approaches

The technique that separates of the peptides by liquid chromatography coupled to mass spectrometry known as shotgun proteomics. Shotgun proteomics is a method of identifying proteins in complex mixtures using a combination of high performance liquid chromatography combined with mass spectrometry. In this proteomics approach, a complex mixture of proteins are digested firstly. Then the resulting peptides are seperated by liquid chromatography and tandem mass spectrometry is used to identify the peptides.

2.9. The Aim of the Study

The purpose of this study was to identify the salt-stress tolerant proteins in wild type of sugar beet (*Beta maritima*) and to explain the tolerance mechanism in salt-tolerant genotype of sugar beet by using proteomic approach. After growing the sugar beet, 250 mM NaCl was applied to salt treatment plant for seven days. Control plants received no salt treatment during this period. Total proteins of leaf and root tissues were extracted from control and salt treated plants. In-solution digestion procedure was done using trypsin and whole proteins were fragmented into peptides. The obtained peptides were analyzed by mass spectrometry and each protein was identified by the help of database search programs. Proteomic study provides an excellent opportunity to identify salt-stress responsive proteins and to explain the defense mechanism in salt-tolerant sugar beet to salt toxicity. The understanding of plant stress physiology is well correlated with the changes in proteome content of cells. In addition, newly synthesized, up-regulated, down-regulated, or totally disappeared proteins were compared in salt treated and control plants of sugar beet.

CHAPTER 3

EXPERIMENTAL

3.1. Plant Growth Conditions and Salt Treatment

In this study, sugar beet, Beta maritima was used. It is salt-tolerant wild species of sugar beet. Seeds were sown into the pots that were filled with soil. They grown under controlled environmental condition (23±2 °C with 16-h light/8-h dark photoperiod) approximately for two months. After germination, seedlings were watered with half-strength Hoagland solution. Growing plants were taken plastic beakers containing half-strength Hoagland solution. This solution is a special mixture that contains essential nutrients for growing plants. It includes 3.5 mM $Ca(NO_3)_2.4H_2O$, 2.5 mM KNO_3, 1 mM KH_2PO_4, 1 mM $MgSO_4.7H_2O$, 22 μM H_3BO_3, 4.5 μM $MnCl_2.4H_2O$, 0.35 μM $ZnSO_4.7H_2O$, 0.2 μM $CuSO_4.5H_2O$, 0.07 μM $NaMoO_4$, mixture of 15 μM EDTA.2Na, 14 μM $FeSO4.7H_2O$ and 0.5 mM KOH whose pH was adjusted to 5.5.

- To prepare of the Hoagland solution for 2 L: 7 ml $Ca(NO_3)_2.4H_2O$, 5 ml KNO_3, 2 ml KH_2PO_4, 2 ml $MgSO_4.7H_2O$, 1 ml of trace elements (2.8 g H_3BO_3, 1.8 g $MnCl_2.4H_2O$, 0.2 g $ZnSO_4.7H_2O$, 0.1 g $CuSO_4.5H_2O$, 0.025 g $NaMoO_4$ were dissolved in 1 L of water)

Root of the plants were in the solution, stalk and leaves were on the sponge. They were covered with aluminum foil. And Hoagland solution added regularly into the beaker to prevent lossing of water by evaporation. At the seventh day of growth in the beaker, mixture of half-strength Hoagland and 250 mM NaCl was prepared. This solution was applied only one beaker. Other one was the control plants that it continued to grow with half-strength Hoagland solution. Control plants (no salt treatment) and salt treatment plants had the same physical conditions and grown with for additional seven

days. After the seven days, the leaf and the root of *Beta maritima* control and *Beta maritima* salt tissues (54 days old) were harvested and wind with an aluminum foil quickly, and froozen in liquid nitrogen to minimize proteolytic activity. Samples were stored at -80 °C for the protein extraction.

3.2. Protein Extraction from Leaves and Roots

TRIzol (phenol/guanidine thiocyanate) reagent that is a quick and convenient reagent used for the simultaneous isolation of RNA, DNA, and protein. And plant sample isolated successfully with TRIzol. So TRIzol was used for protein extraction in this study.

TRIzol includes 38 % phenol in saturated buffer (Merck), 0.8 M guanidine thiocyanate (AppliChem), 0.4 M ammonium thiocyanate (AppliChem), 0.1 M sodium acetate (Merck), 5 % glycerol (AppliChem) and water. To preparation of 100 ml TRIzol; firstly 38.0 ml phenol, 11.816 g of guanidine thiocyanate, 7.612 g of ammonium thiocyanate, 0.8203 g of sodium acetate, 5.0 ml glycerol were mixed. Then pH was adjusted to 5. Lastly, the mixture was fulfilled to 100 ml with ultra pure water. It can be stored at 2-8 °C for several months.

Approximately 3 grams of frozen control and salt leaf or root samples were weighted. After they were mashed by a mortar and pestle with liquid nitrogen to a powder, they were taken in centrifuge tubes. And for homogenization 30 ml of TRIzol reagent added for 3 grams of mashed leaf or root tissues. Waited for 5 minutes at room temperature to ensure complete dissociation of nucleprotein complexes. Then added 6.0 ml of chloroform (AppliChem) (0.2 ml of chloroform per ml of TRIzol) into the sample, shaken for 15 seconds and waited for 2-15 minutes at room temperature. Centrifuged at 13,000 x g for 17 minutes at 4 °C. After doing centrifugation, the mixtures were separated into the 3 phases: a upper aqueous phase (colorless) containing RNA, an interphase containing DNA, and lower phase containing protein. Aqueous protein phases were taken to a new and clean centrifuge tubes and 45 ml of isopropanol (AppliChem) (1.5 ml of isopropanol per 1 ml of TRIzol) were added and waited for at least 10 minutes at room temperature. And then, the sample mixtures centrifuged at

12,000 x g for 10 minutes at 4 °C. The supernatants were discarded and the pellets were washed 3 times with 0.3 M guanidine hydrochloride/95 % ethanol solution. For each washing 60 ml of 0.3 M guanidine hydrochloride/95 % ethanol solution (use 2 ml per 1 ml TRIzol) was used.

- To prepare of 0.3 M guanidine hydrochloride/95 % ethanol solution for 100 ml: 2.866 g of guanidine hydrochloride (AppliChem) weighted and diluted to the 100 ml with 95 % of ethanol solution (AppliChem).

Samples were stored in wash solution for 20 minutes at room temperature during each washing. Centrifuged at 7,500 x g for 5 minutes at 4 °C. At the end of this step, pellets were taken to the eppendorf tubes and 2 ml of ethanol (AppliChem) added in each tubes. Pellets can be stored -80 °C for further usage or it can be dissolved with rehydration buffer for done the next step of isoelectric focusing (IEF).

3.3. Protein Solubilization with Rehydration Buffer

Pellets were centrifuged at 14,000 x rpm approximately for 5 minutes at 4 °C. The excess ethanol above the pellets were taken carefully. Then the pellets were vacuum-dried in SpeedVac (Thermo Electron Corporation) maximum 5 minutes, until all ethanol was completely evaporated. Then pellets were dissolved in rehydration buffer. This buffer consists of 7 M urea, 2 M thiourea, 4 % (w/v) CHAPS, 65 mM DTT (AppliChem), and 2.5 % Ampholyte pH 3-10 (Fluka).

- To prepare of rehydration stock solution for 10 ml: 4.2 g of urea, 1.52 g of thiourea, and 0.4 g of CHAPS were weighted and dissolved in ultra pure water to a final solution volume of 10 ml. This stock solution can be stored at 4 °C up to one month.

Just prior to use, 1 ml of stock solution (explained above) was taken, and added 0.01 g of DTT and 25 µl of ampholyte. DTT and ampholyte must be added just before use.

The rehydration buffer was added to the pellet in a sufficient amount for complete solubilization. After addition enough buffer to pellet, it was thoroughly mixed and vortex for 10 minutes. Approximately 400 µl of rehydration buffer was used for solubilization of one leaf (or root) sample pellet. Then the eppendorf tubes were centrifuged at 14,000 x g for 5 minutes at 4 °C and the supernatants were taken for IEF experiment. Before IEF, protein concentrations were determined by Bradford method.

3.4. Bradford Protein Assay for Protein Determination

The Bradford protein assay is the spectroscopic analytical methods that is used to determine the total protein concentration of a sample (Bradford 1976). This method is based on the absorption shift from 470 nm to 595 nm. This absorption occurs when the Coomassie brilliant blue G-250 (CBB G-250) dye binds the protein. It binds to protein from its sulfonic groups. So it can binds to the arginine, lysine, and histidine residues. Additionally, the dye also binds weakly to the tyrosine, tryptophan, and phenylalanine resulting of hydrophobic interactions.

After addition of the sample, the dye binds protein and colour of mixture change from gren to blue. Increases of the protein concentration, the color of the sample become darker.

The Bradford assay has a linear dynamic range, from 2µg/ml to 120 µg/ml. To determine the sample protein concentration, standard curve must be produced. Bovine serum albumin (BSA) was used to produce standard curve.

- To prepare of Coomassie reagent for 100 ml: 10.0 mg of CBB G-250 (AppliChem) dissolves in 5 ml of 95 % ethanol and 10.0 ml of 85 % phosphoric acid (AppliChem) added and the mixture was diluted to 100 ml with ultra pure water. The final solution was filtered through filter paper (Whatman No. 1) and was stored in an darker bottle at 4 ºC.

- To prepare of 0.2 mg/ml stock BSA Standard for 1 ml: 0.0200 g of BSA was weighted and dissolved in water to a final volume of 1.0 ml.

Table 3.1. Preparation of the Test Sample for the Bradford Protein Assay

Test Sample	Sample Volume, µl	Water Volume, µl	Coomassie Reagent Volume, µl
Blank	0	800	200
BSA Standard – 1 µg/ml	5	795	200
BSA Standard – 2 µg/ml	10	790	200
BSA Standard – 4 µg/ml	20	780	200
BSA Standard – 6 µg/ml	30	770	200
BSA Standard – 8 µg/ml	40	760	200
Protein Sample	2	798	200

Blank, BSA standards, and protein samples were prepared according to Table 3.1 in disposable cuvettes and absorbance measurements readed by using UV-visible spectrophotometer at 595 nm. The solution prepared by mixing these reagents. Firstly water added, then BSA or sample protein, and lastly Coomassie solution added. And waited for 5 minutes at room temperature. Then, absorbance of each sample was measured at 595 nm using a UV-visible spectrophotometer.

The standard curve was draft by plotting the reading of absorbance versus µg of protein in BSA standard samples. The best straight line was determined. And the equation is such as "y=mx + b" where y is absorbance reading at 595 nm and x is protein concentration. Using this equation, the concentration of the protein sample that measured absorbance at 595 nm was calculated.

Following table shows the absorbance values for various BSA standards.

Table 3.2. Absorbance Values for BSA Standards

Concentration (µg/ml)	Absorbance at 595 nm
1	0.0820
2	0.1412
4	0.238
6	0.3120
8	0.3852

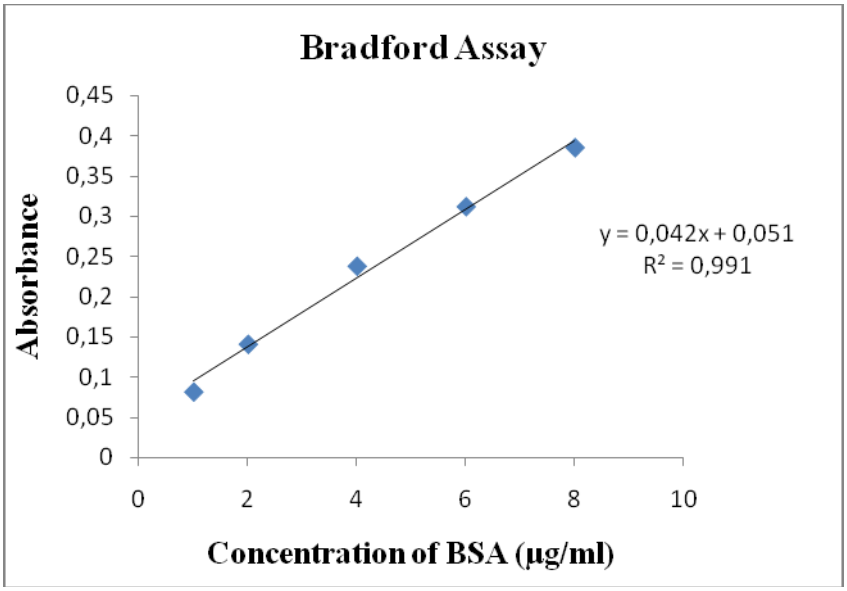

Figure 3.1. Standard Curve for BSA

3.5. In Solution Digestion

The main part of this procedure is in solution digestion. After produced the pellet of the protein sample, it dissolves in special buffer as described below. And trypsin added to cleavage of protein into peptides and sequencing of these fragments. In solution digestion procedure was taken two days in this study. In the first day, samples were prepared for overnight digestion. After an overnight reaction samples are prepared for MS analysis.

The preparation of the mixture for in solution digestion:

- To prepared of Tris stock for 25 ml: 1.21 g of Tris-base was dissolved in 20 ml of water, ph was adjusted to 7.8 using HCl (~6 M). And the total volume was adjusted to 25 ml with water. The final Tris concentration is 0.4 M.
- To prepare of urea and Tris buffer for 5 ml: Placed 2 g of urea in a 15-ml centrifuge tube. Added 1.25 ml of Tris stock. Adjusted to final volume 5 ml with water. The final concentrations are 6 M Urea, 100 mM Tris Buffer.
- To prepare reducing agent for 1 ml: 30 mg dithiothreitol (DTT) was dissolved in 750 µL water. 250 µL of Tris stock was added and mixed by gentle vortex. The final concentrations are 200 mM DTT and 100 mM Tris.
- To prepare of alkylating reagent for 1 ml: 36 mg of iodoacetamide dissolved in 750 µL of water. 250 µL of Tris stock was added and mixed by gentle vortex. The final concentrations are 200 mM iodoacetamide and 100 mM Tris.
- To prepare of 100 mM iodoacetamide: 18 mg of iodoacetamide was placed in a 1.5 ml plastic centrifuge tube followed by addition of 1 ml of 100 mM ammonium bicarbonate for complete dissolving of iodoacetamide. The final concentration
- To prepare of trypsin solution: 100 ml of acetic acid added to 20 µg of sequencing-grade modified trypsin (V5111; Promega). And dissolved the trypsin by drawing the solution into and out of the pipette. The solution was kept on ice until use. The final concentration of trypsin is 200 ng/µl.

Day One :

- Place a 100-µL aliquot of the protein sample in the 6 M Urea, 100 mM Tris Buffer, containing 1 mg of total protein, in a 1.5-ml plastic microcentrifuge tube.
- 5 µL of reducing agent was added and the sample mixed by gentle vortex.
- Reduced the protein mixture for 1 h at room temperature.
- Added 20 µL of the alkylating reagent and the sample mixed by gentle vortex.
- Alkylated the protein mixture for 1 h at room temperature.
- 20 µL of the reducing reagent added to consume any unreacted iodoacetamide. Mixed the sample by gentle vortex. Allow the reaction to stand at room temperature for 1 h.
- The urea concentration was reduced by diluting the reaction mixture with 775 µLof water. Mixed the solution by gentle vortex.
- The 100-µL trypsin solution containing 20 µg of trypsin was added. Mixed the sample by gentle vortex and carried out the digestion overnight at 37 ºC.

Day Two :

- The reaction was stopped. And the pH of the solution was adjusted to <6 by adding concentrated acetic acid. The digest was been analyzed concentrated to 200 µL by evaporation.

