CHAPTER 18

GENETICS

Doctoral Theses

01. ISRANI (Bhawna)
Development of Insect Resistant Cauliflower by RNAi-Mediated Knock-Down of Important genes of Plutella Xylostella.
Supervisor : Prof. M. V. Rajam
Th 22977

Abstract
(Not Verified)

Plutella xylostella is a serious pest of Brassicaceae, affecting the yield and quality of the produce. The pest is highly adaptive and has become increasingly difficult to control owing to emergence of resistance to a broad range of chemical insecticides and bio-pesticides. RNAi is a widely used tool for functional genomics studies and has been successfully applied against a host of agriculturally important pests. The present study makes an attempt to develop an RNAi based strategy for pest control. Three candidate genes- Ecdysone Receptor (EcR), Insect Intestinal Mucin (IIM) and Sericotropin (STP) were chosen. In vitro feeding assays were carried out to ascertain the importance of these genes for the pest. Bacterially produced dsRNA was chosen as the silencing molecule of choice. Both Helicoverpa armigera and Plutella xylostella were employed as the three target genes show a high degree of conservation across Lepidoptera. RNAi mediated knock-down of target genes led to impairment of molting process, reduced reproductive potential, insect mortality and overall stunted growth. Expression analysis revealed a significant reduction in gene expression in treated insects with respect to control. Bacterially produced dsRNAs were efficiently processed in insect gut and a likely involvement of a systemic component in spread of RNAi signal was noted. For stable and continuous expression, cauliflower transformations were carried out using Agrobacterium cultures harboring RNAi constructs. Detailed molecular analysis was done to check the event of transgene integration and expression. To evaluate the resistance conferred by these transgenics, detached leaf feeding assays were carried out with P. xylostella. Down-regulation of target transcripts was seen in larvae fed with cauliflower transgenics, and a developmental arrest was noted. The study successfully reports the application of HIGS to the area of insect pest management in cauliflower and adds value to the existing insect management practices to achieve pest control.

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02. CHANU (Soram Idiyasan)
Downregulation of dymc (a Homolog of Human c-myc Proto-Oncogene)
Supervisor : Dr. Surjit Sarkar
Th 22976

Abstract
(Not Verified)

Human neuronal tauopathies such as Alzheimer's disease (AD), Frontotemporal Dementia (FTD), etc. represent a group of neurodegenerative disorders which are characterized by abnormal hyperphosphorylation of microtubule associated protein, tau. The present study focuses on

understanding the pathogenesis of human neuronal tauopathies and also aims to identify the genetic suppressor which could be utilized as a potential drug target. Drosophila tauopathy models recapitulate several cellular and behavioral features of human tauopathies. Formation of Neurofibrillary Tangles (NFTs) in neuronal tissues has been implicated as the hallmark of the tau mediated toxicity in human and mammalian models but not found in Drosophila. Though, a recent report suggests formation of NFTs like structures only in dopaminergic neurons in Drosophila, the present study demonstrates distinct and recurrent formation of NFTs in Drosophila neuronal tissues upon expression of wild type or mutant isoforms of human tau and this appears as the key mediator of the disease pathogenesis. Further, it was found that tissue specific downregulation of dmyc (Drosophila homolog of human c-myc proto-oncogene) suppresses tau mediated morphological and functional deficits by restricting the formation of NFTs through stabilized tau phosphorylation and abated heterochromatin loss. Comprehensive investigations revealed that reduced level of dMyc in tauopathy background stabilizes the level of tau phosphorylation through PP2A-GSK-3β signaling pathways, and this appears to be the possible mechanism which suppresses the pathogenesis of neurodegenerative tauopathies in Drosophila. Therefore, the present study provides a very critical and novel insight about pathogenesis of human neuronal tauopathies in Drosophila disease models, and also demonstrates that inherent chromatin remodeling ability of myc proto-oncogenes could be exploited to limit the pathogenesis of such devastating human neurodegenerative disorders. Interestingly, recent reports on successful uses of some anti-cancer drugs against Alzheimer's and Parkinson's diseases in clinical trials and animal models strongly support the present findings and proposed possibility.

