CHAPTER 47

PLANT MOLECULER BIOLOGY

Doctoral Theses

534. BUSHRA SAEED
Comparative Transcriptomics for Marker Development and Characterization of Novel Stress responsive Genes in Mulberry.
Supervisor : Prof. Paramjit Khurana
Th 22293

Contents

1. Comparative transcriptomics and comprehensive marker resource development in mulberry 2. Identification and characterization of lectin gene superfamily in mulberry 3. Isolation and functional characterization of novel early responsive to dehydration genes from mulberry 4. Characterization of B-Carotene hydroxylase over expression lines of mulberry 5. Summary and conclusions. References and annexure.

535. FAUZIA ZARREEN
RNAi Defence Response Against Virus Infection in Rice and Comprehensive Analysis of Transcription Regulation in Sri Lankan Cassava Mosaic Virus.
Supervisor : Prof. Indranil Dasgupta
Th 22640

Contents

1. General introduction 2. Review of literature 3. High throughput sequence analysis of small RNAS in Rice affected by rice Tungro disease 4. Screening of rice tungro spherical virus genome for viral suppressors of RNAi 5. Construction of a stable intron-containing cDNA clone for rice tungro sherical virus 6. Transcript mapping of Sri Lankan cassava mosaic virus 7. Summary and conclusions. References and appendices.

536. HAIRAT (Suboot)
Evaluation of Members of Triticeae Family for thermotolerance, and Transcriptome analysis and Functional Characterization of Lipid Transfer Proteins in Bread Wheat (Triticum Aestivum).
Supervisor : Prof. Paramjit Khurana
Th 22287

Contents

1. Identification of thermotolerance in members of the triticeae 2. Role of lipid transfer protein (LTP) gene family in abiotic stress 3. NGS-Based comparative transcriptome profiling of the awn and lodicule tissues 4. Summary and conclusions. Literature cited.

537. JIWANI (Gitanjali)
Studies on Mechanism of Fruit ripening for Improving Fruit Quality of Tomato.
Supervisor : Dr. Arun K. Sharma
Th 22288

Abstract

Tomato is considered both a fruit and a vegetable. It is of great importance to human beings as it can be consumed raw without any processing. Tomato is a rich source of antioxidants and vitamins. The major carotenoid pigment lycopene is beneficial to human health as it prevents risk of cancer and cardiovascular diseases. Tomato is also a good source of pro-vitamin A, β-carotene. It is seen that tomato is prone to post harvest damage. Major loss in tomato production occurs at the post-harvest stage. As ripening progresses, the fruit becomes softer, as a result it is suffers mechanical damage during transportation. Delaying fruit ripening and increasing shelf life of tomato has been one of the major goals of scientists. It is also important to realize the fact that with ripening the fruit accumulates nutrients and important health promoting factors beneficial to humans. Therefore delaying ripening along with maintaining the fruit quality is the focus point in tomato research. In order to address this requirement the present study aimed at four major objectives. First objective was to identify ripening related genes by RNA–Seq analysis, which deals with fishing out genes which regulate ripening in a positive or negative manner. The second objective was to attain delayed ripening and increasing shelf life by expressing the mutant Arabidopsis receptor and silencing the SlMADSRIN gene. The third objective deals with identification and characterization of class II PLP dependent decarboxylase gene family in tomato. Following this the fourth objective was focused on improving fruit quality of tomato by overexpression of Arabidopsis genes to achieve folate biofortification and studying various aspects of folate pathway genes in tomato. The results obtained by this study shall be of great interest and add value to the goal of improving the fruit quality and shelf life of tomato.

Contents

538. KUSHAWAHA (Akhilesh Kumar)
Sequence Analysis, Infectivity and Gene Functions in Sri Lankan Cassava Mosaic Virus.
Supervisor : Prof. Indranil Dasgupta
Th 22666

