CHAPTER 19

GENETICS

Doctoral Theses

01. CHOUDHURY (Aparajita)
Host Rnai- Mediated Silencing of Chitin Synthase Gene of Maruca Vitrata for Insect Resistance in Cowpea (Vigna Unguiculata).
Supervisors: Prof. Aruna Naorem and Prof. M.V. Rajam
Th 26662

Abstract

Cowpea is a climate-resilient crop having high protein content and it has the ability to fix the atmospheric nitrogen. These properties make cowpea a crop of interest in the changing climatic conditions. However, the yield is severely affected by insect pest infestation every year. Maruca vitrata is one of the destructive pests of cowpea and many other legumes. The conventional methods of pest control such as the use of insecticides or natural enemies are not very effective and some are detrimental to environment and human health. Genetic engineering technologies have exhibited promising results in insect pest control in the past two decades. Bt technology and RNA interference (RNAi) technology have revolutionized the agricultural realm. Our study aimed to generate transgenic cowpea plants using RNAi technology, targeting chitin synthase (CHS) gene of Maruca vitrata to confer insect resistance. CHS is a vital gene of insects and plays a pivotal role in the development and metamorphosis. Initially, we have optimized the efficient in vitro plant regeneration and Agrobacterium-mediated genetic transformation system using cotyledonary node explants. A regeneration frequency greater than 90% and transformation efficiency up to 4% was achieved. For gene expression analysis by qRT-PCR, selection of suitable reference gene is of utmost importance in order to avoid the misinterpretation of the results. As there were no previous reports on the suitable reference genes that can be used for qRT-PCR experiments in M. vitrata, we have identified RP49 and RPL13 as the suitable reference genes across developmental stages. RP49 and RPL24 were found to be the stable reference genes across diets and GAPDH and RPL24 were the stable genes across male and female moths of M. vitrata. As rearing and procurement of M. vitrata larvae were the limiting factors in our study, we checked the efficacy of CHS of M. vitrata on the growth and development of a related insect pest, Spodoptera litura. MvCHS dsRNA coated artificial diet was fed continuously to the S. litura larvae and results showed a significant delay in the growth and development. Reduction in the transcript level of CHS attributed to the results of developmental delay and mortality in S. litura. Furthermore, MvCHS-RNAi cowpea transgenic plants were generated by Agrobacterium-mediated transformation. Transgenic nature of the plants was validated by PCR analysis. Further molecular characterization possibly opens up promising insect pest control strategies.

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1. Introduction 2. Review of literature 3. Materials and methods 4. Results amd Discussion 5. Summary and conclusions 6. References and annexure. List of publication.

02. PANT (Pratibha)
Evaluation of RNA Interference (RNA)- Based Strategies Targeting Sclerotinia Sclerotiorum.
Supervisor: Prof. Jagreet Kaur
Th 26660

Abstract

Sclerotinia sclerotiorum (Lib) de Bary, a broad host range aggressive Ascomycete causes Sclerotinia Stem Rot (SSR) in over 600 dicots. Control is hampered by the ineffectivity of fungicides, the rapid development of resistance to fungicides, the long-term viability of sclerotia in the soil and a lack of robust natural resistance against the pathogen. RNA Interference (RNAi)-based strategies have emerged as a viable alternative for controlling S. sclerotiorum. In this study, two different RNAi-based strategies: i. Host-Induced Gene Silencing (HIGS) and ii. Spray-Induced Gene Silencing (SIGS) were evaluated for their potential in controlling SSR in Nicotiana benthamiana, Arabidopsis thaliana and Brassica juncea. Genes involved in cell wall synthesis, (SsChs2/3, SS1G_03857 & SS1G_08265 respectively), cell membrane integrity (SsCyp51, SS1G_04805), OA synthesis (SsOah1 & SsPac1, SS1G_08218 & SS1G_07355, respectively), development (SsSmk1, SS1G_11866), reactive oxygen species (ROS) detoxification (SsSod1, SS1G_00699) and dsRNA biogenesis (SsDcl1/2, SS1G_13747 & SS1G_10369 respectively) were selected based on their involvement in diverse developmental and pathogenesis pathways. A transient dsRNA expression-based pipeline in N. benthamiana using Agrobacterium and the Tobacco Rattle Virus (TRV) viral vectors was proposed to identify S. sclerotiorum pathogenesis genes. While HIGS-based strategies failed to restrict the developed disease lesions despite in planta dsRNA expression, SIGS of selected candidate genes provided significant protection during the initial stages of infection. Topical dsRNA application delayed disease initiation and significantly reduced lesion development. However, the protection conferred by SIGS was concentration and host-dependent. Further, silencing could not be maintained long-term and thus requires enhanced dsRNA persistence. This study also implicates SsCyp51 and SsChs2/3 as novel candidates for S. sclerotiorum development and pathogenesis and identifies them as viable anti-fungal targets. SIGS of the selected candidate genes also altered fungal hyphal morphology, indicating the utility of SIGS as a robust methodology for preliminary functional genomics.

