# CHAPTER 45

# PLANT MOLECULER BIOLOGY

## Doctoral Theses

01. BALAJI M.
**Genome-wide Analysis of Tetraspanin (TET) Gene Family and Functional Characterization of OsTET2 and OsTET5 in Oryza Sativa L.**
Supervisor: Dr. Surekha Katiyar Agarwal
Th 24757

*Abstract*
*(Not Verified)*

Tetraspanins (TETs) belong to a superfamily of evolutionarily conserved, integral membrane proteins with four transmembrane domains, two extracellular loops, a small intracellular loop, short N-, C-terminal cytoplasmic tails and a signature motif in EC2. Being membrane localized, their role in congregating other proteins, passing the community-generated signal across the membrane and transducing it into biological relevant processes has been envisaged in animal systems. However, in plants there is a limited understanding on their biological functions. Comprehensive expression profiling revealed that the transcripts of several rice tetraspanins are differentially accumulated in various tissues, abiotic stresses, nutrient deprivation and in response to phytohormones. Like animals, several rice tetraspanin proteins were observed to be localized to plasma membrane and interact among themselves to form homomeric and/or heteromeric complexes. Phenotyping of the transgenic rice plants generated for overexpression and amiRNA-mediated silencing of OsTET2 and OsTET5, revealed their key role in tolerance to increased salinity. Additionally, overexpression of OsTET5 imparted growth advantage and superior yield-related traits to the transgenic rice plants. Besides being salt tolerant, OsTET5 overexpression rice plants were extremely tolerant to drought stress. The increased tolerance was, to an extent, attributed to lower accumulation of Na+ in salt stress and ROS (reactive oxygen species) in both salinity and drought stress. Transcriptome sequencing of the overexpression plants revealed enhanced expression of transporters, antiporters, ROS homeostasis enzymes and signaling components of stress-related genes. The present study has provided valuable insights into the possible biological function of TETs in regulating development and response to abiotic stresses in plants.

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02. BHATNAGAR (Akanksha)
**Funtional Analysis of bZip Transcription Factor Genes, OsbZIPI and OsbZIP48, in Light-Mediated Development in Rice.**
Supervisor: Prof. Jitendra P. Khurana
Th 24759

*Abstract*
*(Verified)*

One of the largest and most diverse transcription factor families in eukaryotes is the basic leucine zipper (bZIP) transcription factor family. Phylogenetic analysis of the bZIP transcription factors across plant species has predicted a majority of the rice bZIP transcription factors to be orthologs of Arabidopsis bZIP transcription factors. Out of the 89 bZIP transcription factors identified in rice, OsbZIP1, OsbZIP18 and OsbZIP48 have been predicted to be homologs of AtHY5, which is known to be a positive regulator of photomorphogenesis in Arabidopsis. In rice, the overexpression of OsbZIP48 under the control of a constitutive promoter causes a semi-dwarf phenotype while adversely affecting the plant fertility. Thus, we have overexpressed OsbZIP48 under a promoter having maximum expression in the vegetative tissues of rice. The transgenic plants thus obtained show a semi-dwarf phenotype without affecting plant fertility. Further analysis of OsbZIP48 at the protein level reveals that it is not regulated by light. OsbZIP1, another AtHY5 homolog in rice, has also been functionally characterized in this study. It has been shown to interact with OsCOP1, an E3 ubiquitin ligase, subsequent to which it undergoes faster degradation in dark as compared to light in-vitro. It has also been shown to interact with OsCK2, a protein kinase, responsible for its phosphorylation, due to which it becomes less susceptible to degradation. OsbZIP1 has been shown to be a functional homolog of AtHY5 in rice as it can complement the Athy5 mutant in Arabidopsis with respect to hypocotyl length, chlorophyll content and cotyledon angle. Analysis of the overexpression and RNAi transgenics of OsbZIP1 indicate its role in regulation of plant height, flag-leaf and Y-leaf length, flowering time and seedling development in rice.