Abstract

Cassava mosaic disease (CMD), caused by small single stranded DNA viruses (geminiviruses) severely affects the production of cassava tubers in African countries, in India and Sri Lanka. In this study, three full length SLCMV and one ICMV sequence, known to be the causative agents of CMD in India, were cloned and analyzed for its structure, diversity, phylogenetic relationship with other begomoviruses and infectivity analysis by biolistic method. RCA-RFLP technique

was applied to study the distribution and variability of all cassava-infecting begomoviruses from 80 symptomatic cassava samples collected from various districts of Tamil Nadu. Alignment of partially sequenced fragments showed that minor variability exists in SLCMV DNA and there were no hotspots of sequence variability. It was found that SLCMV is more numerous as compared to the ICMV. Sequence analysis of cloned SLCMV DNA (SLCMV-Attur) indicated the conservation of all known functional motifs of begomoviral genes and that SLCMV-Attur was most closely related to Indian begomoviruses infecting solanaceous plants but distantly related to begomoviruses infecting legumes and those from outside Indian subcontinent. The standardization of biolistic inoculation showed a relationship between the amount of DNA used for biolistic inoculation and pressure on symptom development. Infectivity analysis showed that 1000ng of SLCMV-Attur had a higher efficiency of infection (56 out of 66 plants infected) and symptoms appeared between 21 to 28 dpi (stunting and downward leaf curling) at the pressure of 200 psi. For analysis of the effect of introduced C-terminal truncations in the AV2 protein of SLCMV-Attur, it was seen that the mutated clones were non-infectious, suggesting that the truncated protein could not perform the functions which the intact protein performs. It was concluded that for infection, full expression of AV2 protein is required for symptom development and viral accumulation.

Contents

539. LAVANIA (Dhruv)
Hsf-Hsp Circultry Regulating the Transcription of OsClpB-C/Hsp 100 Gene in Rice (Oryza Sativa L.)
Supervisor : Prof. Anil Grover
Th 22639

Contents

540. PANDEY (Agni Shekhar)
Functional Characterization of Some Rice bZIP Transcription Factor Genes Involved in Stress Tolerance and Seed Development.
Supervisor : Prof. Jitendra P. Khurana
Th 22290

Abstract

To understand how plants adapt to adverse environmental conditions, three dehydration stress inducible genes in rice, namely OsbZIP16, OsbZIP76 and

OsbZIP80, encoding bZIP transcription factors, were characterized. Phylogenetic analysis showed presence of all three genes in both monocots and dicots with a likely conserved function. Real-time PCR analysis revealed that the transcript levels of OsbZIP16 are up-regulated when rice seedlings are subjected to dehydration and salt stress. OsbZIP76 had very specific expression during early stages of seed development, whereas OsbZIP80 had high expression during later stages of seed maturation; its expression was transiently elevated during early panicle development. Particle bombardment in onion epidermal cells confirmed nuclear localization of only OsbZIP16 protein. OsbZIP16 also had the transcription activation potential but the results were negative for OsbZIP76 and OsbZIP80, possibly, because the other two bZIPs require some modification or a chaperone to drive them to the nucleus and for enabling their transcriptional activity. For functional characterization, these three genes were ectopically over-expressed in Arabidopsis thaliana Col-0, as well as over-expression and RNAi transgenics were raised in rice. The OsbZIP16 and OsbZIP76 over-expressing Arabidopsis transgenics performed better in seed germination assays, the adult plants survived dehydration stress, and young seedlings accumulated less reactive oxygen species under oxidative stress; essentially confirming the ability of both genes in conferring dehydration and oxidative stress tolerance. Preliminary analysis of rice transgenics provided evidence that both OsbZIP16 and OsbZIP76 over-expression rice transgenics too were tolerant to mannitol, salt and ABA. In comparison to wild type and the OsbZIP80 orthologous mutant of Arabidopsis, the OsbZIP80 over-expression transgenic Arabidopsis plants were delayed in initial growth and flowering with their seeds slightly large and pale in color, less viable and more sensitive to salt and dehydration stress, emphasizing that OsbZIP80 may acts as a negative regulator of reproductive development.

Contents

541. PANDEY (Ritu)
A. Genome-Wide Study of Tissue- and Abiotic stress-Specific Small Non-Coding RNAs in Wheat (Triticum Aestivum L.)
B. Comparative Transcriptomics of Wheat Cultivars with Contrasting Response to High Temperature Stress.
Supervisor : Dr. Surekha Katiyar-Agarwal
Th 22277

