CHAPTER 40

PLANT MOLECULER BIOLOGY

Doctoral Theses

01. BOORA (Neelima)
Comprehensive Analysis of Interaction Between OsMADS29, Calmodulin and Calmodulin- Like Proteins and Factors Influencing MADS29'S Nuclear Transport.
Supervisor: Prof. Sanjay Kapoor
Th 25781

Abstract

OsMADS29 (M29) is a seed- specific MADS-box transcription factor involved in regulating multiple aspects of early seed development in rice. It is involved in hormone homeostasis during seed development its expression is tightly regulated at transcriptional and post-transcriptional levels. Its interaction with 19 other seed-expressing MADS-box proteins influences the nuclear localization of the resultant homo/heteto-dimers. It is also shown to interact with the calcium sensor protein calmodulin. This interaction is specifically restricted to the cytoplasm, indicating that CaM could have a regulatory function in nuclear localization and oligomerization of M29. In the present study, we have further dissected the structural components involved in the M29-CaN interacts and those that may influence its localization into the nucleus. We also show that besides CaM, M29 interacts with 22 other CaM-like (CML) genes. Sixteen of the resultant dimers localizen in the cytoplasm; however, six are localized in the nucleus, suggesting that cytoplasmic retention of the dimers could be a function of variability in the interacting domains. By swapping the conserved domains between CaM and CMLs that cause differential localization of the M29-CaM/CML dimers in the nucleus or cytoplasm, we have found that the C-terminal domain in CaM/CMLs is important for the cytoplasmic retention function. Using BiFC-FRET-FLIM assays, to we demonstrate the existence of a trimeric complex where one CaM may interact with two M29 monomers, strengthening our hypothesis of the involvement of CaM in M29 oligomerization. To gain insights into the other function of CaM/CMLs, i.e. regulation of nuclear transport of M29 oligomers, we searched for candidates that could provide anchorage to the M29-CaM dimers to a cytoplasmic entity. These analyses revealed that two ER membrane-associated autoinhibited calcium ATPases (ACAs) could provide a tether to the M29-Cam complex. The bi-and tri-partite interactions between ACAs,CaM,and M29 have been validated by FRET-FLIM and BiFC-FRET-FLIM, respectively. By using Y2H, we also show that besides M29,CaM/CMLs interact with at least four other M29-interacting seed-expressing MADS proteins. We also demonstrate that the MADS protein oligomers may utiliza the nuclear import machinery (including importing) to transport to the nucleus. In conclusion, the results presented in this thesis indicate that calmodulin may play a vital role in regulating the dimerization of M29 with itself and other parteners and coordinate its eventual nuclear transport by importin beta nuclear import machinery.

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1. Review of literature 2. Material and Methods 3. Results 4. Discussion. Bibliography and Publications.

02. GUPTA (Kanika)
Infectivity of Okra Enation Leaf Curl Virus and Promoter Analysis of Viruses Infecting Okra and Cassava.
Supervisor: Prof. Indranil Dasgupta
Th 25776

Abstract

Geminiviruses are highly destructive, insect-transmissible viruses that infect a wide variety of crops and cause serious threat to their production worldwide. This work deals with the geminiviruses infecting two important crops, okra and cassava. The first crop under study is okra, Abelinoschus esculentus (L.) which is one of the important vegetables widely cultivated in tropical, subtropical, and warm temperate regions of the world. The production of okra is greatly affected by large number of viral diseases. In India, the major yield loss in cultivated okra is caused by Bhendiyellow vein mosaic virus (BYVMV) and Okra enation leaf curl virus (OELCuV) which causes Bhendi yellow vein mosaic disease (BYVMD) and okra enation leaf curl disease (OELCuD) respectively. The second subject of this study is cassava infecting single- stranded DNA containing, Sri Lankan cassava mosaic virus (SLCMV) a geminivirus which causes cassava mosaic disease (CMD). Chapter I of this thesis gives a general introduction about the crop plants okra and cassava, the above-mentioned viruses infecting them and their importance. Chapter Il gives the literature review about the molecular biology of the geminiviruses, the significance of infectivity studies in okra and promoter analysis of viruses infecting okra and cassava. Chapter Ill represents the first objective which was undertaken to develop an inoculation system for OELCuD in okra and to gain more insights on the infectivity of cloned geminiviruses and betasatellite sequences infecting three plant system, viz. Nicotiana benthamiana, cotton and okra by agrobacteriummediated inoculation. Chapter IV forms the second objective that emphasize on the study of promoters of OELCuV. The third objective of this study is represented as Chapter V which describes the experiments conducted to study the inducibility of SLCMV promoter by viral proteins and upon geminivirus infection.