3.6. Protein Identification and Mass Spectrometric Analysis

After in solution digestion procedure, leave samples were identified by LC-MS/MS, Synapt High Definition Mass Spectrometry (HDMS) System (Waters Corporation). And both leave and root samples were identified by LTQ Orbitrap XL (Thermo Fisher Scientific). And sequence of the proteins were found by using NCBInr protein database (National Center for Biotechnology Information, Bethesda, USA) and properties of the proteins were found by using The ExPASy (**Ex**pert **P**rotein **A**nalysis **Sy**stem) that proteomics server of the Swiss Institute of Bioinformatics (SIB).

CHAPTER 4

RESULTS AND DISCUSSION

In this study, wild salt-tolerant sugar beet, *Beta maritima* was chosen to observe the effect of salinity at the proteome level. Shotgun approach was used. Means that whole protein digested by using trypsin (in-solution digestion). It cleavage of protein into peptides and sequencing of these fragments. These fragments were separated by liquid chromatography (LC) and tandem mass spectromerty (MS/MS) was used for identified the proteins. Synapt High Definition Mass Spectrometry (HDMS) System (Waters Corporation) was used for leave samples. Then both leave and root samples were analyzed by LTQ Orbitrap XL (ThermoFisher Scientific).

4.1. Effects of Salt Toxicity on Growth

In this study, 250 mM NaCl applied to *Beta maritima*. And observed that salinity affected the growing of *Beta maritima.* It reduced leaf chlorophyll, photosynthetic rates decreased. And so growing of the leave and root affected. Observed that some leaves of the salt-treatment plant were smaller than the control ones and sometimes salt-treated plants were fewer leaves. And salinity altered leaf color and leaves were wilted after applying salt. These changes of leaves were shown in the Figure 4.1, after applying the 250 mM NaCl.

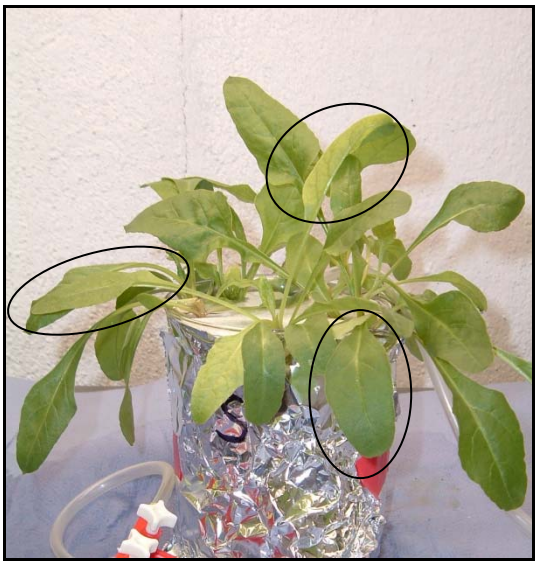

Figure 4.1. *Beta maritima* Leaves after Applying 250 mM NaCl

4.2. Salt-Stress Responsive Proteins in *Beta maritima* Leaves

Salt tolerant sugar beet of *Beta maritima* were grown in the presence or absence of 250 mM NaCl for seven days. Total proteins were extracted from leaves and roots and the first experimental part is analyzed them by using 2-DE. The main aim was to see the different spots between salt-treated and control plants and to identified salt-specified proteins under salt stress. Plant culture, protein extraction and 2-DE experiments, was repeated more than three times in our laboratory. Proteins were separated in the first dimension on an IPG Strip pH 3.0-10.0 and in the second dimension on a 12 % acrylamide SDS-gel. The gels were stained with Coomassie colloidal blue staining. But there was poor reproducibility among the replicate gels. The reason of this result can be caused by IPG Strips, they could be cause some problems with dating time or the loaded protein sometimes burned the IPG Strip or second dimension of 2-DE could be caused this problem. Before attached the IPG Strip to the SDS-gel, to produced the solid acrylamide gel was very important. In this part could cause this problem or there was some problems with PROTEAN II xi Cell (Bio-Rad) that was used for separation in the second dimension by SDS-PAGE. Produced one of the good gel result was shown in Figure 4.2.

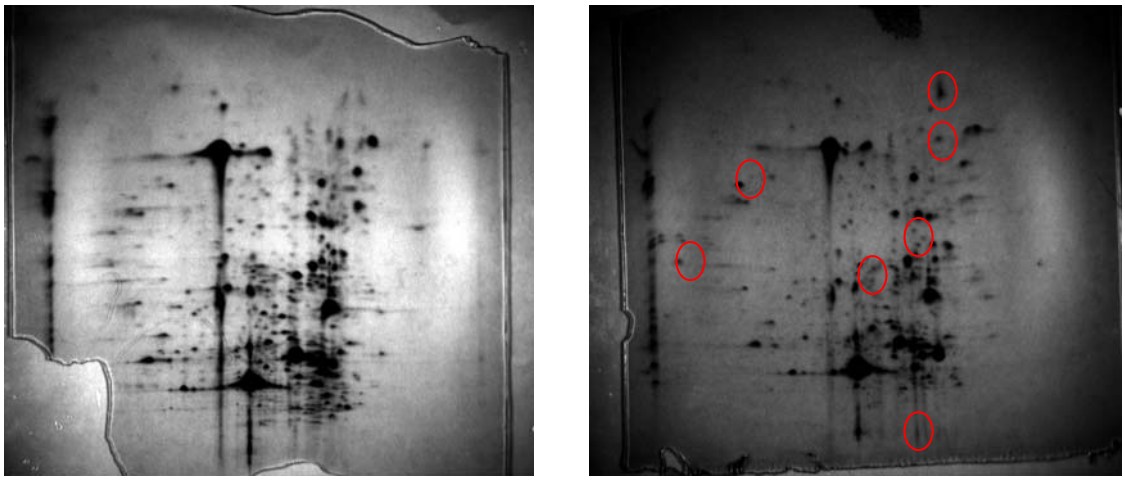

Figure 4.2. 2D-PAGE Gels of *Beta maritima* Leaf Proteins, Left one is Control, Right one is Salt Responsive Gels

In the second experimental part of this study, in-solution digestion procedure was applied after extraction the samples. The crude protein extract was digested directly by using trypsin. Then samples were analysed by separation of the peptides by liquid chromatography coupled to tandem mass spectrometry, LC-MS/MS. LC-MS/MS results gave information about both qualitative and quantitative analysis.

In this study two different instruments used for analyzed the samples. First one was the Synapt High Definition Mass Spectrometry (HDMS) System (Waters Corporation). Firstly, Uniprot green plant entries was used for this analysis. It contains 22,000 entries. Triplicate runs were done for each sample, with the specified database search criteria Ion Accounting. Control sample identified 98, 103, and 100 proteins respectively. The average loading of sample for the triplicate analyses was calculated to be 47.8 ng using the absolute quantification function of Identity^{E} analysis. Results for salt-treatment sample was; in the triplicate runs, with the specified database search criteria Ion Accounting identified 152, 124, and 99 proteins respectively. The average loading of sample for the triplicate analyses was calculated to be 40.8 ng using the absolute quantification function of Identity^{E} analysis. Salt and control samples showed statistical differences which were represented graphically. Injection comparisons between the control and salt triplicate analyses using Log/log normal intensity plots show that the injections are reproducible. All of these information can be shown in the below figures.

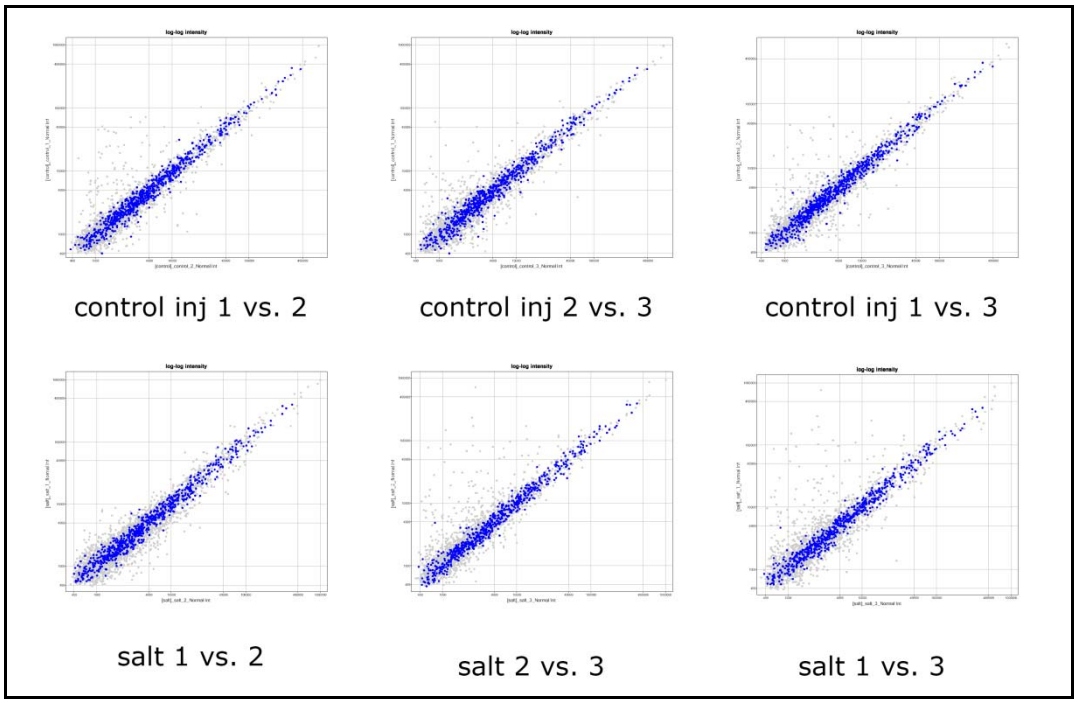

Figure 4.3. Comparing Injection Intensity Reproducibilities of Control and Salt

All of these analysis, totally 288 proteins were identified by Synapt High Definition Mass Spectrometry (HDMS) System. 152 proteins were non-regulated proteins so nonidentified and. 19 proteins were up-regulated, 93 proteins were down-regulated. 18 proteins were unique to control sample and 6 proteins were unique to salt sample. Schematic representation of the results was shown in Figure 4.4.

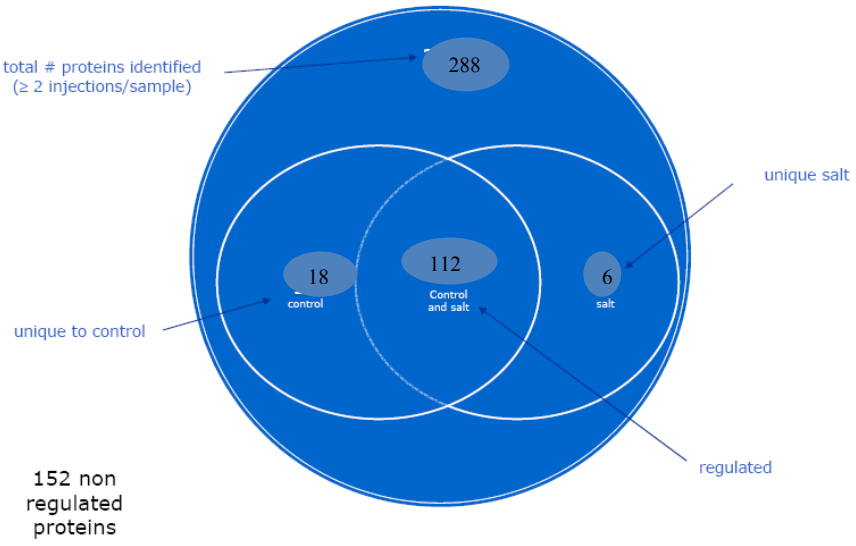

Figure 4.4. Schematic Representation of The Results

The found proteins are shown in the tables. Except their name (they were given by Water Corporation), other informations were found from The ExPASy (**Ex**pert **P**rotein **A**nalysis **Sy**stem) that proteomics server of the Swiss Institute of Bioinformatics (SIB).

In the following table, proteins which were control-responsive in *Beta maritima* were shown. The C is the shorthand notation stands for control leave proteins.

Table 4.1. Control-Responsive Proteins, Identified by LC-MS/MS

Spot No	Protein Name	Sequence	Query ID (gi|NCBI) and Reference Organism	Molecular weight (average) (Da)	Theoretial pI
C 1	Ribulose bisphosphate carboxylase/oxygenase activase,chloroplast precursor	MAASVSTIGAASKAPLSLNNSVAGTSVPSTAFFGKSLKKVYAKGVSSPKVSNRNLRVVAQEVDETKEDRWKGLYDNTSDDQQDIARGKGLVDSLFQAPTGTGTHHAIMNSYEYVSQALKTYQLDNKLDGFYIAPAFMDKLVVHITKNFLTLPNIKVPLILGVWGGKGQGKSFQCELVFRKMGINPIMMSAGELESGNAGEPAKLIRQRYREAAEIIRKGNMCCLFINDLDAGAGRMGGTTQYTVNNQMVNATLMNIADNPTNVQLPGMYNKQENARVPIIVTGNDFSTLYAPLIRDGRMEKFYWAPTREDRIGVCKGIFRTDNVPEEAVVKIVDSFPGQSIDFFGALRARVYDDEVRKWVSGTGIELIGEKLLNSRDGPPTFEQPKMTLEKLLEYGNMLVQEQENVKRVQLAETYLKEAALGDANADAINTGISKNFTNLKSRLNNEEAKKARHVNFQE	10720247 Solanum pennellii	50700.8	8.61
C 2	60S ribosomal protein L23a-1	MSPAKVDTTKKADPKAKALKAAKAVKSGQAFKKKDKKIRTKVTFHRPKTLTKPRTGKYPKISATPRNKLDHYQILKYPLTTESAMKKIEDNNTLVFIVDIRADKKKIKDAVKKMYDIQTKKVNTLIRPDGTKKAYVRLTPDYDALDVANKIGII	73914091 Arabidopsis thaliana	17440.68	10.20
C 3	Photosystem I reaction center subunit VI-2, chloroplast precursor	MASFATIAAVQPSAAVKGLGGSSLAGAKLFIKPSRQSFKTKSTRAGAVVAKYGDKSVYFDLEDLGNTTGQWDVYGSDAPSPYNPLQSKFFETFAAPFTKRGLLLKFLILGGGSLLTYVSANSTGDVLPIKRGPQEPPKLGPRGKL	17369623 Arabidopsis thaliana	15273.57	9.90
C 4	Ribulose bisphosphate carboxylase/oxygenase activase 2, chloroplast precursor	MATSVSTIGAANKAPLSLNNSVAGTSVPSTAFFGKTLKKVYGKGVSSPKVTNRSLRIAAEEKDADPKKQTYSDRWKGLVQDFSDDQQDIARGKGMVDSLFQAPTGTGTHHAVLQSYEYVSQGLRQYNMDNTLDGFYIAPSFMDKLVVHITKNFLKLPNIKVPLILGVWGGKG	12643758 Nicotiana tabacum	48343.09	8.14

(cont. on next page)

Table 4.1. Control-Responsive Proteins, Identified by LC-MS/MS (cont.)