Abstract

Wheat (Triticum aestivum L.) is one of the world's leading food crops in terms of its cultivation area and consumption. However there is an enormous gap between the production and demand of wheat grains, which is contributed by several biotic and abiotic factors. Plants utilize diverse strategies to adapt to the imposed environmental challenges, of which reprogramming of gene expression is one such strategy. It is now known that the components of non-coding transcriptome (miRNA and siRNAs), drive alteration in expression of coding transcriptome. Hence, we performed an exploratory study utilizing next generation sequencing-based methodology for genome-wide

identification of miRNAs and mRNAs that are differentially expressed during stress and development in wheat. The major objectives and salient findings of this study are as follows: Expression catalogue of wheat miRNAs was prepared across three abiotic stresses and four tissues specific libraries. A dataset of abiotic-stress responsive and tissue-preferential known and novel miRNAs was established. Putative targets were computationally predicted and three predicted targets (SPL, ARF, NAC1) were experimentally confirmed by RLM-RACE along with expression analysis. The regulation of NAC1 by tae-miR164 was established by transient assay in N. benthamiana plants. We generated transgenic rice lines for over-expression of wheat miR164, NAC1 and mutated NAC1. In miR164-overexpressing lines the expression of one rice NAC gene was downregulated. Comparative miRNAomics of wheat cultivars with contrasting response to high temperature stress was performed employing high throughput sequencing. Digital expression analysis of identified miRNAs revealed that 46 miRNAs were differentially regulated in response to heat stress between the tolerant and susceptible cultivars. RNA-Seq Analysis of wheat cultivars with contrasting response to high temperature stress was performed. Validation of few transcripts was performed using qPCR and it was found that many genes were induced in tolerant cultivar as compared to susceptible.

Contents

542. PAREEK (Amit)
Overexpression of LeMPK3, LeAOXIa & Le AOX1b Genes in Tomato Plants for Improvement of Cold Tolerance.
Supervisor : Dr. Arun K. Sharma
Th 22289

Abstract

One of the way to analyse the function of gene is by over-expression in transgenic plants. These plants provide a tool to characterize the activity of gene for abiotic stress tolerance. Transgenic plants are useful to understand molecular physiology and gene networks of plants in response to abiotic stresses. For improvement of cold tolerance in tomato plants, LeMPK3, LeAOX1a and LeAOX1b genes had been over-expressed in tomato plants. Physiological, biochemical and molecular characterization of transgenic tomato plants showed that these plants perform much better under cold, drought and salt stresses. Further LeCAT01 and LeAOX1b promoters were also characterized as stress inducible promoters in tomato plants. Deletion analysis of LeAOX1b promoter, suggested that -1533 to -1106 promoter region was required for basal expression under cold, heat and salt stress conditions. Overall, the present research work improves the knowledge about the physiological and molecular characteristics of cold stress response, in terms of sensitivity and tolerance.

Contents

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

543. MUTUM ROSEETA DEVI
Identification and Characterization of MicroRNAs from Indica Rice Var. Nagina 22 and Orthologous MicroRNAs from Pisum Sativum Var. Arkel.
Supervisor : Dr. Saurabh Raghuvanshi
Th 22641

Abstract

MicroRNAs (miRNAs) are important regulators of plant development and metabolism. The study was undertaken to identify novel miRNAs in rice and orthologous miRNAs in garden pea as well as to characterize known miRNAs (miR408 and miR1425) in rice. While both the plants are agro-economically important, the genome of rice is known and well-studied while that of pea is un-sequenced yet. With the utilization of deep sequenced small RNA libraries and the integration of computational biology, expression analysis and target validation by degradome libraries generation, many novel miRNAs targeting important genes have been identified in rice. The study was extended with the identification of miRNAs in Pisum sativum var. Arkel. It was carried out to address an important issue in miRNA gene identification (i.e. requirement of a reference genome sequence). However, whole genome sequencing for species with large genome size and rich repeat elements is difficult to assemble. In order to address this issue, we have developed a novel technique wherein only the miRNA coding genomic loci from the pea genome have been enriched without whole genome sequencing. Sequencing and subsequent analysis of these enriched fragments led to identification of several miRNA genes in pea. In addition, two known miRNAs viz. miR408 and miR1425 were characterized in rice. Interestingly, miR408 demonstrated a variety-specific drought stress response at both the seedling and adult stages of rice development (N22 versus PB1). The target genes of miR408-3p i.e., plastocyanin-like family members showed anti-correlation with miRNA expression. On the other hand, miR1425-5p targets Rf1 (fertility restorer 1), an important player involved in pollen fertility, which prevents cytoplasmic male sterility. Rf1 is reported to be induced by cold stress and thus provide cold tolerance at booting stage in hybrid rice. Over-expression lines of precursor miR1425 exhibited right helically twisted phenotype at the culm region.

