CHAPTER 19

GENETICS

Doctoral Theses

179. CHOUBEY (Ami)
Functional Analaysis of Polyamines in Tobacco by RNAi-Mediated Down-Regulation of Polyamine Biosynthesis Genes.
Supervisor : Prof. M. V. Rajam
Th 22521

Abstract

Polyamines (PA) are ubiquitously present polycationic compounds that play a critical role in various biological processes. In the present study, three key PA biosynthesis genes, arginine decarboxylase (ADC), ornithine decarboxylase (ODC) and spermidine synthase (SPDS), have been targeted by RNA interference (RNAi) to downregulate their expression and manipulate intracellular PA titres in order to study the effects on various morphological and physiological aspects of tobacco plant development. Significant physiological and morphological alterations seen in response to ODC knockdown included poor regeneration response from leaf explants, reduced leaf size, reduced chlorophyll and carotene content, increased vulnerability to abiotic stress conditions, delayed flowering, pollen sterility, reduced seed setting and seed viability and delayed seed germination. Downregulation of ADC gene resulted in pronounced decrease in chlorophyll and carotenoid levels along with reduced reproductive potential of RNAi lines as pollen viability was found to be reduced as were the seed setting and seed viability. Decreased SPDS transcript levels did not appear to have much impact on the normal vegetative growth and development of SPDS-RNAi lines but pollen viability, seed setting and seed viability were adversely affected just like they were in case of ADC- and ODC- RNAi lines. Also, there was an early onset of senescence in ADC- and ODC- RNAi lines. Abiotic stress assays were performed for checking salt stress tolerance in PA-RNAi lines through floating leaf disc assay and whole plant salt stress assay. While ADC and ODC-RNAi lines were found to be susceptible to salt stress, SPDS lines showed better tolerance than untransformed tobacco plants as they had elevated levels of Put in free fraction. Also, for the first time, microarray analysis has been attempted to study genome-wide gene expression changes in response to lowered PA titres in an ODC knockdown line, revealing a probable list of PA-responsive genes.

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180. DHAKA (Namrata)
Genetic Dissection of Some Agronomically Important Characters with Emphasis on Seed Size in Brassica Juncea.
Supervisor : Prof. Akshay K. Pradhan
Th 22522

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1. Introduction. 2. Literature review. 3. Materials and methods. 4. Results. 5. Discussion. 6. Summary and conclusions. References and annexures.

181. GUPTA (Aditi)
Fine Mapping and Functional Analysis of Select Novel Genes Associated with Ulcerative Colitis.
Supervisor : Prof. B. K. Thelma
Th 22526

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1. Review of literature & introduction. 2. Materials & methods. 3. Fine mapping of susceptibility genes for ulcerative colitis. 4. Functional characterisation of CFB & ALC44A4, two novel GWAS hits for ulcerative colits in north Indians. 5. A cross-ethnic survey of CFB and SLC44A4 identifies allelic heterogeneity and underscores their potential role in ulcerative colitis biology. 6. Summary & perspectives. Appendices.

182. JAIWAL (Anjali)
Engineering of Insect Resistance in Transgenic Tabacco and Cotton by Host-Induced RNAi Silencing of Vital Genes of the Target Insect Pest, Helicoverpa Armigera.
Supervisor : Prof. M. V. Rajam
Th 22528

Abstract

The insect pests are considered to be a great threat for the production of crops. To control the growing population of insects, various conventional and non- conventional methods have been applied but with limited success. Although the Bt technology has revolutionized the agriculture sector by protecting many commercial crops like cotton, corn etc. against the insect pests but resulted in the development of resistance of the insects against the Bt protein. RNA interference (RNAi) is a sequence-specific gene silencing mechanism and has been recognized as a potential tool in combating insect pests. Helicoperva armigera is the most notorious pest damaging important food and non-food crops like cotton, tomato, chickpea, sorghum etc. For host induced RNAi (HI-RNAi) to be effective in controlling various insect pests, the selection of potential target gene is an important requirement. Screening of seven genes of H. armigera, viz, CHS, PTTH, PBAP, JHAMT, AP-4, EHP and HR3 by feeding gene-specific dsRNA to target insect via artificial diet was done. The silencing of these genes led to larval mortality, reduction in larval weight and target gene transcript levels. These genes are really important for the normal growth and development of the target pest and silencing of these genes provides complete abnormality to the pest. The CHS gene of H. armigera was considered for generating HaCHShp tobacco transgenics. The larvae

fed on both T_0 and T_1 transgenics showed mortality, significant reduction in larval weight, delay in pupation and pupal deformalities without showing any morphological abnormalities in the transgenics. After studying the host-induced gene silencing in tobacco, the idea was further extended to cotton, a commercial crop plant, which is a major host of this insect pest. An efficient protocol for cotton regeneration has been standardized and an attempt was made for cotton transformation with HaCHS and HaAChE RNAi constructs.

