

CHAPTER 43

PLANT MOLECULAR BIOLOGY

Doctoral Theses

01. BARKHA RAVI
Functional Characterization of Calcium Signaling Kinases Regulation Abiotic and Biotic Stress Responses in Plants.
Supervisor: Prof. Girdhar K. Pandey
Th 26857

Abstract

Calcium (Ca²⁺) signaling is versatile communication network implicated to regulates a diverse array of biological responses. Stimuli perceived by cells are transposed through the Ca²⁺-signature, and decoded by the plethora of Ca²⁺ sensors present in the cell. Therefore, Ca²⁺ sensor proteins are crucial components of stress signaling pathways. CBL (calcineurin B-like proteins) are Ca²⁺ sensors that interact and form modules with CIPKs (CBL-interacting protein kinases) to regulate a multitude of signaling processes. This study intends to investigate a fundamental pathway linking the CBL-CIPK module to oxidative and biotic stress signaling. The first two chapters of this work aimed to characterize the roles of CIPK9, VDAC3 (voltage-dependent anion channel 3), and CIPK3 to get a better understanding of how these signaling components mediate oxidative stress responses in Arabidopsis. A novel function for these kinases and how they regulate their common downstream target, VDAC3 is investigated in this study. This study shows how CIPK9 regulates VDAC3 positively, whereas CIPK3 was observed as a negative regulator of VDAC3 during oxidative stress conditions. Further, the third chapter focuses on identifying the role of VDAC3 in response to pathogens and the interplay of plant hormone signaling during defense as an outcome of plant-pathogen interaction. Finally, we aim to understand the function of CIPK21 with its downstream interactor DSP4 (Dual specificity phosphatases 4) during biotic stress responses in Arabidopsis in the last chapter of this thesis. Overall, this study focuses on deciphering the role of Ca²⁺ signaling kinases and how they control stress signaling pathways in plants. The knowledge gained from this detailed mechanistic study of Ca²⁺ signaling will be very relevant to pave the path for future biotechnological intervention, where stress resilient crop varieties can be generated.

Contents

1. Organization of the thesis 2. Review of literature 3 CIPK9 regulates VDAC3 and modulates oxidative stress responses in arabidopssis 4. CIPK9 negatively regulates VDAC3 and modulates oxidative stress signaling in arabidopsis 5. VDAC3 positively regulates plant defense responses during pseudomonas syringae infection 6. CIPK21 negatively regulates plant immune responses during pseudomonas syringae infection 7. Materials and methods 8.Summary and Conclusion. References. Annexures

02. BHIMRAJKA (Sanchi)
Understanding Transcriptional and Post-Transcriptional Regulatory Mechanisms Affecting Reproductive Development in Rice and their Applications.

Supervisor: Prof. Sanjay Kapoor

Th 26858

Abstract

Rice is a major cereal crop that is consumed around the world as a primary source of carbohydrates in the form of starch. We have been studying different aspects of reproductive biology in rice to characterize vital regulatory components involved. Here, we aimed to utilize that knowledge to develop a starch bio-factory system in Bright Yellow-2 (BY-2) tobacco cell line by introducing few essential components of the cereal seed starch biosynthesis pathway into this dicot cell line. For this, our primary candidate was OsMADS29 (M29), a seed-specific transcription factor that has been shown to perform significant roles in starch accumulation during seed development. This work was initiated by developing technologies to understand the working mechanism of M29, for which we first developed a cytokinin sensor-reporter system in BY-2 cells and rice, which could be further exploited to decipher M29's effect on cytokinin levels in individual cells or tissues. Subsequently, the transcriptional regulation of M29 was analyzed, and besides several positive elements, we found a negative regulatory element in M29 URR, which imparted tissue and developmental-stage specificity to M29 expression. To analyze the effect of post-transcriptional regulation on gene expression by the 26S proteasome pathway during reproductive development, a comprehensive interaction study was performed between the members of SKP1-like and Cullin1 proteins by yeast two-hybrid assay to determine their involvement in SCF-E3 ligase complexes. Further, we also attempted functional characterization of a meiotic anther stage-specific F-box protein, FBX350 by the CRISPR technique, and we concluded redundancy in its function by another, yet unknown, F-box protein. Finally, we utilized the concept of cellular agriculture in order to create an alternative source of starch production that is independent of agricultural practices. For this, along with M29, we selected two cereal grain-specific genes, ADP-glucose pyrophosphorylase (AGPase) and Brittle1 (BT1), which are known to mediate rate-limiting steps in the starch synthesis pathway, and expressed them in BY-2 cells and successfully increased starch accumulation by 19-folds.

