CHAPTER 44

PLANT MOLECULER BIOLOGY

Doctoral Theses

01. AMRAPALI

Study if Mechanism of Action of Methyl-CpG-Binding Domain Proteins AtMBD 1 and AtMBD 13 in Gene Regulation in Arabidopsis.

Supervisor : Prof. Arun K. Sharma

Th 24030

Abstract
(Verified)

Epigenetic modifications are heritable modifications of the core components of chromatin i.e. DNA and histones that do not involve changes in the underlying DNA sequence. DNA methylation in the form of 5-methyl cytosine is the most common epigenetic modification of DNA in eukaryotes. DNA methylation is usually associated with silencing of the associated locus. Though the establishment and maintenance of DNA methylation has been studied in great details, not much is known about the mechanism by which it is translated into biological processes. Methyl-CpG-binding domain proteins (MBDs) are proteins capable of specifically recognizing and binding methyl CpGs through their MBD domain. Because of this ability, MBD proteins are also known as interpreters of the DNA methylation code. MBD domain is about 70-85 amino acids in size. MBD proteins were initially identified in mammals. Based on homology with mammalian MBD proteins have been recognized in many plant species as well. The Arabidopsis MBD (AtMBD) family consists of 13 members. These thirteen members have been classified into eight different subclasses. Some of the members of the AtMBD have been characterized. AtMBD5 and AtMBD6 have been reported to play a role in gene silencing. But the mechanism of action of most AtMBDs is still unknown. The present study focuses on the studying the role of two members of the AtMBD family: AtMBD1 and AtMBD13. Yeast- two-hybrid screening has been used to find out the interacting partners of both the proteins. Both the genes have been functionally characterized in this study using knock-out mutants. The genes were found to be involved in two different pathways. The study sheds light on the mechanism of action of AtMBD1 and AtMBD13 in gene regulation.

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02. CHAUDHARY (Chanderkant)

BAC End Sequencing (BES) of Wheat Chromosome 2AS and Role of Spike Photosynthesis in Grain Filling.

Supervisor : Prof. ParamjitKhurana

Th 24029

Abstract
(Verified)

Bread wheat (Triticum aestivumL.) is grown the world over and is cultivated more than any other cereal crop and feeds the approx. 30% of the human population. Being a part of IWGSC,

University of Delhi South Campus was assigned with the sequencing of BAC-ends of wheat chromosome 2A. BAC end sequences are important in the construction of physical maps, genome organization and architecture, marker development, and molecular evolution studies. In this work, we have sequenced 55,648 clones encompassing 102,255 sequences for the short arm of wheat chromosome 2A. We were able to identify 2,080 SSRs and 17223 CDS were predicted in the chromosome 2A. Syntenic maps were also constructed between model grass genomes (Hordeum vulgare, Brachypodium distachyon, Oryza sativa, Sorghum bicolor, and Zea mays) and wheat BESs using the Circos program. In Chapter II, role of spike photosynthesis in grain filling were evaluated. Part A consist of transcriptome profile of wheat awns and effect of Rubisco Activase overexpression in enhancing photosynthetic efficiency and yield under heat stress. Contribution of photosynthetic organs (flag leaf, awn, and spike) in photosynthesis and grain filling were evaluated. This analysis suggests the crucial role of awns in photosynthesis other than flag leaf. Further, transcriptome of wheat awn was analysed using RNA-seq under heat stress (HS) conditions. Ectopic expression of TaRCA-B in rice transgenic indicates a direct correlation with tolerance under HS conditions. In part B Wheat ATP synthase was amplified and cloned from spike zadok stage 65. Arabidopsis (Arabidopsis thaliana) transgenics overexpressing TaATP-Bwere raised. Photosynthetic efficiencies were measured for Arabidopsistransgenics overexpressing TaATP-Bgene under heat stress, which shows the enhancement of NPQ levels during elevated HS conditions. This attribute of the photosynthetic machinery under HS conditions helps the plant in evading photodamage conditions, and thus preventing the photosynthetic apparatus from degradation.

