CHAPTER 17

GENETICS

Doctoral Theses

01. AAKANSHA
Genetic Analysis of Heterosis for Seed Yield and Yield Component Traits in Brassica Juncea.
Supervisors: Prof. Jagreet Kaur and Prof. Akshay K. Pradhan
Th 26146

Abstract

The exploitation of heterosis through hybrid breeding is one of the major breeding objectives for productivity increase in crop plants. This research analyses the genetic basis of heterosis in *Brassica juncea* by using a doubled haploid (DH) mapping population derived from F1 between two heterotic inbred parents, one belonging to the Indian and the other belonging to the east European gene pool, and their two corresponding sets of backcross hybrids. An Illumina Infinium Brassica 90K SNP array-based genetic map was used to identify yield influencing quantitative trait loci (QTL) related to plant architecture, flowering, and silique- and seed-related traits using five different data sets from multiple trials, allowing the estimation of additive and dominance effects, as well as digenic epistatic interactions. In total, 695 additive QTL were detected for the 14 traits in the three trials using five data sets, with overdominance observed to be the predominant type of effect in determining the expression of heterotic QTL. The results indicated that the design in the present study was efficient for identifying common QTL across multiple trials and populations, which constitute a valuable resource for marker-assisted selection and further research. In addition, a total of 637 epistatic loci were identified, and it was concluded that epistasis among loci without detectable main effects plays an important role in controlling heterosis in yield of *B. juncea*.

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02. BHATTACHARYYA (Upasana)
Genetic Landscape of Schizophrenia – Witnessing Change with New Leads from Evolutionary and Structural Variants.
Supervisors: Prof. T. Srivastava and Prof. B. K. Thelma
Th 26147

Abstract

Schizophrenia, a highly heritable disorder, has persisted throughout human history despite reduced fecundity and higher mortality rates in patients, posing itself as a "Darwinian paradox". Its genetic underpinnings however remain elusive despite long-drawn global research efforts. Recent large-scale transethnic GWASs report the association of a substantial number of loci from non-coding genomic regions, presumably regulatory, but severely understudied, further obscuring genotype-phenotype correlations. Quad-SNPs with the potential to alter DNA secondary structure, and thereby gene expression, despite being implicated in neurological conditions, are unexplored in schizophrenia.

Enrichment of schizophrenia risk loci near evolutionarily relevant genomic regions is documented but their role in increasing disease susceptibility is unclear. Human accelerated regions (HARs) are one such important human specific regulatory genomic regions, whose target genes and role in schizophrenia etiology warrant investigation. Using a north Indian schizophrenia case-control study cohort and an integrated analysis of publicly available PGC3-SZ-GWAS and functional genomics data, we demonstrated that **a)** Quad-SNPs could alter gene expression, probably via modulating dynamic G-quadruplex DNA secondary structure formation and thereby contribute to i) schizophrenia symptoms, i.e, positive and negative (Bhattacharyya *et al*.,2021; Schizophrenia Research), ii) neurocognition (manuscript submitted) and iii) Tardive dyskinesia (Ms submitted) in patients; **b)** HARs and genes regulated by them may create a large species-specific regulatory network partially accounting for human-specific phenotypes (manuscript submitted); **c)** variants within HARs could interfere with such regulation thus contributing to i) risk of schizophrenia (Bhattacharyya et al., 2021; Schizophrenia Bulletin) and ii) neurocognition in cases and controls (manuscript submitted); and **d)** schizophrenia-associated variants at evolutionarily relevant genomic regions, probably alter expression of genes involved in immune response and/or neurodevelopment and interfere in the cross-talk between these two systems. Our finding provides new insights into i) the poorly understood regulatory regions; ii) long-debated immune system involvement and iii) human evolution, in schizophrenia etiology.

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03. CHAUHAN (Sambhavana)

Characterization of Fusarium Oxysporum f. sp. Lycopersici Specific Fasciclin-Like Proteins (FoFLPs) in Fungal Virulence and Development of Transgenic Tomato Resistant to Fusarium Wilt.