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03. BABBAR (Richa)
**Production of Heat Tolerant Transgenic Arabidopsis Thaliana Plants by Over-Expression of CIpB-C/Hsp101 Protien.**
Supervisor: Prof. Anil Chopra
Th 24767

*Abstract*
*(Verified)*

Clp/Hsp100 family proteins are considered critical players in acquisition of tolerance to heat stress conditions.The under-expression of ClpB-C/Hsp100 proteins has been linked with increased sensitivity of plants to heat stress in several studies. We asked the inverse question in this thesis work: how important the over-expression of ClpB-C /Hsp100 protein is in raising heat tolerance in plants?We worked with the model plant Arabidopsis thaliana in this study. We used 10 C lines (Arabidopsis plants transformed with CaMV35S-driven OsClpB-C) and10 IN lines (Arabidopsis transformed with AtClpB-C promoter-driven OsClpB-C) in this study. We produced 14 GF lines (transgenic Arabidopsis lines transformed with the genomic fragment of AtClpB-C gene in entirety including its structural and regulatory regions) in this work.The lines were analysed for ClpB-C transcript separately for the native AtClpB-C transcript and trans-gene OsClpB-C transcript for the C and IN and AtClpB-C transcript for the GF types.The protein expression was scored using antiAtClpB-C antiserum.Western analysis of the transgenic lines showed that the appropriate titre of AtHsp101 protein is important for the survival of plants under heat

stress. The phenotyping work was done in respect of basal thermotolerance-seed (BT-seed), basal thermotolerance-seedling (BT-seedling) and acquired thermotolerance-seedling (AT-seedling) assays.Out of diverse transgenic lines tested in this study, GF30-7 line showed significantly high ClpB-C protein as against the Col-0 plants transformed with empty vector sequence. GF30-7 line was analysed for heat-induced transcript changes by RNAseq method. We showed that OsClpB-C, AtClpB-C, OsClpB-P, and OsClpB-M proteins have the ability in protection of substrate proteins from heat stress.

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04. CHEENI VIJAYA KUMAR

**Functional Characterization of Heat Shock Transcription Factors, HSFA1e and HSFC1, in Arabidopsis Thaliana and Geonome-wide Polymorphism Studies in Rice Cultivars.**

Supervisor: Dr. Surekha Katiyar-Agarwal

Th 24766

*Abstract*
*(Verified)*

Plants, being sessile in nature, are exposed to plethora of biotic and abiotic stresses, either sequentially or in combination. Heat Shock Transcription Factors or HSFs are known to be crucial regulators of stress response in plants. In the present study efforts were made to functionally characterize two Arabidopsis HSFs, AtHSFA1e and AtHSFC1. It was observed that overexpression of AtHSFA1e conferred enhanced basal thermotolerance in Arabidopsis by activating various stress-responsive genes, including AtHSP101. The AtHSFA1e overexpressing transgenic Arabidopsis lines also exhibited tolerance to salt and oxidative stress. Analysis of the interactome network showed that AtHSFC1 interacts with few members of class A HSFs, but not with any member of class B HSFs. Yeast-two-hybrid based library screening approach identified an autophagy protein, ATG8i, as an interacting partner of AtHSFC1. Transient luciferase assays showed that AtHSFC1 might act as co-activator of AtHSFA1d in inducing AtHSFA2 promoter. Overexpression of AtHSFC1 resulted in mild sensitivity in transgenic Arabidopsis plants. Genomic organization study revealed that a gene, At3g24518, overlaps with AtHSFC1 and possibly forms natural antisense transcript, may be involved in regulating AtHSFC1. Genetic polymorphism studies showed that ~72% Indian rice cultivars possess truncated form of OsHSFB2b. Genome-wide association study of salt tolerance trait in 129 Indian rice cultivars led to the identification of 18 accessions to be highly salt tolerant and 7 SNPs associated with the trait. The present study has contributed towards the elucidation of function of two HSFs in Arabidopsis. Further, efforts were made to provide a platform to perform a more detailed study for genome-wide association analysis of salt tolerance trait in rice.

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05. JYOTISH M. S.