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1. General Introduction 2. Review of literature 3. Infectivity analysis of okra infecting geminiviruses and associated betasatellte in different host systems 4. Promoter analysis of okra enation leaf curl virus 5. Study of inducibility of slcmv promoter by viral proteins and upon geminivirus infection 6. Summary and Conclusions. References and Appendices.

03. KAMLESH KUMARI
Gene Silencing in Monocots Using a Vector Derived From Rice Tungro Bacilliform Virus.
Supervisor: Prof. Indranil Dasgupta
Th 25784

Abstract

Remarkable developments have been made in the construction of viral vectors and viral vector delivery systems in plants. Virus Induced Gene Silencing (VIGS) is a gene silencing technology for plants, where modified viral vectors carrying a portion of a target gene to be silenced, are introduced into plants. Several VIGS vectors have been utilized for gene functional analysis in dicot plants including N. benthamiana, soybean, potato, and tomato but very few VIGS systems are available for monocots. Rice, barley, foxtail millet, maize, and wheat are economically important crops. Genome complexity, vector-host specificity and insert stability confine the use of developed VIGS vectors for functional genomics. Thus, VIGS vectors which can overcome all these hurdles and can be used efficiently for gene silencing in monocots are needed. Rice tungro bacilliform virus (RTBV) a parareterovirus has been previously modified into RTBV-derived VIGS system for gene silencing studies in the natural host, rice. RTBV-derived VIGS vector has been used to silence genes such as phytoene desaturase (pds), magnesium chelatase (chlH subunit), which give rise to a visual phenotype and the disease resistance gene Xa21 in rice. The pRTBV-MVIGS system has not yet been assessed as a VIGS vector for non-rice cereals. In the first part of this thesis, pRTBV-MVIGS system was tested on four non-rice cereals, which contribute significantly to the food security of the world, namely barley, foxtail millet, maize and wheat for gene silencing. For this study, marker genes pds and chlH responsible for the chlorophyll biosynthesis were used, whose silencing gives rise to visible changes in the chlorophyll contents, and hence, appearance of a silencing phenotype in the leaves. Results of this study revealed approximately 30-50 % reduction in transcript accumulation of pds and chlH upon silencing the corresponding genes. The total chlorophyll content in all four cereal plant species were seen to decrease 20-50 % at 10-20 days post-inoculation (dpi). Newly emerging leaves displayed mild chlorosis and white streaking phenotype in comparison to empty vector inoculated control plants, indicating that pRTBV-MVIGS has the potential to be used as a gene silencing system in these important crops. The visible phenotype of gene silencing of pds was not seen in wheat plants. When the accumulation of RTBV-DNA replicative molecules was studied, it revealed that RTBV-DNA molecules did not accumulate in the inoculated plants. When compared with the previous studies mentioned above, the current studies on non-rice cereals also showed similar gene silencing efficiency as in rice, which was in the range of 30-40 %. In the second part of this thesis, pRTBV-MVIGS system was used to silence rice genes in a locus (Xa38) known to confer resistance to bacterial blight (BB) disease, followed by an assessment of the resistance in the silenced plants. This was the first time that the system has been used to silence a poorly characterized biotic resistance locus. The locus Xa38 confers resistance to the bacterial pathogen Xanthomonas oryzae pv. oryzae, (Xoo) responsible for BB. This has been demonstrated earlier by the introgression of this locus to the susceptible variety PR114, making it resistant to BB. The introgressed rice line is named PR114-Xa38. The locus Xa38 is known to carry several putative openreading frames (ORFs), the functions of which are unknown. In this study, pRTBV-MVIGS system was used to silence two ORFs within Xa38 locus variety PR114-Xa38. The silenced plants were then challenged with Xoo. Results showed that upon silencing of both genes in Xa38 locus PR114-Xa38 plants become susceptible for the Xoo pathogen and resulted in BB disease symptoms. The results indicated that both the ORFs contributed towards the function of the Xa38 locus. All the above studies on pRTBV-MVIGS system shows that it can be a good future candidate for gene silencing studies on a variety of cereal species, opening the way for functional genomic studies on them.