Contents

1. Review of literature 2. Materials and methods 3. Results 4. Discussion. 5. Bibliography and Publication.

03. GAMBHIR (Priya)
Regulation of Tomato Fruit Ripening by Ethylene Response Factors (ERFs) and Glyoxalase Enzymes.

Supervisor: Prof. Arun K. Sharma

Th 26859

Abstract

The fruit quality of tomato can be improved by preventing the post-harvest losses and by generating tomato fruit with enhanced levels of useful metabolites. Ethylene plays a major role in ripening of fruits and can affect both aspects. Ethylene action involves a signal transduction cascade which exerts its action at the terminal end via ethylene response factors (ERFs). Despite the obligatory nature of ethylene to

climacteric fruit ripening and the identification of 77 Ethylene Response Factors (ERFs) in the tomato genome, the role of only limited ERFs has been validated in the ripening process. In the present study, using a comprehensive morphophysiological, molecular, and biochemical approach, we demonstrate the regulatory role of SlERF.9, SlERF.11, SlERF.72 and SlERF.81 in tomato fruit ripening. Over-expression of SlERF.72 resulted in reduced lycopene accumulation coupled with enhanced fruit firmness. However, over-expression of SlERF.81, also annotated as SlERF.D7 promotes ripening and its silencing has the opposite effects. Alterations in its expression modulated ethylene production, pigment accumulation, and fruit firmness. This study uncovers that SlERF.D7 positively regulates the transcript abundance of an auxin response factor, SlARF2, to amalgamate auxin and ethylene signalling pathways for controlling tomato fruit ripening. Furthermore, the present study also investigates the potential role of SlGLY/4 mediated methylglyoxal (MG) detoxification during later stages of fruit maturation in tomato. Silencing of this gene leads to drastic MG overaccumulation at ripening-stages in the transgenic fruits and interferes with the ripening process. Further investigations show that MG plausibly glycates and inhibits key enzymes such as methionine synthase (MS) and S-adenosyl methionine synthase (SAMS) of ethylene biosynthesis pathway, thereby indirectly affecting fruit pigmentation and cell wall metabolism. Apart from evaluating the ripening associated function of glyoxalase detoxification system, the third objective uncovers the comparative analysis of the MG detoxification potential of the two glyoxalase pathways in response to multiple stresses in tomato, indicating a prominent role of the glutathione-independent GL VIII catalysed MG detoxification pathway in this species.

Contents

1. Characterization of ethylene response factors (ERFS) during tomato fruit ripening
2. Methylglyoxal controls tomato fruit ripening by regulating ethylene biosynthesis
3. Comparative analysis of the methylglyoxal detoxification potential of the two glyoxalase pathways in response to multiple stresses in tomato. Summary and Conclusion. Appendix.

04. GAWANDE (Gautam)

Characterization of Os MADS29 Function in Arabidopsis, OsAGO3 on Rice and Determination of OSL19 and 22 as Components of the anther-specific E2 Ligase SCF Complex.