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183. KAJLA (Sachin)
Reduction of Antifeedant Compound Sinapine from the Seeds of Brassica Juncea Through Transgenic Approach.
Supervisor : Prof. Akshay K. Prdhan
Th 22407

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1. Introduction and review of literature 2. Materials and methods 3. Results 4. Discussion 5. Summary and conclusions. Bibliography and annexures.

184. MAMTA
Silencing of Chitinase and Cathepsin L Genes in Helicoverpa Armigera by Host Induced RNAi for Insect Resistance in Tobacco and Tomato.
Supervisor : Prof. M V Rajam
Th 222404

Helicoverpa armigera (Lepidoptera: Noctuidae) is a polyphagous insect pest, causing million dollars crop loss annually. Present conventional and transgenic approaches met with certain limitations such as unavailability of resistant genotypes, harmful effects on environment and development of resistance by insect pests. Hence, novel alternate strategies are required to combat insect pests in an eco-friendly manner. Host induced RNA interference (HI-RNAi) is a sequence-specific gene silencing mechanism emerged as a novel tool for control of insect pests. In the present study, we have selected chitinase (HaCHI) and cathepsin L (HaCL) genes as potential targets for HI-RNAi. Chitinase and cathepsin L are involved in various processes such as molting, metamorphosis, digestion and immune response, etc. Suppression of HaCHI /CL gene found to be detrimental for insect growth, development and survival. Through in silico analysis, partial off-target free sequences were selected from CDS and UTR regions of HaCHI and HaCL genes respectively. Then, these sequences were used to prepare hair-pin RNAi constructs. Several HaCHI/CL-RNAi tobacco and tomato lines were generated which showed transgene integration, expression and presence of siRNAs. Continuous feeding on detached leaves of HaCHI/CL-RNAi tobacco and tomato lines showed detrimental effects on larval growth, development and survival with significant reduction in target gene transcripts. Various developmental deformities occurred in pupa and adults developed from larvae fed on

HaCHI-RNAi lines. Reduction in fertility/ fecundity was also observed in adults whose larvae were fed on HaCL-RNAi lines. Thus, RNAi plants expressing the HaCHI/CL dsRNA produced efficient and potent siRNA molecules, which upon feeding enter into insect body and down-regulate the target genes, interfere with its functions and induce growth retardation, developmental deformities and mortality. These results demonstrate that the chitinase and cathepsin L are essential for insect growth and development and HI-RNAi holds a great potential for effective management of H. armigera.

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185. MANDAL (Sayanti)
Characterization of Defense Components in Arabidopsis Thaliana - Alternaria Brassicae Pathosystem.
Supervisor : Dr. Jagreet Kaur
Th 22523

Abstract

Alternaria blight caused by Alternaria brassicae is one of the major yield constrain of the oil yielding varieties of Brassica worldwide. In this work we have analyzed the interaction of Arabidopsis accessions that show differential reactions to A. brassicae infection to identify the underlying basis of genetic resistance in Arabidopsis at cytological levels. The pathogen growth and plant responses in the compatible interaction with B. juncea or A. thaliana were comparable suggesting the A. thaliana when used as a model host would provide insightful information regarding pathogenesis. Microscopic observation showed that both compatible and incompatible A. thaliana accession support the pathogen growth in the early events. Attempted infection of compatible and incompatible accessions induced similar defense responses, including callose deposition, accumulation of reactive oxygen species in epidermal and mesophyll cells during early stages of infection. The differential reactions between the compatible and incompatible interactions are apparent from 4 days post inoculation when the levels of ROS production and correspondingly cell death is extensive in the compatible interaction and significantly reduced in the resistant accessions. It suggested that A. brassicae actively triggers an extensive H_2O_2 response in the susceptible accessions whereas the resistant accessions are somehow able to restrict the spread of H_2O_2 and thus restrict the corresponding cell death. In the present study, 208 independent transgenic lines of B. juncea cv. Varuna overexpressing Arabidopsis NPR1 gene were generated, and 39 single locus integration events were further evaluated by detached leaf assay for improved resistance against A. brassicae. Two lines each for GN (GN68_5 and GN85_4) and HN (HN32_2 and HN22_8) showed significant reduction in disease ranging from 21.9% to 31.1%. Further in planta evaluation under controlled conditions and molecular analysis is required. Our preliminary analysis suggest that overexpression of AtNPR1 could improve resistance against A. brassicae.

