CHAPTER 40

PLANT MOLECULAR BIOLOGY

Doctoral Theses

344. GHOSH (Ratna)
Characterization and Functional Analysis of Methylated DNA Binding Proteins in Plants.
Supervisor: Dr. Arun K. Sharma
Th 14726

Abstract

Deals with the identification and characterization of methylated DNA binding proteins in plants. Work was initiated with an aim to identify methylated DNA binding proteins in tomato. Identification of proteins from nuclear and crude extracts of tomato that bind to methylated CpG rich DNA indicates the presence of such proteins in plants. Next, database search carried out using MBD domain of MeCP2 from mouse, led to the identification of one putative gene encoding methylated DNA binding protein from Arabidopsis, which was later named as AtMBD 11 by chrom DB. The AtMBD11 gene was isolated from genomic DNA of Arabidopsis by PCR amplification using gene specific primers. To study the functional role of AtMBD11, binary vector constructs were transformed into totacco. Transgenic plants raised showed some phenotypic abnormalities thus pointing towards a crucial role of this gene in plants. Promoter analysis was also carried out for this gene. Full promoter showed maximum activity in leaf, flower and stem with minimum activity in the roots. Deletion analysis identified the region of the promoter, which is crucial for its activity. Further, biochemical characterization of AtMBD10 protein (which is a methylated DNA binding protein similar to AtMBD11) and AtMBD 11 was performed by expressing these proteins in bacteria, followed by their purification. Assays were performed for studying their affinity to bind to methylated DNA. While binding of AtMBD 10 was found to be indifferent to the methylation status of DNA and showed affinity only for CpG rich DNA, AtMBD 11 showed affinity to methylated DNA. Further. to characterize the possible role of AtMBD 11 in gene silencing, an experimental model was developed. Attempts were made to silence stably integrated gus gene, using agroinfiltration-mediated transient

gene expression, targeted at silencing of gus gene by co-suppression or RNA-Interference. Expression of AtMBD 11 in antisense orientation was found to interfere with the silencing of gus gene stably integrated in the plant genome.

Contents

1. Review of literature on DNA methylation. 2. Materials and methods. 3. Results. 4. Discussion. 5. Summary, Conclusions, Bibliography and Appendix.

345. JAIN (Mukesh)
Functional Analysis of Genes Encoding Topoisomerase 6 Homologues and Genome-wide Analysis of Early Auxin-and Cytokinin-responsive Gene Families in Rice (Oryza sativa).
Supervisor : Prof. Jitendra P. Khurana
Th 14730

Abstract

Describes the identification and characterization of some of the signal transduction pathway genes in rice as a part of the rice functional genomics program. The first part includes the "Functional analysis of genes encoding topoisomerase 6 homologues in rice". The DNA topoisomerase 6 represents the prototype of type II DNA topoisomerases present only in archaebacteria other than plants. The homologues of topoisomerase 6 among plants have been characterized so far only in Arabidopsis and found to play a crucial role in meiotic recombination, endoreduplication, and plant growth and development in general. The present study provides the evidence for the presence of putative topoisomerase 6 genes in rice. Their detailed quantitative tissue specific expression, hormonal regulation and subcellular localization have been reported. The overexpression of these genes conferred tolerance to various abiotic stresses in transgenic Arabidopsis plants as revealed by their extensive physiological and molecular analyses. This study provides the first evidence for the involvement of a chromatin modulation factor, topoisomerase 6, in stress signaling in rice. The second part includes the "Genome-wide analysis of early auxin and cytokinin-responsive gene families in rice". The components of auxin and cytokinin signal transduction pathways have been very well characterized in Arabidopsis, the model dicot plant; however, the knowledge about these components in rice, the model monocot plant, remains largely untapped. The availability of complete rice genomic sequence combined with the full-length

cDNA sequences from KOME provides a unique opportunity to identify components in rice. This study describes the genome-wide identification of early auxin-responsive GH3, AuxlIAA, and SAUR gene families, and cytokinin responsive type-A response regulators, in rice by screening the available databases. This work involves the study of their genomic organization, chromosomal distribution, sequence homology, evolutionary expansion and expression patterns.

