

CHAPTER 44

PLANT MOLECULAR BIOLOGY

Doctoral Theses

01. ALKA SHANKAR

Transcriptome Profiling of Rice Under Potassium and Calcium Deficiency and Functional Characterization of Rice Non-Symbiotic Hemoglobin and Chloroplast Localized Rice Dual Specificity Phosphatases.

Supervisor : Prof. Girdhar K. Pandey

Th 22855

*Abstract
(Not Verified)*

Plant nutrition is one of the important areas for improving the yield and crop quality as well as non-crop plants. K^+ and Ca^{2+} deficiency directly affects the plant growth and hence crop yield and production. A balance homeostasis of these ions is required to be maintained for normal plant physiology and adaptation during stress conditions. Therefore, understanding the molecular aspects of various components regulating this balance is of great importance. In objective 1 and 2, we report a combined physiological and whole genome transcriptomic study of rice seedlings exposed to K^+ and Ca^{2+} deficiency respectively. Our findings highlight altered expression patterns of K^+ and Ca^{2+} responsive genes majorly involved in metabolic processes, stress responses, signaling pathways, transcriptional regulation, and transport of multiple molecules including K^+ and Ca^{2+} . In objective 3, we found that rice non-symbiotic hemoglobin genes OsnsHb1 and OsnsHb2 are differentially regulated under nutrition deficiency and osmotic stress conditions and localized to cytosol and nucleus. Promoter analysis of OsnsHb1 and OsnsHb2 exhibited that these are ubiquitously expressed in plant tissues and induced by nutrition deficiency as well as abiotic stresses. Objective 4, deals with the biochemical characterization of rice dual specificity phosphatases (DSPs). Starch degradation is a very important process of plants, which helps to convert starch into sucrose during the night. It is known that in Arabidopsis chloroplast localized AtDSP4, and AtDSP5 are involved in starch degradation. In this study, we have identified their homolog in rice and their sub-cellular localization site. Our biochemical results showed that these putative laforin like OsDSPs can bind to starch and involved in starch metabolism.

Contents

1. Review of literature 2. Material and method 3. Results and discussion. Summary and conclusions. References. List of Publications.

02. AGARWAL (Preeti)

Functional Characterization of Some Heat Stress Associated Transcription Factors (TaHSF, TabZIP, TaZnF and TaMADS) from Wheat (Triticum aestivum L.).

Supervisor : Prof. Paramjit Khurana

Th 23163

Contents

1. Introduction 2. Functional characterization of TaHSFs from wheat in response to heat and other abiotic stress conditions 3. Genome-wide structural and evolutionary diversity of bZIP transcription factors and functional characterization of a TabZIP

under salinity, drought and heat stress 4. Characterization of a novel zinc finger transcription factor (TaZnF) from wheat conferring abiotic stress tolerance in Arabidopsis 5. Functional characterization of TaMADS box transcription factor from wheat (*Triticum aestivum* L. Summary and conclusions.

03. ARCHANA
Novel RNA Interference Based Resistance Against Tomato Leaf Curl New Delhi Virus and Characterization of a New Virus-Induced TAS Locus in Tomato.
 Supervisor : Prof. Indranil Das Gupta
Th 22852

Contents

1. Introduction of objectives 2. Review of literature 3. Material and methods 4. Results 5. Discussion. Conclusions. List of publications.

04. CHAWLA (Mrinalini)
Identification and Functional Characterization of Components Involved in Ubiquitin-Mediated Proteasome Pathway During Male Gametophyte Development in Rice.
 Supervisor : Prof. Sanjay Kapoor
Th 22853

*Abstract
(Verified)*

The life cycle of flowering plants alternates between a dominating sporophytic and a much shorter gametophytic phase. This transition from diploid sporophytic phase to haploid gametophytic phase is marked by meiosis. A comprehensive transcriptomics analysis involving our in house data representing different stages of vegetative and reproductive development in rice, had revealed an important role of gene inactivation along with that of activation of a set of genes during male gametophyte development. We also found a marked increase in the activity of genes involved in ubiquitin-mediated protein degradation pathway. Central to this protein turnover machinery is the Skp1-Cullin-F-box (SCF) class of E3 ligases, which identify and prepare specific proteins for degradation by adding ubiquitination tags. Our investigations revealed 23 F-box and five OSK encoding genes expressing specifically in meiotic anthers in rice. Based upon the expression data, three genes, FBDUF27, FBX350 and OSK22, were selected for functional characterization by using knock-down an approach in rice. The silencing of FBDUF27 and FBX350 exhibited delayed tapetum degeneration as a major cause for pollen death. Besides tapetum degeneration, the OSK22 knock-down also resulted in anther indehiscence associated with failure of interocular septal degeneration and absence of lignified striations in endothelial wall layer at the time of anther maturity. The silencing lines also displayed defects in all anther wall cell-types including delayed tapetum degeneration, which resulted in premature pollen abortion and epicuticular nanoridges formation in anther epidermal wall layer. Further elucidation of the function of these components will unravel the mysteries of male gametophyte development and open the gateways for our understanding about the regulatory networks underlying the Sporophytic to Gametophytic transition in plants. This will not only add to our knowledge of basic reproduction biology but will also aid in improving crop yield and generation of male sterile varieties in crop plants.