Supervisor: Prof. Sanjay Kapoor

Th 27142

Abstract

The transition from the sporophytic to gametophytic phase in angiosperm requires regulating certain factors that could have adverse effects during gametophyte development. Such regulation can be achieved on the mRNA level or protein level or protein level. Argonautes are major players in the RNA-Induced silencing complex (RISC), and are known for their involvement in post-transcriptional gene silencing via RNA interference. Here, as the first objective I analyzed the anther-specific argonaute Argonaute OsAGO3 with regards to its gene structure, expression and function. This analysis showed that there exists a longer and spliced transcript of OsAGO3, and based on sequence homology among rice orthologs, I predict the entire exon 1 to be part of the 5' UTR. I also successfully established the CRISPR/Cas3 technique in rice using OsAGO3 as a target and achieved mutations at both target sites as validated by restriction digestion and sequence of the target sites. OSK proteins are part of E3 ligase SCF complex that promotes protein degradation. Here,

I show the interaction of two anther-specific OSKs, OSK19 and 22 with an F-box protein and cullin-1 protein, thus, confirming their role in forming SCF complex and mediating protein degradation and they might regulate the activity of target proteins during anther development. OsMADS29 is a seed-specific AMDS-box (M29) transcription factor reported to a shift in auxin:cytokinin homeostasis in rice, and its ectopic expression leads to starch accumulation in BY2 cell lines mimicking the exogenous addition of cytokinins. Here, I ectopically expressed rice MADS29 in *Arabidopsis thaliana* and showed that it affects various developmental processes. Also, the phenotypes observed were similar to those obtained in other studies related to cytokinin.

Contents

1. Review of literature 2. Materials and methods 3. Results 4. Discussion. References. Publication.

05. KHUNGAR (Lisha)

Insights into Function and Regulation of ClpB1/Hsp101 Protein in *Arabidopsis thaliana* (L.) Heynh, *Oryza Sativa* (L.) and *Solanum Lycopersicum* (L.).

Supervisor: Prof. Anil Grover

Th 26860

Abstract

ClpB1/Hsp101 protein is critically important in acquisition of thermotolerance in plants. Recent work has shown that the overexpression of Hsp101 using heat inducible promoter is a highly suitable approach for improving thermotolerance capacity in *Arabidopsis* (Babbar, R. et al., 2023, *Plant Science* 330: 111639). We aimed at generating transgenic rice and tomato plants by the similar strategy of using heat inducible promoter driven Hsp101 gene. We produced transgenic rice IR64 plants overexpressing full length genomic sequence of OsHsp101 (GS lines) and transgenic tomato Pusa Ruby plants overexpressing 1.1KbSIHsp101promoter driven OsHsp101 CDS (IN lines) and lines overexpressing full length genomic fragment of SIHsp101 (GF lines). Importantly, the transgenic rice and tomato lines produced in our work showed improved thermotolerance capacities than their respective wild type (WT) plants in basal thermotolerance (BT) and short-term acquired thermotolerance (SAT) analysis at seedling and potted plant stages. Previous work has shown that AtHsc70-1 negatively regulates AtHsp101 expression (Tiwari, L.D. et al., 2020, *The Plant Journal* 103: 2069-2083). We analyzed the AtHsc70-1 overexpression lines raised using constitutive CaMV35S promoter and using the native promoter (by using the genome fragment of AtHsc70-1). AtHsc70-1 lines developed by expressing the genome fragment of AtHsc70-1 failed to accumulate Hsp101 protein and were more heat sensitive as against the WT Col-0 seedlings. The extended work showed that AtHsc70-1 attenuates AtHsfA1d during non-stress conditions and controls Hsp101 transcription. Further, we noted that AtHsfA1d and AtHsfA2 overexpression in *Arabidopsis* influences BT-seed response. Our work shows that Hsp101 is an important candidate in the regulation of heat tolerance across diverse plant types and it is potentially a suitable candidate for genetically engineering heat tolerance in crops.

Contents

1. Introduction 2. Review of literature 3 Materials and methods 4. Results 5. Discussion. 6. Summary and Conclusions. Literature cited. Annexures. Appendix.