Contents

1. Introduction 2. Literature review 3. To study the silencing efficiency of pRTBV-MVIGS system in non-rice cereals namely barley, foxtail millet, maize and wheat 4. Silencing of rice Xa38 using pRTBV-MVIGS system to determine its function against BB disease 5. Summary and Conclusions. References and Appendices.

04. KAUR (Kanwaljeet)
Functional Characterization of Rice Dual- Specificity Phosphatases in Rice and Arabidopsis.
Supervisor: Prof. Girdhar K. Pandey
Th 25783

Abstract

Dual- specificity phosphatases (DSP) dephosphorylate both Serine/Threonine and tyrosine residues. Arabidopsis DSPs have been previously characterized in various aspects like starch degradation, abiotic stresses, ROS homeostasis and biotic stresses. Rice DSPs, closely related to AtDSP4, AtDSP5 and AtDSP6 possess conserved amino acids required for starch binding and showed starch binding activity in vitro. These DSPs possess phosphatase activity and were shown to be localized in the chloroplast, indicating their involvement in the starch degradation process. Rice overexpression lines of OsDSPs showed better growth as compared to the wild type. Increased seed size was observed in OsPP42-OE lines and less in RNAi lines. Seed size alteration was observed in case of OsPP100 and OsPP124. Scanning electron microscopy (SEM) analysis of seeds of OsPP42-OE lines showed larger starch granules as compared to wild type, while RNAi lines showed smaller starch granules. However, seed specific expression lines of OsPP42 did not show much difference in seed size and starch granule morphology. In case of OsPP100, no significant difference in starch granule morphology was observed. The OsPP42-OX lines showed tolerance to iron and calcium deficient condition, OsPP100-OE lines showed tolerance to iron deficient conditions and OsPP124-OE lines showed tolerance to iron and potassium deficient stress. Overexpression of OsPP42 and OsPP117 leads to better plant development and larger seeds. Phenotypic analysis of OsPP42-OE and OsPP117-OE lines showed tolerance to oxidative stress (in both rice and Arabidopsis) and nutrient deficiency (in Arabidopsis). The OsPP42-OE and OsPP117-OE lines showed sensitivity to osmotic, abscisic acid and PEG induced desiccation stress. The knowledge generated from this study could be a step forward in developing tools and methodologies for generating crops with enhanced stress tolerance and higher starch content.

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1. Organization of thesis 2. Review of literature 3. To unravel the contribution of rice DSPs in starch degradation pathway in plants 4. DSPs in conferring nutrient deficiency tolerance in rice 5. Elucidation of functional role of rice DSPs in abiotic stress 6. Summary and Conclusions and References.

05. KHURANA (Ridhi)
Analysis of Factors Contributing to Transcriptional Regulation of OsMADS29 Exprssion and Interaction of MADS29 with Calmodulin-Like Proteins.
Supervisor: Prof. Sanjay Kapoor
Th 25782