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03. JOHN (JIBIN)

Identification of Rare and Ultra-Rare Exonic Variants in Multiplex Families With Schizophrenia Using Contemporary Genomic Tools.

Supervisor : Prof: V. L. Nimgaonkar and B. K. Thelma

Th 23172

Abstract
(Not Verified)

Schizophrenia (SZ) is a common neuropsychiatric disorder, characterised by cognition and behavioural abnormalities and the prevalence is ~1%. Contribution of genetic and environmental factors in the development of the disorder is well demonstrated. Due to the complex mode of inheritance and variable phenotypes, understanding the genetic determinants of SZ remains a challenge. In this study we tested i) the association of MiRSNPs with sporadic SZ using a case-control (n=1017 cases, n=1073 controls) approach and ii) the contribution of rare or ultra-rare protein coding variants to SZ using multiplex families (n=16) and whole exome sequencing strategy. Of the 35 MiRSNPs prioritised from SZ candidate genes, significant association (p=0.001) of MiRSNP rs7430 at PPP3CC with SZ was observed and in a subset of samples tested for association of MiRSNPs with associated phenotypes, five SNPs were nominally associated with tardive dyskinesia (P=0.04–0.004) and 12 SNPs were associated with one or more of the eight cognitive domains (P=0.05–0.003) (John et al., 2016). Using whole exome sequencing (WES) approach, we identified a rare heterozygous variant (p.Cys182Phe) in TAAR1 in one family. TAAR1 is a modulator of monoaminergic and glutamatergic pathways. Screening of TAAR1 in sporadic SZ cases, identified four additional protein altering variants (MAF<0.001) in north Indian (n=475) and two in Caucasian/African-American patients (n=310) which were absent in controls (n=410) (John et al., 2017). Most notable observation from the remaining 15 families was the presence of several rare/ultra-rare heterozygous variants in dominant and even in otherwise seemingly autosomal recessive families reiterating the polygenic/oligogenic nature of SZ. Compound heterozygotes in two different genes in two different families; most of the prioritised heterozygous variants identified being unique to each family; and enrichment of glutamatergic pathway genes despite varying genetic background of the study families, are other salient findings which warrant functional studies and computational analysis.

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04. KAHALI (Madhurima)

Studies to Delineate Region(s) of Cryl Ac Protein Ressponsible for its Adverse Effects on Regeneration and Growth of Transgenic tobbacco (Nicotiana tabacum cv. Xanthi) Plants

Supervisor: Prof. Pradeep Kumar Burma

Th 23100

Abstract
(Not Verified)

Observations from our laboratory showed that cytosolic expression of the insecticidal protein Cry1Ac, led to unintended abnormalities on growth and regeneration of transgenic plants (Rawat et al 2011). It was further observed that targeting this protein to chloroplast alleviated the problem. The main objective of this work was to identify region(s) of the Cry1Ac protein responsible for the adverse effects on in vitro regeneration and growth of transgenic plants caused by its cytosolic expression. Several constructs expressing different cry genes, individual domains of cry1Ac gene and modified cry genes were developed, and their effect on tobacco regeneration was studied. We observed many interesting results which ultimately indicated that domain III of the Cry1Ac protein, is responsible for the adverse effects. Though the metal-binding sites seem to have a major role to play in the overall detrimental effects caused by Cry1Ac, other regions in the protein could also add on the effect, as even after mutating the sites, we could never achieve the kind of regeneration we observe with our control gene, gus (β-glucuronidase). The domain III of Cry1Ac is a CBM-like domain (carbohydrate binding module), which is a present in many sugar-active enzymes. This domain functions by binding to sugar substrates. The binding could cause disturbance in the internal balance between sugars and sugar-metabolizing enzymes. The other possibility could be perturbation of Ca^{2+} or Mg^{2+} regulated pathways, due to binding of Cry1Ac protein to these ions. Although literature points to the importance of domain III in receptor binding, the effects of mutation of the metal-binding sites have not been explored. Another observation made in the earlier work was the silencing of the cry1Ac gene, while the neighboring marker gene (nptII) was still active. An attempt has been made to understand this phenomenon using available transgenic tobacco lines.