Contents

1. Introduction and Review of literature 2. Materials and methods 3. Results and Discussion. Conclusions and Prospects. List of publication.

03. SANDHU (Gurvisha)
Lead Molecule Development Directed by Synovial Biology for rheumatoid Arthritis Using Fragment Quantum Mechanical Approach.
Supervisors: Prof. P.K Burma and B.K. Thelma
Th 27033

Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disease in ~1% worldwide population with a complex and unknown etiology, leading to progressive articular damage of small joints and disability. Early diagnosis and treatment with disease-modifying antirheumatic drugs (like methotrexate), and inclusion of targeted therapies (like TNF inhibitors) have improved prognosis/remission. However, inadequate response to available therapies witnessed in a subset of patients warrants discovery of new therapeutics. This study aimed to i) prioritize and identify potential drug targets based on molecular insights from synovial biology; ii) assemble simulation pipeline for efficient lead molecule evaluation; iii) design and optimize lead molecules using CADD approach with assessment of their binding affinities using ii); and iv) develop a cheminformatics tool for predicting off-target profiles of lead candidates. Based on available integrated-omics leads and functional evidence of a regulatory role in RA, MAP3K8 having unique kinase domain architecture, was selected for lead molecule development. Combining protein fragmentation into tractable sub-systems with continuum solvation method, a quantum mechanical simulation pipeline was assembled for incorporation of electronic polarization into protein-inhibitor binding dynamics. This enabled reliable evaluation of binding affinities, thereby ranking inhibitors having close nanomolar range activity for the same target. With rational design of novel molecular series using de novo fragment growth method, 13 lead molecule candidates from the generated SAR were synthesized based on pipeline evaluation and synthesizability criteria. A kinase class-specific cheminformatics tool was developed based on similarity ensemble approach for off-target prediction of small molecule inhibitors. This validated tool predicted 6/13 synthesized compounds as specific MAP3K8 binders, reiterating the suitability of MAP3K8 as a drug target for selective lead design. With the ongoing cell line-based evaluation of their inhibitory potential, in vitro and in vivo pharmacokinetic/pharmacodynamic assessments are warranted to establish this series as a novel class of MAP3K8 inhibitors.

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04. TANDON (Shweta)
Deciphering the Mechanistic in- Depths of the Insulin Signalling Ppthway and Exploring the therapeutic Interventions to Mitigate Human Polyglutamine Disorders in Drosophila.
Supervisor: Prof. Surajit Sarkar
Th 26661

Abstract

Human polyglutamine/poly(Q) disorders, such as Huntington's disease (HD) and Spinocerebellar ataxias (SCA) 1, 2, 3, etc., are characterised by an abnormal expansion of CAG repeats in the causative gene. The insulin/insulin-like signalling