Contents

1. Review of literature 2. Materials and methods 3. Results. 4. Discussion. 5. References. 6. Publications.

05. KANSAL (Shivani)
Investigation on Regulatory Dynamics of microRNA Genes in Rice.
 Supervisor : Dr. Saurabh Raghuvanshi
Th 22854

*Abstract
(Verified)*

MicroRNA mediated post-transcriptional regulation is involved in almost all the critical biological processes in plants. Characterization of miRNome at anthesis stage of rice indica var Nagina 22 (drought-tolerant) revealed that transition from heading to anthesis caused significant differential regulation in one-fifth of the miRNA population. Majority of differentially regulated miRNAs appeared to regulate transcription factors while some could be implicated in regulation of development and fertilization. Drought at anthesis induced significant change in over 25% of the miRNA population which primarily target transcription factors and catalytic enzymes. It was possible to identify the drought response of several miRNAs for the first time. Some of drought regulated miRNAs co-localize with QTLs related to drought tolerance and associated traits. Upon comparing expression profiles of miRNAs between N22 and PB1 (drought sensitive), we found many miRNAs with variety-specific expression patterns from heading to anthesis as well as drought at anthesis. Variety specific expression trend of the miRNAs was somewhat transferred to their target genes as well. Further, we found that miRNAs respond to changes in light conditions such as etiolation and dark treatment. To understand how these miRNAs themselves are regulated we studied the effect of $[Ca^{2+}]_{cyt}$ on miRNA expression. With the help of expression profiles generated with Ca^{2+} channel blockers and a calcium ionophore A23187, we found differentially regulated miRNAs in N22 and PB1. Hereby, some miRNAs displayed opposite as well as exclusive expression response to $[Ca^{2+}]_{cyt}$ in variety specific manner. We further dissected calmodulins and calmodulin binding transcription activators having influence on miRNA expression. Further, dehydration and ABA response of many miRNAs was found to be mediated by $[Ca^{2+}]_{cyt}$ signaling. Also, the drought response of many miRNAs in heading stage flag leaf were found to be seemingly ABA-dependent. We also found evidences of miRNAs targeting signal transduction components such as CDPKs and CAMKs.

Contents

1. Review of literature 2. Materials and methods 3. Results 4. Discussion 5. Summary and conclusions. References. Annexure. List of publications and poster.

06. RASHMI RISHISHWAR
Analysis of Gene Functions and Infectivity of Geminiviruses Infecting Okra.
 Supervisor : Prof. Indranil Dasgupta
Th 23164

*Abstract
(Verified)*

Okra is an important vegetable crop in the tropical and sub-tropical regions of the world, India being the highest producer. Okra production is severely affected by the occurrence of two diseases bhendi yellow vein mosaic disease (BYVMD) and okra enation leaf curl disease (OELCuD). Several small DNA viruses, known as geminiviruses, namely Bhendi yellow vein mosaic virus (BYVMV), Mesta yellow vein mosaic virus (MeYVMV), Okra enation leaf curl virus (OELCuV) and several viral satellite molecules have been found to be associated with these diseases in okra. The first objective of this study was to gain more information about viruses and betasatellites associated with YVMD and OELCuD. The second objective was to check infectivity and replication of okra-associated viruses and betasatellite by constructing agro infectious clones. The third objective of this work was to construct hairpin RNA producing plasmids against BYVMV and associated betasatellites and testing their ability to produce small RNA in the model plant *Nicotiana benthamiana*. The fourth objective was to check whether any gene of okra-associated geminiviruses and satellites possess activities which suppress the anti-viral RNAi defence pathway of plants. The BYVMD affected samples, from seven different locations in India, were subjected to rolling circle amplification, followed by restriction digestion. Through this exercise, random sized fragments were cloned and end-sequenced. It was found that most viral and satellite-like molecules resembled previously reported sequences from okra but a few sequences were found resembling alphasatellites reported from wild cotton and other weeds. Upon agroinoculation of cloned MeYMV and BYVMV clones, viral replicative forms were observed in *N. benthamiana* by Southern hybridization. Small RNA production against hairpin RNA producing constructs was detected in transient expression assay in *N. benthamiana* by Northern hybridization. Mild RNAi suppressor activity could be observed for BYVMV encoded V2 and betasatellite encoded β C1 gene products.

Contents

1. General introduction 2. Review of literature 3. Cloning and sequence analysis of geminivirus sequences associated with YVMD and OELCuD 4. Infectivity analysis of okra associated geminiviruses and betasatellite by agroinoculation in *Nicotiana benthamiana* and *Abelmoschus esculentus* 5. Construction of hairpin RNA producing constructs for RNAi-mediated resistance against geminiviruses infecting okra 6. Screening of okra associated geminiviruses and Betasatellite genome for viral RNAi suppressors 7. Summary and conclusions. References. Appendices.