		QGKSFQCELVFRKMGINPIMMS AGELESGNAGEPAKLIRQRYRE AAEIIRKGNICCLFINDLDAGAG RMGGTTQYTVNNQMVNATLM NIADNPTNVQLPGMYNKQENA RVPIIVTGNDFSTLYAPLIR DGRMEKFYWAPTREDRIGVCK GIFRTDNVPEEAVIKIVDTFPGQ SIDFFGALRARVYDDEVRKWV SGTGIEAIGDKLLNSFDGPPTFE QPKMTVEKLLEYGNMLVQEQE NVKRVQLAETYLKEAALGDAN ADAINTGNF			
C 5	60S ribosomal protein L23a	MSPAKVDVTKKSDAKAQALKT AKAVKSGTTKFKKVKKIRTSVT FHRPRTLTKDRNPKYPRISATPR NKLDQYQILKYPLTTESAMKKI EDNNTLVFIVDIRANKKKIKDA VKKMYDIQTKKVNTLIRPDGT KKAYVRLTPDYDALDVANKIGI I	49036456 Daucus carota	17521.67	10.27
C 6	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRNRL VNMLVNRILKHGKKSLAYQIIY RAMKKIQQKTETNPLSVLRQAI RGVTPDIAVKARRVGGSTHQV PVEIGSTQGKALAIRWLLGASR KRPGRNMAFKLSSELVDAAKG SGDAIRKKEETHRMAEANRAF AHFR	134002 Glycine max	17361.22	11.28
C 7	60S ribosomal protein L12	MPPKFDPSQVVDVYVRVTGGE VGAASSLAPKIGPLGLSPKKIGE DIAKETANDWKGLRVTVKLTV QNRQAKVSVVPSAAALVIKAL KEPERDRKKTKNIKHSGHISLD DVIEIAKIMKHRSMAKELAGTV KEILRTCVSVGCTVDGKDPKDL QQEIADGDVEIPLD	6094002 Prunus armeniaca	17882.79	9.02
C 8	Photosystem I reaction center subunit VI, chloroplast precursor	MASFATIAAVQPYSAVKGLGG SSLTGAKLFIKPSRQSFKPKSTR AGAVVAKYGDKSVYFDLEDLG NTTGQWDLYGSDAPSPYNPLQ SKFFETFAAPFTKRGLLLKFLIL GGGSLLTYVSASSTGDVLPIKR GPQEKPKLGPRGKL	3914442 Brassica rapa	15409.77	9.91
C 9	Chloroplast 30S ribosomal protein S7	MSRRGTTEEKTAKSDPIYRNRL VNMLVNRILKHGKKSLAYQIIY RALKKIQQKTEKNPLSVLRQAI RGVTPDIAVKARRVGGSTHQV PIEIGSAQGKALAVRWLLGASR KRPGRNMAFKLSSELVDAAKG SGDAIRKKEETHRMAEANRAF AHFR	122166133 Citrus sinensis	17370.26	11.29
C 10	60S ribosomal protein L23a	MAPAKADPSKKSDPKAQAAKV AKAVKSGSTLKKKSQKIRTKVT FHRPKTLKKDRNPKYPRISAPG RNKLDQYGILKYPLTTESAMK KIEDNNTLVFIVDIKADKKKIKD AVKKMYDIQTKKVNTLIRPDG TKKAYVRLTPDYDALDVANKI GII	585876 Nicotiana tabacum	17281.41	10.18
C 11	Chloroplast 30S ribosomal protein S7	MSRRGTAKGKTAKYDPIYRNR LVNMLVNRILKHGKKALAYKI LYGAVKKIQQNTKTNPLSILRQ AIRGVTPDIAVKARRKSGSTRQ VPIEIGSTQGKTLAIRWLLGASR KRPGQNMAFKLSSELVDAAKG RGGAIRKKEETIKMAEANRAFA HFR	62287273 Sagittaria latifolia	17269.37	11.57

(cont. on next page)

Table 4.1. Control-Responsive Proteins, Identified by LC-MS/MS (cont.)

C 12	Photosystem I reaction center subunit VI, chloroplast precursor	MASLAAVSVKPVAIKGLAGSSI SGRKLAVARPSARSIRRPRAAA VVAKYGDKSVYFDLDDIGNTT GQWDLYGSDAPSPYNPLQSKFF ETFAAPFTKRGLLLKFLLLGGG SLLAYVSASASPDLLPIKKGPQE PPQPGPRGKI	3914465 Zea mays	14929.30	10.10
C 13	Ribulose bisphosphate carboxylase/oxygen ase activase, chloroplast precursor	MATAVSTIGSVNRAPPNLNGSS SSASVPSSTFLGSSLKKVNSRFT NSKVSSGSLRIVASVDEDKQTD KDRWKGLAFDTSDDQQDITRG KGKVDSLFQAPQGSGTHFAIMS SYEYISTGLRQYNFDNNMDGY YIAPAFMDKLVVHITKNFMTLP NMKVPLILGIWGGKGQGKSFQ CELVFAKMRISPIMMSAGELES GNAGEPAKLIRQRYREAADIIR KGKMCALFINDLDAGAGRLGG TTQYTVNNQMVNATLMNIADN PTNVQLPGMYNKEENPRVPIIV TGNDFSTLYAPLIRDGRMEKFY WAPTREDRIGVCIGIFRSDNVA KEDIVKLVDTFPGQSIDFFGALR ARVYDDEVRKWITGVGVDSIG KKLVNSKEGPPTFEQPKMTIEK LLEYGNMLVQEQENVKRVQLA DKYLSEAALGDANSDAMNTGT FYG	3914605 Malus domestica	48076.64	8.20
C 14	Ribulose bisphosphate carboxylase/oxygen ase activase 1, chloroplast precursor	MATSVSTIGAVNKTPLSLNNSV AGTSVPSTAFFGKTLKKVYGK GVSSPKVTNKSLRIVAEQIDVD PKKQTDSDRWKGLVQDFSDDQ QDITRGKGMVDSLFQAPTGTGT HHAVLQSYEYVSQGLRQYNLD NKLDGFYIAPAFMDKLVVHITK NFLKLPNIKVPLILGIWGGKGQ GKSFQCELVFRKMGINPIMMSA GELESGNAGEPAKLIRQRYREA AEIIRKGNMCCLFINDLDAGAG RMGGTTQYTVNNQMVNATLM NIADNPTNVQLPGMYNKQENA RVPIIVTGNDFSTLYAPLIRDGR MEKFYWAPTREDRIGVCTGIFR TDNVPAEDVVKIVDNFPGQSID FFGALRARVYDDEVRKWVSGT GIEKIGDKLLNSFDGPPTFEQPK MTIEKLLEYGNMLVQEQENVK RVQLADKYLKEAALGDANAD AINNGSFFAS	12643757 Nicotiana tabacum	48753.64	8.43
C 15	60S ribosomal protein L23a-2	MSPAKVDVTKKADPKAKALK AAKAVKSGQIVKKPAKKIRTK VTFHRPKTLTVPRKPKYPKISAT PRNKLDHYQILKYPLTTESAMK KIEDNNTLVFIVDIRADKKKIKD AVKKMYDIQTKKVNTLIRPDG TKKAYVRLTPDYDALDVANKI GII	73914092 Arabidopsis thaliana	17395.77	10.23
C 16	Photosystem I reaction center subunit VI-1, chloroplast precursor	MASLATVAAVKPSAAIKGLGG SSLAGAKLSIKPSRLSFKPKSIR ANGVVAKYGDKSVYFDLEDLG NTTGQWDVYGSDAPSPYNPLQ SKFFETFAAPFTKRGLLLKFLIL GGGSLLTYVSATSTGEVLPIKR GPQEPPKLGPRGKL	20143886 Arabidopsis thaliana	15216.65	9.95

(cont. on next page)

Table 4.1. Control-Responsive Proteins, Identified by LC-MS/MS (cont.)

C 17	Photosystem I reaction center subunit VI, chloroplast precursor	MASLATLAAVQPTTLKGLAGS SIAGTKLHIKPARQSFKLNNVRS GAIVAKYGDKSVYFDLEDIANT TGQWDVYGSDAPSPYNSLQSK FFETFAAPFTKRGLLLKFLILGG GSLLTYVSANAPQDVLPITRGP QQPPKLGPRGKI	131199 Spinacia oleracea	15324.66	9.89
C 18	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRNRL VNMLVNRILKHGKKSLAYQIIY RAVKKIQQKTETNPLSVLRQAI RGVTPDIAVKSRRVGGSTHQVP VEIGSTQGKALAIRWLLGASRK RPGRNMAFKLSSELVDAAKGS GDAIRKKEETHRMAEANRAFA HFR	17367684 Ceratophyllu m demersum	17345.16	11.28

In the following table, proteins which were unchanged in *Beta maritima* under salt stress were shown in Table 4.2.

Table 4.2. Unchanged proteins, Identified by LC-MS/MS

Protein No	**Protein Name**	**Molecular weight (average) (Da)**	**pI (pH)**	**Reference Organism**	**Peptides**	**Coverage (%)**
1	Calmodulin	16836	3,9106	Daucus carota	6	43,6242
2	Chlorophyll a b binding protein 1 chloroplast Fragments	6166	5,3846	Populus euphratica	1	18,8679
3	Cytochrome c	12029	10,025	Abutilon theophrasti	1	7,2072
4	Phosphoprotein	28777	4,8347	Daucus carota	1	2,7132
5	Glycine cleavage system H protein mitochondrial precursor Fragment	16185	5,1398	Flaveria pubescens	1	13,1579

(cont. on next page)

Table 4.2. Unchanged proteins, Identified by LC-MS/MS (cont.)

6	Glutathione S transferase 103 1A	25983	5,2934	Arabidopsis thaliana	1	3,5714
7	Histone H2A	16417	11,029	Zea mays	2	24,5283
8	Histone H2A variant 1	14532	10,7802	Arabidopsis thaliana	1	6,6176
9	Histone H2 B5	13958	10,5198	Arabidopsis thaliana	1	7,1429
10	Adenylate kinase 2	27318	7,1743	Arabidopsis thaliana	2	8,0645
11	Glycogen phosphorylase muscle form Myophosphorylase	97096	6,7941	Oryctolagus cuniculus Rabbit	7	9,1449
12	Peroxidase 67 precursor	34685	10,155	Arabidopsis thaliana	1	2,5316
13	Peroxiredoxin Q chloroplast precursor Fragment	20638	9,9115	Sedum lineare	4	19,8925
14	Peroxiredoxin Q chloroplast precursor	23574	9,7502	Suaeda salsa	3	20,5607
15	Wound induced basic protein	5447	10,0331	Phaseolus vulgaris	1	23,4043
16	Proteasome subunit alpha type 6	27374	5,7874	Glycine max	1	8,5366
17	Photosystem I reaction center subunit II chloroplast precursor	21328	10,7267	Chlamydomonas reinhardtii	1	7,6531

(cont. on next page)

Table 4.2. Unchanged proteins, Identified by LC-MS/MS (cont.)

18	Photosystem I reaction center subunit VI chloroplast precursor	14920	10,4707	Zea mays	1	7,7465
19	Photosystem I reaction center subunit N chloroplast precursor	18417	9,2411	Arabidopsis thaliana	4	16,3743
20	Photosystem I reaction center subunit N chloroplast precursor Fragment	12613	8,2571	Zea mays	4	29,4643
21	Oxygen evolving enhancer protein 1 chloroplast precursor	34847	6,2369	Fritillaria agrestis	5	10,3343
22	Oxygen evolving enhancer protein 1 chloroplast precursor	35148	5,3919	Spinacia oleracea	4	27,7108
23	Oxygen evolving enhancer protein 1 chloroplast precursor	35206	5,6975	Nicotiana tabacum	4	20,4819
24	Oxygen evolving enhancer protein 2 1 chloroplast precursor	28077	7,2198	Arabidopsis thaliana	1	5,3232
25	Oxygen evolving enhancer protein 3 chloroplast precursor	24826	10,0172	Onobrychis viciifolia	1	6,0606
26	Oxygen evolving enhancer protein 3 2 chloroplast precursor	22829	9,618	Zea mays	2	5,6338
27	U box domain containing protein 51	90166	6,3038	Arabidopsis thaliana	1	1,005

(cont. on next page)

Table 4.2. Unchanged proteins, Identified by LC-MS/MS (cont.)

28	Cytochrome b c1 complex subunit 7	14461	9,6313	Solanum tuberosum	3	18,6992
29	60S ribosomal protein L23a 1	17429	10,6384	Arabidopsis thaliana	1	8,4416
30	50S ribosomal protein L12 chloroplast precursor	19921	5,3558	Spinacia oleracea	5	25,9259
31	60S ribosomal protein L40	6118	11,0817	Brassica rapa	1	21,1538
32	40S ribosomal protein S28 1	7365	11,2582	Arabidopsis thaliana	1	18,75
33	Superoxide dismutase Cu Zn chloroplast Fragment	14426	5,0299	Pinus sylvestris	2	19,8582
34	Thylakoid lumenal 15 kDa protein 1 chloroplast precursor	23763	7,6291	Arabidopsis thaliana	1	4,9107
35	Thioredoxin M type chloroplast precursor	19827	8,2504	Spinacia oleracea	2	11,0497
36	Cytochrome b6 f complex iron sulfur subunit 1 chloroplast precursor	24136	7,5954	Nicotiana tabacum	1	6,1404

In the following table, proteins which were down-regulated in *Beta maritima* under salt stress were shown. The DR is shorthand notation stands for down-regulated proteins.

Table 4.3. Down-Regulated Proteins, Identified by LC-MS/MS

Spot No	Protein Name	Sequence	QueryID (gi | NCBI) and Reference Organism	Molecular weight (average) (Da)	Theoretial pI
DR 1	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH KMAXANRAFAHFR	62287250 Aristolochia macrophylla	17297.4	11.31
DR 2	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTPKSDPIYRNR LVNMLVNRIMKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	62287228 Ananas comosus	17387.3	11.28
DR 3	Photosystem I iron-sulfur center -	MSHSVKIYDTCIGCTQCVRA CPTDVLEMIPWDGCKAKQIA SAPRTEDCVGCKRCESACPT DFLSVRVYLWHETTRSMGLG Y	131160 Spinacia oleracea	9024.4	6.68
DR 4	Oxygen-evolving enhancer protein 1, chloroplast precursor	SSDGTVKFEEKDGIDYAAVT VQLPGGERVPFLFTIKQLVAS GKPESFSGEFLVPSYRGSSFL DPKGRGGSTGYDNAVALPAG GRGDEEELQKENVKNTSSST GKITLSVTQSKPETGEVIGVFE SIQPSDTDLGAKVPKDVKIQG IWYAQLE	11134054 Nicotiana tabacum	35227.8	5.89
DR 5	Chloroplast 30S ribosomal protein S7	MSRRGAAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDITVKARRVGG STHQVPIEIGSTQGKALAIRW LLAASRKRPGRNMAFKLSSE LVDAAKGSGDAIRKKEETHR MAEANRAFAHFR	122153665 Coffea arabica	17357.2	11.28
DR 6	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QILYRAMKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	62287247 Euonymus alatus	17375.2	11.28
DR 7	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRALKKIQQKTETNPLSVL RQAIRGVTPDIAVKARRVGG STHQVPIEIGSTQGKALAIRW LLGASRKRPGRNMAFKLSSE LVDAAKGSGDAIRKKEETHR MAEANRAFAHFR	48429101 Arabidopsis thaliana	17357.2	11.28
DR 8	Photosystem I iron-sulfur center	MSHSVKIYDTCIGCTQCVRA CPTDVLEMIPWDGCKAKQIA SAPRTEDCVGCKRCESACPDF LSVRVYLWHETTRSMGLAY	150403927 Aethionema	9038.5	6.68

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Table 4.3. Down-Regulated Proteins, Identified by LC-MS/MS (cont.)

