CHAPTER 20

GENETICS

Doctoral Theses

01. BHAYANA (Latika)
Mapping White Rust Resistance in Brassica Juncea Line Tumida and B. Nigra.
Supervisor :Prof. A.K. Pradhan
Th 24484

Abstract
(Not Verified)

White rust disease, caused by oomycete Albugo candida, is one of the most significant diseases of crop Brassicas. Almost all the varieties of Brassica juncea (mustard), cultivated in India are highly susceptible to the pathogen. A total of 96 F1DH lines derived from Tumida (resistant) × Varuna (susceptible) cross were used for genetic analysis and mapping of the resistance trait. Initially, a framework map was developed using 535 – IP, genic SSR, and genic SNP markers. A high marker density map was developed by adding 8,303 GBS based SNP markers and was used for mapping the disease resistance trait. Disease assays were carried out using A. candida isolate AcB1. The resistance trait was mapped using qualitative and quantitative approaches. Qualitative mapping suggested the presence of single dominant gene involved with resistance. A single major locus AcB1-A6.1, controlling 80% of phenotypic variance mapped to an interval of 63.0 – 70.8 cM on the genetic map using quantitative approach. Genome assemblies of Tumida and Varuna were used to identify the most probable candidate gene in the mapped interval. A single CNL type R gene, BjuA046215, was identified in Tumida. The corresponding allele in susceptible parent Varuna had a five bases duplication in the exon3 leading to the formation of a stop codon in the ORF – resulting in a truncated protein with a deletion in the LRR region. The second aim of the research work was to map the resistance in B. nigra germplasm. An F1DH population derived from Sangam DH4 (susceptible) × 2782 (resistant) cross was developed using microspore culture; 92 lines of the DH population were randomly picked for construction of a linkage map. Phenotyping could not be accomplished as DH lines could not be maintained; developed linkage map could be used for assembly of B genome of B. juncea.

Contents

02. KAUR (Prabhjot)
Relevance of Tumor Hypoxia in Signalling Networks of Metastasis and Cancer Progression in Cancer Cell Line Models.
Supervisor :Dr. Tapasya Srivastava
Th 24481

Abstract
(Verified)

Metastasis and relapse of cancers are the leading cause of mortality in cancer patients. Studies have shown that cells in tumor mass attain metastatic potential and chemo-resistance, often leading to cancer stem cell like properties. Heterogeneous tumor microenvironment contributes to genomic instability leading to selection of aggressive cells. Hypoxia is one such important microenvironmental factor in which metabolic adaptations and altered gene expression regulates pathways of invasion and metastasis, and others. In this work, we have studied mechanistic and therapeutic aspects of hypoxia mediated alterations in actin signalling network. We have investigated hypoxia mediated regulation of stemness and metastasis in leukaemia. Wiskott Aldrich Syndrome protein (WASP), an actin nucleation promoting factor, was observed to be downregulated in hypoxia. Downregulation of WASP was observed to regulate stemness and TH1 lineage commitment via changing dynamics of actin. Our molecular studies suggest that hypoxia primed naïve CD4+ cells towards TH1 lineage. An attempt to study Rho GTPases as a therapeutic target was made by employing inhibitors of three well known Rho GTPases; RhoA, Rac1, and Cdc42 in glioma. We have employed cellular assays to check their efficacy w.r.t. cytotoxicity, migration potential, adhesion capacity, and effect on the actin cytoskeleton in normoxia and hypoxia. Our results have been exciting and inhibitor of Cdc42 and RhoA appeared to work effectively in hypoxia. However, inhibitor of Rac1 was observed to be ineffective in hypoxia. We have also developed a gene switch to study the disparate regulation of HIF isoforms. HIF-1 and HIF-2 were cloned into two commercially available inducible expression system, Tet ExpressTM inducible expression system and Gene SwitchTM mammalian expression system, to be able to induce the expression of HIF-1 and HIF-2, and study the cellular and molecular aspects of the cell behaviour in different stress conditions.

Contents

03 NISHA
Studies on the Role of Drosophila Globin1 in Neurogenesis and Modulation of Human Tauopathies.
Supervisor :Dr. Surajit Sarkar
Th 24934

Abstract
(Not Verified)