Supervisors: Prof. Jagreet Kaur and Prof. M. V. Rajam

Th 26148

Abstract

Fusarium oxysporum f. sp. *lycopersici* (*Fol)* is responsible for instigating Fusarium wilt disease in tomato and causes a significant yield loss. The pathogen invades and colonizes within the host via roots, resulting in transpirational blockage leading to severe wilting symptoms. Fasciclin-like proteins (FLPs) were shown to be involved in cell-to-cell adhesion and virulence. Therefore, we have investigated the role of putative *Fol*-specific FLPs (*FoFLPs*) in virulence using RNA interference (RNAi). The *in vitro* studies showed the down-regulation of endogenously expressed *FoFLP1, FoFLP3, FoFLP4 and FoFLP5* genes in their respective *Fol* transformants expressing dsRNAs specific to *FoFLP* genes. As a result, the fungal transformants showed significant reduction in spore count and spore germination frequency, thereby suggesting role of *FoFLPs* in conidiation. The fruit invasion and plant infection assays using *FoFLP* fungal transformants showed the late onset of disease with significant reduction in disease symptoms in the infected plants. Although the spores of fungal transformants entered host plant by penetrating root cortex, the infected plants showed minimum mycelial colonization as a result of siRNA-mediated targeting of *FoFLPs*. This confirmed the role of *FoFLPs* in conidiation and virulence of *Fol.* Further, we have also performed Host Induced Gene Silencing (HIGS) of *FoFLPs* to investigate the disease severity in the infected tomato RNAi

transgenic lines expressing dsRNAs specific to *FLP* genes. Consequently, we have observed a significant reduction in *FoFLP1*, *FoFLP4* and *FoFLP5* transcript levels in *Fol* upon infection of their respective tomato RNAi transgenic lines. Post-infection, the tomato RNAi transgenic lines expressing the intended siRNA molecules in T1 generation exhibited the enhanced Fusarium wilt resistance with a delay in disease onset. Overall, these results have supported the cross-kingdom transfer of either dsRNAs or siRNAs from tomato to *Fol* and targeting its *FLP* genes in a sequence-specific manner. Hence, these results also demonstrated HIGS as a potential approach in rendering resistance to *Fol* in tomato plants.

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04. JONITA CHONGTHAM

Investigating the Role of Protein Cornichon Homolog 1 (CNIH1) in the Hypoxic Microenvironment of Solid Tumours.

Supervisor: Prof. Tapasya Srivastava

Th 26143

Abstract

Stomach cancer and Glioblastoma are among the most aggressive cancer types; both with a very poor survival rate evading standard treatment procedures. To aggravate the complications, solid tumours are associated with tumour hypoxia, a condition of low oxygen condition that overwhelms all cancer management efforts. The upregulation of gene expression has been widely studied in hypoxia but repressive signals in hypoxia have only been recently identified. In our study, we have analysed the expression of Protein cornichon homolog 1 (CNIH1) in many solid tumour models and elucidated interesting gene regulation mechanisms responsible for the repression of CNIH1 that can regulate the expression of the mitogenic TGFα. We have correlated the expression of CNIH1 with disease prognosis and deduced the anti-proliferative, anti-migratory and pro-apoptotic property of the gene by using cellular and molecular assays. We have also shown that CNIH1 inhibits the stemness characteristics of the hypoxic cells and sensitizes them to respond to cisplatin treatment which is one of the standard chemotherapeutic drugs. Through transcriptomic analysis, the pathway(s) that are affected by CNIH1 overexpression, and can explain the role of CNIH1 have been investigated. *In vivo* studies also confirmed the anti-tumour potential of CNIH1. Therefore, the ability of CNIH1 to affect Notch signalling along with EGFR signalling will open new avenues for gene therapy for the treatment of solid tumours. As an additional project, we have also analysed the effect of rs9387478, an SNP between two very important genes ROS1 and DCBLD1 and the chances of getting lung cancer in the North Indian population. The relationship between smoking and lung cancer has been studied widely and 29% of the Indian population (+15 years) are known to be exposed to tobacco smoke. Therefore, in this pilot project, we studied the effect of the genetics of rs9387478 and smoking on lung cancer in North India.

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05. MATHUR (Shikha)

Identification of Candidate Genes Influencing Seed Size/Weight using Comparative Transcriptomics and Genetic Analysis of Plant Architectural Traits in Brassica Juncea.

Supervisors: Prof. Jagreet Kaur and Prof. Akshay K. Pradhan

Th 26144

Abstract

An understanding of the genetic basis of seed size/weight is of great interest in the improvement of seed yield and quality in mustard. We performed a global transcriptome analysis at the initial stages of seed development in a small-seeded (EH-2), and a large-seeded (PJ) *Brassica juncea* line. Pairwise comparisons at each developmental stage identified 5,974 differentially expressed genes (DEGs) between the two lines, including 954 transcription factors. Two modules were found to be significantly correlated with an increase in seed size using weighted gene coexpression network analysis. The DEG and coexpression datasets were integrated with eight thousand seed weight quantitative trait locus/loci (QTL) mapped earlier in the EPJ (EH-2 × PJ) F1-derived doubled haploid (DH) population, which identified forty candidate genes for seed size. Plant architecture is of high agronomic importance as it influences the suitability of a plant for cultivation, its yield, and harvesting efficiency. The DH population resulting from a cross between an Indian oleiferous line, Varuna and Chinese stem type mustard, Tumida (TUV population) showed significant variability for several plant architectural traits including, stem strength, stem diameter, plant height, branch initiation height, number of primary branches (*Pbr*), and days to flowering (*Df*). A multi-environment QTL analysis of these traits in the TUV population identified twenty Stable QTL. The study revealed that Tumida can contribute some significant QTL to improve these traits in the Indian gene pool lines. We found a QTL cluster on LG A10 harboring Stable QTL for seven architectural traits, including antagonistic overlapping major QTL for *Df* and *Pbr*. Conditional QTL analysis for *Pbr* identified the QTL for improvement of *Pbr* without negative correlated effects on *Df*. Finally, the candidate genes underlying the Stable QTL intervals were identified for each trait. These results would find application in breeding for improved ideotypes of *B. juncea.*