DR 9	Oxygen-evolving enhancer protein 3-1, chloroplast precursor	MASMGGLHGASPAVLEGSLK INGSSRLNGSGRVAVAQRSRL VVRAQQSEETSRRSVIGLVAA GLAGGSFVQAVLADAISIKVG PPPAPSGGLPAGTDNSDQAR DFALALKDRFYLQPLPPTEAA ARAKESAKDIINVKPLIDRKA WPYVQNDLRSKASYLRYDL NTIISSKPKDEKKSLKDLTTKL FDTIDNLDYAAKKKSPSQAE KYYAETVSALNEVLAKLG	193806375 Arabidopsis thaliana	23866.2	9.64
DR 10	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAMKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMVFKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	122166753 Morus indica	17403.3	11.28
DR 11	Chloroplast 30S ribosomal protein S7	MSRRGTTEEKTAKSDPIYRNR LVNMLVNRILKHGKKSLAYQ IIYRAVKKIQQKTETNPLSVL RQAIRGVTPDIAVKARRVGG STHQVPIEIGSTQGKALAIRW LLWASRKRPGRNMAFKLSSE LVDAAKGSGDAIRKKEETHR MAEANRAFAHFR	24638192 Saururus cernuus	17502.4	11.28
DR 12	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMALKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	62287241 Saruma henryi	17309.2	11.28
DR 13	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLVASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	62287224 Asparagus officinalis	17385.3	11.28
DR 14	Plastid 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKALAY QIIYRAMKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLAASRKRPGRDMAFKLSS ELVDAAKGSGDAIRKKEETH KMAEANRAFAHFR	24638189 Lathraea clandestina	17346.2	11.03
DR 15	Photosystem I iron-sulfur center	MSHSVKIYDTCIGCTQCVRA CPTDVLEMIPWDGCKAKQIA SAPRTEDCVGCKRCESACPDF LSVRVYLWHETTRSMGLSY	122245679 Phalaenopsis aphrodite subsp. formosana	9054.5	6.68
DR 16	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRK RPGRNMAFKLSSELVDAAKG SGDAIRKKEETHRMAEANRA FAHFR	62288949 Cercidiphyllum	17343.2	11.28

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Table 4.3. Down-Regulated Proteins, Identified by LC-MS/MS (cont.)

DR 17	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTEANPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	62287272 Schisandra chinensis	17313.2	11.28
DR 18	Chloroplast 30S ribosomal protein S7	MSRRGTVEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QILYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGAARKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	24638196 Beta vulgaris	17355.2	11.28
DR 19	Glycine cleavage system H protein, mitochondrial precursor	MALRLWASSAANALKISCSG ATRAAPAYSISRYFSTVLDGL KYSSSHEWVKNDGSVATIGIT DHAQGHLGEVVFVELPEAGA KVSQGGAFGNVESVKATSDI NSPISGEVVEVNDKLSETPGLI NSSPYEDGWMIKVKPSSPSEL DALLDPAKYTKHCEEEDAH	152032493 Oryza sativa subsp. indica	17367.3	4.92
DR 20	Photosystem I reaction center subunit II, chloroplast precursor	MAMATQASLFTPPLSVPKSTT APWKQSLVSFSTPKQLKSTVS VTRPIRAMAEEAPAATEEKPA PAGFTPPQLDPNTPSPIFGGST GGLLRKAQVEEFYVITWESP KEQIFEMPTGGAAIMRQGPN LLKLARKEQCLALGTRLRSK YKINYQFYRVFPNGEVQYLH PKDGVYPEKVNPGREGVGQN FRSIGKNKSAIEVKFTGKQVY DI	131166 Solanum lycopersicum	22918.4	9.71
DR 21	Chloroplast 30S ribosomal protein S7	MSRRGTAEKKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDITVKARRVGG STHQVPIEIGSTQGKALAIRW LLAASRK RPGRNMAFKLSSELVDAAKG SGDAIRKKEETHRMAEANRA FAHFR	51338627 Atropa belladonna	17386.3	11.41
DR 22	Oxygen-evolving enhancer protein 1-1, chloroplast precursor	MAASLQSTATFLQSAKIATAP SRGSSHLRSTQAVGKSFGLET SSARLTCSFQSDFKDFTGKCS DAVKIAGFALATSALVVSGA SAEGAPKRLTYDEIQSKTYM EVKGTGTANQCPTIDGGSETF SFKPGKYAGKKFCFEPTSFTV KADSVSKNAPPEFQNTKLMT RLTYTLDEIEGPFEVASDGSV NFKEEDGIDYAAVTVQLPGG ERVPFLFTVKQLDASGKPDSF TGKFLVPSYRGSSFLDPKGRG GSTGYDNAVALPAGGRGDEE ELVKENVKNTAASVGEITLK VTKSKPETGEVI 300GVFESLQPSDTDLGAKVP KDVKIQGVWYGQLE	19883896 Arabidopsis thaliana	35142.4	5.55
DR 23	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRALKKIQQKTETNPLSVL RQAIRGVTPDIAVKSRRVGGS THQVPIEIGSTQGKALAIRWL	24638195 Acorus calamus	17359.2	11.17

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Table 4.3. Down-Regulated Proteins, Identified by LC-MS/MS (cont.)

		LAASRK RPGRNMAFKLSSELVDAAKG SGDAIRKKEETHKMAEANRA FAHFR			
DR 24	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QILYRAMKKIQQKTETNPLSV LHQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRK RPGRNMVFKLSSELVDAAKG SGDAIRKKEETHRMAEANRA FAHFR	122247653 Populus alba	17384.3	11.16
DR 25	Glycine cleavage system H protein, mitochondrial precursor	MALRMWASSTANALRLSSAT RPHFSPLSRCFSSVLDGLKYA NSHEWVKHEGSVATIGITDH AQDHLGEVVFVDLPEAGGSV TKATGFGAVESVKATSDVNS PISGEIVEV NSKLSETPGLINSSPYEDGWM IKVKPSNPSELDSLMGAKEYT KFCEEEDAAH	2499417 Flaveria anomala	17354.4	5.04
DR 26	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRIMKHGKKSLA YQIIYRAVKKIQQKTETNPLS VLRQAIRGVTPDIAVKARRV GGSTHQVPIEIGSTQGKALAI RWLLGASRKRPGRNMAFKLS SELVDAAKGSGDAIRKKEET HRMAEANRAFAHF	62287244 Nelumbo lutea	17361.2	11.28
DR 27	Photosystem I iron-sulfur center	MSHSVKIYDTCIGCTQCVRA CPTDVLEMIPWDGCKAKQIA SAPRTEDCVGCKRCESACPT DFLSVRVYLWHETTRSMGIA Y	122244026 Helianthus annuus	9038.5	6.68
DR 28	Photosystem I iron-sulfur center	MAHSVKIYDTCIGCTQCVRA CPTDVLEMIPWEGCKAKQIA SAPRTEDCVGCKRCESACPT DFLSVRVYLWHETTRSMGLA Y	172048631 Cycas taitungensis	9036.5	6.68
DR 29	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QILYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLAASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	62287248 Cornus mas	17357.2	11.28
DR 30	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIHGVTPGIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLAASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKREETH RMAEANRAFAHFR	122248117 Helianthus annuus	17308.2	11.40
DR 31	Plastid 30S ribosomal protein S7	MSRRGTAEEKTAKPDPIYWN RLVNMLVNRILKHGKKSLAY QIIYRALKKIQQKTEKNPLYV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAVR WLLVASKKRPGQNMAFKLSS ELVDAAKGSGDAIRKKEETH KMAEASRAFAHLR	266979 Epifagus virginiana	17383.4	10.70

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Table 4.3. Down-Regulated Proteins, Identified by LC-MS/MS (cont.)

DR 32	Glycine cleavage system H protein, mitochondrial precursor	MALRIWASSTANALRLSSAT RPHFSPLSRCFSSVLDGLKYA NSHEWVKHEGSVATIGITDH AQDHLGEVVFVDLPEAGGSV TKATGFGAVESVKATSDVNS PISGEIVEVNSKLSETPGLINSS PYEDGWMIKVKPSNPSELDS LMGAKEYTKFCEEEDSAH	1346118 Flaveria pringlei	17352.3	5.04
DR 33	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYQAVKKMQQKTETNPLS VLRQAIRGVTPDIAVKARRV GGSTHQVPIEIGSTQGKALAI RWLLGASRKRPGRNMAFKLS SELVDAAKGSGDAIRKKEET HRMAEANRAFAHFR	68052921 Allium textile	17333.2	11.16
DR 34	Photosystem I iron-sulfur center	MAHSVKIYDTCIGCTQCVRA CPTDVLEMIPWEGCKAKQIA SAPRTEDCAGCKRCESACPT DFLSVRVYLWHETTRSMGLA Y	1172660 Pinus thunbergii	9008.4	6.68
DR 35	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QILYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	62287274 Magnolia stellata	17343.2	11.28
DR 36	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRALKKIQQKTETNPLSVL RQAIRGVTPDIAVKARRVGG STHQVPIEIGSTQGKALAIRW LLGASRKRPGRNMAFKLSSE LVDAAKGNGDAIRKKEETHR MAEANRAFAHFR	17367676 Amborella trichopoda	17384.2	11.28
DR 37	Oxygen-evolving enhancer protein 1, chloroplast precursor	MAASLQAAATVMPAKIGGR ASSARPSSHVARAFGVDAGA RITCSLQSDIREVASKCADAA KMAGFALATSALLVSGATAE GAPKRLTFDEIQSKTYMEVK GTGTANQCPTIDGGVDSFPFK AGKYEMKKFCLEPTSFTVKA EGIQKNEPPRFQKTKLMTRLT YTLDEMEGPLEVRRRRTLKF EEKDGIDYAAVTVQLPGGER VAFLFTVKQLVATGKPESFRP FLVPSYRGSSFLDPKGRGGST GYDNAGALPRGGRGDEEELA KENVKNASSSTGNITLSVTKS KPETGEVIGVFESVQ 300PSDTDLEAPKDVKIQGVW YAQLESN	131388 Triticum aestivum	34740.4	8.73
DR 38	Oxygen-evolving enhancer protein 3-2, chloroplast precursor	MAQAMASMTGLSQGVCPAA ADSRTRTAVVVVRASAEGDR CAGGPRCDRLVATASSPPLSQ AVHAETVKTIKIGAPPPPSGG LPGTLNSDQTRDFDLPLKERF YLQPLPPAEAVARVKTSAQDI INLKPLIDKKAWPYVQNDLR LRASYLRYDLKTVIASKPKEE KKSLKELTGKLFSTIDDLDHA AKMKSTPEAEKYFAATKDAL GDVLAKLG	11134066 Zea mays	22843.3	9.30

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Table 4.3. Down-Regulated Proteins, Identified by LC-MS/MS (cont.)

DR 39	Oxygen-evolving enhancer protein 3-2, chloroplast precursor	MAQAVTSMAGLRGASQAVL EGSLQINGSNRLNISRVSVGS QRTGLVIRAQQNVSVPESSRR SVIGLVAAGLAGGSFVKAVF AEAIPIKVGGPPLPSGGLPGTD NSDQARDFSLALKDRFYIQPL SPTEAAARAKDSAKEIINVKS FIDKKAWPYVQNDLRLRASY LRYDLNTVISAKPKEEKQSLK DLTAKLFQTIDNLDYAARSKS SPDAEKYYSETVSSLNNVLA KLG	18206249 Arabidopsis thaliana	24643.0	9.72
DR 40	Oxygen-evolving enhancer protein 1-2, chloroplast precursor	MATSLQAAATFLQPAKIAASP SRNVHLRSNQTVGKSFGLDS SQARLTCSLHSDLKDFAGKC SDAAKIAGFALATSALVVSG AGAEGAPKRLTYDEIQSKTY MEVKGTGTANQCPTIDGGSE TFSFKAGKYTGKKFCFEPTSF TVKADSVSKNAPPDFQNTKL MTRLTYTLDEIEGPFEVGSDG SVKFKEEDGIDYAAVTVQLP GGERVPFLFTVKQLEASGKPE SFSGKFLVPSYRGSSFLDPKG RGGSTGYDNAVALPAGGRG DEEELSKENVKNTAASVGEIT LKITKSKPETGEVIG VFESLQPSDTDLGAKVPKDV KIQGVWYGQIE	11134146 Arabidopsis thaliana	35019.3	5.92
DR 41	Photosystem I iron-sulfur center	MSHSVKIYDTCIGCTQCVRA CPTDVLEMIPWDGCKAKQIA PAPRTEDCVGCKRCESACPT DFLSVRVYLWHETTRSMGLA Y	122239936 Liriodendron tulipifera	9048.5	6.68
DR 42	Oxygen-evolving enhancer protein 1, chloroplast precursor	MASSLQAAATLIPAKVGAPA RTHLRSNSHLSKAFGFDNSTA GRLTCSINSDLRDIAQKCTDA AKLAGFALATSALVISGASAE GVPKRLTFDEIQSKTYMEVK GSGTANQCPTIEGGTESFGYK TGKYTLKKLCLEPTSFTVKAE GINKNAPPEFQKTKLMTRLT YTLDEIEGPFEVAPDGTVKFE EKDGIDYAAVTVQLPGGERV PFLFTVKQLVATGKPESFSGS YLVPSYRGSSFLDPKGRGGSA GYDNAVALPAGGRGDEEELV KENIKDVSSSTGKITLSVTKS KPETGEVIGVFESIQPSDTDLG SKAPKDVKIQGIWYAQLE	11133881 Fritillaria agrestis	34869.4	6.26
DR 43	Oxygen-evolving enhancer protein 3-1, chloroplast precursor	MAQAMASMTGLSQGVLPSR RADSRTRTAVVIVRASAEGD AVAQAGRRAVIGLVATGIVG GALSQAARAETVKTIKIGAPP PPSGGLPGTLNSDQARDFDLP LKERFYLQPLPPAEAAARVK TSAQDIINLKPLIDKKAWPYV QNDLRLRASYLRYDLKTVIA SKPKEEKKSLKELTGKLFSTI DDLDHAAKIKSTPEAEKYFA ATKDALGDVLAKLG	11134057 Zea mays	23132.7	9.77
DR 44	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLAASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	122246293 Daucus carota	17357.2	11.28

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Table 4.3. Down-Regulated Proteins, Identified by LC-MS/MS (cont.)

DR 45	Chloroplast 30S ribosomal protein S7	MSRRGTTEEKTAKSDPIYRNR LVNMLVNRILKHGKKSLAYQ IIYRAVKKIQQKTETNPLSVL RQAIHGVTPDIAVKARRVGG STHQVPIEIGSTQGKALAIRW LLWASRKRPGRNMAFKLSSE LVDAAKGSGDAIRKKEETHR MAEANRAFAHFR	122164309 Piper cenocladum	17483.3	11.16
DR 46	Chloroplast 30S ribosomal protein S7 Silene latifolia	MSRRGTVEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGAARKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	68052866 Silene latifolia	17355.2	11.28
DR 47	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVTMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	17367679 Trochodendron aralioides	17330.2	11.28
DR 48	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRSVKKIQQKTETNPLSVL RQAIRGVTPDIAVKARRVGG STHQVPIEIGSTQGKALAIRW LLGASRKRPGRNMAFKLSSE LVDAAKGSGDAIRKKEETHR MAEANRAFAHFR	122246608 Vitis vinifera	17359.2	11.28
DR 49	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRALKKIQQKTETNPLSVL RQAICGVTPDIAVKARRVGG STHQVPIEIGSTQGKALAIRW LLWASRKRPGRNMAFKLSSE LVDAAKGSGDAIRKKEETHR MAEANRAFAHFR	62287291 Hydrastis canadensis	17433.	11.02
DR 50	Oxygen-evolving enhancer protein 1, chloroplast precursor	MAASLQAAATLMQPTKLRSN TLQLKSNQSVSKAFGLEHYG AKVTCSLQSDFKELAHKCVE ASKIAGFALATSALVVSGASA EGAPKRLTFDEIQSKTYLEVK GTGTANQCPTIDGGVDSFSFK PGKYNAKKLCLEPTSFTVKSE GVTKNTPLAFQNTKLMTRLT YTLDEIEGPFEVSADGSVKFE EKDGIDYAAVTVQLPGGERV PFLFTIKQLVASGKPDSFSGEF LVPSYRGSSFLDPKGRGASTG YDNAVALPAGGRGDEEELGK ENNKSAASSKGKITLSVTQTK PETGEVIGVFESIQPSDTDLGA KAPKDVKIQGVWYAQLES	131384 Pisum sativum	34893.4	6.25
DR 51	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMAFKLSS ELVDAAKGSGDSIRKKEETH RMAEANRAFAHFR	62287249 Canella winterana	17359.2	11.28
DR 52	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIVYQAVKKIQQKTETNPLSV LRQAICRVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR	62287301 Calycanthus fertilis var	17347.2	11.02

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Table 4.3. Down-Regulated Proteins, Identified by LC-MS/MS (cont.)

		WLLGASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR			
DR 53	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAMKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH RMAEANXAFAHFR	62287276 Gunnera chilensis	17330.4	11.16
DR 54	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKNGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMDFRLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	122249055 Phalaenopsis aphrodite subsp. formosana	17392.2	11.25
DR 55	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMALKLSS ELVDAAKGSGDAIRKKEETH KMAEANRAFAHFR	17367693 Asarum canadense	17281.2	11.17
DR 56	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH KMAEANRAFAHFR	62287229 Butomus umbellatus	17315.2	11.17
DR 57	Chloroplast 30S ribosomal protein S7	MSRRGTVEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QILYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGAARKRPGRNMAFKLSS ELVDAAKGSGDAVRKKEETH RMAEANRAFAHFR	17380275 Spinacia oleracea	17341.2	11.28
DR 58	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRTVKKIQQKTETNPLSVL RQAIRGVTPDIAVKARRVGG STHQVPIEIGSTQGKALAIRW LLGASRKRPGRNMAFKLSSE LVDAAKGSGDAIRKKEETHR MAEANRAFAHFR	24638194 Dioscorea bulbifera	17373.2	11.28
DR 59	Chloroplast 30S ribosomal protein S7	MSRRGTAKEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAMKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLAASRKRPGRNMAFKLSS ELVDAAKGSGEAIRKKEXTH RMAEANRAFAHFR	62287240 Stewartia pseudocamellia	17384.6	11.53
DR 60	Photosystem I iron-sulfur center	MSHSVKIYDTCIGCTQCVRA CPTDVLEMIPWGGCKAKQIA SAPRTEDCVGCKRCESACPT DFLSVRVYLWHETTRSMGLA Y	73620988 Cucumis sativus	8980.4	7.50

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Table 4.3. Down-Regulated Proteins, Identified by LC-MS/MS (cont.)

DR 61	Photosystem I reaction center subunit II, chloroplast precursor	MAMATQASLFTPALSAPKSS APWKQSLASFSPKQLKSTVS APRPIRAMAEEAATKEAEAP VGFTPPQLDPNTPSPIFGGSTG GLLRKAQVEEFYVITWESPKE QIFEMPTGGAAIMREGANLL KLARKEQCLALGTRLRSKYK INYRFYRVFPNGEVQYLHPK DGVYPEKVNAGRQGVGQNF RSIGKNKSPIEVKFTGKQVYD L	131167 Nicotiana sylvestris	22423.8	9.78
DR 62	Chloroplast 30S ribosomal protein S7	MSRRGTVEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLWAARKRPGRNMAFKLS SELVDAAKGSGDAIRKKEET HRMAEANRAFAHFR	62287243 Phytolacca americana	17484.4	11.28
DR 63	Glycine cleavage system H protein, mitochondrial precursor (Fragment)	MALRMWASSTANALRLSSAT RPHYSPLSRCFSSVLDGLKYA NSHEWVKHEGSVATVGITDH AQDHLGEVVFVDLPEAGGSV TKATGFGAVESVKATSDVNS PISGEIVEVNSKLSETPGLINSS PYEDGWMIKVKPSNPSELDS LMGAKEYT	1346119 Flaveria pubescens	16196.1	5.28
DR 64	Chloroplast 30S ribosomal protein S7	MSRRGTAEKKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAMKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	17367635 Lotus japonicus	17374.3	11.41
DR 65	Chloroplast 30S ribosomal protein S7	MSRRGTAEKKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH KMAEANRAFAHFR	68052959 Spathiphyllum wallisii	17314.2	11.32
DR 66	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QILYRAVKKIQQKTETNPLSV LRQAIRGVTPDIVVKARRVG GSTHQVPIEIGSTQGKALAIR WLLVASRKRPGRNMAFKLSS ELVDAAKGGGDAIRKKEETH KMAEANRAFAHFR	24638193 Lactoris fernandeziana	17355.3	11.17
DR 67	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRALKKIQQKTETNPLSVL RQAIRGVTPNIAVKARRVGG STHQVPIEIGSTQGKALAIRW LLGASRKRPGRNMAFKLSSE LVDAAKGGGDAIRKKEETHR MAEANRAFAHFR	62287275 Lilium superbum	17326.2	11.41
DR 68	Oxygen-evolving enhancer protein 1, chloroplast precursor	MAASLQAAATLMQPTKVGG VSARNNLQLRSSQSVSKAFG LEPSASRLSCSLQTDLKDFAQ KCTDAAKIAGFALATSALVV SGANAEGVPKRLTFDEIQSKT YMEVKGTGTANQCPTIDGGV DSFAFKPGKYNAKKFCLEPTS FTVKAEGVSKNSAPDFQKTK LMTRLTYTLDEIEGPFEVSPD	131385 Solanum tuberosum	35388.9	5.84

(cont. on next page)

Table 4.3. Down-Regulated Proteins, Identified by LC-MS/MS (cont.)

		GTVKFEEKDGIDYAAVTVQL PGGERVPFLFTIKQLVASGKP ESFSVDFLVPSYRGSSFLDPK GRGGSTGYDNAVALPAGGR GDEEELQKENVKNTASLTGK ITFTVTKSNPQTGEVIGVFESI QPSDTDLGAKTPKDVKIQGI WYAQLES			
DR 69	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAMKKIQQKTETNPLSV LRQAIRGVTPDIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRK RPGRNMAFKLSSELVDAAKG SGDAIRKKEETHRMAEANRA FAHFR	122249136 Eucalyptus globulus subsp. globulus	17375.2	11.28
DR 70	Chloroplast 30S ribosomal protein S7	MSRRGTAEEKTAKSDPIYRN RLVNMLVNRILKHGKKSLAY QIIYRAVKKIQQKTETNPLSV LRQAIRGVTPNIAVKARRVG GSTHQVPIEIGSTQGKALAIR WLLGASRKRPGRNMAFKLSS ELVDAAKGSGDAIRKKEETH RMAEANRAFAHFR	17367690 Cabomba caroliniana	17342.2	11.41
DR 71	Photosystem I iron-sulfur center	MSHSVKIYDTCIGCTQCVRA CPTDVLEMIPWNGCKAKQIA SAPRTEDCVGCKRCESACPT NFLSVRVYLWHETTRSMGLS Y	172048702 Dioscorea elephantipes	9052.5	8.10
DR 72	Thylakoid lumenal 15	GADFSLANVTK	–	1122.58	–
DR 73	Photosystem I reaction center subunit II, chloroplastic [Precursor]	EQIFEMPTGGAAIMR	–	1305.65	–
DR 74	Phosphoprotein	GLFDFMK	–	857.4	-
DR 75	Oxygen-evolving enhancer protein	AWPYVQNDLRLR	–	1530.8	–
DR 76	Oxygen-evolving enhancer protein	AWPYVQNDLR	–	1261.6	–
DR 77	Oxygen-evolving enhancer protein	TNTDFLPYNGDGFK	–	1588.7	–
DR 78	Oxygen-evolving enhancer protein	NAPPDFQNTK	–	1131.5	–
DR 79	Oxygen-evolving enhancer protein	FCLEPTKFAVK	–	1339.7	–
DR 80	Oxygen-evolving enhancer protein	LTYTLDEMEGPFEVSSDGTV K	–	2318.1	–

(cont. on next page)

Table 4.3. Down-Regulated Proteins, Identified by LC-MS/MS (cont.)

DR 81	Oxygen-evolving enhancer protein	QLVASGKPESFSGEFLVPSYR	–	2281.1	–
DR 82	Oxygen-evolving enhancer protein	LTFDEIQSK	–	1080.6	–
DR 83	Oxygen-evolving enhancer protein	DGIDYAAVTVQLPGGER	–	1760.9	–
DR 84	Oxygen-evolving enhancer protein	VPFLFTVK	–	950.6	–
DR 85	Histone H2B5	LVLPGELAK	–	939.6	–
DR 86	Glycogen phosphorylase	VIFLENYR	–	1053.6	–
DR 87	Glycogen phosphorylase	EIWGVEPSR	–	1072.5	–
DR 88	Glycogen phosphorylase	LLSYVDDEAFIR	–	1440.7	–
DR 89	Glycogen phosphorylase	VFADYEEYVK	–	1262.6	–
DR 90	Glycine cleavage system H	LSETPGLINSSPYEDGWMIK	–	2237.1	–
DR 91	Cytochrome b c1 complex	SYLQEMLALVKR	–	1450.8	–
DR 92	Cytochrome b c1 complex	EALGALPLYQR	–	1230.7	–
DR 93	Calmodulin	EADVDGDGQINYEEFVK	–	1927.9	–

In the following table, proteins which were up-regulated in *Beta maritima* under salt stress were shown. The UR is shorthand notation stands for up-regulated proteins.

Table 4.4. Up-Regulated Proteins, Identified by LC-MS/MS

Spot No	Protein Name	Sequence	Query ID (gi | NCBI) and Reference Organism	Molecular weight (average) (Da)	Theoretical pI
UR1	Oxygen-evolving enhancer protein 1, chloroplast (Fragments)	RLTYDEIQSKAEGINKNSPP DFQKTKLMTRDGIDYAAVT VQLPGGERVPFLFTIKGGST GYDNAVALPAGGRSKPETG EIIGVFESLQPSDTDLGAKTP K	158562857 Populus euphratica	10700	5.36
UR2	Superoxide dismutase [Cu-Zn], chloroplast precursor	MAVNTLLSTAPSRVFLSFPN PSPNPSPQLHSQFHGLSLKL TRQSIPLATAPKPLSVVAVT KKAVAVLKGTSSVEGVVTL SQEDDGPTTVSVRITGLTPG NHGFHLHEFGDTTNGCMST GAHFNPNGMTHGAPEDDV RHAGDLGNIIANAEGVAEA TIVDTQIPLSGPNAVIGRAL VVHELEDDLGKGGHELSLT TGNAGGRLACGVVGLTPI	12230570 Vitis vinifera	21700	5.87
UR3	Oxygen-evolving enhancer protein 1 (Fragments)	LTYDEIQSKGGSTGYDNAV ALPAGGRLTYDEIQSKGGS TGYDNAVALPAGGRLTYD EIQSKGGSTGYDNAVALPA GGR	39932634 Pinus pinaster	2640	4.56
UR4	Wound-induced basic protein	MIYDVNSPLFRSFLSQKGGS SDKRKTEEQKPKEHRPKAS ENKPIMTE	1172597 Phaseolus vulgaris	5400	9.52
UR5	Peroxiredoxin Q, chloroplast precursor	MASISLPKHSLPSLLPTLKPI TSSSQNLPILSKSSQSQFYGL KFSHSTSLSIPSSSSVKNTIF AKVNKGQAPPSFTLKDQDG KTLSLSKFKGKPVVVYFYP ADETPGCTKQACAFRDSYE KFKKAGAEVVGISGDDPSS HKAFAKKYRLPFTLLSDEG NKIRKEWGVPADLFGTLPG RQTYVLDKKGVVQLIYNN QFQPEKHIDETLKLLQSL	75127599 Populus jackii	23400	9.62
UR6	Putative oxygen-evolving enhancer protein 1 (Fragments)	DGIDYAAVTVQLPGGERGG STGYDNAVALPAGGRGSS MLDPKELGQMNIVFEGVSK SYHDTNAENEFVTIKKAVA LVLPSLKASTYYEESLYKVI NTWADIINRALTEAVAAEA AAAEDPEMETMYTK	109892868 Pinus strobus	13600	4.46
		MAAICLPVAKHSFPSLLNTQ TPKPLFSQNLHTIPLSSQSQI			

(cont. on next page)

Table 4.4. Up-Regulated Proteins, Identified by LC-MS/MS (cont.)

UR7	Peroxiredoxin Q, chloroplast precursor	CGLKFLISSPSSLPPPPSYSARISVFAKVSKGSVPPQFTLKDQDGKNVSLTEFKGKPVVVYFYPADETPGCTKQACAFRDSYEKFKKAGAEVIGISGDDPSSHKAFAKKYRLPYTLLSDEGNKIRREWGVPADLFGTLPGRQTYVLDKNGTVQLIYNNQFQPEKHIDETLKFLQSA	75138338 Gentiana triflora	23900	9.27
UR8	Peroxiredoxin Q, chloroplast precursor (Fragment)	QTLQTSSQSQFHGLKFSHASSFKSPSAPLRKNSIFAKVTKGSTPPPFTLKDQEGRPVSLSKFKGKPVVVYFYPADETPGCTKQACAFRDSYEKFKKAGAEVVGISGDSSESHKAFAKKYKLPFTLLSDEGNKVRKEWGVPSDLFGTLPGRETYVLDKNGVVQLVYNNQFQPEKHIDETLKLLQSLK	75336180 Sedum lineare	20651.5	9.54
UR9	Oxygen-evolving enhancer protein 1, chloroplast precursor	MAASLQAAATLMQPTKVGVAPARNNLQLRSAQSVSKAFGVEPAAARLTCSLQTELKDLAQKCTDAAKIAGFALATSALVVSGANAEGVPKRLTYDEIQSKTYMEVKGTGTANQCPTIEGGVGSFAFKPGKYTAKKFCLEPTSFTVKAEGVSKNSAPDFQKTKLMTRLTYTLDEIEGPFEVSPDGTVKFEEKDGIDYAAVTVQLPGGERVPFLFTIKQLVASGKPESFSGEFLVPSYRGSSFLDPKGRGGSTGYDNAVALPAGGRGDEEELQKENVKNTASLTGKITLSVTQSKPETGEVIGVFESIQPSDTDLGAKVPKDVKIQGIWYAQLE	12644171 Solanum lycopersicum	34947.6	5.91
UR10	Peroxiredoxin Q, chloroplast precursor	MATLSLPNHSPTFALPSQTPKPHSSQNLSIISKSAHSQFCGIKLSHSSSLSPPLYPRSYKASIVAKVSEGSMPPAFTLKDQDGKNVSLSKFKGKPVVVYFYPADETPGCTKQACAFRDSYEKFKKAGAEVIGISGDDSSSHKAFKQKYKLPYTLLSDEGNKVRKDWGVPSDLFGALPGRQTYVLDRNGVVRLVYNNQFQPEKHIDETLKFLQSL	75324751 Suaeda salsa	23600	9.45
UR11	Superoxide dismutase [Cu-Zn], chloroplast (Fragment)	QVEGVVTLSQEDNGPTTVKVRLTGLTPGKHGFHLHEFGDTTNGCMSTGSHFNPKKLTHGAPEDDVRHAGDLGNIVAGSDGVAEATIVDNQIPLSGPDSVIGRALVVHELEDDLGKGGHELSLTTGNAGGRLACGVVGLTPI	134685 Pinus sylvestris	14400	5.16
UR12	Superoxide dismutase [Cu-Zn], chloroplast precursor	MAAHTIFTTTSTTNSFLFPIASSNTNSAPSLSSSFHGVSLKVKSKTPQSLTLSSVTSPKPFIVFAATKKAVAVLKGTSNVEGVVTLTQDDDGPTTVKVRITGLAPGLHGFHLHEFGDTTNGCMSTGPHFNPNGLTHGAPGDEVRHAGDLGNIEANASGVAEATLVDNQIPLSGPNS	134684 Petunia hybrida	22300	6.17

(cont. on next page)

Table 4.4. Up-Regulated Proteins, Identified by LC-MS/MS (cont.)

