

## CHAPTER 48

### PLANT MOLECULAR BIOLOGY

#### Doctoral Theses

01. AMARJOT KAUR

**Transcriptome Analysis of Cold and Submergence Responsive Genes and Functional Analysis of Osbzip62 in Regulating Reproductive Development in Rice**

Supervisor : Prof. Jitendra P. Khurana

Th 23495

*Abstract  
(Verified)*

Rice has a major contribution in the global food security as it serves as a source of energy for millions of people. Improving the yield potential of rice is a necessity to meet the global demand. Exposure of plants to a number of abiotic stresses adversely affects rice productivity. The present study focussed on the transcriptome analysis of different rice varieties under cold and submergence stress using microarray. Major shift in the expression pattern of the genes could be observed in response to 3 h of cold stress in both Nonglowai (tolerant) and IR50 (susceptible) rice varieties but Nonglowai was able to achieve homeostasis earlier than IR50. A number of unique metabolic pathways for the biosynthesis of amines, polyamines and sugars along with genes involved in detoxification signalling were uniquely activated in Nonglowai, probably to achieve redox homeostasis. Similarly, transcriptome profiling of FR13A (tolerant) and IR42 (susceptible) rice varieties in response to 1d, 4d and 8d of submergence stress was carried out. In FR13A, the expression of genes involved in the biosynthesis of trehalose and starch along with detoxification signalling and gibberellin inactivation pathway got activated earlier than IR42. The control of flowering time in rice is essential for reproductive success and seed set. To identify genes involved in floral transition, microarray analysis of shoot apical meristem and early panicle stages in rice was carried out earlier in our lab. *OsbZIP62*, an ortholog of *FD* in *Arabidopsis*, was selected for its functional validation. RNAi rice transgenics of *OsbZIP62* displayed slightly delayed flowering phenotype and its overexpression in *fd-1* mutant of *Arabidopsis* could partially complement its late flowering phenotype. *OsbZIP62* interacted with OsFTL, OsGF14 and OsCAMK proteins, which might be essential for activation of downstream floral meristem identity genes in rice.

*Contents*

1. Transcriptome analysis of cold and submergence responsive genes 2. Functional analysis of *osbzip62* in regulating reproductive development in rice.

02. GAURAV KUMAR

**Molecular Analysis of Stress Response Dynamics in Rice (*Oryza sativa* L.) Against Rice Tungro Viruses and Assessment of RNAi Mediated Viral Resistance in Transgenic and Backcrossed Rice Varieties .**

Supervisor : Prof. Indrani Dasgupta

Th 23496

### Contents

1. General introduction 2. Review of literature 3. Study of differentially expressed genes (DEGs) in rice upon tungro infection by RNA-seq mediated transcriptome profiling and high throughput sequence analysis of small RNA respectively 4. Elucidation of status of RNAi components in rice upon tungro infection 5. Study of cell wall and ROS metabolomics in rice upon tungro infection 6. Estimation of resistance in transgenic backcrossed lines containing a double-stranded RNA construct against rice tungro bacilliform virus and validation of dual RNAi strategy in pusa basmati transgenics against tungro virus complex. Summary. References. Appendix I Appendix II

#### 03. SHARMA (Manisha)

##### **Identification and Functional Analysis of Armadillo Repeat Containing Proteins in Rice and Arabidopsis: and Detailed Expression Analysis of CIP K9 in Response to Potassium Deficiency.**