06. NEELAM
Molecular Basis of Virus Induced Gene Silencing in Rice and Gene Expression Landscape of Virus Infected Nicotiana Benthamiana.
 Supervisor: Prof. Indranil Dasgupta
Th 26861

Abstract

The thesis presents the finding of research on the molecular basis of virus-induced gene silencing (VIGS) in rice and the gene expression landscape of virus-infected nicotiana benthamiana. The research aims to explore the relationship between viral resistance/tolerance and VIGS-mediated gene silencing in the plant monocot plants typically lag behind in reports on VIGS. Compared to dicot plants. Here, a VIGS system, mean for monocots has been used to address the above question. Rice tungro bacilliform virus (RTBV), a rice- infecting DNA virus, which has been earlier modifies to serve as a VIGS vector (RTBV-MVGS) has been used in this study to assess the degree of silencing of two genes affecting chlorophyll levels in the leaves of the rice plant. The putative role of a small RNA binding protein (SRBP), recently reported to be important in small RNA trafficking in the model plant Arabidopsis, has been studied in this thesis for its involvement in VIGS in rice. Additionally, the thesis examines the changes induced by Sri Lankan cassava mosaic virus (SLCMV) DNA in its laboratory host, N. benthamiana, with the goal of understanding how the viral DNA accumulates in its host with time and how host transcriptome changes in response to viral infection. SLCMV, a bipartite Begomovirus in its natural host cassava, can exist as a monopartite entity in n. benthamiana, giving unique opportunity to study the transcriptomic change due to DNA-B, which is not possible with begomoviruses which are strictly bipartite.

Contents

1. Introduction 2. Literature review of literature 3 Extent of VIGS in rice lines with varying virus susceptibility 4. Analysis the role of SRBPI (Ossrbp1) in VIGS in rice 5. Transcriptomic study of N. benthamiana plants inoculated with cloned SLCMV DNA 6. Conclusion. References. Appendix.

07. PRUSTY (Ankita)
Functional Analysis of Mediator Subunit Genes, OsMED14_2 and Os MED26_2, in Rice Development.
 Supervisors: Prof. Sanjay Kapoor and Prof. Akhilesh Kumar Tyagi
Th 27143

Abstract

The mediator complex may regulate traits by acting as a co-activator or co-repressor in RNA polymerase II-mediated transcription in rice, the knowledge about structure and function of mediator complex and its subunits is in its infancy. The present work involves the function of characterization of two rice mediator subunits, OsMED14_2 and OsMED26_2 in development by adopting reverse genetics approaches, OsMED14_2 and OsMED26_2 were found to influence plant development during vegetative and reproductive stages the investigation revealed that OsMED14_2 controls yield potential in rice by regulating panicle length/branching and pollen development. Overexpression of OsMED14_2 affects pollen viability, pollen morphology and pollen development indicating that OsMED14_2 acts as a critical player in pollen development OsMED14_2 was also found to regulate the grain size and weight. Analysis suggested that OsMED14_2 is involved in maintaining overall JA homeostasis by affecting accumulation of bioactive JA the expression of several

OsJAL Genes and by interacting with several JA signaling components. The present study proposes that OsMED14_2 may provide an additional components of JA repression machinery and involves in regulating expression of JA responsive genes by interaction with OsHDAC6 and OsIAZ repressors to mediate plant growth and development.

Contents

1. Review of literature 2. Materials and methods 3. Results 4. Discussion. 5. Summary and Conclusion. References. List of Publication. Conferences attended and poster presentations.

08. ROHIT KUMAR

Functional Studies of Sri Lankan Cassava Mosaic Virus AC5 Protein and Transgenic Expression Analysis of Geminiviral Promoter.

Supervisor: Prof. Indranil Dasgupta

Th 26862

Abstract

Certain plant-infecting viruses (begomoviruses) of the monopartite and bipartite class encode a protein referred to as ACS/C5 from the complementary- sense DNA strand. These ACS/C5 proteins, which are composed of 50-270 amino acids, are transcribed from an open reading frame (ORF) downstream of the AC3 ORF. In this thesis, a study has been carried out on the AC5 gene encoded by Sri Lankan cassava mosaic virus (SLCMV), in which it is shown by means of mutagenesis and ectopic expression analysis via the PVX vector, that this protein is essential for the virus to infect. The intracellular localization of the AC5 protein was shown to be in the plasma membrane using confocal microscopy, utilizing a fluorescent labelling method. It is also shown that the AC5 protein interacts with ABI1, a major director of ABA signalling, using yeast two-hybrid, bimolecular fluorescent complementation and Co-IP assays. These results indicate that AC5 plays a role in suppressing ABA-activated defence pathways in plants. Using AC5 over-expressing plants, it is shown that AC5 induces the expression of AGO proteins and other defence-related proteins. Hence, the role of AC5 of SLCMV is elucidated for the first time in this thesis which also shows a novel method by which plant viruses suppress host defence pathways.