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1. Introduction and review of literature. 2. Characterizing the arabidopsis - Alternaria brassicae interaction at the cellular and molecular level. 3. Development and analysis of Brassica juncea transgenic lines overexpressing arabidopsis NPR1. Bibliography and annexures.

186. MEHTA (Reetu)
Genetic and Functional Analysis of ent Locus in Enterococcus faecium Strain LR/6.
Supervisor : Prof. Sheela Srivastava
Th 22406

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1. Introduction 2. Materials and methods 3. Results 4 Discussion 5. Summary and Conclusions. References. Annexures.

187. PRADHAN (Shrikant)
Studies on Cisplatin Based Combination Treatment of Lung Cancer Cells in Hypoxic Stress.
Supervisor : Dr. Tapasya Srivastava
Th 22524

Abstract

Cisplatin (CDDP)-based chemotherapy is the most common form of treatment for non-small cell lung cancer (NSCLC). However, majority of patients gain resistance to CDDP during therapy and the hypoxic tumor micro-environment is a significant contributor to chemo/radio resistance. In my research, I used a novel histone deacetylase inhibitor– Scriptaid (SCP) - in combination with CDDP to address two questions: (a) Whether combination of CDDP and SCP prove effective and (b) Can the combination overcome hypoxia-induced chemo-resistance. Cytotoxicity assays were first performed on lung cancer cell lines with CDDP and SCP. Combination treatments, using sub-lethal doses of both, were found to have a synergistic effect in all three environmental conditions- normoxia, 1% hypoxia and 0.2% hypoxia. Under hypoxic conditions, cancer cells exhibited highly invasive phenotype. The combination treatment, however, significantly inhibited the proliferative and migratory properties of cancer cells. We further confirmed the mechanism of cell death to be caspase-dependent apoptosis as opposed to necrosis. While p38MAPK-dependent apoptosis was observed in normoxia, the same was not true in hypoxia. To further understand the role of hypoxia and chemo-resistance, two stable mutants were generated in A549 cell line. Both the cell lines- hypoxia inducible factor 1-α (HIF1-α) overexpressing mutant and p53-dominant negative expressing mutant- were resistant to CDDP. Doublet treatment successfully overcame CDDP-resistance in these stable cell lines, with elevated DNA damage and apoptosis compared to monotherapy. Thus the combination therapy was effective in overcoming hypoxia-induced cisplatin resistance as well as proves beneficial in over-expressing HIF1α or dominant-negative p53 mutant backgrounds.

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1. Introduction. 2. Review of literature. 3. Materials and methods. 4. Evaluating the efficacy of cisplatin and scriptaid, alone and in combination, in overcoming chemotherapeutic resistance in hypoxia. 5. Studying the effectiveness of combination treatment in a p53 mutant background and the role of hypoxia in integrin signaling in A549 cells. References and appendixes.

188. SHARMA (Manisha)
Molecular Cloning and Isolation of the Gene Responsible for Propyl glucosinolate in Brassica Juncea.
Supervisor : Prof. Akshay K Pradhan
Th 22405

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1. Introduction 2. Materials and methods 3. Results 4. Discussion 5. Summary and conclusions. References. Annexures and publication.

189. SUDHAMAN (Sumedha)
Discovery of PODXL and RIC3 as Causal Genes for Parkinson's Disease, by Whole Exome Sequencing.
Supervisor : Prof. B. K. Thelma
Th 22527

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1. Review of literarutre and introduction. 2. Material and methods. 3. VPS35 and EIF4G1 mutations are rare in Parkinson's disease among Indians. 4. Discovery of PODXL as a causal gene for autosomal recessive juvenile Parkinsonism. 5. Discovery of RIC3 as a causal gene for autosomal dominant Parkinson's disease. 6. Summary and perspectives. Appendixes.