Contents

346. PUSHPA KUMARI
Studies on Mechanisms of Gene Silencing in Transgenic Plants.
Supervisor: Dr. Arun K. Sharma
Th 14728

Abstract

The role of DNA methylation is well understood in both plants and animals. Mutant for MET1 gene, encoding DNA methylase and plants with down regulation of this gene by antisense method have helped in understanding the role of DNA methylation in plants. Methyl-CpG binding proteins are known for a long time and their role is established beyond doubt in gene repression. The existence of such proteins was reported recently in plants. This study was initiated to know whether plants also have proteins similar to well characterized vertebrate proteins, mouse MBD2 and rat MeCP@. Genes for these two mammalian proteins and Arabidopsis protein AtMBD10 were over and under expressed in tobacco to characterize the function of AtMBD10 gene and other genes which might code for proteins similar to mammalian

proteins MeCP2 and MBD2. Functional characterization of these genes was performed by Agrobacterium mediated transient gene expression assay in plants. Plants expressing MBD2, and AtMBD10 in sense and antisense orientation and MeCP2 in only sense orientation in addition to gus reporter gene were used to find out whether MBD proteins interfered in silencing of gus gene.

Contents

1. Review of previous work on mechanisms of gene silencing. 2. Materials and methods. 3. Results. 4. Discussion. 5. Summary and Conclusions.

347. RAVI (V)

Sequencing and Analysis of 15 BAC/PAC Clones from Chromosome 11 of Rice and Structural Organization of the Mulberry Chloroplast Genome.

Supervisor: Prof. Paramjit Khurana

Th 14731

Abstract

The field of deciphering the letters of life, known as structural genomics, not only paves the path for gene discovery and characterization (functional genomics), but also provides the raw materials for analyzing evolutionary history of the organism (molelcular phylogeny). The study revolves around this powerful field called structural genomics. The first half is part of an international effort to sequence the genome of the world's most important crop - rice. Rice is considered the model crop amongst the monocots just as Arabidopsis is amongst the dicots. Decoding its genome would uncover secrets lying within its DNA which can in turn be manipulated for varied requirements and utilities. In total, 2.04 Mb of high quality assembled sequence data were generated by sequencing 15 BAP/PAC clones of rice. Out of these, 13 were from chromosome 11 while the remaining two were located to chromosome 3 and 9. Finished sequence was generated for 12 clones of chromosome 11 and these were used for gene prediction and duplication analysis. Plant cells have two more genomes other than the central nuclear genome. These genomes reside inside two very important organelles of the plant cell. One is the powerhouse of the cell, mitochondria, having originated from a free-living oxygen-consuming proteobacterium through endosymbiosis. The second, chloroplast, is the unique feature about plant and algal cells, possibly having its origin from a free-living oxygen producing cyanobacterium.

This organelle gives the plant the capacity to utilize Sun's energy to produce food for itself and in turn for others and is the focus of the second part of the work.

Contents

1. Review of literature. 2. Materials and methods. 3. Results. 4. Discussion. 5. Summary, Conclusions and Bibliography.

348. TANDON (Vidhu)
Sequence Analysis of Rice Tungro Spherical Virus, Engineering Viral Resistance and Development of a Self Replicating Vector for Rice.
Supervisor: Dr. Indranil Dasgupta
Th 14729

Abstract

It has been estimated that half the world population is dependent wholly or partially on rice for dietary calories (Coffman and Juliano, 1987). During the past few yuears global rice production has remained stagnant, while the population in the rice consuming countries is still growing at 1.8 percent (Leung 1996). At present Thailand leads in the world rice trade closely followed by Vietnam and the United States of America. Other countries such as India and Myanmar are gradually emerging in the world rice trade scenario. India and Pakistan are the only two countries, who monopolize the export of aromatic "Basmati Rice" (International Rice Commission., 2001). Ninety percent of the world rice is grown and consumed in Asia. Both aboitic (Salinity, drought, low pH etc.) and biotic stresses (insect pests, bacterial, fungal and viral pathogens) affect the productivity of rice adversely. Scientists are trying to make plants resistant to various stresses. Biotic stresses mainly comprise of pathogen which infect plants and pests which feed on plants and suck their sap. Out of 85 diseases that occuar in rice, one of prime economic importance is Tungro disease of rice. Tungro is the most widely distributed and devastating viral disease of rice (Hibino and Cabauatan, 1987) which is caused by similtaneous infection of rice plants with two viruses, RTSV and RTBV. In order to contain the damages due to Tungro, it is essential to incorporate transgenic resistance in the rice improvement programs of the country. In addition, understanding the genetic structure of the natural populations of Tungro viruses and the vector is also important. Viral genomes can often be used as gene silencing vectors in host plants because

of their property of triggering gene silencing during the infection process Suitably modified viral vectors have thus been very useful in the targeted silencing of genes and are an important tool for the study of the functions of kunknown genes.