		VVGRALVVHELEDDLGKG GHELSLTTGNAGGRLACGV VGLTPI			
UR13	Superoxide dismutase [Cu-Zn], chloroplast precursor	MAAHSIFTTTSTTNSFLYPIS SSSSSPNINSSFLGVSLNVNA KFGQSLTLYAVTTPKPLTVF AATKKAVAVLKGNSNVEG VVTLSQDDDGPTTVNVRIT GLAPGLHGFHLHEYGDTTN GCMSTGAHFNPNKLTHGAP GDEIRHAGDLGNIVANADG VAEVTLVDNQIPLTGPNSV VGRALVVHELEDDLGKGG HELSLTTGNAGGRLACGVV GLTPI	134682 Solanum lycopersicu m	22200	5.77
UR14	Ribosomal protein L12, chloroplast precursor	MAAHSIFTTTSTTNSFLYPIS SSSSSPNINSSFLGVSLNVNA KFGQSLTLYAVTTPKPLTVF AATKKAVAVLKGNSNVEG VVTLSQDDDGPTTVNVRIT GLAPGLHGFHLHEYGDTTN GCMSTGAHFNPNKLTHGAP GDEIRHAGDLGNIVANADG VAEVTLVDNQIPLTGPNSV VGRALVVHELEDDLGKGG HELSLTTGNAGGRLACGVV GLTPIMAATTTMATLNLPSL TSHPNSSTFPKHPQPLQFPF RTTTNPISLSSTRTTRLRPIA AVEAPEKIEQLGTQLSGLTL EEARVLVDWLQDKLGVSA ASFAPAAAVAAPGAPADAA PAVEEKTEFDVSIDEVPSNA RISVIKAVRALTSLGLKEAK ELIEGLPKKLKEGVSKDDA EDAKKQLEDAGAKVSIV	133085 Spinacia oleracea	19900	5.50
UR 15	Thioredoxin M type chloroplast	LIAPWDELAK	–	1167.7	–
UR 16	Thioredoxin M type chloroplast	SIPTVLFFK	–	1051.6	–
UR 17	Superoxide dismutase Cu	ALWHELEDDLGK	–	1437.7	–
UR 18	Peroxiredoxin Q chloroplast	FKGKPWVYFYPADETPGCT K	–	2403.2	–
UR 19	Cytochrome b6 f complex	DALGNDVIASEWLK	–	1530.8	–

Results shown that wound-induced basic protein, ribosomal protein L12, oxygen-evolving enhancer protein 1, peroxiredoxin Q, thioredoxin M type chloroplast, cytochrome b6 f complex and superoxide dismutase (SOD) proteins were up-regulated.

Known that oxidative stress is an important stress that caused by salinity. Oxidative stress is produced by the formation of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and singlet oxygen ($^1O_2$). ROS is produced is a normal function of aerobic metabolism and under

normal conditions the negative effects of ROS can be eliminated. But stress conditions such as salinity increases the ROS production. To eliminated the ROS effect, plants produced ROS scavenging enzymes and so produced the defense mechanism. Known that the first step of defense mechanism is conversion of superoxide anions to hydrogen peroxide. This step is produced by superoxide dismutase (SOD). If the superoxide anion is not neutralized at this step, it reacts with reduced transition metals such as Fe^{2+} to produce highly reactive hydroxyl radicals by the Fenton reaction. Therefore, SOD is the very important enzyme in the defence mechanism against oxidative stress. And in our results shown that two of salt-responsive proteins are superoxide dismutase (SOD) shown in Table 4.1. It proved that salt-treated *Beta maritima* plants produced the SOD to defence itself against salinity (Jithesh, et al. 2006).

Another important enzyme is peroxiredoxin Q. Peroxiredoxins (Prxs) are the antioxidative enzymes. They are characterized as peroxidases and they present in all organisms. Prxs convert hydroperoxides into the corresponding alcohol or water. Based on the sequence of amino acid comparisons, Prxs are grouped in four classes. The first subgroup is the 1-Cys peroxiredoxin (1-Cys Prx). 1-Cys Prx is localized in the nucleus and is suggested to protect macromolecules from oxidative damage. The second member of the Prx family is 2-Cys peroxiredoxin (2-Cys Prx), it is localized in the chloroplast and it protects the photosynthetic membrane from oxidative damage. The third one is the Prx II. It shown antioxidant activity in *Arabidopsis thaliana* and became a new member of the peroxiredoxin family (type II Prx). Finally, the fourth subgroup is Prx Q. Prx Q is localized in the chloroplast and it was identified initially in *Sedum lineare*. Prx Q use Trx as a proton donor to catalyze H_2O_2 to H_2O. However, in Prx Q, the two conserved cysteine residues can form intramolecular disulphide bond rather than intermolecular bonds (Karl-Josef Dietz 2007).

For many peroxiredoxins, it has been established that thioredoxins act as an electron donor for redeveloping the active form. And suggesting that the Trx is essential for the redeveloping of oxidized Prx Q.

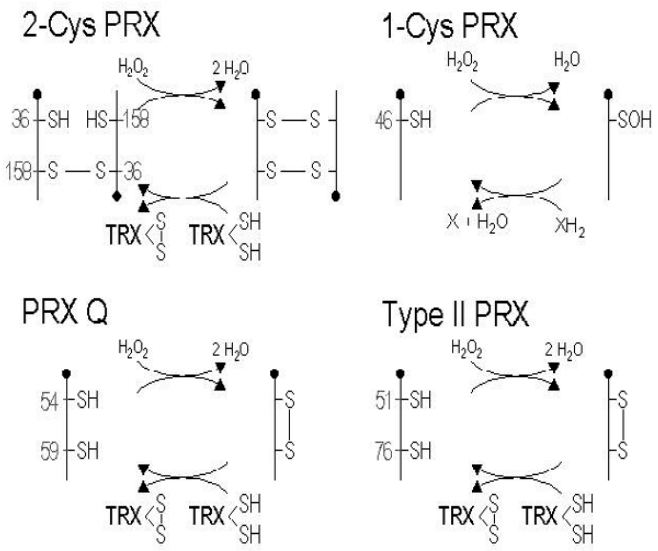

Figure 4.5. Shown of Peroxide Reduction Mechanism and Prx Regeneration for The Four Clans of Peroxiredoxins Found in The *Arabidopsis* Genome (Source Frank Horling, et al. 2002)

Ribosomal protein L12 is the one of the up-regulated protein. The function of ribosomal protein L12 is known. It binds to ribosomal RNA during gathering and preservation of ribosome structure and function. However, their role in salt stress response is not clear in plants (Dea-Wook Kim, et al. 2005).

In the following table, proteins which were salt responsive in *Beta maritima* under salt stress were shown. The SL is shorthand notation stands for salt leave proteins.

Table 4.5. Salt-Responsive Proteins, Identified by LC-MS/MS

Spot No	Protein Name	Sequence	Query ID (gi | NCBI) and Reference Organism	Molecular weight (average) (Da)	Theoretial pI
SL 1	Superoxide dismutase [Cu-Zn], chloroplast precursor	MASHSLMSPSPLTSHSLLRSSFS GVSVKLSPQFSTLSRSKFQPLSV VAAAKKAVAVLKGNSTVEGV VTLTQENESPTTVNVRITGLTPG LHGFHLHEYGDTTNGCISTGPH FNPNQLTHGAPEDEIRHAGDLG NIIADANGVAEATIVDNQIPLTG PNSVIGRALVVHELEDDLGKGG HELSLSTGNAGGRLACGVVGL TPV	12230569 Medicago sativa	20800	6.02
SL 2	Superoxide dismutase [Cu-Zn], chloroplast precursor	MAAHTILASAPSHTTFSLISPFSS TPTNALSSSLQSSSFNGLSFKLS PTTQSLSLSTSAASKPLTIVAAT KKAVAVLKGTSNVEGVVTLTQ EDDGPTTVNVRISGLAPGKHGF HLHEFGDTTNGCMSTGPHFNP DKKTHGAPEDEVRHAGDLGNI VANTDGVAEATIVDNQIPLTGP NSVVGRALVVHELEDDLGKGG HELSPTTGNAGGRLACGVVGL TPV	134686 Spinacia oleracea	22600	5.88
SL 3	U-box domain-containing protein 51	MGDGALIVAVAIKGNNSKTKG VVRWALQEFASQEHVVFKLLH VQPRDSNSVSTTRKDLTTSVYK KDVDRKTREMLLPSRDMFVHR EVQLDIMVLESDDIADAISKAV QDHGISELVIGASSSIIFSWKLK RSNLSSRIADATPRFCSVHVISK GKLLNVRKSDMDTETSIADDRS ESRFSSDSHSGTVSSTSSHQFSS TPLLFQRIQALTTVNQKVGTNI GKQNNEPHHHHHNRAGSLDVD ESKLLNQKGFYRTSSSGIGYGG SDISSWRSSQMEEASSSSTYSDP TSSSSQIHKDFELEKLKIELRHIK GMYAVAQSEVIDASKKMQDLN QRRSEEATRLKNLTIREEEADE VVEMERERQEDAENEAELVRE CIERETEERLEAEAAEEVRKEK QRLEDALEGGPLQRQQYMKFE WEEIVEATSSFSDELKGVGGYG SVYRCNLHHTTVAVKVLHSDK SSLTKQFHQELEILSKIRHPHLL LLLGACPERGSLVYEYMHNGS LEERLMKRRPNVDTPQPPPLRW FERFRIAWEIASALYFLHTNEPR PIVHRDLKPANILLDRNNVSKIG DVGLSKMVNLDPSHASTVFNE TGPVGTFFYIDPEYQRTGVVTP ESDIYAFGIILLQLVTARSAMGL AHSIEKALRDQTGKFTEILDKT AGDWPVKEAKEMVMIGLRCA EMRKRDRPDLGKEILPVLERLK EVASIARNMFADNLIDHHHNAP THFYCPITKDVMENPCVASDGY TYEKRAIKEWLQKNHKSPMTD LPFPSDSLLPNHSLLSAIKEWRS QLIK	172045896 Arabidopsis thaliana	90223	6.3

(cont. on next page)

Table 4.5. Salt-Responsive Proteins, Identified by LC-MS/MS (cont.)

SL 4	Photosystem I Supercomplex	GVIEEYLEKSKTNKELNDKKRL ATTGANFARAYTVEFGSCKFPE NFTGCQDLAKQKKVPFLSDDL DLECEGKDKYKCGSNVFWKW	149242539 Phaseolus vulgaris	9752	7.80
SL 5	Glycogen phosphorylase	MSRPLSDQEKRKQISVRGLAGV ENVTELKKNFNRHLHFTLVKD RNVATPRDYYFALAHTVRDHL VGRWIRTQQHYYEKDPKRIYY LSLEFYMGRTLQNTMVNLALE NACDEATYQLGLDMEELEEIEE DAGLGNGGLGRLAACFLDSMA TLGLAAYGYGIRYEFGIFNQKIC GGWQMEEADDWLRYGNPWE KARPEFTLPVHFYGRVEHTSQG AKWVDTQVVLAMPYDTPVPG YRNNVVNTMRLWSAKAPNDF NLKDFNVGGYIQAVLDRNLAE NISRVLYPNDNFFEGKELRLKQ EYFVVAATLQDIIRRFKSSKFGC RDPVRTNFDAFPDKVAIQLNDT HPSLAIPELMRVLVDLERLDWD KAWEVTVKTCAYTNHTVLPEA LERWPVHLLETLLPRHLQIIYEI NQRFLNRVAAAFPGDVDRLRR MSLVEEGAVKRINMAHLCIAGS HAVNGVARIHSEILKKTIFKDFY ELEPHKFQNKTNGITPRRWLVL CNPGLAEIIAERIGEEYISDLDQL RKLLSYVDDEAFIRDVAKVKQ ENKLKFAAYLEREYKVHINPNS LFDVQVKRIHEYKRQLLNCLH VITLYNRIKKEPNKFVVPRTVMI GGKAAPGYHMAKMIIKLITAIG DVVNHDPVVGDRLRVIFLENY RVSLAEKVIPAADLSEQISTAGT EASGTGNMKFMLNGALTIGTM DGANVEMAEEAGEENFFIFGM RVEDVDRLDQRGYNAQEYYD RIPELRQIIEQLSSGFFSPKQPDL FKDIVNMLMHHDRFKVFADYE EYVKCQERVSALYKNPREWTR MVIRNIATSGKFSSDRTIAQYAR EIWGVEPSRQRLPAPDEKIP	6093713 Oryctolagus cuniculus (rabbit)	97289	6.77
SL 6	Gluthathione-S-transferase	MALKLKGINYDYVEEKFESKSS LLLALNRIHKKVPVLVHNGKTI LESHVILDYIEETWPHNPILPQD PDERSKTRFLAKLVDEHVTNV GFVSMPKADEKGRQVLVEQIR ELIMYLEKELIGKDYFGEEKFPE YNKWVKNLEKVEIVKDCIPPRE KHVEHMNYMAKRIRSS	158828318 Arabidopsis cebennensis	20042	7.07

As known that SOD is very important enzyme in the defence mechanism against oxidative stress and explained in the up-regulated proteins part.

The up-regulated and down-regulated proteins are graphed according to their salt to control ratio versus protein index, showing below.

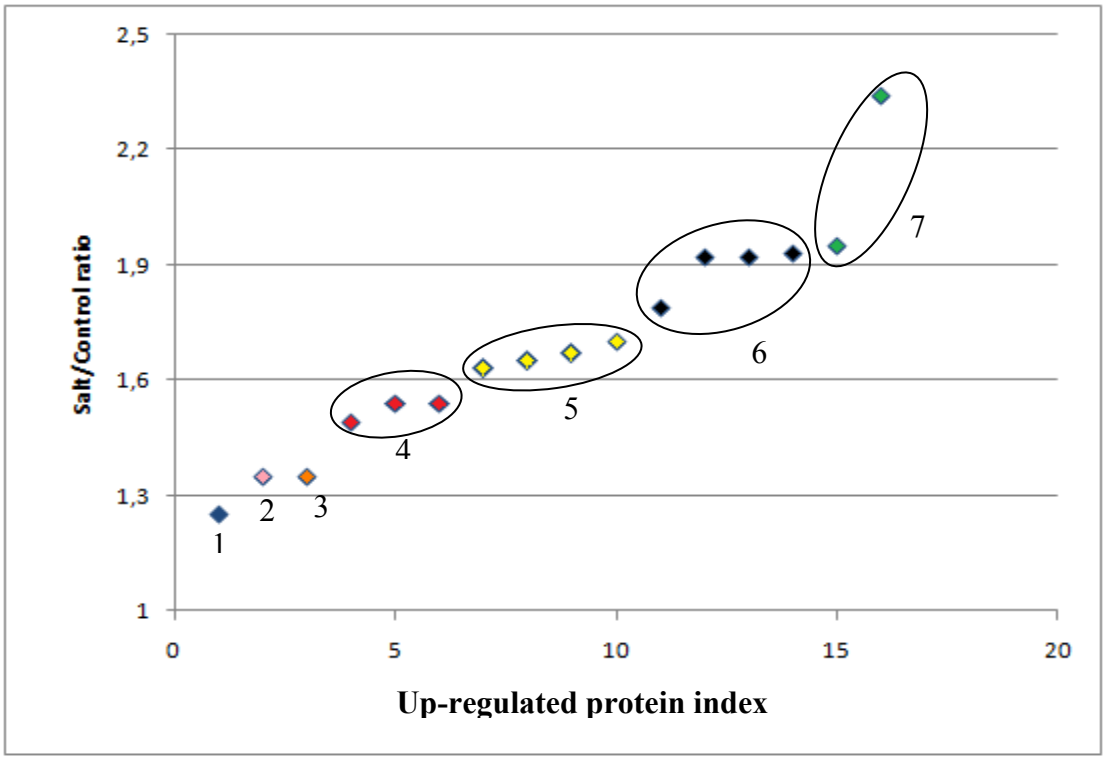

Figure 4.6. Graph of Index of Up-Regulated Proteins Versus Salt to Control Ratio (Ribosomal protein L12 (1), Cytochrome b6 f complex (2), Wound-induced base protein(3), Peroxidoxin Q (4), Oxygen-evolving enhancer protein 1 (5), Superoxide dismutase (6),Thioredoxin M type chloroplast (7))