Contents

1. Introduction 2. Review of literature 3 Functional studies of Sri Lankan cassava mosaic virus AC5 protein. 4. Transgenic expression analysis of geminiviral promoter 5. Summary and Conclusion. Bibliography.

09. SHARMA (Aishwarye)

Functional Analysis of OsATL53 in Lignin Biosynthesis and OsbZIP45 in Drought Tolerance in Rice.

Supervisors: Prof. Arun K. Sharma and Paramjit Khurana

Th 27144

Abstract

Rice is an important crop in terms of food security and the economy of our country. Many high yielding indigenous varieties of rice are susceptible to biotic and abiotic stresses. In this study, two important aspects of growth and development of rice

plants are investigated lignin biosynthesis and abscisic acid (ABA) mediated drought stress tolerance. In the first chapter, regulation of cinnamoyl-CoA reductase enzyme OsCCR+4 by F-box protein OsFBK1 and RING-H2 domain containing protein OsATL53 along with functional characterization of OsATL53 and OsCCR14 was investigated. This includes in-depth protein cellular localization and movement of OsFBK1, OsAYL53 and OsCCR14 proteins in response to jasmonic acid, protein degradation kinetic of OsATL53 and OsCCR14 by SCF-OsFBK1 complex, and generation of rice transgenics of OsATL53 –knock down (OsATL53^{RNA}) and OsCCR14-overexpression (OsCCR14^{OX}) transgenics. Also, lignin accumulation was studied and compared in OsCCR14^{OX}, OsCCR14^{OX} and OsATL53^{RNA} transgenics by Microscopy in anthers, spectrophotometrically in roots, and by RT-PCR in the leaf tissues. OsATL53^{RNA} plants resembled OsCCR14^{OX} plants in accumulation of lignin in roots, anthers and transcripts of OsCCR14. Thus, OsATL53 was found to be a negative regulator of lignin biosynthesis.

Contents

1. Functional characterization of OsCCR14 and OsATL53 in oryza sativa 2. Functional characterization of OsZIP45 in oryza sativa. Appendix List of Publication and posters.

10. SHARMA (Deepika)
Characterization of miRNAs Regulating the Flowering Time in Rice.
 Supervisors: Prof. Arun K.Sharma and Prof. Saurabh Raghuvanshi
Th 26863

Abstract

For plants, flowering is a crucial biological process that promotes successful reproduction. MicroRNA (miRNA) is one of the endogenous non-coding RNAs that is crucial for controlling plant development. The current study, further explores this dimension and characterizes two miRNAs that impact various yield related traits in rice. While one miRNA is an uncharacterized novel miRn-001 that was previously identified by our group, the other is a monocot specific miRNA (miR528). Interestingly, miRn001 targets the gene HD16 (Heading date 16) which is directly involved in regulating flowering time in rice. Hd16 is known to phosphorylates Ghd7 that inhibits the expression of Ehd 1 (a floral activator) hence suppressing flowering. It promotes the expression of the downstream flowering genes Hd3a and RFT1 in both LD and SD conditions. To determine the functional impact of miRn-001, we generated the overexpression plants of miRn-001. Analysis of the miRn001 overexpression lines indicated that there was an increase in the effective grains per main panicle and panicle branching without any significant change in grain size. More importantly, it was found that over-expression of miR-n001 led to early flowering probably due to down-regulation of HD16 transcript levels.

Contents

1. Review of literature 2. Materials and methods 3. Results 4. Discussion. Summary and Conclusions. References and Appendix.