190. UPADHYAY (Anamika)
Mechanisms of Zinc Management in Plant Growth Promoting Fluorescent Pseudomonas Strains : Psd and Pft-1.
Supervisors : Prof. M. V. Rajam, Prof. Sheela Srivastava and
Dr. Mandira Kochar
Th 22529

Abstract

Zinc is an essential micronutrient involved in the activity of more than 300 enzymes, wherein it plays, either catalytic, co-catalytic or structural roles. The present study was taken up to investigate the mechanisms involved in the management of Zn^{2+} by two strains of fluorescent Pseudomonas, Psd and PfT-1. The two strains have been earlier characterized to possess multiple plant growth promoting properties and biocontrol potential. Both the strains exhibited differential growth responses towards Zn^{2+} in the medium. Whereas strain Psd was able to tolerate a high Zn^{2+} concentration (>10mM), strain PfT-1 was found to resist a low (0.3mM) Zn^{2+} concentration in the medium. Zn^{2+}distribution studies in strain PfT-1 pointed towards an apparent role of the periplasm in Zn^{2+} acquisition. Zn^{2+}sequestration in the periplasm was studied by identification of a periplasmic Zn^{2+} binding protein, ZnuA. Over-expression of znuA

in cells growing in the presence of Zn^{2+} indicated towards the role of this protein in Zn^{2+} management by the strain. This aspect was dissected further by heterologous expression of the protein in E. coli. Majority of the Zn^{2+} accumulated by strain Psd was confined to the outer membrane, which emanated from biosorption of the metal ion by alginate exopolysaccharides secreted by the strain. The effect of Zn^{2+} biosorption on the PGP potential of strain Psd was worked out and it was found that the strain exhibited a better PGP potential in the presence of Zn^{2+} in the medium. Because of the high Zn^{2+} accumulation potential of strain Psd, the effect of metal-laden biomass of the strain was established in Zn^{2+} biofertilization of a Zn^{2+}-deficient soil. Overall, this study highlighted the different mechanisms (Biosorption and Periplasmic sequestration) of Zn^{2+} management operating in two fluorescent Pseudomonas strains.

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191. YADAV (Renu)
Deciphering the Role of Drosophila Glob1 in Development and Fertility.
Supervisor : Dr. Surajit Sarkar
Th 22525

Abstract

Globin(s) have been classically studied as oxygen binding protein(s) without any noted implication(s) in development. Drosophila possesses three globin genes (glob1, glob2, glob3) located at different cytogenetic positions. A comprehensive investigation was performed to study the developmental expression profile and functional significance of Drosophila glob1. Dynamic expression of glob1 was evident during embryogenesis and in most of the larval tissues. Reduced expression of glob1 leads to various impairments and lethality during embryogenesis and larval development. Moreover, a substantial increase in level of cellular ROS was also evident; which consequently leads to locomotor impairments and early aging in surviving adult flies. In view of significantly reduced fecundity of P-glob1/P-glob1 females, potential role of glob1 was studied in progression of oogenesis. Substantial overlap was evident between expression of Glob1 and F-actin cytoskeletal structures during development and oogenesis. However, in mutant tissues, such overlapping pattern was missing and the cytoskeletal integrity was also impaired. Reduced and/or altered distribution pattern of F-actin, DE-cadherine, Phophotyrosine, Dlg, α-spectrin, Fas-III in the follicle cells of mutant egg chambers suggest poorly formed cellular junctions and unstable adhesion. It appears that defective signalling events originating from the posterior follicle cells along with abnormal cytoskeletal remodelling leads to partial or irregular localization of Gurken and oocyte nucleus in mutant egg chambers. Further, near complete absence of “F-actin cage” in mutant egg chambers affects the cytoplasmic dumping process resulting in formation of the “dumpless” and abnormal eggs. Clonal analysis performed clearly established cell autonomous specific role of

glob1 in maintenance of F-actin based cytoskeleton during development and oogenesis. The present study demonstrates, for the first time, that in addition to its distinguished role in O2 management, globin gene(s) are equally essential to regulate various aspects of development and fertility in Drosophila.

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