Abstract

OsMADS29 (M29) has been identified as a seed-specific MADS-box transcription factor implicated in embryo and endosperm development by regulating the degradation of the nucellus via nucellar programmed cell death. It is also involved in plastid biogenesis and starch filling via its influence on maintaining auxin/cytokinin homeostasis. Stringent control over M29 function at the transcriptional and translational level has been reported previously. The present study focuses on gaining a deeper understanding of the same. Our analysis of the upstream regulatory region (URR) of M29 revealed the presence of auxin-responsive and seed-specific elements. Expression analysis of the ß- glucuronidase reporter gene fusions with deletions of the URR based on conserved elements in stable rice transgenics revealed the presence of a strong distal repressor element and proximal auxin-responsive elements. Supplementing URR-GUS fusion transgenic seedlings with exogenous indole-3-acetic acid (IAA) induced the transcription of the M29 URR, strengthening the role of auxin in guiding early seed development in rice. Previous studies in our lab have shed light on the possible role of calmodulin in regulating the nuclear entry of M29. The analyses involving interactions of M29 with 27 seed-expressed calmodulin-family proteins carried out as part of the present study show that M29 interacts with at least 15 calmodulin-family proteins. M29 has been previously reported to interact with 19 seed-expressed MADS-box proteins. The possible combinatorial interactions between MADS-box and calmodulin-family proteins might be instrumental in shaping up the M29- mediated transcriptional regulation of its target genes. The findings of this study provide insights into another layer in the already complex regulation of M29 function and highlight the complexity of gene regulatory networks orchestrating aspects of plant growth and development. Understanding the M29 regulatory modules would be beneficial for developing molecular strategies targeted towards augmenting grain filling and grain quality traits rice and other cereals.

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06. RAGHUVANSHI (Utkarsh)
Transcriptome Analysis and Genetic Manipulation for Improving Tomato Fruit Quality.
Supervisors: Prof. Arun Kumar Sharma
Th 26507

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07. SAMTANI (Harsha)
Genome Wide Indentification and Expression Anslysis of Hsfs in Aegilops tauschii and Functional Characterization of TaHsfA5, TaOBF1 and HvHVA1 Genes in Bread Wheat for Thermotolerance.
Supervisor: Prof. Paramjit Khurana
Th 25779

Abstract

Temperature affects the survival of nearly all life forms on earth. Plants often suffer from heat stress and therefore, in its response synthesize Heat shock proteins (HSPs) inside the cell. These functions as chaperones by preventing protein aggregation and facilitating refolding of heat stressed damaged proteins. HSPs are in turn controlled by heat stress transcription factors (Hsfs), which act as master regulators of Heat Shock Response (HSR). The earlier reports on mechanisms involved in acquiring therrnotolerance in higher plants is presented as a review. To understand the molecular mechanisms involved in HSR, Hsfs were identified in Aegilops tasuchii and functional roles of TaHsfA5, TaOBF1 and HvHVA1 were investigated for thermotolerance. In the first chapter, Hsfmembers were identified in Aegilops tasuchii and their expression analysis under various stresses and light conditions was investigated. In chapter Il, an in-depth analysis of TaHsfA5 was carried out by studying its expression profiling in wheat. Its functional role in HSR was deduced by finding its interaction with TaHsfA4, TaHsfA3 and TaHSP2 proteins. Overexpression of TaHsfA5 in Arabidopsis and Oryza sativa promoted thermotolerance. In the third chapter, TaOBF1 homeologs were identified in wheat and TaOBF15B was found to be regulated by heat stress. Interaction of TaOBF1-5B with TaSTI and TaHSP90 indicated its role in HSR. Overexpression of TaOBFl-5B in Arabidopsis and Oryza sativa promoted thermotolerance which was attributed to the high expression levels of the heat stress marker genes in the transgenic lines. In chapter IV, doubled haploid transgenic wheat plants overexpressing HvHVA1 gene were analyzed for drought and heat stress tolerance. Transcriptome analysis was undertaken to identify the key genes contributing towards the drought and heat tolerance mechanism. Taken together the present study highlight different approaches that could be used for engineering heat stress tolerance in various plants and are worth investigating.

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08. SHARMA (Nikita)
Molecular Investigation During Somatic Embryogenesis and Genome-Wide Analysis of TaSERKs, TaBRI1, TaARFs and TaAUXs Genes in Wheat.
Supervisors: Prof. Anil K. Grover and Prof. Paramjit Khurana
Th 25780

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09. SINGH (Shipra)
Functional Characterization of OsCRY2 and Os FBO10 in rice and AtBTF3 and AtHY5 in Arabidopsis in Regulation of Photomorphogenesis.
Supervisors: Prof. Arun Kumar Sharma
Th 26506

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