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05. MUKHI (Nitika)

Structural and Functional Characterizationol Arabidopsis Thaliana Nonsymbiotic Globins

Supervisor : Dr. Jagreet Kaur

Th 22978

Abstract
(Verified)

Genomes of all land plants are endowed with multiple nonsymbiotic hemoglobins (nsHbs), viz. class I nsHbs, class II nsHbs and plant truncated hemoglobins (ptrHbs). Precise physiological role of these novel plant hemoglobins have not been elucidated, though their involvement in plant physiology and various stress responses mainly hypoxia is being speculated. Although, the role of plant nsHbs as potential NO dioxygenases is well recognized, mechanistic insights into the same is lacking. The rationale behind the execution of the current work was to carry out coordinated structure-function studies

in the model plant Arabidopsis thaliana with an aim to understand its physiological significance. The first part of the thesis focuses on detailed structural imprints of Arabidopsis nonsymbiotic globins (AHbs). Crystal structure of two classes of Arabidopsis nonsymbiotic globins - class I (AHb1) and ptrHbs (AHb3) was successfully solved. Each class displays some unique structural signatures suggesting their role in NO dioxygenase reaction. The second part elaborates the role of Arabidopsis nonsymbiotic hemoglobins in pathogen defense using Sclerotinia sclerotiorum. Globin overexpression and knockdown were generated and analysed for their response to S. sclerotiorum. Attributed to high susceptibility of AHbs RNAi/TDNA lines, these globins seems to play a crucial role in plant defense against S. sclerotiorum. Furthermore, additional insights into peroxidase-like activity of these globins are highlighted. Subcellular localization of AHbs indicates their preferential accumulation in guard-cells and nuclei, suggestive of yet unrecognized facet of their physiological presence. Moreover, using information available from publically microarray datasets, novel insights into diverse physiological roles of these multi-facet proteins are presented. Taken together, the current investigation provides a holistic view towards understanding the physiological significance of this conserved family of proteins. However, there are still many missing links in the chain towards understanding their physiological importance and significance of structural features of nsHbs in vivo.

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06. PAREEK (Manish)

Functional Validation of Map Kinase Genes in Fusarium Oxysporum and Targeting of fmk1 Gene by RNA interference for Engineering Fungal Resistannce in Tomato.

Supervisor : Prof: M. V. Rajam

Th 23173

Abstract
(Not Verified)

Fusarium oxysporum is a ubiquitous soil-borne fungal pathogen, which causes a major loss in productivity of several crops. The control measures mainly include the use of harmful fungicides. RNA interference (RNAi) has emerged as a novel and alternative strategy to functionally analyze the uncharacterized genes and to develop fungal resistance in plants by host-induced gene silencing. In present work, fungal RNAi transformants were developed with the MAP kinase genes to study their role in fungal pathogenesis. Further, tomato RNAi lines of Fusarium MAP kinase (Fmk1) gene have been developed for resistance against Fusarium wilt. In present work, the hairpin RNAi constructs of three MAP kinase genes namely, Fmk1, Hog1 and Pbs2 were prepared to knock-down their expression in F. oxysporum. Agrobacterium-mediated transformation was done to generate RNAi transformants of F. oxysporum, and the positive colonies were selected on hygromycin containing selection medium and presence of transgenes was confirmed by PCR analysis. qPCR analysis revealed that the Fmk1, Hog1 and Pbs2 transformants showed reduced target transcript levels. Fmk1 RNAi transformants exhibited the loss of surface hydrophobicity, loss of invasive growth on tomato fruits and reduced virulence on tomato seedlings as compared to untransformed fungi. Hog1 and Pbs2 RNAi fungal transformants showed reduced invasive growth, but colony surface hydrophobicity was unchanged. Hog1 RNAi transformant showed hypo-virulence on tomato seedlings when compared to untransformed fungi. Further, host-induced gene silencing approach has been used to develop fungal resistant tomato plants. Putative transgenic tomato plants expressing dsRNA of Fmk1 gene were generated by Agrobacterium-mediated transformation. The transgenic status of tomato was confirmed by PCR and Southern analysis. Fmk1 T_1 tomato transgenic lines were screened using marker-specific PCR, and analyzed for enhance fungal resistance against Fusarium wilt. RNAi tomato lines Fmk1-2 and Fmk1-8 showed increased resistance as compared to untransformed seedlings.