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06. PRAGATI
Elucidating the Role of Insulin Signaling and Its Downstream Candidates in Mitigation of Human Neuronal Tauopathies in Drosophila Disease Models.
Supervisor: Prof. Surajit Sarkar
Th 26519

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07. THIYAM LAKSHMI DEVI
Studies on the wee Genes of Dictyostelium Discoideum.
Supervisor: Dr. Aruna Naorem
Th 26149

Abstract

Wee1 is a kinase identified in fission yeast *Schizosaccharomyces pombe* as a regulator of cell size by inhibiting Cdk1 function at G1 to M phase entry, preventing premature entry into mitosis. Loss of Wee1 function produces smaller than normal daughter cells and overexpression of Wee1 causes cell cycle arrest. Besides its function in cell cycle regulation, it is reported to function in chromatin remodeling in humans. *Dictyostelium discoideum*, the model organism used in the current study has a vegetative (unicellular) and a developmental (multicellular) phase, which is composed of only two cell types. As the phase of cell cycle phase is one of the factors for cell fate determination and that multicellularity is by aggregation and not division, it prompted us to investigate the function of Wee in *Dictyostelium*. In this study, we observed that *D. discoideum* has three copies of the *wee* genes, i.e. *weeA*, *weeB*, and *weeC*. Analysis of the protein sequence revealed that the catalytic domain of these proteins carried all the important signatures found in known Wee proteins. Interestingly, the WeeA catalytic domain was observed to be split into two parts, which is probably unreported. Further, WeeA was found to have diverged from the WeeB and WeeC both in sequence as well as in expression profiles. Spatial analysis of *wee* gene expression showed that the three genes are expressed in a prestalk specific manner. Based on the analysis of knockout strains of *weeA* gene, it was observed that there was a reduction in generation time (~1-2 hours) during vegetative growth. It was also seen that the *weeA* knockout strain developed faster than the wild-type in the initial stages. The current work provides leads that can be used to understand the role of Wee proteins in processes other than as a sentinel of cell cycle.

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08. VERMA (Yash)
Regulation of Mitochondrial Protein Synthesis by a Member of the YihA Family of GTPases, MRX8, in Saccharomyces Cerevisiae.
Supervisor: Dr. Kaustuv Datta
Th 26145

Abstract

Saccharomyces cerevisiae shows an interesting metabolic plasticity during its life cycle. When grown aerobically in glucose, yeast cells predominantly use fermentation for ATP production. Upon exhaustion of glucose yeast cells enters the diauxic growth phase which involves a switch in ATP synthesis predominantly via oxidative phosphorylation (OXPHOS). This metabolic switch during a yeast growth cycle requires extensive cellular reprogramming where specific pathways get upregulated in order to support increased requirement for OXPHOS subunits. Complex IV of the mitochondrial OXPHOS machinery couple reduction of oxygen to water. Cox1 forms the catalytic-core of complex IV is conserved in all aerobic organisms sequenced. How Cox1 synthesis is regulated in response to environmental changes has remained unexplored. We have shown that novel protein Mrx8 helps in cellular adaptation to utilize non-fermentable carbon source by regulating the optimal rate of Cox1 translation initiation and elongation at reduced temperatures (16°C). *MRX8* is a YihA class of GTPase with orthologues in bacteria, yeast, and vertebrates including humans but none in invertebrates. We have shown that nucleotide binding is dispensable for its association with the mitoribosomes. Our results suggest that it functions *in vivo* to communicate state of nucleotide availability within the mitochondria to the mitochondrial translation system. *MRX8* function is conserved between yeast and humans as human Mrx8 (hMrx8) or *GTPBP8* complements loss of cellular respiration in Δ*mrx8* yeast cells and localizes to the mitochondria in mammalian cells. In yeast besides Mrx8, at least two additional translational regulators, Pet309 and Mss51 are required for Cox1 synthesis which are not conserved in humans. Overexpression of neither Pet309 nor Mss51 rescues the glycerol growth defect observed in Δ*mrx8* cells and Mss51 association with the ribosome was not dependent on Mrx8

presence. Thus, we believe *MRX8* represents an essential regulator of Cox1 expression conserved in yeast and humans.

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