11. SHIMPHRUI (Rinchiula)
Landscaping Transcriptomic and Metabolomic Alterations Underlying Development of Heat Tolerances in Arabidopsis Thaliana (L) Heynh.
 Supervisor: Prof. Anil Grover
Th 26864

Abstract

HSP101 plays the role of ‘disaggregase’ in solubilizing non-functional polypeptides produced because of heat stress (HS) in bacteria, yeast, and plants. HSP101 is critical for controlling heat-tolerance in plants. To elucidate the molecular mechanism(s) underlying HSP101-mediated development of heat tolerance in *Arabidopsis thaliana*, we examined the transcriptome and metabolome landscapes in GF30-7 (an AtHSP101 overexpressing line exhibiting superior heat tolerance phenotype) as against empty vector transformed Col-0 [Col-0(V)] (Babbar, R. et al., 2023, *Plant Science* 330: 111639) and *hot1-3* (AtHSP101 null mutant line; shows extreme heat sensitivity) seedlings, under non-HS (NHS), HS and post-HS recovery conditions. In the analysis of global transcript profiling data (RNA-seq; Babbar, R. et al., 2020, *Frontiers in Plant Science* 11:617779), chaperone-mediated protein folding, and ROS-scavenging genes were more abundant whereas genes involved in cell wall organization, secondary metabolism and biotic stress response were repressed, suggesting that HS affects these processes as an early response. Under NHS, GF30-7 line expressed more AtHSP101 transcript and protein, indicating that GF30-7 line is thermo-primed. GF30-7 line showed repression of secondary metabolism and biotic stress-related genes during early response. Metabolomic changes under HS and recovery conditions were examined using gas and liquid chromatography techniques coupled with mass spectrometry. Under long term recovery (8 days post-HS), GF30-7 line showed high accumulation of phenylpropanoid and anti-oxidative compounds along with glucosinolates as against Col-0(V) and *hot1-3*, suggesting that during late recovery, these pathways contributed to promoting higher thermotolerance in GF30-7 whereas, steroid and glutathione metabolism and fatty acid biosynthesis were negatively affected in Col-0(V) and *hot1-3*. Additionally, high performance liquid chromatography analysis demonstrated a higher accumulation of glucosinolates in GF30-7 line. Overall, our study revealed the battery of differential transcriptomic and metabolomic responses which were associated with the development of heat tolerance state achieved through HSP101 overexpression.

Contents

1. Introduction
 2. Review of literature
 3. Materials and methods
 4. Results
 5. Discussion
 6. Summary and Conclusions. Literature Cited.
12. SINGH (Vijendra)
Characterization of the Roles of AtMBDI Gene and its Tomato Homolog SIMBD8 in Salinity Stress.
 Supervisor: Prof. Arun K. Sharma
Th 26865

Abstract

Methyl CpG binding domain (MBD) – containing proteins, as the name suggests bind methylated DNA and facilitate the decoding of information encoded in methylation codes. The role of these proteins in mammals has mostly been implicated in gene silencing by recruiting chromatin-modifying factors to the target loci. In plants, physiological functions of most of these proteins, on the other hand, are yet to be explored. Therefore, the present study is imperative to investigate physiological roles of these proteins in abiotic stress responses in *Arabidopsis* and tomato. Salt stress is one of the environmental factors that negatively affect plant growth and development. This study provides evidences that salt stress increases the expression of AtMBD1. And the overexpression of AIMBD1 gene under salt stress

conditions reduces seed germination and negatively affect plant growth through enhancing oxidative stress. Overexpression of AMBD1 also enhances the expression of ABA inducible salt responsive genes. The present study also uncovers the also uncovers the full complement of MBD proteins in tomato I e , 18 MBDs, further grouped into seven classes, on the basis of protein sequence homology and motif conservation.

Contents

1. Review of literature 2. Materials and methods 3. Results 4. Discussion. Summary and Conclusions. References and Appendix.