**Exploration of the Transcriptome of Flag Leaf and Coleoptile Senescence and Investigation of the Role of Heat Shock Transcription Factors in Regulating Senescence in Oryza Sativa L.**

Supervisor: Dr. Surekha Katiyar-Agarwal

Th 24765

*Abstract*
*(Not Verified)*

Senescence is the final stage of development that involves a highly-orchestrated degradation process for nutrient recycling in plants. Apart from age, several external and internal factors induce premature senescence, thereby reducing the yield of plants. Therefore, it is of utmost importance to study senescence programme in plants. Even though attempts to study the molecular basis of senescence have been carried out, little is known about its regulation in plants. Therefore, the present study was designed to identify the regulatory components of senescence pathway(s) in rice. Comprehensive expression profiling of HSF (Heat Shock Factor) genes in flag leaf, coleoptile and dark-induced leaf senescence revealed that several HSFs were differentially expressed. Based on these results, OsHSFA7a and OsHSFC1b were selected for functional characterization studies. OsHSFA7a overexpression (OX) plants were retarded with inferior yield-related traits, while silenced (KD) plants exhibited higher culm number and seed weight. However, no significant difference in flag leaf senescence was observed. The transgenic rice lines for OX of OsHSFC1b displayed enhanced growth and seed length, whereas its KD lines exhibited severely retarded growth. Significant changes in flag leaf and dark-induced senescence were also observed in OsHSFC1b lines. Further, we sequenced small RNAs (sRNAs) from different stages of flag leaf senescence in rice and identified 34 known and 494 novel miRNAs. RNA-seq analysis revealed 3166 differentially expressed genes during coleoptile senescence and their functional categorization showed enrichment of TFs, transporters, hormone pathway and stress-responsive genes. sRNA analysis identified 50 known and 280 novel miRNAs, several of which were differentially expressed during coleoptile senescence. Inverse correlation in expression profile of several miRNAs and their target genes was observed. Overall, our study has led to the identification of new components that could potentially be used for manipulating the process of senescence and eventually increase grain yield in rice.

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06. NEGI (Nisha)
**Comparative Leaf Transcriptomics for Finctional Characterization of Growth and Biotic Stress Related Genes in Indian Mulberry .**
Supervisor: Prof. Paramjit Khurana
Th 24762

*Abstract*
*(Verified)*

Mulberry is an economically important plant on account of its leaves being the sole source of food for silkworm (Bombyx mori); the producer of silk. Plants being sessile are unable to flee away from any environmental stresses. Mulberry is highly impacted by stress caused by plant pathogens (biotic stress) which results in yield losses in mulberry. On the other hand, growth of mulberry is crucial for the development of high quality leaves with high nutrition for the production of good quality silk from silkworm. Keeping these two major aspects of mulberry development and enhancement in mind we studied comparative analysis of the leaf transcriptomes of two wild varieties; Morus laevigata and Morus serrata and two cultivated varieties; Morus indica varieties K2 and V1 alongwith the sequenced haploid variety Morus notabilis. In this analysis, important growth and stress related genes were mined and

validation by real-time PCR was also carried out. In addition to this, functional characterization of some growth related genes viz. arfs and bch1 and biotic stress related genes viz. Miisolectin and MiWRKY53 reveals the involvement of these crucial genes in growth of mulberry and stress management in particular biotic stress against fungal pathogens.

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1. Comparative leaf transcriptomics of five mulberry accessions and cataloguing structural and expression variants 2. Characterization of growth related genes of mulberry 3. Characterization of biotic stress related genes of mulberry. Summary and conclusions. Annexures. List of publications.

07. RITESH KUMAR
**Natural Variation in Thermotolerance of Rice and Arabidopsis and Linking it with ClpB-C/Hsp101 Gene Sequence and Expression.**
Supervisor: Prof. Anil Gover
Th 24761

