Chapter 6

Summary
6.0 Summary

The identification of predicted gene products at the protein level bridges the gap between genome sequencing data and protein function, and is referred to as "functional genomics". Proteome analysis has become an indispensable source of information about protein expression, splice variants, and erroneous or incomplete prediction of gene structures in databases. Furthermore, the proteomes of organelles comprise a focused set of proteins that fulfills discrete but varied cellular functions. The analyses of cell organelle proteomes provide additional important information about protein localization and pathway compartmentalization.

Nuclear proteins constitute a highly organized, complex network that plays diverse roles during cellular development and other physiological processes. It is known that the yeast nuclear proteome corresponds to about one-fourth of the total cellular proteins suggesting the involvement of the nucleus in a number of diverse functions. Legumes are valuable agricultural and commercial crops, mostly cultivated in the arid and semi-arid part of the world and are particularly important as nutrient sources for human diet and animal feed (Baker et al., 1990). Chickpea is one of the most important food legumes and its susceptibility to dehydration severely reduces the yield. In an attempt to understand the complexity of plant nuclear proteins, we have developed a proteome reference map of the legume, chickpea using two-dimensional gel electrophoresis (2-DE). Approximately, 600 protein spots were detected and LC-ESI-MS/MS analyses led to the identification of 150 proteins that have been implicated in a variety of cellular functions. The largest percentage of the identified proteins were involved in signaling and gene regulation (36%), while 17% were involved in DNA replication and transcription. The chickpea nuclear proteome indicates presence of few new nuclear proteins of unknown functions vis-à-vis many known resident proteins.

Water-deficit or dehydration impairs almost all physiological processes and greatly influences geographical distribution of many crop plants. Although dehydration induced changes in gene expression have been investigated widely in many plant species, reports on identifying and understanding the role of their functional products are rare. The alteration of protein synthesis and/or degradation is one of the fundamental metabolic processes that may influence dehydration tolerance in plants. The power of proteomics is not necessarily in the mapping of complete
proteomes, but rather in the comparison of proteomes, aiming at the identification of differentially expressed proteins in response to various stress and the subsequent changes taking place in the system. To understand the molecular mechanism of dehydration response in plants better, we have developed a comparative nuclear proteome in chickpea. Dehydration responsive temporal changes of nuclear proteins in JG-62, a relatively tolerant chickpea variety were monitored using 2-DE. Approximately, 205 protein spots were found to be differentially regulated under dehydration. Mass spectrometry analysis allowed the identification of 147 differentially expressed proteins, presumably involved in a variety of functions including gene transcription and replication, molecular chaperones, cell signaling and chromatin remodeling. The dehydration responsive nuclear proteome of chickpea revealed a coordinated response, which involves both the regulatory as well as the functional proteins.

High genetic variability of dehydration tolerance indicates that some plant species evolved special mechanisms to survive stress conditions. The study of genetic variation for dehydration tolerance, in plants, is crucial for the development of more tolerant varieties because divergent varieties with contrasting physiochemical traits aid in the identification of key cellular components that confer dehydration tolerance. To understand the molecular mechanism for dehydration response better, a nucleus-specific proteome was developed in ICCV-2, a dehydration-sensitive variety of chickpea. This proteome was then compared with that of JG-62, a dehydration tolerant chickpea variety. The comparative expression profile of nuclear proteome between the two varieties displayed a great degree of divergence. The comparison of different functional classes of identified DRPs in two varieties did not show a significant variation, except for ROS pathway related proteins. Though, though there were many common DRPs between JG-62 and ICCV-2, the kinetic profile for these proteins was contrastingly dissimilar, which might be responsible for the difference in dehydration sensitivity of the two varieties.

The analysis of nuclear proteome in chickpea revealed the importance of various proteins in conferring dehydration tolerance. One such protein was CaN-8, which showed homology to a 14-3-3 like protein from *Pisum sativum*. Since the protein is known to regulate the activities of a wide array of targets via direct protein–protein interactions, it might play a decisive role in activating the dehydration
responsive network. Primers were designed from the protein sequence 'tag' obtained from MS/MS analysis and a 0.97 partial clone was obtained using 3' RACE. Further, 5' RACE was used to obtain the N-terminal sequence for this gene. A full-length cDNA, Ca14-3-3a of 1.013 Kb, was obtained by combining the results from 3' and 5' RACE, with an ORF of 783 bp. The dehydration-responsive profile of this gene was analysed by Northern blotting. Ca14-3-3a was expressed as GFP fusion protein and was shown to reside in the nucleus. Further, the protein was overexpressed in E. coli. The fusion protein was purified and was used to identify 14-3-3 binding partners by Far-Western blotting. Future efforts would focus on getting a clearer picture of how this protein functions in dehydration response.