

## CHAPTER 38

### PLANT MOLECULAR BIOLOGY

#### Doctoral Theses

01. JAJO (Ringyao)  
**Study of Stress Responsive Methylome Dynamics In Rice.**  
Supervisor: Prof. Saurabh Raghuvanshi  
Th 27719

#### *Abstract*

Drought stress triggers extensive genome-wide modifications of DNA methylation in rice. The current study delves into the changes and distinctions in the methylome dynamicity profiles of panicle, flag leaf, and root tissues in IR64 and N22, comparing control and drought stress conditions in field during the “heading” stage. Despite the prevailing hyper-methylation trend in N22 compared to IR64 in control, there is a distinct preference for CHH site hypo-methylation in both panicle and root tissues of the tolerant cultivar (N22). Drought stress increases methylation levels in IR64, while N22 response is contextually dependent on the tissue type. The study highlights the variation in sequence preferences for methylation dynamicity between the two cultivars. Drought-induced differentially methylated regions (dDMR) show hyper-methylation in both cultivars, with N22 exhibiting unique preference for CHH in panicle and root tissues. Approximately 90% of the dDMR are cultivar-specific, and about 70% of the cultivar differences (cDMR) under stress are unique compared to control condition. Likewise, more than 60% of total tissue-DMR differ between the growth conditions. There is higher prevalence of hyper-methylated dDMR that co-localized with differentially expressed genes. dDMR of CHH sequence exhibit stronger negative correlation with expression compared to other sequences. Examination of DEG with DMR highlights functional relevance under drought stress, especially with DMR found in gene bodies and promoter regions. Notably, in panicle, methylation divergence influences flowering regulation genes. Additionally, we developed a web-based data visualization and analysis application called PlotS. Certain limitations in visualization of multivariate data and statistical analysis using non-proprietary tools necessitate a refined approach. PlotS, a versatile tool for diverse datasets, offers multiple graphs and customization options for multivariate visualization and statistical analysis in a unified workflow, providing a robust platform for data exploration and analysis.

#### *Contents*

1. Stress Responsive Methylome Dynamics in Rice 2. Development of Web-based Application for Data Visualization and Statistical Analysis. References. Annexures. Appendices. Publication and Posters.

02. SHARMA (Shivam)  
**Functional Characterization of a Calcium-dependent Protein Kinase, OsCPK29, and a General Transcription Factor, OsTFIIB, During Development in Rice.**  
Supervisors: Prof. Sanjay Kapoor and Akilesh Kumar Tyagi  
Th 27720

*Abstract*

The present study explored the functional significance of two rice genes, OsCPK29 and OsTFIIB, in rice developmental processes by utilizing reverse genetics approaches. These genes encode distinct proteins with different molecular functions: OsCPK29 encodes a nuclear calcium-dependent protein kinase, while OsTFIIB (TRANSCRIPTION FACTOR II B) encodes a general transcription factor (GTF). Silencing OsCPK29 resulted in pollen grains with a defective intine layer and reduced pollen germination efficiency, whereas its overexpression negatively impacted only pollen germination. Interaction studies suggested a potential interaction between OsCPK29 and OsMADS68, a transcription factor involved in pollen maturation and germination. Yeast one-hybrid analysis revealed that OsMADS68 can bind to the OsCPK29 promoter, thereby regulating its transcription. These findings indicate that OsCPK29 plays a regulatory role in intine development and pollen germination in rice. Additionally, OsCPK29 overexpression led to a reduction in grain size, possibly due to alterations in cell proliferation and expansion in spikelet hull. On the other hand, OsTFIIB has been found to influence various stages of plant growth and development. Overexpression of OsTFIIB resulted in phenotypic changes, such as altered seedling growth, reduced plant height, delayed heading, altered panicle architecture, decreased yield, and changes in the levels of seed storage substances. The study indicated that OsTFIIB overexpression affects expression of genes related to gibberellin (GA) biosynthesis, signalling, and deactivation, suggesting an impact on GA homeostasis and signalling in rice. Overexpression of OsTFIIB also altered the expression of genes involved in the photoperiodic flowering pathway and accumulation of seed storage proteins (SSPs). Consequently, the implications of OsTFIIB overexpression provided insights into its diverse functions as a transcriptional regulator during rice development. Overall, the present work unveiled the functional relevance of OsCPK29, a component of Ca<sup>2+</sup> signaling, and OsTFIIB, a general transcription factor, in various aspects of rice growth and development.