*Abstract*
*(Verified)*

Three plant species namely rice (Oryza sp.), Arabidopsis (Arabidopsis sp.) and faba bean (Vicia faba) were analyzed. Sequence comparison of different cultivated and wild rice types showed that ClpB-C/Hsp100 protein was of variable lengths in these rice types. Moroberekan (japonica) was found to be highly sensitive in basal thermotolerance-seedling (BT-seedling) assays than IR64 (indica) and N22 (aus) rice types. However, Moroberekan performed better in short term acquired thermotolerance (SAT-seedling) assays on relative basis. The three cultivar types were almost comparable in long term acquired thermotolerance (LAT-seedling) assays. The introduction of priming treatment in SAT-seedling and LAT-seedling assays and the absence of this treatment in BT-seedling assays thus appears a critical factor in governing heat stress response in these three rice types. In priming stress, Moroberekan showed higher expression of ClpB-C/Hsp100 in western blotting experiment. Further, anther proteomes of three rice types namely Moroberekan, IR64 and N22 were analyzed at specified heat stress regimes. Higher number of Hsps were noted in N22 as compared to Moroberekan and IR64 when plants were subjected to heat stress. Also, genes related with carboxylic acid metabolism and vitamin E biosynthesis process were notably enriched in short-term heat stress treatment given to N22 (NST). These processes may contribute to higher heat tolerance of N22 at reproductive stage than other two rice types. Further, nine different Arabidopsis accessions were compared for heat response. Cvi-0 accession of Arabidopsis (found at higher altitudes) was notably more sensitive in basal and acquired thermotolerance assays. Cvi-0 accession was found to have higher ClpB-C/Hsp100 protein accumulation during priming phase in acquired thermotolerance-seedling response. In Vicia faba, ClpB-C/Hsp100 and sHsp17.9 genes were cloned and sequenced. Transcripts of both of these genes were found to be heat inducible. Further, these transcripts were found to accumulate in pollen under heat stress.

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1. Introduction 2. Review of literature 3. Materials and methods 4. Results 5. Discussion. Summary and conclusion. Literature cited. Appendix.

08. SANTOSH KUMAR
**Study of Epigenetic Regulatory Mechanisms in Rice.**
Supervisor: Dr. Saurabh Raghuvanshi
Th 24760

*Abstract*
*(Not Verified)*

H3K4me3 modification is an active histone mark and positively correlates with gene expression ranging from plants to animals. In order to study the involvement of H3K4me3 mark in regulation of drought response, whole genome ChIP-seq (Chromatin immunoprecipitation followed by high-throughput sequencing) was performed in two contrasting rice cultivars, N22 (drought tolerant) and IR64 (drought sensitive) at the heading stage in the flag leaf and spikelets tissue for plants grown under control and drought conditions. ChIP-seq tags generated were mapped on genome with the help of 'Bowtie2' software and in overall, more than 90% of the reads mapped and out of these 55-65% of the tags mapped uniquely. Associated peaks were called with the help of 'MACS' software and on average, 15-30 thousands peaks were identified (p 1e-05). The number of identified peaks changed dynamically depending upon growth conditions, tissue types as well as cultivar used. The average peak lengths ranged from 800 bp to 1100 bp depending on cultivar, tissue types as well as growth conditions. H3K4me3 histone mark was found to be associated with about 25% to 45% of total rice genes. Further analysis for peaks genomic distribution shown that peaks were localized both in genic as well as intergenic loci in both cultivar. However most of the peaks were found to be enriched mostly within 1kb of the transcription start sites (TSSs) of genes and varied dynamically with growth conditions, tissue types as well as cultivar specific manner. Analysis of ChIP-seq and RNA seq data under similar conditions suggested that a distinct subset of genes were positively correlated with H3K4me3 mark. This study clearly demonstrates the drought mediated and variety-specific dynamism of the H3K4me3 histone mark in indica rice.

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09. SHARMA (Komal)
**Cellular Characterization of Channelrhodopsins from Green Algae.**
Supervisor: Prof. Giridhar K. Pandey
Th 24764