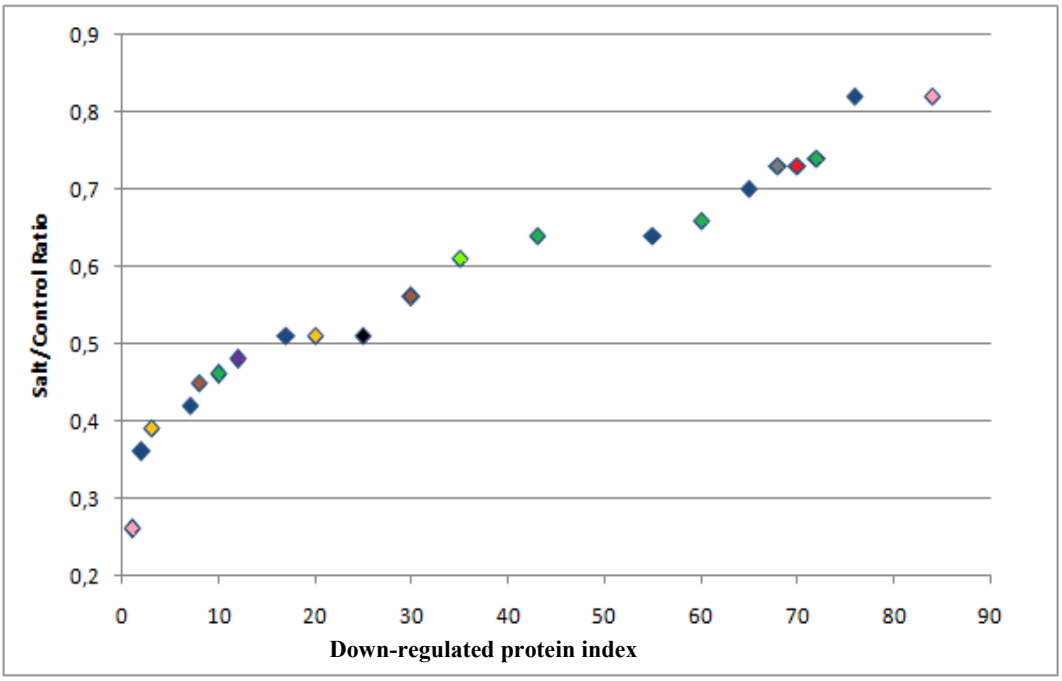

Figure 4.7. Graph of Index of Down-Regulated Proteins Versus Salt to Control Ratio
Cytochrome bc1 complex(spot 1,21), Glycogen phosphorylase (spot 7), Oxygen-evolving enhancer protein (spot 2,4,8,14,16,20), Phosphoprotein (spot 10), Glycine cleavage system H protein (spot 3,9), Calmodulin (spot 12), Chloroplast 30S ribosomal protein S7(spot 5,11), Thylakoid lumenal (spot 17), Photosystem I iron-sulfur center (spot 6,13,15,19), Histone H2 B5(18)

Although they have enough peptide sequences they were not identified with the database search due to incomplete of the database of *Beta maritima*. All of these proteins were shown in the below tables.

Table 4.6. Unspecified Salt and Control Proteins, Identified by LC-MS/MS

Spot No	Decription	Peptide	Molecular weight (average) (Da)	Unique
1	Unspecified	VMYLDRVARGLFLLGDLL DLLLRGSSQNSWGR	3645.82	Control
2	Unspecified	DMLKNTLDLNGFWWR	1908.89	Salt

Table 4.7. Unspecified Up-regulated Proteins, Identified by LC-MS/MS

Spot No	Description	Peptide	Molecular weight (average) (Da)	Salt:Control
1	Unspecified	QWGYYEAYQYQTEEPPR	2371.02	1.60
2	Unspecified	KDFWQEWAADQAK	1622.76	2.61
3	Unspecified	GPQWPTTTPWRALVLVLVLVPWWMEFR	3278.64	1.77
4	Unspecified	CAWCLGRLPHNADLTSLCVNLLNCAMQ	3187.34	1.60
5	Unspecified	LRSAASFMPQDAPGAAVVASAPRGVETR	2812.43	1.88
6	Unspecified	HVSETLLEEVDEMLR	1799.89	1.65
7	Unspecified	DWVELAAQNTARWMK	1818.88	4.44

Table 4.8. Unspecified Down-regulated Proteins, Identified by LC-MS/MS

Spot No	Decription	Peptide	Molecular weight (average) (Da)	Salt:Control
1	Unspecified	LFDSLNNLDHAAK	1457.73	0.75
2	Unspecified	AMRDRTAAAAPETAAWFVG PDPR	2457.19	0.56
3	Unspecified	AWLENLVCANWEWK	1818.85	0.74
4	Unspecified	WFSGFEGWEAR	1383.67	0.25
5	Unspecified	PTEEGEVAAAAGAAPK	1468.73	0.47
6	Unspecified	LQSEHVELESLWAR	1696.89	0.49

As described before, samples were analyzed with two different instruments. Results of the first instrument that only leave samples were analyzed was discussed. The LTQ Orbitrap XL (Thermo Fisher Scientific) is the second instrument that analyzed

both leave and root samples. The results of the leave samples were parallel with the first instrument, same proteins were identified in both instrument. The other results were including the root samples. While 124 proteins were identified in the control root samples, 135 proteins were identified in the salt root. Most of the proteins were same in both root samples except the eleven salt responsive root proteins. These different of eleven salt responsive root proteins were shown in table 4.9.

Table 4.9. Salt Responsive Root Proteins, Identified by LC-MS/MS

Protein Name	Reference Organism	Peptides	Coverage
Calreticulin precursor	Beta vulgaris	9	22,84
26S protease regulatory subunit 6B homolog	Arabidopsis thaliana	2	4,66
Cysteine synthase, chloroplast/chromoplast precursor	Capsicum annuum	4	10,96
Vacuolar ATP synthase catalytic subunit A	Zea mays	5	9,27
SKP1-like protein 1B	Arabidopsis thaliana	14	18,71
2,3-bisphosphoglycerate-independent phosphoglycerate mutase	Zea mays	9	7,87
Probable rhamnose biosynthetic enzyme 3	Arabidopsis thaliana	2	4,97
Outer mitochondrial membrane protein porin	Oryza sativa subsp. japonica	3	4,38
Protein At5g10860, mitochondrial precursor	Arabidopsis thaliana	4	9,22
GTP-binding nuclear protein Ran1A	Lotus japonicus	2	10,05
Putative DNA repair protein RAD23-3	Arabidopsis thaliana	2	4,77

Some of the these eleven proteins were described. 26S protease regulatory subunit 6B homolog protein; is the members of the 26S proteasome subunit P45 family. It may be phosphorylated within the proteasome. This phosphorylation event may play a key role in ATP-dependent proteolysis. SKP1-like protein 1B; is an important protein for cell elongation and division. It expressed specially in tips, cortical layer and epidermis of roots. Identified in whole seedling, vascular tissues of young stem, leaves, flowers, etc. Outer mitochondrial membrane protein porin; porin is the voltage-dependent anion channel and it is the most founding protein of the mitochondrial outer membrane. The inserted porin was resistant to trypsin treatment after detergent solubilization. Results indicate that, unlike proteins that are imported to the inner membrane and matrix of the mitochondria. GTP-binding nuclear protein Ran1A; is the member of the RAN (nuclear import/export) family of GTPases. It is important role in the nuclear transporter. Putative DNA repair protein RAD23-3; this protein involved in the repair of the damaged DNA by producing the various biochemical processes. Therefore, damaged DNA can be restored.

On the other hand, the most important identified protein was the vacuolar ATP synthase catalytic subunit A that is identified as vacuolar proton-translocating ATPase (H+ V-ATPases or V-ATPase) subunit A. V-ATPase is a primary-active proton pump. Protons are pumped out of the cytoplasm either into the organelle or out into the extracellular space (Harvey, et al. 1998). It located at the vacuolar membrane (tonoplast). V-ATPases affect several cellular processes such as cell expansion, acidity of organelles, cytoplasmic pH and ion homeostasis. V-ATPase genes are highly regulated under environmental stress; specially salt stress induced an increase in V-ATPase activity.

V-ATPases are multi-subunit enzyme complexes that they consist of two main parts, the head region or the peripheral sector is called as (V_1) domain that translocate protons across membranes using the free energy of ATP hydrolysis and the Vo domain that includes the part of the protein necessary for proton translocation (Wieczorek, et al. 1999). And totally V-ATPases consists of 14 different polypeptide subunits and they work together as a rotary machine. While the V_1 domain includes eight subunits (A, B, C, D, E, F, G and H), the V_0 domain contains six subunits (a, c, c', c'', d and e). The head region (V_1 domain) includes three A and three B subunits, and two isoforms of the E and G subunits, one or two of subunit of H and remaining subunits are single. Three copies of subunit A (catalytic subunit) and subunit B (noncatalytic ATP binding). The

other remaining subunits are found between fixed peripheral stalk and central rotational stalk, they connect the V_1 with V_0 domain. The V_0 domain composed of four or five subunits of proteolipid (c, c', and c") and single subunit of the remaining subunits which involved in proton translocation. The V-ATPase membrane protein shown in the Figure 4.8.

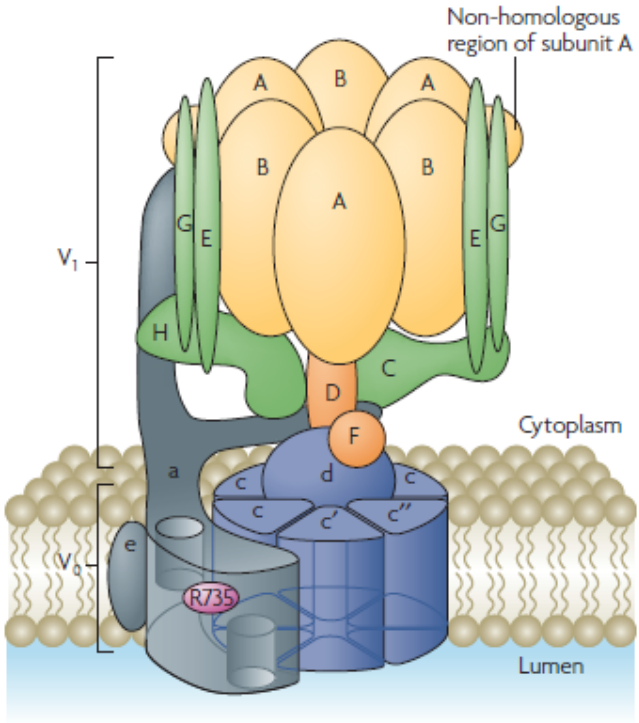

Figure 4.8. The Structure of V-ATPase
(Source Forgac 2007)

V-ATPases operate by a rotary mechanism. V-ATPase consumes ATP for producing the energy for the rotation and for the translocates the protons across the membrane.

We proposed that the excess salt in the cell is transported into the vacuole of the plant cell by the V-ATPase. And during proton translocation across the membrane of the vacuole, electrochemical gradient provides excess salt to transfer from cytoplasm into the vacuole of the plant cell. Therefore, there is no toxic effects caused by salinity and wild salt tolerant species of *Beta maritima* can be survive using this defensive mechanism under salinity conditions.

CHAPTER 5

CONCLUSION

The main purpose of this study was to identify salt-stress tolerant proteins in wild salt-tolerant beet, *Beta maritima* by proteomic approach. And V-ATPase subunit A is identified. It is very important protein for producing the defense mechanism under salinity conditions. The excess sodium ion in the cytoplasm of the cell is translocated into the vacuole by the help of V-ATPase subunit A. As mentioned before, V-ATPase has the rotary mechanisms. During rotation of head group of V-ATPase (subunits A and B), two-protons are transferred into the vacuolar lumen through channels. This generates an electrochemical gradient across the membrane which helps the transport sodium ion into the vacuole of the plant cell. So, plant can survive by the help of this defensive mechanism under salinity conditions.

In addition of V-ATPase protein, some important antioxidant enzymes were identified in the salt responsive leave samples and they were also up regulated. These proteins were; superoxide dismutase (SOD), peroxiredoxin Q. Other important proteins were oxygen-evolving enhancer protein 1 (OEE1), ribosomal protein L12, chloroplast 30S ribosomal protein, wound-induced basic protein. Some of these proteins increased capacity for oxygen radical and the relationship between salt tolerance and antioxidant defense system. So they protect of the cellular membrane (Bor, et al. 2003) and means that *Beta maritima* protects itself against salt stress using antioxidant enzymes.

REFERENCES

Abigail Frost, Juan Carlos Lopez-Gutierrez and Colin B.Purrington. 2003. Fıtness of *Cuscuta Salina* (Convolvulaceae) parasitizing *Beta vulgaris* (Chenopodiaceae) grow under different salinity regimes. *American Journal of Botany* 90:1032-1037.

Adessi, C., Miege, C., Albrieux, C., and Rabilloud, T. 1997. Two-dimensional electrophoresis of membrane proteins: A current challenge for immobilized pH gradients. *Electrophoresis* 8:127-135.

Alaina J. Garthwaite, Roland von Bothmer and Timothy D. Colmer. 2005. Salt tolerance in wild *Hordeum* species is associated with restricted entry of Na^+ and Cl^- into the shoots. *Journal of Experimental Botany* 56:2365-2378.

Alscher RG, Erturk N, Heath LS. 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *Journal of Experimental Botany* 53:1331-1341.

Anna Maria Salzano and Marco Crescenzi. 2005. Mass spectrometry for protein identification and the study of post translational modifications. *Ann Ist Super Sanita* 41:443-450.

Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology* 55:373-399.

Bae, M.S., Cho, E.J., Choi, E.Y., and Park, O.K. 2003. Analysis of the arabidopsis nuclear proteome and its response to cold stress. *Plant Journal* 36:652-663.

Banaras Hussain Niazi, Mohammad Athar and Jelte Rozema. 2004. Salt tolerance in fodderbeet and seabeet: Analysis of biochemical relations. *Bulg. J. Plant Physiology* 30:78-88.

Banaras Hussain Niazi, Jelte Rozema and Mohammad Athar. 2005. Effect of pre-germination and post-germination treatment with growth hormones (kinetine and abscisic acid) on ion concentration and biochemical contents of fodderbeet and seabeet under saline conditions. *Gen. Appl. Plant Physiology* 31:89-104.

Barbier-Brygoo, H. and Joyard, J. 2004. Focus on plant proteomics. *Plant Physiology and Biochemistry* 42:913-917.

Bıology Department of Davidson University, 2008. www.bio.davidson.edu/.../strategies/2dgels.html, (accessed, June 13, 2008).

Borderies, G., Jamet, E., Lafitte, C., Rossignol, M., Jauneau, A., and Boudart, G. 2003. Proteomics of loosely bound cell wall proteins of *Arabidopsis thaliana* cell suspension cultures: A critical analysis. *Electrophoresis* 24:3421-3432.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72:248-254.

Bruno Domon and Ruedi Aebersold. 2007. Mass spectrometry and protein analysis. *Science* 312.