*Contents*

1. Part A: Functional characterization of OsCPK29 in rice development
  2. Part B: Functional characterization of OsTFIIB in rice development. General summary and conclusions.
  - References
  5. List of publications, conferences attended and work presentations.
03. SINGH (Arunima)  
**Molecular Characterization of Universal Stress Proteins in Wheat and their Functional Characterization under Abiotic Stress.**  
 Supervisors: Prof. Arun Kumar Sharma and Paramjit Khurana  
Th 27721

*Abstract*

Universal Stress Proteins are stress responsive proteins present in a variety of life forms ranging from bacteria to multicellular plants and animals. In this study we have identified 85 TaUSP genes in the wheat genome and have characterised their abiotic stress responsive members in yeast under different stress conditions. Localization and Y2H studies suggest that wheat, USP proteins are localized in the ER complex, and extensively crosstalk amongst themselves through forming hetero and homodimers. Expression analysis of these TaUSP genes suggests their role in adaptation to multiple abiotic stresses. TaUSP\_5D-1 was found to have some DNA binding activity in yeast. Certain abiotic stress responsive TaUSP genes are found to impart tolerance to

temperature stress, oxidative stress, ER stress (DTT treatment) and LiCl<sub>2</sub> stress in the yeast heterologous system. TaUSP\_5D-1 overexpression in *A. thaliana* imparts drought tolerance via better lateral root network in transgenic lines. We also found a novel interacting partner, TaGolS, which physically interacts with another USP protein, TaUSP\_3B-1 and colocalizes in the endoplasmic reticulum. TaGolS is a key enzyme in the RFO (Raffinose oligosaccharides) biosynthesis which is well reported to provide tolerance under water deficit conditions. TaUSP\_3B-1 overexpression lines showed an early flowering phenotype under drought stress. Moreover, at the cellular levels ER stress induced TaUSP\_3B-1 transcription and provides tolerance in both adaptive and acute ER stress via less ROS accumulation in the overexpression lines. TaUSP\_3B-1 overexpression plants had increased silique numbers and a denser root architecture as compared to the WT plants under drought stress. We have also characterized a heat stress upregulated TaUSP\_6D-5 gene via overexpression in *Arabidopsis thaliana* and Rice.

### Contents

1. Identification of *Triticum aestivum* USPs and their functional characterization in *Saccharomyces cerevisiae* and *Arabidopsis thaliana* under abiotic stress  
 2. Functional characterization of *Triticum aestivum* Universal stress protein, TaUSP\_3B-1 under drought stress  
 3. Functional characterization of *Triticum aestivum* Universal stress protein, TaUSP\_6D-5 under heat stress. Summary and Conclusions. Appendices. List of Publications. Posters Presented