Contents

1. Review of literature. 2. Material and methods. 3. Results and discussion. Summary, conclusions, references and annexures.

544. RAVI KANT
Functional Analysis of Rice Genes Through Virus Induced Gene Silencing and Study of RNA Silencing Suppressors in Rice Tungro Bacilliform Virus.
Supervisor : Prof. Indranil Dasgupta
Th 22292

Abstract

Rice is the most important staple food crop in the world and more than half of the world population relies on it as a dietary source. Rice yields in south and Southeast Asia, one of the most productive rice growing regions of the World, is compromised by Rice tungro disease, caused by the joint infection of Rice tungro bacilliform virus (RTBV) and Rice tungro spherical virus (RTSV). Virus induced gene silencing (VIGS) is a novel reverse genetics tool for functional genomics studies in plants. Here, RTBV derived VIGS tool has been utilized to determine the function of reporter genes in rice such as Phytoene desaturase (pds), chlH subunit of Magnesium chelatase and Bacterial leaf blight (BLB) disease resistant gene Xa21. In addition, effect of sense, antisense and hairpin orientations of chlH gene fragment in the VIGS vector was conducted to elucidate the dependence of silencing efficiency on gene orientation. Phenotypic expression and quantitative real time PCR analysis in inoculated rice plants displayed significant silencing phenotype and down-regulation of target gene expression as compared to control plants. On the other hand, during plant-virus interactions, viruses encode proteins known as viral suppressors of RNA silencing (VSRs), as a counter defense mechanism to inhibit the host RNA silencing pathway. To screen the RTBV encoded silencing suppressor protein(s), agrobacterium mediated transient agroinfiltration assay in GFP expressing Nicotiana benthamiana (16c) uncovered PRT and ORF-IV of RTBV as silencing suppressor proteins. Gene expression and transcript abundance of some of the host RNAi components were also detected to be correlated to PRT and ORF-IV expression in infiltrated plants, indicating these suppressors to likely have strong effect on expression pattern of RNAi components.

Contents

545. SONIA
Comparative Mirnome of Drought Tolerant and Sensitive Indica Rice Cultivars.
Supervisor : Dr. Saurabh Raghuvanshi
Th 22291

Abstract

The present study exploits the comparative miRNomics to delineate the tissue and drought mediated dynamism of miRNAs in different tissues of drought tolerant N22 and sensitive PB1 indica rice cultivars. The result highlights the critical role of flag leaf in N22 development and stress response. Clearly under non-stressed conditions N22 shows high number of flag leaf preferential miRNAs while PB1 has high number of spikelet preferential miRNAs. Secondly, N22 flag leaf responds very dynamically

to drought as compared to other tissues and very high number of miRNAs displayed drought responsive differential regulation including several miRNAs that showed enrichment in flag leaf. Further the study identified a unique mode of operation of a miRNA-mediated network in the drought tolerant rice cultivars, during drought. This group of miRNAs (miR408-3p, miR528-5p, miR398b, miR397, miR1871, miR159f and miR2878-5p) referred as Drought Trait Associated (DTA)-miRNAs are upregulated in the flag-leaves of tolerant cultivar, N22 and Vandana, but downregulated in the sensitive cultivars PB1 and IR64, under similar field drought conditions. Interestingly, most of them target copper containing protein genes such as plantacyanins, laccases, ascorbate oxidase and copper-zinc superoxide dismutases. The DTA-miRNAs were also found to be copper-responsive. Further, as compared to PB1, Cu levels were significantly decreased in the N22 flag-leaves, during drought. Investigations into the functional impact of the cultivar-specific regulation revealed a marginally higher accumulation of reactive oxygen species (ROS) in N22 flag-leaves as compared to PB1. Further, over-expression of a DTA-miRNA osa-MIR408 revealed its role as a positive regulator of rice vegetative growth and water deficit stress response. Over-expression of MIR528 suggests its role in root and seed development. Detailed analysis of miRNA 5' start site variability identified several isomiRs that also regulate unique to similar targets via mRNA cleavage and their processing is also regulated by drought.

Contents