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07. PRASAD (Pankaj)
Studies on the Genome Biology of Glioma in the Hypoxic Microenvironment
Supervisor : Dr. Tapasya Srivastava
Th 22979

Abstract
(Verified)

Activation of pluripotency regulatory circuit is an important event in solid tumor progression and the hypoxic microenvironment is known to enhance the stemness feature of some cells. This distinct population of cancer stem cells (CSCs)/ tumor initiating cells (TICs) exist in a niche and augment invasion, metastasis and drug resistance. Previously, studies have reported global hypomethylation and site-specific aberrant methylation in gliomas along with other epigenetic modifications as important contributors to genomic instability during glioma progression. Here, we have demonstrat- ed the role of hypoxia-mediated epigenetic modifications in regulating ex- pression of core pluripotency factors, OCT4 and NANOG, in glioma cells. We observe hypoxia-mediated induction of demethylases, TET1 and 3, but not TET2 in our cell-line model. Immunoprecipitation studies reveal active demethylation and direct binding of TET1 and 3 at the Oct4 and Nanog regulatory regions. Tet1 and 3 silencing assays further confirmed induction of the pluripotency pathway involving Oct4, Nanog and Stat3, by these paralogues, although with varying degrees. Knockdown of Tet1 and Tet3 inhibited the formation of neurospheres in hypoxic conditions. We ob- served independent roles of TET1 and TET3 in differentially regulating pluripotency and differentiation associated genes in hypoxia. Overall this study demonstrates an active demethylation in hypoxia by TET1 and 3 as a mechanism of Oct4 and Nanog overexpression thus contributing to the formation of CSCs in gliomas.

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08. TETORYA (MEENAKSHI)
Development of Tomato Plants for Resistance Against Fusarium Wilt by host-induced RNA Interference of Vital Genes of Fusarium Oxysporum.
Supervisor : Prof. M. V. Rajam
Th 23174

Abstract
(Not Verified)

Abstract of the Ph. D. Thesis Entitled Development of Tomato Plants for Resistance Against Fusarium Wilt by Host-induced RNA Interference of Vital Genes of Fusarium oxysporum Submitted by MEENAKSHI TETORYA Department of Genetics, University of Delhi South Campus, New Delhi - 110021 Fusarium wilt, caused by Fusarium oxysporum f.sp. lycopersici (Fol) is among the most destructive diseases of tomato, and therefore the development of sustainable and environment friendly methods to improve resistance against Fusarium wilt is crucial. In this study, it was investigated on whether hairpin RNA (hpRNA) mediated expression of small interfering RNAs (siRNAs) targeted against vital fungal genes (Chorismate synthase, Peroxisomal biogenesis factor and β -1, 3-Glucanosyltransferase) could achieve effective resistance against Fol. Potential partial gene fragments of all the three genes were assembled individually in the form of hpRNAs in suitable silencing vectors (hpFoCSRNAi, hpFoPEX6RNAi, hpFoGAS1RNAi) and transferred into F. oxysporum as well as tomato by glass-bead and Agrobacterium-mediated genetic transformation, respectively. It was

observed that the mRNA levels in all three target genes in all fungal transformants showed variable degree of reduction, and also the silencing of target genes had a clear negative effect on the pathogenicity of Fol. Similarly, all transgenic lines of tomato, expressing dsRNA specific to target fungal genes displayed enhanced resistance against Fol with delayed disease symptom development. The rate of survival of tomato transgenic lines after fungal infection were better as compared to untransformed tomato plants. The present study demonstrates an efficient role of RNA interference (RNAi)-based approach in developing resistance against Fol in tomato.

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