#### 04. SINGHAL (Chanchal)

#### **Molecular and Functional Characterization of Growth-Related Genes in Indian mulberry (*Morus indica*).**

Supervisors: Prof. Arun Kumar Sharma and Paramjit Khurana

Th 27722

### Abstract

The mulberry plant (*Morus* spp.), belonging to the genus *Morus* in the family Moraceae, is a globally cultivated deciduous tree renowned for its economic and ecological significance. It is primarily valued for its role as a primary food source for silkworms (*Bombyx mori*), making it a cornerstone of the sericulture industry. In addition to this crucial role, mulberry plants are also valued for their nutritious berries and their use in traditional medicines. The mulberry plant exhibits moderate tolerance to salinity and drought stresses. However, its susceptibility to adverse weather conditions, environmental cues, and vulnerability to pests frequently lead to reduced growth and productivity. With the recent release of the genome sequences of three *Morus* species—*Morus notabilis*, *Morus alba*, and *Morus indica* cv K2—we aimed to elucidate the roles of growth-related genes in mulberry in these species. This thesis details the genome-wide identification, evolutionary significance, and expression analysis of Auxin Response Factors (ARFs), Cytokinin oxidases (CKXs), and Isopentenyl transferases (IPTs) across three chapters. The first chapter investigates ARFs, highlighting the generation of overexpression lines for MiARF13 in *Arabidopsis thaliana* and *M. indica* cv K2, which demonstrated superior performance compared to wild-type (WT) under drought stress induced by abscisic acid and under biotic stress induced using flagellin and *Pseudomonas syringae* pv. Tomato (Pst) DC3000. The second chapter explores the function of CKXs under drought stress, revealing that MiCKX4 overexpression significantly enhanced root growth under drought conditions compared to WT. The third chapter addresses the role of IPTs in drought stress, with elevated IPT expression levels underscoring their critical function in stress response. Altogether, our research provides crucial

insights into the roles of ARFs, CKXs, and IPTs in mulberry growth and development, with implications for enhancing crop productivity and yield.

#### *Contents*

1. Genome-wide identification, structural, and evolutionary analysis of auxin response factor (ARF) gene family in *Morus* species and functional characterization of MiARF13 under drought and pathogen attack 2. Genome-wide identification, structural, functional, and evolutionary analysis of cytokinin oxidase (CKX) gene family in *Morus indica* cv K2 and functional characterization of MiCKX4 during drought stress 3. Identification, structural, functional, and evolutionary analysis of Isopentenyl transferase (IPT) gene family in *Morus* species. Summary and Conclusions. Appendices. List of Publications and Posters Presented.

05. VASHISTH (Vishal)  
**Investigation on the Mechanism of Action of Stress Associated Protein (SAP) Genes of Rice.**  
 Supervisors: Prof. Sanjay Kapoor, Arun Kumar Sharma and Akhilesh Kumar Tyagi  
Th 27723

#### *Abstract*

Stress Associated Proteins (SAPs) are a group of proteins characterized by the presence of A20/AN1 zinc finger domains and their genes respond to various abiotic stresses. SAPs have been identified in a range of plant species, with 18 and 14 members reported in rice and Arabidopsis, respectively. The aim of this study is to explore the roles of three stress-inducible rice genes, OsSAP6, OsSAP10, and OsSAP18, under water-deficit stress and to examine the sub-functionalization among these members of the rice SAP gene family. Earlier, it was observed that rice OsSAP6, OsSAP10, and OsSAP18, are upregulated under dehydration stress and their encoded proteins contain A20 and/or AN1 zinc finger domain(s). The selected genes, OsSAP6, OsSAP10, and OsSAP18, have been found to act as positive regulators of water-deficit stress response in transgenic Arabidopsis. They influence the physiological, biochemical, and molecular response of transgenic plants, thereby enhancing their tolerance to water-deficit stress. Moreover, overexpression of these genes in Arabidopsis protects yield loss under water-deficit stress. These findings also indicate that OsSAPs enhance water-deficit stress tolerance by variably regulating the expression of certain endogenous genes. The differential protein-protein interactions observed for three OsSAPs indicate that rice SAP gene family members may have undergone sub-functionalization at the level of protein interactions. The study highlighted OsSAP6, OsSAP10, and OsSAP18 as potential candidates for enhancing water-deficit stress tolerance in plants.

#### *Contents*

1. Review of Literature 2. Materials and Methods 3. Results 4. Discussion 5. Summary and Conclusions 6. References. List of Publications, Conferences Attended and Work Presentations.