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03. SANYAL (Sibaji Kumar)
Role of Alternative Splicing in Modulating CBL-CIPK Network and Characterization of VDAC Isoforms in Arbidopsis.
Supervisor : Prof. Girdhar K. Pandey
Th 24032

Abstract
(Verified)

Signal transduction process is very important for an organism to sense the signal and generation of response. Plants being sessile need a robust and efficient signaling system. The role of Ca and its information decoders have been rigorously investigated in the last two decades. Ca signaling today is one of the most intricate signaling mechanisms extensively studied in both animals and plants. In this regard the Ca sensors and responders play major role, which decrypt the messages encoded in the Ca signature. A Ca sensor with EF-hands and subsequently termed calcineurin B-like (CBL) and a responder kinase that is activated by CBL binding also known as CBL-interacting protein kinase (CIPK) together form a sensor relay module. In this thesis, I proved the interaction between CIPK3 and ABR1 is required for negative regulation of ABA signaling. I was also able to show that CBL9 plays a crucial role in enhancing the CIPK3 mediated phosphorylation of ABR1. Taking this story further, I could show that alternately spliced

forms of CIPK3 (CIPK3.1 and CIPK3.2) could interact with ABR1 and other two variants CIPK3.3 and CIPK3.4 interacted with CBL6. This proved that alternative splicing in CIPK3 leads to distinct target selection. Analysing the effect of alternative splicing on the CBL-CIPK gene family in Arabidopsis and rice, I could show that alternative splicing increased the number of interaction possibilities for CBLs and CIPKs. The quantitative real time analysis proved that their expression is differentially regulated. In my endeavor to find a specific interactor of CIPK3.3 and CIPK3.4, I found mitochondrial Ca transporters VDAC3, which itself is a member of multi-gene family (comprising six members). My analysis on using yeast VDAC mutant proved that Arabidopsis VDACs are functionally different and VDAC6 might act as a death signal in plants under stress condition.

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04. SHARMA (Eshan)

Comparative Transcriptome Analysis for Frought Stress Responsive Pathways in Rice and Functional Characterization of Stress Responsive Genes Encoding F-Box Protiens in Rice and Arabidopsis.

Supervisor : Prof. Jitendra P. Khurana

Th 24028

Abstract
(Verified)

Under adverse environmental conditions, like drought, plants require synchronous regulation of a large number of genes. To understand the mechanisms involved in conferring drought tolerance in rice, physiological and transcriptome analyses of two contrasting cultivars, drought tolerant Dhagaddeshi and susceptible IR20 were performed. Further, a bioinformatics approach was also used to identify gene groups and associated pathways from microarray and RNA-seq experiments that are restricted in their gene expression amplitude within fold change intervals (FCIs) under drought stress. It could be shown that the expression of genes as functional groups is coordinated quantitatively, in a fold change specific manner, and differs among three rice cultivars distinct in their drought stress response. One of the F-box protein coding genes, *OsFBX*257, is also differentially expressed under drought stress in rice. Strikingly, its expression profile is different in various rice cultivars especially in the drought tolerant cultivar Nagina22. The expression of *OsFBX257* gene is developmentally regulated too. *OsFBX257* further shows expression correlation with kinases/phosphatases and OsFBX257 can also interact with OsPP2C08 *in vitro* and *in vivo*. Conserved among land plants, OsFBX257 can be a component of the SCF complex and it interacts with rice 14-3-3 proteins (GF14b and GF14c). While its homologous genes, *AtFBS2* and *AtFBS3*, are important for response towards ABA and osmotic stress in *Arabidopsis*, *OsFBX257* increases sensitivity towards drought stress response in rice. These F-box protein coding genes are also important for stress associated ROS production and signaling responses in rice and *Arabidopsis*. *OsFBX257* in rice can modulate developmental responses such as plant height, transition to reproductive phase and root development as evident from the phenotype of its over-expression and knock-down transgenic lines of rice. *OsFBX257* serves as a key modulator of drought stress and developmental responses acting within a network of co-expressed genes, thus influencing multiple pathways in rice.

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encoding F-box protiens in rice and Arbidopsis 4.Summary and conclusion 5. References.