Contents

1. Review of literature. 2. Cloning and nucleotide sequence analysis of RSTV genomes from Orissa and West Bengal. 3. Genetic transformation of rice with different viral gene constructs to engineer resistance against RTSV, Molecular analysis of transgenic rice plants and virus resistance assays. 4. Deleted version of an infectious clone of rice Tungro bacilliform virus for independent replication in rice. 5. Summary and Conclusion.

349. TYAGI (Himani)
Transformation of a Popular Indica Rice Variety ADT 39 and Engineering Pathogen Derived Resistance Against Tungro Viruses in Pusa Basmati 1.
Supervisor: Dr. Indranil Dasgupta
Th 14732

Abstract

Established an efficient system for embryogenic calli production and regeneration by optimizing carbohydrate sources and concentration, and supplementing cytokinins, auxin and amino acids. For rice variety ADT 39, J3 medium was the most suitable callusing medium, whereas MS was the better regeneration medium. Obtaining sufficient good quality embryogenic callus for indica rice is difficult, most studies utilize MS or NB medium for callusing. ADT 39 responded better to J3 callusing medium, sufficient callus was obtained in 21 days as against MS medium which yield transformable callus in 45 days. Regeneration efficiency of 67% was obtained on MS medium. Thus, we used well-structured embryogenic calli derived from MS medium for transformation with A. tumifaciens strain EHA 105 harbouring a binary plasmid containing gus reporter gene being driven by a constitutive RTBV promoter. The molecular analyses of the putative transgenic plants were performed by PCR, southern and northern. Several single copy insertions were observed. Gus expression was confirmed by Northern as well as histochemical staining analyses. The studies of transformation of rice suggested that numerous factors including genotype of plants, age of tissues inoculated, and selective

agents, and various conditions of tissue culture, are of critical importance. Standardization of Agrobacterium-mediated transformation protocol for ADT 39 is particularly important because this variety is extensively cultivated in Southern India and is popular among the farmers because of its inherent resistance to economically devastating diseases like bacterial blast and sheath blight. Availability of this transformation protocol for ADT-39 will allow the introduction of several agronomically important genes, leading to further genetic enhancement of this rice variety.

Contents

1. Review of literature. 2. Materials and Methods. 3. Results and Discussion. 4. Summary, Conclusions and Bibliography.

350. VIJ (Shubha)
Sequencing and Annotation of 1.7 Mb of Chromosome 11 and Functional Characterization of OSISAP 1-Related Abiotic Stress-Responsive Genes from Rice.
Supervisor: Prof. Akhilesh K. Tyagi
Th 14727

Abstract

Studies to inter-related aspects of rice genomics. The first involved sequencing of -1.7 Mb of chromosome 11 of rice as part of an international effort to sequence the rice genome. This sequence was generated from eleven BAC/PAC clones using a clone-by-clone shotgun approach along with high throughput sequencing and brought to phase III (finished level). The finished sequence was annotated to identify several important genes such as those involved in disease resistance abiotic stress response and transcriptional control. Out of 37,544 genes identified from the validation is still required. The second part of the work involved characterization of three abiotic stress-responsive genes from rice. OSISAP1, a stress-associated zinc-finger protein gene from rice, had been previously characterized in the lab and shown to be inducible to multiple stresses like cold, salt and dehydration stress at the seeding stage. The OSISAP1 promoter was also characterized in transgenic rice and was found to be involved in regulation of the cold stress response. During an extension of the work, two other novel abiotic stress-responsive genes, OSISAP2 (the closest homologue of OSISAP1 in the rice genome) and OSIRPK homologous proteins with characteristic A20-AN1 zinc-fingers have also been

identified in rice and Arabidopsis. OSISAP2 and OSIRPK, like OSISAP1, were found to be inducible to abiotic stresses like cold, salt and dehydration. Detailed interaction analysis of OSISAP1, OSISAP2, OSIRPK as well as A20 and AN1 type zinc-fingers using yeast two hybrid analysis revealed complex interaction patterns of each of these proteins/domains with itself and with the other proteins. These proteins are localized in the cytosol and may form a crucial component of stress signal transduction in rice.

Contents