Supervisor : Prof. Girdhar K. Pandey

Th 23497

#### *Abstract (Not Verified)*

ARM repeat proteins display ubiquitous presence across eukaryotes and expanded dramatically in the land plant lineage. In the present study, extensive database searches facilitated identification of 158 Armadillo repeat proteins in rice genome. An in-depth analysis of gene expression through microarray and Q-PCR, revealed a number of ARM proteins expressing differentially in abiotic stresses and developmental conditions, suggesting a potential role of this superfamily in development and stress signaling. Based on microarray expression analysis, *OsPUB75/ARM* was selected for further functional characterization in plants. The phenotypic assay under mannitol and salt stress revealed that the *35S:OsPUB75/ARM* overexpression lines are sensitive to these stress conditions. High salinity and mannitol induced oxidative stresses are extremely inhibitory for their growth in comparison to the wild type. The interaction analysis between *OsPUB75/ARM* and *AtSK41* ortholog in rice (LOC\_Os03g62500) *OsSK41* has confirmed them to be interacting in yeast two-hybrid system. The interaction of full-length *OsPUB75/ARM* was confirmed by BiFC assay. In conclusion, our results suggest that the U-box E3 ubiquitin ligase *OsPUB75/ARM* is an important negative regulator of drought and salt stress responses in Arabidopsis. Following the characterization of a rice PUB protein, we have selected one Arabidopsis U-box/ARM protein (*AtPUB2*) to elucidate its function in plants. This gene is highly expressed under different biotic and abiotic stress conditions. Here, we show that the transcripts of this gene are accumulated rapidly in response to various stress treatments that arise due to oxidative stress. *AtPUB2* overexpression lines are highly tolerant to methyl viologen induced oxidative stress condition. Subsequently, to study nutrient deficiency response in plants, a detailed expression analysis of *CIPK9* (CBL-interacting protein kinase 9) was performed under different nutrient deficiency conditions using detailed phenotypic assay, transcriptome analysis and promoter analysis.

### Contents

1. Introduction 2. Review of literature 3. Identification of armadillo (ARM) repeat containing proteins and their expression analysis under abiotic stress conditions in rice 4. Interaction and functional analysis of rice ARM protein *OsARM/PUB75* in response to abiotic stress conditions 5. Functional analysis of Arabidopsis ARM containing U-box protein (*atpub2*), in response to oxidative stress 6. Detailed expression analysis of CBL-interacting protein kinase 9 (*cipk9*) in Arabidopsis 7. Summary. References. List of publications.

04. SHARMA (Naveen)  
**Bac end Sequencing (BES) of Wheat Chromosome 2a1 and Functional Characterization of tamips for heat stress tolerance.**  
 Supervisor : Prof. ParamjitKhurana  
Th 23494

*Abstract*  
*(Not Verified)*

Wheat is the second most produced cereal. Genome availability of crop plants has been seen to accelerate breeding by understanding of genome structure and function at molecular level and understanding phenotypic variations of genetic diversity. Thus, leading to development of new varieties with increased yield and enhanced resistance to biotic and abiotic stresses. Therefore, an international body i.e. International Wheat Genome Sequencing Consortium (IWGSC) was established. India being a part of IWGSC was assigned with physical mapping and sequencing of 2A chromosome of wheat. My work includes BAC end sequencing of wheat chromosome 2A long arm. BAC end sequencing gives an early insight into the genomic organization and accurate assembly of contigs. Our work indicated presence of maximum retrotransposons followed by DNA transposons and dinucleotide SSRs. Gene ontology analysis suggested the enrichment of regulation transcription, DNA-dependent in 2AL BES. Comparative genomic analysis revealed its close homology with *Hordeum vulgare*. Second part of my work includes functional characterization of *TaMIPS* for heat stress tolerance. We have characterized *TaMIPS-B* OE Rice T2 transgenics and *Atmips1* mutant and analysed the whole transcriptome. Expression of *TaMIPS-B* in rice transgenics showed heat tolerance phenotype and activation of ET/JA response. Further we performed far-western and pull-down analysis which revealed the interaction of *TaMIPS-B* with ethylene signaling and synthesis pathway proteins. Our observation on etiolated seedlings with Myo-inositol (MI) supplementation resulted in decreased hook angle and hook formation in presence of ethylene signaling inhibitor. Further, experiments suggest MIPS to be a central regulator of Ethylene, Auxins and the BR network. Hence, the quantitative levels of these phytoeffectors have an important role in regulating plant growth, development and response to biotic and abiotic stresses.

*Contents*

1. Bac end sequencing (BES) of wheat chromosome 2a1
2. Functional characterization of tamips for heat stress tolerance. References. Appendices. List of publications.