Chapter 6

Summary
6.0 Summary

The nucleus, the regulatory hub of the eukaryotic cell, is a dynamic system and a repository of various macromolecules that serve as modulators of cell signaling dictating the cell fate decision. Rice (*Oryza sativa* L.) is one of the most important crops of the world and it serves as the staple food source for more than half the world’s population. Rice is generally considered to be sensitive to dehydration and salinity, where dehydration is one of the most severe limitations on the productivity of rice. To understand the function of nuclear proteins, the nucleus proteome of model crop rice was developed. The proteome was determined using a sequential method of organellar enrichment followed by two-dimensional electrophoresis-based protein identification by MALDI-TOF-MS and LC-ESI-MS/MS. Proteomic analyses revealed new nuclear proteins of unknown functions vis-à-vis the presence of many known nuclear proteins. The data also unraveled the presence of unexpected proteins with known biochemical activities, which have never been associated with nucleus.

Water deficit or dehydration is the most crucial environmental constraint on plant growth and development and crop productivity, influences geographical distribution of many crop plants. It has been postulated that plants respond and adapt to dehydration by altering their cellular metabolism and by activating various defense machineries. Most of the earlier understanding of dehydration responsive cellular adaptation has come from transcriptome analyses. By contrast, comparative analysis of dehydration responsive proteins (DRPs), particularly proteins in the subcellular fraction is limiting. To better understand the molecular mechanisms of dehydration-responsive adaptation in plants, a proteomic approach was initiated to identify dehydration-responsive nuclear proteins in rice. Several commercial rice varieties and parental lines were screened for the status of dehydration tolerance using different physiological and biochemical indexes like RWC, proline content, cell membrane stability and status of photosynthetic pigments and established their relative dehydration tolerance. Dehydration-responsive temporal changes of nuclear proteins in Rasi, a relatively tolerant rice variety were monitored using 2-DE and protein identification by LC-ESI-MS/MS. The proteomics analysis led to the identification of 109 differentially regulated proteins presumably involved in a variety of functions, including transcriptional regulation and chromatin remodeling, signaling and gene
regulation, cell defense and rescue, and protein degradation. The dehydration-responsive nuclear proteome revealed a coordinated response involving both regulatory and functional proteins, impinging upon the molecular mechanism of dehydration adaptation. Further, dehydration responsive nuclear proteome was also studied in IR-64, a relatively dehydration susceptible rice variety. The comparative expression profile of nuclear proteome between the tolerant and susceptible varieties displayed a group of differentially expressed proteins among which some were common to both the varieties while a number of proteins were specific to either of the varieties. This suggests that although a large part of the differences between susceptibility and resistance might be due to the quantitative differences of common regulatory pathway, there might be some separate branches in this network that make a plant susceptible or resistant. The protein expression dynamics also differed between the varieties suggesting that temporal and spatial changes might lead to variety specific response. These results might help in understanding the spectrum of nuclear proteins and the biological processes they control under dehydration as well as having implications for strategies to improve dehydration tolerance in plants.

The analysis of nuclear proteome in rice revealed the presence of various novel proteins in this compartment. Three proteins belonging to proteasome pathway were cloned and their presence in nucleus was confirmed by GFP localization studies. One of the proteins identified in rice nucleus was WD repeat containing protein (OsNWD), found to be differentially regulated in both tolerant and susceptible variety indicating its role in dehydration. OsNWD was unique from other known WD proeins in having U-box and PrP19 domain. WD repeats containing protein is important for a variety of cellular functions and its transcript analysis suggest OsNWD is inducible to abiotic stresses may in part depend on ABA. Hence, it is concluded that OsNWD might play an important role under osmotic stress. Thus it will be quite interesting to study the functional role of OsNWD and their interacting partners involved in regulatory network in dehydration tolerance.