CHAPTER VI

SUMMARY

The main objective of the study was identification of differentially expressed proteins in *Vigna radiata* under salinity stress substantiated by the study of the metabolic and antioxidant status of the seedlings at different stages of growth i.e., one week, three weeks and seven weeks after sowing and treatment. Seventh week was considered for experimentation and analysis because it is the major vegetative and productive stage in the life span of *Vigna radiata*. The differentially expressed proteins may be those proteins that are expressed in response to stress and hence stress responsive proteins. They may help the plants to withstand/ tolerate stress overcoming the effects of stress.

The major effects of salinity stress are considerably noted in all stages of plant growth, development and yield. These effects include delay in germination as the salinity stress modifies imbibitions of water due to osmotic effects. Accordingly an increase in time period was noted for germination of Vigna radiata seeds with increase in salt concentration from 50 to 200mM sodium chloride comparing to water control.

The hindrance in development of root and shoot system was another major effect of salinity due to osmotic effects. The variations in
shoot and root lengths were observed in the seedlings throughout the various stages of plant growth and was noted on seventh, twenty first and forty fifth days after sowing and treatment. Though the average root and shoot lengths vary based on the cultivar types, it was observed a gradual decline in both root and shoot length with increasing concentrations of sodium chloride. Measurements of other metric parameters like leaves size, leaf area index, number of pods per plant, number of nodules in the roots might be important in assessing the actual status of the plant under stress. Only parameters to validate the effects stress are measured, as the main objective was on stress responsive protein identification.

Assessments of pigment levels are an important indicator of photosynthetic efficiency of plants under stress. Plants under salinity stress with increase in salt concentration show chlorosis and necrosis of leaves. This is due to decrease in chlorophyll pigments and production of reactive oxygen species. A decline in carotenoids was also observed. It is meaningful to note that carotenoids also have antioxidant potentials.

The major markers for any type of stress in plants enhanced production of reactive oxygen species (ROS). ROS are produced in plants generally as byproducts of metabolic reactions for example, photosynthesis and respiration. ROS are generated in different cellular compartments as mitochondria, peroxisomes, chloroplasts, cytoplasm or
in the extracellular space, known as apoplast. The easily measurable ROS is hydrogen peroxide. The effects of increase in ROS is directly observed as peroxidation of lipid membranes and hence the production of malondialdehyde, a major product of membrane lipid peroxidation. A corresponding increasing level of hydrogen peroxide was noted for increasing concentrations of sodium chloride and the same is the case for malondialdehyde.

Plants have well defined, structured and organized self defence mechanism for counteracting and foraging the reactive oxygen species and hence the oxidative stress. Such systems comprise of enzymic antioxidants and non enzymic antioxidants. Enzymic antioxidant system includes catalase (CAT), peroxidase (POX), polyphenol oxidase (PPO) and superoxide dismutase (SOD). The major non enzymic antioxidants in plants include ascorbic acid, α-tocopherol, β-carotene, glutathione, phenolic compounds and so on. However in this study, the focus was only on enzymic antioxidants.

With increased salinity and hence increased production of ROS, increase in the activities of all the four enzymes monitored in this study, were observed. Nevertheless a small decline in Catalase activity was observed at later stages and this may be due to enormous accumulation of hydrogen peroxide at levels that are toxic to the cellular components.
An inline increase in SOD activity was observed may be combating the superfluous hydrogen peroxide.

Proline and glycine betaine are the major osmoprotectants in plants that accumulate in conditions of drought and salinity to maintain the osmotic potential of the system. Hence these osmolytes serve as markers for assessing the status of plants under these stress conditions.

Reflective increase in the concentrations of both proline and glycine betaine were observed with increasing concentrations of sodium chloride. It meant that the plants were subjected to stress with the supplied levels of sodium chloride, also as already noted from the literature.

Despite the fact that the recent advances in proteomic technologies are wide, the basic approach using two dimensional gel electrophoresis and MALDI-TOF MS followed with MASCOT database search was followed in identification of proteins.

The various parameters for two dimensional gel electrophoresis technique were optimized for better resolution of proteins on the gel. The proteins were resolved on the gel to detect spots and hence distinguish protein spots from the control and the treated, which was reproducible. Nevertheless, higher protein resolution may still be achieved. The two
dimensional gel patterns of the protein spots were analyzed with PD Quest software.

Total protein spots observed were approximately 371 in the control, 761 in the treated and highly upregulated 22 spots in common, with actual matched spots around 231 and unmatched spots 140. The number of protein spots included the hidden spots and excluded the cancelled spots. The more number of protein spots in the treated sample, may be due to slightly higher concentration of the protein loaded wherein, proteins at very lower level of expression might have been spotted. Only highly upregulated proteins were chosen for peptide mass fingerprinting and identification.

From the investigations of this project, The proteins identified were the Ribosomal protein L17 of Ribosomal L17 superfamily, TMV resistance protein like protein a member of the superfamily of TIR and /or ABC-ATPase, belonging to NB-ARC multidomain group, defensin like proteins, pentatricopeptide repeat containing mitochondrial protein, peptidyl-prolyl cis-trans isomerase (PPIases) of cyclophilin family and kunitz type soybean trypsin inhibitor of STI family.

Through literature, it was found that all the six proteins identified have some role to be played in salinity stress response. Some proteins have been found to have major roles in biotic stress too, and presence of
such proteins may be due to secondary biotic stress, as a consequence of severe salinity stress. The attempt made to establish the participation of PPR domain containing protein in salinity stress response through sequence analysis and gene ontology predictions, as one of the pots matched to it, enhances the probability that the differentially expressed proteins are stress responsive proteins.

The exploration of regulation of synthesis of these proteins and establishment of plausible key roles of the proteins either in metabolic process and regulation or in networking signalling pathways or as a part of signal transduction pathways, in combating abiotic and/or biotic stress in crop plants needs investigations aiming at genetic improvement of crops for stress resistance and/or tolerance either improving the quality of yield or without affecting the quality of yield.