13. TRIPATHI (Gayatri)
Hsp101 Sequence Variations and Plant Heat Tolerance.
 Supervisor: Prof. Anil Grover
Th 26866

Abstract

Protein aggregation under heat stress is the major cause of heat injury in plants. Cytosolic Hsp101 helps in solubilization of such protein aggregates. Hsp101 protein diversity was analyzed in Arabidopsis and rice plants, in this thesis work. Hsp101 protein sequences were downloaded from 855 Arabidopsis accessions (sequenced in 1K Arabidopsis genome project) and 435 Indian rice types (sequenced in 3K rice genome project). Overall, 63 haplotypes of AtHsp101 and 41 haplotypes of OsHsp101 were identified. In Arabidopsis, the notable features observed included the absence of conserved histidine (at 33rd position) in a few accessions and absence of Walker A motif in Bay-0 and Fjae1-5 accessions. The HS phenotype analysis of 29 accessions showed considerable variations in the thermotolerance response and the Hsp101 protein accumulation on western blots was positively related with the survival. Importantly, a trade-off existed between the Hsp101 levels and growth of seedling under non-heat stress. In rice, an InDel was noted leading to an in-frame insertion of TCC nucleotides, causing an addition of glutamic acid at 907th position in indica as against japonica rice sequences. Specific mutations in OsHsp101 gene were created through site-directed mutagenesis using PCR and the mutant sequence versions were genetically transformed in yeast cells lacking ScHsp104 and hot1-3 Arabidopsis lacking AtHsp104. As in AtHsp101 protein, the functionalities of histidine at 33rd and threonine at 600th positions were conserved in OsHsp101: their mutations in OsHsp101 sequence abolished the acquired heat stress response. The NBD1 and NBD2 sequences were non-redundant in their function. The indica Hsp101 showed a higher protein aggregate resolving ability than japonica Hsp101 in experiment with curing of yeast aggregate protein, RNQ tagged with GFP expression in yeast cells. We developed a DNA marker using dCAPS primer to distinguish indica and japonica Hsp101 sequences.

Contents

1. Introduction 2. Review of literature 3. Materials and methods 4. Results 5. Discussion. 6. Summary and Conclusions. 7. Literature cited 7. Annexure.

14. VAISHALI
Cytosolic Calcium Level Mediated Regulation of MicroRNA Expression Level in Plants.
 Supervisors: Prof. Arun K. Sharma and Saurabh Raghuvanshi
Th 26867

Abstract

MicroRNA genes regulate diverse molecular processes by regulating gene expression. Thus, understanding of the regulatory schema governing miRNA expression becomes important. $[Ca^{2+}]_{cyt}$ is one of the most universally implicated signalling molecules, however its involvement in the regulation of miRNA expression in plants has not been explored. In the current study we establish that the expression of several miRNA genes in rice and Arabidopsis is mediated by $[Ca^{2+}]_{cyt}$ levels. Calcium responsive miRNA expression was established by studying expression in plant seedlings pre-treated with various internal/external calcium channel inhibitors as well as ionophores. Arabidopsis mutants were used to establish the role of CAMTA TFs. Analysis of Arabidopsis mutants also identified the role of CAMTA TFs in the regulation of miRNA genes in rice. Subsequent, yeast-one-hybrid studies confirmed the binding of the CAMTA TFs on miRNA promoter in rice. Role of calmodulin in mediating the calcium response was also investigated and miRNAs responsive to calmodulin inhibitor were also identified. To further explore the regulatory schema, yeast-two-hybrid assay was done to confirm calmodulin-CAMTA TF interaction. The study identifies mediator components (e.g., calmodulin and CAMTA) that are integral component of the signalling cascade of the $[Ca^{2+}]_{cyt}$ mediated regulation of miRNA genes in plants. The findings are significant since they unravel a relatively less explored dimension of miRNA mediated regulation of plant processes. Thus, it would enable a more detailed understanding of the miRNA biology in plants.

Contents

1. Review of literature 2. Materials and methods 3. Results 4. Discussion. Summary and Conclusions. References and Appendix. List of publications and posters.