*Abstract*
*(Not Verified)*

Chlamydomonas reinhardtii is a unicellular, bi-flagellated, green alga. It consists of a specialized organelle called eyespot that scans the surrounding environment for the quantity or quality of the light by virtue of different photoreceptors. Rhodopsins are the photoreceptor responsible for the photomotility behavior of C. reinhardtii. It contains twelve different rhodopsins among which channelrhodopsin1 (ChR1) and channelrhodopsin2 (ChR2) controls the photomotile responses. Recently, the light dependent trafficking of ChR1 to eyespot and flagella involving intraflagellar transport (IFT) machinery has been elucidated. However, vesicular targeting or sorting of ChR1 from Golgi bodies to the basal bodies is still unknown. We identified proteins like CrARL11 and phototropin proposing their involvement in IFT machinery modulated

trafficking of ChR1 to flagella or eyespot. Sumoylation, a well-known post-translational modification process, can alter the fate of a target protein in the cellular system by its ubiquitination leading to its degradation or signaling. Variations in ChR1 expression during light/dark cycle was previously reported, depicting its regulation either at RNA or protein level. This differential expression of ChR1 might be associated with its regulated degradation. Experimental results suggested presence of sumoylation machinery proteins in the ChR1 interactome and sumoylation of the ChR1 was confirmed. Ca2+-dependent change in phosphorylation of ChR1 has been reported, suggesting a Ca2+-dependent activation/deactivation of ChR1 similar to that of vertebrate rhodopsins. Here, two Ca2+-sensing proteins, CrCBL1 and CrCBL2, homologue of calcineurin B like proteins (CBL) of plants, were identified and characterized. CrCBL1 interacts with ChR1 and VGCC (voltage gated Ca2+-channel). This interaction suggests that the signaling of ChR1 might be Ca2+-mediated in C. reinhardtii. Overall, present research shows the evolutionary conservation of rhodopsin trafficking machinery for both the animal and bacterial type rhodopsins. Moreover, this would be the first report suggesting sumoylation-regulated control of cellular turnover of the bacterial type rhodopsins in algal systems.

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10. SINGH (Garima)
**Analysis of Rice Heat Stress Trenscription Factort OsHsfA6a Networks involved in OsCIpB-C/Hsp101 Gene Expression.**
Supervisor: Prof. Anil Grover
Th 24763

*Abstract*
*(Not Verified)*

High temperature stress profoundly affects growth and reproduction of crop plants especially rice. A forecast by Intergovernmental Panel on Climate Change (IPCC) says that global average temperature will increase by 1.8 to 4 ˚C by 2100. Identification of master regulatory genes for thermotolerance in rice is imperative for generation of high temperature tolerant rice plants. ClpB-C/Hsp101 protein, a major molecular chaperone is necessary for plant survival under HS. ClpB-C/Hsp101 proteins mediate solubilisation of proteins aggregates formed during HS. In rice, heat stress transcription factor binds to the promoter of OsClpB-C/Hsp101 gene. This thesis deals with understanding the networks of OsHsfA6a involved in transcriptional regulation of OsClpB-C/Hsp101 expression. Phylogeny analysis of Hsf sequences from different plant species revealed that rice HsfA6a has functionally diverged from dicot HsfA6a and there are no expressologs of OsHsfA6a in Arabidopsis and tomato. Rice HsfA6a is a close paralogue to A2 sub-family and should be re-classified as HsfA2 member. DBD and OD regions of rice class A Hsfs are highly conserved and all class A Hsfs of rice possess an AHA domain and NES in their CTD. Gene organisation of OsHsfA6a showed that it occurs as a single isoform with no alternative variants. Using several deletion constructs high TA potential of OsHsfA6a was mapped to the CTD of the Hsf. Role of post-translational modifications especially phosphorylation in regulating the activity of OsHsfA6a was studied. Interactors of OsHsfA6a were

identified by library scale screening and were analysed for their functional significance on OsClpB-C/Hsp101 gene expression. Yeast based assays, transient system using rice protoplasts and in-vitro phosphorylations are some of the techniques used for the present work. OsHsfA6a anti-sense rice transgenics were raised and analysed for their HS response.

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1. Introduction 2. Review of literature 3. Materials and methods 4. Results 5. Discussion. Summary and conclusion. Literature cited. Annexures.

11. VERGISH (Satyam)
**Role of Zeitlupe Family Proteins, OsFBO8, OsFBO9 and OsFBO10, in Regulating Plant Development and Aboitic Stress Responses.**
Supervisor: Prof. Jitendra P. Khurana
Th 24758

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1. Introduction 2. Review of literature 3. Materials and methods 4. Results and discussion 5. Summary and conclusion 6. References 7. Appendices