CHAPTER 40

PLANT MOLECULAR BIOLOGY

Doctoral Theses

288. AGARWAL (Sangeeta)
Molecular Studies on Rice (Oryza Sativa L.) Flooding Stress Response Using Transgenic - and Comparative Genomics-Based Approaches.
Supervisor: Prof. Anil Grover
Th 14299

Abstract

The work is aimed at (1) production and characterization of transgenic rice plants that over-express Adh protein, (2) production and characterization of transgenic rice plants that over-or under-express Pdc protein, (3) optimization of yeast expression system for examing roles of plant stress responsive genes and (4) generation of subtraction cDNA libraries of clones that are differentially expressed in flood-tolerant FR13A and flood-sensitive Pusa Basmati 1 rice types and characterization of expression profiles of the clones isolated from such libraries. The study suggests that while over-expression of pyruvate decarboxylase and alcohol dehydrogenase may be useful for increasing early seedling vigor under flooded conditions, there is a need to look for more candidate genes for future production of transgenic plants with improved submergence tolerance. It further shows that proteins involved in signaling, sugar and ion transport and transcript stability hold promisr for increasing level of flooding stress tolerance in future plant transgenic production programmes. The functional roles of large number of proteins identified in this study in conferring tolerance to low O_2 stress conditions can be further established using yearst and transgenic plant production systems. Protocols for the use of these systems for the genetic transformation-related work were shown to work reasonably well.

Contents

289. ANAND (Saurabh)

Characterization of a Pollen-Preferential Gene (OSIPP2) and an Anther-Specific Gene (OSIPP4) and their Regulatory Elements from Rice.

Supervisor: Prof. Akhilesh K Tyagi

Th 14294

Abstract

Aims at isolation and characterization of organ-specific genes and their regulatory elements. Differential screening of cDNA-libraries specific to various stages of inflorescence development led to the isolation of a pollen-preferential gene, OSIPP2, and an anther-specific gene, OSIPP4, from indica rice. OSIPP2 is single copy intronless gene and codes for a protein with homology to putative arabinogalactan proteins from Arabidopsis. The 5' end of its transcript was mapped to delineate regulatory and transcribed regions. Its expression was found to be pollen-preferential. OSIPP2 also shows developmental and light regulation as well as induction in response to salt stress. The promotor region of OSIPP2 contains several elements known to confer pollen-specific expression. Rice and tobacco plants were transformed with OSIPP2 promoter fused to GUS for studying expression patterns in homologous and heterologous systems. In transgenic tobacco plants, maximum level of the GUS activity was seen in anther, with low levels of expression in other floral organs. The expression level of GUS in anthers increases as bud size increase, maximum being in the mature flower. The promoter is active in the pollen tubes of the transgenic pollen germinated on the vitro germination medium, OSIPP2 promoter in transgenic rice drives the expression of gus in various organs, maximum being in pollen. Its deletion analysis was initiated to unravel regulatory elements with better precision. Antisense strategy was adapted to knockout the expression of OSIPP2. Transgenic plants did produce antisense transcript but not significant phenotypic difference was observed in T0 generation. Probable functions of the gene have been discussed in view of the available information. Another gene, OSIPP4, is anther-specific in expression and northern analysis revealed two transcripts in pre-pollination stage inflrescence. Southern analysis, genomic cloning and sequencing efforts suggest that OSIPP4 is member of small gene family, OSIPP4 shows homology to pollen allergens. The upstream region from OSIPP4 has many cis-acting elements conferring pollen-specific expression. Promoters activity of the gene is yet to be investigated. Such promoters from pollen-

specific genes can be used for expressing genes-of-interest in pollen grains and for raising male-sterile plants for better engineering of crops.

Contents

1. Review of Previous Work on Molecular Basis on Flower Formation with Special Emphasis on Antheir-/Pollen-Specific Genes. 2. Material and Methods. 3. Results. 4. Discussion. 5. Summary and Conclusion. Bibliography and Appendix.

290. DUTT (Nitin)
Sequence Analysis, Infectivity and Investigations on Movement Related Genes of Cloned Cassava Infecting Geminiviruses.
Supervisor: Dr. Indranil Dasgupta
Th 14295

Abstract

The work is a step towards assessing the variability of Cassava Infecting geminiviruses (CIGs) inIndia, full-length, biologically competent clones were obtained using PCR-based cloning strategy. The study led to the identification of a second begomovirus, Sri Lankan cassava mosaic virus (SLCMV), a CIG reported previously only from Sri Lanka, associated with mosaic-diseased cassava plants. The infectious nature of the cloned components was demonstrated on both, the experimental host Nicotiana benthaminana and the original host cassava. This particular study led to the identification of a centrally located stretch of BC1 with the characteristics of an amphipathic alpha helix, characterized by the presence of hyudrophobic, aromatic and basic amino acid residues, responsible for peripheral targeting of the protein; a domain which could serve as a putative plasmodesmal targeting signal (PTS). As SLCMV traces its origin from a hypothetical monopartite progenitor, the potential role of AV2 and AC4 (proteins involved in the movement of monopartite begomoviruses) was studied using the methodology described above. Both AV2 and AC4 displayed perinuclear as well as peripheral localization, reminiscent of the behaviour of BC1, alghough in the case of AV2 fluorescence could be seen to spread to the adjacent cells. The observations indicate a potential role of these proteins in aiding virus movment.