05. TIWARI (Lalit Dev)
Generic Implications of Selected Hsp Genes in Heat Stress and Developmental Responses of Arabidopsis Thaliana (L.)Heynh.
Supervisor : Prof. Anil Grover
Th 24031

Abstract
(Verified)

Most Hsps act as molecular chaperones and play vital roles in protein homeostasis in the cells. Using T-DNA insertional approach, mutants for most of the *Hsp*genes have been raised in *Arabidopsis*. The emphasis in this thesis was on characterization of the role of Hsps in governing heat stress tolerance phenotype using *hsp*mutants of *Arabidopsis*. 54 different mutant accessions belonging to diverse Hsp classes i.e., Hsp20, Hsp40, Hsp60/ Hsp10, Hsp90 and Hsp100 were obtained. Out of 54 accessions, 17 accessions were noted to be homozygous. These 17 mutant types were phenotyped for basal tolerance-seed (BT-seed), basal tolerance seedling (BT-seedling) and acquired tolerance-seedling (AT-seedling). Three mutants namely *salk_064887C* (*cpn60β4*), *salk_087844* (*hsp70-2*) and *cs16284* (*clpB-C*), showed the phenotype which was distinct from the WT. *clpB-C* mutant (also called *hot1-3* mutant) showed highly heat sensitive phenotype at BT-seed, BT-seedling and AT-seedling assay conditions. *cpn60β4*mutant showed phenotype in terms of higher growth rates of seed germination to flowering to seed setting stage. *salk_087844*mutant showed more heat tolerance phenotype than WT plants. It was noted that *salk_087844* mutant has deletion of 6 genes. We showed that *Hsc70-1* is the candidate gene which is responsible for thermotolerance phenotype of the *salk_087844* mutant *Arabidopsis* plants. It was shown that *hsp70-1* single mutant showed thermotolerance phenotype possibly due to higher expression of key regulatory Hsps/ Hsfs like *At*Hsp101 and *At*HsfA2. It was suggested that Hsp101 expression is possibly regulated by Hsp70-1 protein via HsfA1d and HsfA2 proteins. As a whole, this study utilized both loss-of-function and gain-of-function approaches in understanding genes relevant to stress phenotype in *Arabidopsis*.

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06. VERMA (Vibha)
Vharacterization of OsMADS29 Function: Its Effects on Cytokinin Mediated Starch Biosysnthesis and Involvment of Calmodulin in its Nuclear Transport.
Supervisor : Prof. Sanjay Kapoor
Th 24284

Abstract
(Not Verified)

OsMADS29 is a seed-specific transcription factor that regulates different aspects of seed development in rice. It has been shown to affect development of both endosperm and embryo, thereby affecting grain filling as well as seed viability. Earlier studies in our laboratory had indicated that OsMADS29(M29) could target hormone (auxin-cytokinin) homeostasis in favor of cytokinins, thereby affecting divisions and

differentiation of cells in target organs. Here, I show that expression of M29 in dicot tobacco BY-2 cells and an early land plant Physcomitrella patens, leads to alteration of auxin:cytokinin homeostasis as observed earlier in M29 overexpression lines in rice. Q-PCR-based analysis of key genes in these two systems has highlighted the influence of M29 on genes associated with plastid biogenesis, starch biosynthesis and cytokinin biosynthesis pathways. Further experiments would be required to prove if these genes are the direct targets of M29 or M29 regulates their expression by altering the levels of cytokinins. I also investigated molecular aspects related to entry of M29 into the nucleus. The results show that the Ca++ sensor Calmodulin (CaM) sequesters M29 in the cytoplasm, probably at the surface of endoplasmic reticulum and regulates the rate of its nuclear entry. CaM antagonists, W7 and TFP were used to validate the specificity of this interaction, while the use of intracellular Ca++level modifying compounds like EGTA, verapamil, ionophore A23187 indicated the involvement of Ca++in M29-CaM interaction. Fluorescence recovery after photobleaching (FRAP) experiment showed that conditions of low Ca++in the cytoplasm favor M29 sequestration by CaM and downregulate its nuclear import. Furthermore, BiFC-FRET based experiments indicated the formation of a tripartite complex between two M29 monomers and CaM implicating CaM in facilitating interaction between two M29 monomers and thereby facilitating its entry into the nucleus.

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