Campo, S., Carrascal, M., Coca, M., Abian, J., and San Sequndo, B. 2004. The defense response of germinating maize embryos against fungal infection: A proteomic approach. *Proteomics* 4:383-396.

Chernushevich, I.V., Loboda, A.V., and Thomson, B.A. 2001. An introduction to quadrupole time-of-flight mass spectrometry. *Journal of Mass Spectrometry* 36**:**849-865.

Chivasa, S., Ndimba, B. K., Simon, W.J., Robertson, D., Yu, X. L., Knox, J. P., Bolwell, P., and Slabas, A.R. 2002. Proteomic analysis of the *Arabidopsis thaliana* cell wall. *Electrophoresis* 23:1754-1765.

Curt T. Reimann, Axel Mie, and Carina Nilsson. 2005. Introduction to biological mass spectrometry: Determining identity and species of origin of two proteins. *Journal of Chemical Education 82*.

Dani, V., Simon, W.J., Duranti, M., and Croy, R.R.D. 2005. Changes in the tobacco leaf apoplast proteome in response to salt stress. *Proteomics* 5:737-745.

Darbouret, D. and Kano, I. 2000. Ultrapure water blank for boron trace analysis. *Journal of Analytical Atomic Spectrometry* 15:1395-1399.

Dietz, K.J., Rudloff, S., Ageorges, A., Eckerskorn, C., Fischer, K., and Arbinger, B. 1995. Subunit E of the vacuolar H^+-ATPase of *Hordeum vulgare* L.: cDNA cloning, expression and immunological analysis. *Plant Journal* 8:521–529.

Donnelly B. E., Madden R. D., Ayoubi P., Porter D.R., and Dillwith J.W. 2005. The wheat (*Triticum aestivum* L.) leaf proteome. *Proteomics* 5:1624-1633.

Fenn, J.B., Mann, M., Meng, C.K., Wong, S.F., and Whitehouse, C.M. 1989. Electrospray ionization for mass spectrometry of large biomolecules. *Science* 246:64-71.

Ferro, M., Salvi, D., Brugiere, S., Miras, S., Kowalski, S., Louwagie, M., Garin, J., Joyard, J., and Rolland, N. 2003. Proteomics of the chloroplast envelope membranes from *Arabidopsis thaliana*. *Molecular & Cellular Proteomics* 2:325-345.

Fleischer, A., O'Neill, M. A., and Ehwald, R. 1999. The pore size of non-graminaceaous plant cell walls is rapidly decreased by borate ester cross-linking of the pectic polysaccharide rhamnogalacturonan II. *Plant Physiology* 121:829-838.

Foyer CH, Noctor G. 2000. Oxygen processing in photosynthesis: regulation and signalling. *New Phytologist* 146: 359–388.

Francisco M. Canovas, Eliane Dumas-Gaudot, Ghislaine Recorbet, Jesus Jorrin, Hans-Peter Mock, Michael Rossignol. 2004. Plant proteome analysis. *Proteomics* 4:285-298.

Gale, J. H. C. Kohl and R. M. Hagan. 1967. Changes in the water balance and photosynthesis of onion, bean and cotton plants under saline conditions. *Physiologia Plantarum* 20:408-420.

Gallardo, K., Job, C., Groot, S.P., Puype, M., Demol, H., and Vandekerckhove, J. 2001. Proteomic analysis of arabidopsis seed germination and priming. *Plant Physiology* 126:835-848.

Georg H.H. Borner, Kathrynar S. Lilley, Timothy, J. Stevens, and Paul Dupree. 2003. Identification of glycosylphosphatidylinositol-anchored proteins in Arabidopsis. A proteomic and genomic analysis. *Plant Physiology* 132:568-577

Gh. H. Salekdeh, J. Siopongco, L. J. Wade, B. Ghareyazie, J. Bennett. 2002. A proteomic approach to analyzing drought- and salt-responsiveness in rice. *Field Crops Research* 76:199-219.

Golldack, D. and Dietz, K. J. 2001. Salt-induced expression of the vacuolar H^+-ATPase in the common ice plant is developmentally controlled and tissue specific. *Plant Physiology* 125:1643-1654

Görg, A., Postel, W., and Günther, S. 1988. Two-dimensional electrophoresis. The current state of two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* 9:531-546.

Görg, A. 1991. Two-dimensional electrophoresis. *Nature* 349:545-546.

Görg, A., Postel, W., Friedrich, C., Kuick, R., Strahler, J.R., and Hanash, S. M. 1991. Temperature-dependent spot positional variability in two-dimensional polypeptide patterns**.** *Electrophoresis* 12:653-658.

Görg, A., Obermaier, C., Boguth, G., Harder, A., Scheibe, B., Wildgruber, R., and Weiss, W. 2000. The current state of two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* 21:1037-1053.

Greber, S., Lubec, G., Cairns, N., and Fountoulakis, M. 1999. Decreased levels of synaptosomal associated protein 25 in the brain of patients with down syndrome and alzheimer's disease. *Electrophoresis* 20:928-934.

Greenway, H. and R. Munns. 1980. Mechanism of salt tolerance monohalophytes. *Annu. Rev. Plant physiol.* 31:149-190.

Homa Mahboobi, Meral Yucel, and Huseyin Avni Öktem. 2000. Changes in total protein profiles of Barley cultivars in response to toxic boron concentration. *Journal of Plant Nutrition* 23:391-399.

Jian-Kang Zhu. 2003. Regulation of ion homeostasis under salt stress. *Current Opinion in Plant Biology* 6:441–445.

John M. Cheeseman. 1988. Mechanisms of salinity tolerance in plants. *Plant Physiology.* 87:547-550.

Jorrin, J.V., Rubiales, D., Dumas-Gaudot, E., Recorbet, G., Maldonado, A., and Castillejo, M.A. 2006. Proteomics: a promising approach to study abiotic interaction in legumes: A review. *Euphytica* 147:37-47.

Juri Rappsilber and Matthias Mann. 2002. What does it mean to identity a protein in proteomics? *Trend in Biochemical Sciences* 27.

Kang, J.G., Pyo, Y.J., Cho, J.W., and Cho, M.H. 2004. Comparative analysis of differentially expressed proteins induced by K^+ deficiency in *Arabidopsis thaliana*. *Proteomics* 4:3549-3559.

Karas, M. and Hillenkamp F. 1988. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. *Analytical Chemistry* 60:2299-2301.

Karl-Josef Dietz, Simone Jacob, Marie-Luise Oelze, Miriam Laxa, Vanesa Tognetti, Susana Marina Nunes de Miranda, Margarete Baier and Iris Finkemeier. 2006. The function of peroxiredoxins in plant organelle redox metabolism. *Journal of Experimental Botany* 57:1697–1709.

Kav, N.N.V., Srivastava, S., Goonewardene, L., and Blade, S.F. 2004. Proteome level changes in the root of *Pisum sativum* in response to salinity. *Annals of Applied Biology* 145:217-230.

Kawamura, Y. and Uemura, M. 2003. Mass spectrometric approach for identifying putative plasma membrane proteins of arabidopsis leaves associated with cold acclimation. *Plant Journal* 36:141-154.

Klaas J. van Wijk. 2001. Challenges and prospect of plant proteomics. *Plant Physiology* 126:501-508.

Kris Gevaert, Joel Vandekerckhove. 2000. Protein identification methods in proteomics. *Electrophoresis* 21:1145-1154.

Kruft, V., Eubel, H., Jansch, L., Werhahn, W., and Braun, H.P. 2001. Proteomic approach to identify novel mitochondrial proteins in arabidopsis. *Plant Physiol*ogy 127:1694-1710.

Kulakowska, A.B., Krzysik, A.B., Dylag, T., Drabik, A., Suder, P., Noga, M., Jarzebinska, J., and Silberring, J. 2007. Methods for samples preparation in proteomic research. *Journal of Chromatography B* 849:1-31.

Laugesen, S. and Roepstorff, P. 2003. Combination of two matrices results in improved performance of MALDI MS for peptide mass mapping and protein analysis. *Journal of the American Society for Mass Spectrometry* 14:992-1002.

Liebler, Daniel C. 2002. *Introduction to Proteomics-Tools for the New Biology*. Totowa, NJ: Humana Press.

L´opez, L.J. 2007. Two-dimensional electrophoresis in proteome expression analysis. *Journal of Chromatography B* 849:190-202.

Medical College of Georgia Proteomics and Mass Spectrometry Core Laboratory. www.mcg.edu/Core/Labs/pmscf/massspec.htm. (accessed June 13, 2008).

Meng-Cheng Wang, Zhen-Ying Peng, Cui-Ling Li, Fei Li, Chun Liu and Guang-Min Xia. 2008. Proteomic analysis on a high salt tolerance introgression strain of Triticum aestivum/ Thinopyrum ponticum. *Proteomic* 8:1470-1489.

Merril, C. R. 1990. Gel-staining techniques. *Methods in Enzymology* 182:477-488.

Millar, A.H., Sweetlove, L.J., Giege, P., and Leaver, C. 2001. Analysis of the arabidopsis mitochondrial proteome. *Plant Physiology* 127:1711-1727.

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. 2004. The reactive oxygen gene network of plants. *Trends in Plant Science* 9:490–498.

M. M. F. Mansour, K. H. A. Salama, M. M. Al-Mutawa. 2003. Transport proteins and salt tolerance in plants. *Plant Science* 164:891-900.

Nyman, T. A. 2001. The role of mass spectrometry in proteome studies. *Biomolecular Engineering* 18:221-227.

O'Farrell, P.H. 1975. High resolution two-dimensional electrophoresis of proteins. *Journal of Biological Chemistry* 250:4007-4021.

Ole Norregaard Jensen. 2004. Modification-specific proteomics: Characterization of poat-translational modifications by mass spectrometry. *Science Direct* 8:33-41.

Patrick Giavalisco, Eckhard Nordhoff, Hans Lehrach, Johan Gobom, Joachim Klose. 2003. Extraction of proteins from plant tissues for two-dimentional electrophoresis analysis. *Electrophoresis* 24:207-216.

Rabilloud, T., Adessi, C., Giraudel, A., and Lunardi, J. 1997. Improvement of the solubilization of proteins in two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* 18:307-316.

Rana Munns, Mark Tester.2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biology.* 59:651-681

Rose, J.C., Bashir, S., Giovannoni, J.J, Jahn, M.M., and Saravanan, R.S. 2004. Tackling the plant proteome: Practical approaches, hurdles and experimental tools. *Plant Journal* 39:715-733.

Rosenfeld, J.J., Capdevielle, J., Guillemot, C., and Ferrara, P. 1992. In-gel digestion of proteins for internal sequence analysis after one-or two-dimensional gel electrophoresis. *Analytical Biochemistry* 203:173-179.

Salekdeh, G. H, Siopongco, J., Wade, L.J., Ghareyazie, B., and Bennett, J. 2002. A proteomic approach to analyzing drought and salt-responsiveness in rice. *Field Crops Research* 76:199-219.

Salekdeh, G.H., Siopongco, J., Wade, L.J., Ghareyazie, B., and Bennett, J. 2002. Proteomic analysis of rice leaves during drought stress and recovery. *Proteomics* 2:1131-1145.

Shevchenko, A.M., Wilm, O.V., and Mann, M. 1996. Mass spectrometric sequencing of proteins from silver-stained polyacrylamide gels. *Analytical Chemistry* 68:850-858.

Shuji Yokoi, Ray A. Bressan and Paul Mike Hasegawa. 2002. Salt stress tolerance of plants. *JIRCAS Working Report* 25-33.

Shunichi Takahashi and Norio Murata. 2008. How do environmental stresses accelerate photoinhibition? *Trends in Plant Science* 13: 1360-1385.

Strupat, K., Karas, M., and Hillenkamp, F. 1991. 2,5-dihydroxybenzoic acid: A new matrix for laser desorption-ionization mass spectrometry. *International Journal of Mass Spectrometry and Ion Processes* 111:89-102.

Sun Jae Kwon, Eun Young Choi, Yoon Jung Choi, Ji Hoon Ahn and Ohkmae K. Park. 2006. Proteomics studies of post-translational modifications in plants. *Journal of Experimental Batany* 57:1547-1551.

The Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408:796-815.

The University of Bristol, School of Chemistry. 2008. www.chm.bris.ac.uk/ms/theory/esi-ionisation.html. (accessed June 13, 2008).

Tracy A. Prime, D. Janine Sherrier, Piers Mahon, Len C. Packman, Paul Dupree. 2000. A proteomic analysis of organelles from *Arabidopsis thaliana. Electrophoresis* 21:3488-3499.

Van Loon, L.C. and Van Kammen, A. 1970. Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var. "Smsun" and "Samsun NN" II. Changes in protein constitution after infection with tobacco mosaic virus. *Virology* 40:199-206.

Viswanathan Chinnusamy, Andre Jagendorf, and Jian-Kang Zhu. 2005. Understanding and improving salt tolerance in plants. *Crop Science Society of America* 45:437-448.

Whitehouse, C.M., Dreyer, R.N., Yamashita, M., and Fenn, J.B. 1985. Electrospray interface for liquid chromatographs and mass spectrometers. *Analytical Chemistry* 57:675-679.

Wikipedia contributors, "Bottom-up vs Top-down". 2007. Wikipedia, The Free Encyclopedia. http://upload.wikimedia.org/wikipedia/f/f0/Bottom-up_vsTop-down (accessed June 10, 2008).

Wilm, M. and Mann, M. 1996. Analytical properties of the nano-electrospray ion source. *Analytical Chemistry* 68:1-8.

Wilm, M., Shevchenko, A., Houthaeve, T., Breit, S., Schweigerer, L., Fotsis, T., and Mann, M. 1996. Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry. *Nature* 379:466-469.

Wimmer, M.A., Mühling, K.H., Läuchli, A., Brown P.H., and Goldbach, H.E. 2003. The interaction between salinity and boron toxicity affects the subcellular distribution of ions and proteins in wheat leaves. *Plant, Cell & Environment* 26:1267-1274.

Woods, W.G. 1994. An introduction to boron: History, sources, uses, and chemistry. *Environmental Health Perspective* 102:5-11.

Yan, S., Tang, T., Su, W., and Sun, W. 2005. Proteomic analysis of salt stress-responsive proteins in rice root. *Proteomics* 5:235-244.

Yates, J. R. 1998. Mass spectrometry and the age of the proteome. *Journal of Mass Spectrometry* 33:1-19.

W, Chen, X., Wang, Y., Zhang, J., Hu, J., Wang, S., Liu, J., Yang, G., Zhang, Y., Xiong, Z., Li, L., Mao, C., Zhou, Z., Zhu, R., Chen, B., Hao, W., Zheng, S., Chen, W., Guo, G., Li, S., Liu, M., Tao, J., Wang, L., Zhu, L., and Yuan, H. 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp. indica). *Science* 296:79-92.

Yuanqing Jiang, Bo Yang, Neil S. Harris and Michael K. Deyholos. 2007. Comparative proteomic analysis of NaCl stress-responsive proteins in *Arabidopsis* roots. *Journal of Experimental Botany* 1-17.

Zolla, L., Rinalducci, S., Timperio, A. M., and Huber, C. G. 2002. Proteomics of light-harvesting proteins in different plant species. Analysis and comparison by liquid chromatography electrospray ionization mass spectrometry, photosystem I. *Plant Physiology* 130:1938-1950.

Zolla, L., Timperio, A.M., Walcher, W., and Huber, C. G. 2003. Proteomics of light-harvesting proteins in different plant species. Analysis and comparison by liquid chromatography electrospray ionization mass spectrometry, photosystem II. *Plant Physiology* 131:198-214.

Zolla, L., Rinalducci, S., Timperio, A.M., Huber, C.G., and Righetti, P.G. 2004. Intact mass measurements for unequivocal identification of hydrophobic photosynthetic photosystems I and II antenna proteins. *Electrophoresis* 25:1353-1366.