Contents

1. Review of Literature. 2. Cloning, Sequence Analysis and Infectivity of Cassava-infecting Geminiviruses (CIGs) from India. 3. Functional Dissection of some Movement-related Genes of CIGs. 4. Summary and Conclusions. Bibliography and Appendix.

291. KATHURIA (Hitesh)
Investigations on Transgenic Indica Rice for Abiotic Stress Tolerance and an Anther-Specific Rice Gene (OsiPP3) Promoter.
Supervisor : Prof. Akhilesh K Tyagi
Th 14298

Abstract

Investigations have been carried out in indica rice to study basic aspects related to stabilization of the transgene in progenies as well as on transgenics raised for abiotic stress tolerance. The other aspect relates to the activity of a novel anther-specific gene, OsiPP3, promoter in transgenic Arabidopsis. The investigation paves way for engineering abiotic stress tolerance in rice. It also highlights the importance of conducting multi-generation molecular analysis for transgenic lines, prior to release for agronomic purposes, to avoid any rearrangement or silencing of the transgenes. Investigations with rice transgenics show that single gene tranformants have the potential to provide stable expression and stress tolerance ability over generations. promoter activity provides an insight in the expression pattern of a novel rice gene promoter containing anther-specific elements in transgenic Arabidopsis. The smallest deletion showing exclusively anther-specific expression can be used to engineer anther/specific expression can be used to engineer anther/ pollen related traits.

Contents

1. Review of the Previous Work on Genetic Transformation of Rice (Oryza sativa L.). 2. Materials and Methods. 3. Results. 4. Discussion. 5. Summary and Conclusions. Bibliography and Appendix.

292. MATHUR (Saloni)
Cloning, Infectivity and Promoter Analysis of Rice Tungro Bacilliform Virus from India.
Supervisor: Dr. Indranil Dasgupta
Th 14296

Abstract

Showed that Indian isolates of RTBV belong to a separate sub-group from the ones reported from Southeast Asia, but are hightly similar to each other, as assessed by nucleotide sequence analysis. Developed a PCR-RELP based method to identify isolates of RTBV prevalent in certain geographical regions of the country. Produced an infectious clone of RTBV, which can be further developed to investigate viral gene function and as a VIGS vector for rice functional genomics. Mapped the site for transcription intitiation in RTBV-WB. Functionally dissected the promoter region of RTBV in transgenic plants using the host, rice heterologous systems like tabacco, wheat and E. coli. Used EMSA to support observed functional behaviour of the above promoter in rice. Studied the silencing phenomenon of the promoter in a transgenic situation upon RTBV infection in plants. The above findings are expected to be of major importance in control of tungro disease of rice in India, using transgenic approaches. Also, it has provided a new promoter for transgene expression in monocot and dicot plants and also E. coli. the tissue-specific and development-specific behaviour has been characterized to a large extent.

Contents

293. SAHI (Chandan)
Comparative Genomics of Salt Stress Response in Rice (Oryza Sativa L.).
Supervisor: Prof. Anil Grover
Th 14297

Abstract

Shows that differental salt tolerance response as it exists in PB1, CSR27 and Pokkali rice types is constituted by a large

numbner of genes. Differential regulation of protein synthesis; turnover; turnover; folding and RNA metabolism are some of the key components of salt stress response in contrasting rice plants. Selected clones (namely Ospi, OsFKBP20 and Isgr-rbq that encode for specific protease inhibitor protein, FKBP bidnign protein and glycine rich-RNA binding protein, respectively) were taken up for somewhat detailed molecular and functional characterization. Ospi transcript was noted to be strongly inducible by salt stress, ABA and cold stress in the roots of PB1. The corresponding promoter was isolated. Studies with transgenic rice platns showed that this promoter could drive salt and ABA-regulated expression of the β-glucorididase (GUS) gene in transformed call and roots of PB1 seedlings. OsFKBP20, OsRHO (that encodes for specific protein that has hormology to rhodanese enzyme) and RNA binding domain of OsGR-RBP proteins were over expressed in E. coli and HIs-tagged proteins were urified for making antibodies using mice. Functional analysis for OsFKBP20 and OsGR-RBP was undertaken in heterologous systems like yeast and tobacco. OsFKBP20 appeared to be an important stress responsive gene for imparting high-level heat shock tolerance to wild type yeast cells. OsGR-RBP was noted to be important for survival of yeast and tobacco systems at supra-optimal temperatures. Also showed that OsGR-RBP is a nuclear RNA binding protein that is exported to the cytoplasm in response to up-shift in the mabient temperatures. A stress-regulated locus on the rich genome that is transcribed from both the DNA stands in opposite directions by two independent promoters was identified. This locus encoded a Zinc-finger containing protin (ABA-inclucible) in one direction. The gene encoded from the opposite strand showed little homology to rhodanese like proteins (heat stress-inclucible). Provides information on identification and isolation of serveral novels stress associated genes from rice. Salt-regulated gene expression response is a highly complex phenomenon. Salt stress was shown to elicit a multi-genic response of genes. Several known and unknown salt stress associated genes were indentified in the study. cDNA clones isolated in the study represented various functionality classes. This study further revealed that salt-stress response is differentially-regulated in Pusa Basmatil, CSR27 and Pokkali rice types. From this study, it appears that protein and RNA metabolism are the most important pathways that are differentially-affects in contrasting rice types by different abiotic stresses. This study suggested that differential constitutive and / or inducible activity of specific genes might be responsible for relative stress tolerance response in the three

contrasting cultivars employed. Classes of different protein that appear critical in conferring relative advantages in contrasting rice types as emerged from this study are shown in the form of a model.

Contents