Globins are evolutionarily conserved metallo-proteins, characterized by the presence of distinct globin fold. Although, Globins have been classically associated with oxygen managements, however, recent studies have linked globins to other biological and physiological processes as well, such as regulation of reactive oxygen species (ROS), maintenance of cytoskeleton integrity during development, female gametogenesis etc. The first part of the present study was undertaken to investigate the functional relevance of glob1 in development of Central Nervous System (CNS). Expression studies revealed robust accumulation of Glob1 protein in developing neuronal tissues such as Ventral Nerve Cord (VNC) of embryos and outer proliferation region (OPC) of the optic lobes of larval brain. Ubiquitous or CNS-specific downregulation of glob1 leads to poorly developed nervous system and

various neuronal impairments. The in-depth investigation establishes a novel role of glob1 in development and functioning of nervous system in Drosophila. The second aspect of the present study deals with the identification of a novel genetic modifier which could dominantly suppress the pathogenesis of human neuronal tauopathies in Drosophila disease models. Tauopathies, such as Alzheimer's and Parkinson's diseases etc. are neurodegenerative disorders characterized by the accumulation of hyperphosphorylated tau containing Neurofibrillary Tangles (NFTs) in neuronal cells. A genetic screening and subsequent molecular characterization establish that tissue-specific downregulation of glob1 suppresses tau mediated morphological and functional deficits by restricting the NFT formation via restoring the tau hyperphosphorylation and heterochromatin loss. Comprehensive investigations revealed that reduced level of glob1 stabilizes the status of tau phosphorylation by GSK-3β/p-Akt and p-JNK signalling pathways, and also regulates the ROS level by activating Nrf2/Keap1 pathway. The present study establishes glob1 as a novel suppressor of the neuronal tauopathies, which may help in developing effective treatment strategies against devastating human neurodegenerative disorders.

Contents

04. SACHDEV (Mahak)
Host Plant Induced RNA Silencing of Argininosuccinate Lyase Gene of Fusarium Oxysporum For Resistance Against Fusarium Wilt in Tomato.
Supervisor :Prof. M.V. Rajan
Th 24483

Contents

05. SINGH (Priyansha)
Genetic Dissection of Some Important Agronomical Traits in Brassica Juncea.
Supervisor :Prof. A.K. Pradhan
Th 24482

Abstract
(Not Verified)

Brassica juncea is a major oilseed crop of India. Many QTL for important agronomic traits have been identified in F1DH mapping populations derived from crosses between lines belonging to the two divergent gene pools – Indian and east European. In this study an attempt was made to use an F1DH population between two closely related Indian lines – Varuna and Pusa Jaikisan for mapping traits like seed size. It was not possible to develop a linkage map with earlier used genic markers. However, GBS based SNP markers were abundant and allowed the development of a linkage map with 981 markers. Phenotyping of the VPJ population in three environments showed significant level of transgressive segregation for most of the yield traits and a number of major QTL could be mapped. A major QTL cluster was identified on LG A05 (contributed by Varuna) that contained trait

enhancing alleles for six important agronomic traits. Another F1DH population was developed from a cross between recently sequenced Chinese vegetable type line Tumida and Varuna (TUV). Both genic markers and GBS markers were used to develop a linkage map with 5977 markers. Besides the identification of many yield influencing QTL, three novel traits – leaf serration, basal branching, stem strength – available only in this population were mapped. The locus with serrated leaf trait contributed by Tumida was mapped on LG A10 in an interval of 2.7 cM corresponding to 81.88 kb genomic region of B. juncea. Of the 15 genes present in the physical interval – gene BjuA040045 – an orthologue of LMI1 gene of Arabidopsis was marked as the most likely gene encoding the serrated leaf trait. Major QTL could be identified for stem strength and basal branching which will be useful for breeding lodging resistance in pure lines and hybrids in B. juncea.

Contents

06. YADAV (Bal Govind)
Genetic Dissection of Yield and Oil Parameters in Brassica Juncea.
Supervisor :Prof. Akshay K. Pradhan
Th 24485

Abstract
(Not Verified)

The twofold objectives of genetic improvement of seed oil content in mustard (Brassica juncea) are to enhance the nutritional and functional properties with a concomitant overall increase in the oil percentage. Earlier studies have indicated the existence of pleiotropy between erucic acid and oil content, indicating a direct influence of the erucic acid loci on oil content. The present study was therefore undertaken for genetic dissection of seed oil content in B. juncea using eight bi-parental doubled haploid (DH) mapping populations that showed wide phenotypic variation for both oil content and erucic acid. The mapping populations were divided into two sets – one set consisted of DH lines segregating for erucic acid (SE populations) and the other set consisted of zero erucic acid DH lines (ZE populations). We have drawn detailed QTL landscapes of oil content loci describing a pronounced diversity in the allelic effects of lines belonging to the two distinct gene pools of B. juncea. Meta-analysis in SE populations revealed a total of nine consensus QTL. Nine QTL were identified as common to both SE and ZE populations along with six “novel” QTL that were unique to the ZE populations. Also, QTL analysis for the yield associated traits was undertaken in a ZE (EJ8Z) population developed in this study. A total of 76 QTL were identified for the ten yield associated traits with prominent clustering on linkage groups A2 and B8. Co-localization of these traits with oil content QTL led to the identification of three free lying QTL which can be used for improving oil content in ZE populations. Significant QTL x environment interactions and epistasis among 16 pairs of QTL were also observed for the yield associated traits in different environments. These results have been discussed and highlight the scope available for increasing oil content in mustard.

Contents