(IIS) pathway mediated by the insulin receptor (InR) has been found to be a common modifier of different poly(Q) diseases. To harness the InR/IIS pathway in developing effective treatments, it is imperative that its exact mechanism of rescue be deciphered. Therefore, the present study was designed with aims to investigate its mechanistic in-depths that confers rescue against poly(Q) toxicity, to identify insulin pathway specific compatible drugs/molecules to restrict poly(Q) aetiology, and to screen and characterize a suitable genetic modifier(s) which could be utilized along with InR for development of combinatorial strategy against human poly(Q) disorders in Drosophila disease models. The first part of the study identified that the growth promoting S6K/4E-BP pathway is the key contributor of major aspects of InR mediated rescue against poly(Q) pathogenesis. Further, insulin resistance was identified as an important pathogenic aspect of poly(Q) aetiology in Drosophila disease models. Based on current and previous findings, the subsequent study identifies an anti-diabetic drug glipizide, and glutamine as effective molecules which restricts pathogenesis of poly(Q) disorders by stimulating insulin signaling and its growth promoting sub-pathway, respectively. Subsequent and the last part of the study have identified that Myc functions downstream of InR and their concurrent upregulation delivers additive and significant rescue against poly(Q) toxicity. Therefore, InR and Myc can be utilized for development of novel combinatorial approach to comprehensively mitigate the pathogenesis of human neurodegenerative poly(Q) disorders.

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05. TIWARI (Ruby)
RNAi-Mediated Silencing of DISI and PARP Genes for Engineering Drought and Heat Stress Tolerance in Soybean (Glycine max).
Supervisors: Prof. Jagreet Kaur, Prof. M.V. Rajam and Dr. Ajay Kumar Singh
Th 27034

Abstract

An efficient plant regeneration system is a prerequisite for soybean crop improvement through genetic engineering. In this study, an efficient and improved plant regeneration protocol for a popular soybean genotype JS-335 was achieved by using the combination of plant hormones benzyladenine purine (BAP), indole-3-butyric acid (IBA) and kinetin (Kn) at a concentration of 3.0 mg/L, 0.2 mg/L and 0.5 mg/L, respectively, using the whole cotyledonary nodes as explants. Furthermore, there is a necessity to improve the efficiency of transformation for gene function studies and to develop soybean transgenics with new traits. There have been decades of research in the field, nonetheless, due to the recalcitrant nature of soybean, the transformation efficiency continues to remain low. This study used the Agrobacterium-mediated cotyledonary node transformation system and the bar gene as the plant selectable marker coupled with glufosinate as a selective agent. Explants were cultured on Gamborg's B5 medium supplemented with 1.67 mg/L BAP and glufosinate at 3 mg/L for selection of transformants. The

transformation status of the primary transformants (T0) and T1 generation progeny was confirmed by various molecular analyses. Using the cotyledonary node explants and the bar selection system, we have successfully developed an improved transformation system than the earlier reported work on Indian genotypes of soybean, and this established protocol could provide a useful tool for genetic improvement and functional genomics studies in soybean. In addition, the established transformation protocol was used to raise soybean GmDIS1- and GmPARPs-RNAi T0 transformants and T1 transgenic lines, which were confirmed by molecular analyses. Further, the soybean GmDIS1- and GmPARPs-RNAi T1 transgenic lines were evaluated for their tolerance under drought and heat stress conditions. The morphological and various physiological traits showed that the downregulation of GmDIS1 and GmPARP genes in T1 soybean transgenic lines led an enhanced tolerance to drought and heat stress conditions. This suggests that GmDIS1 and GmPARP genes are efficient in improving drought and heat tolerance in soybean, and may also serve as important targets for the improvement of soybean cultivars and other crops.

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06. YADAV (Mavneesh)
Characterization of RIC-3, a Chaperone Protein of Neuronal Receptors, with Emerging Implications for Brain Disorder (S) Using CRISPR/Cas9 Editing in a Cellular Model.
Supervisors: Prof. Tapasya Srivastava and Prof. B.K. Thelma
Th 27035

Abstract

Over 20 disease causal genes have been discovered in Parkinson's disease (PD) to date. RIC3 (OMIM#610509) was identified as a novel PD gene in the laboratory but is poorly studied in humans. Non-availability of brain tissue and an ideal model system have generally hindered gene/variant characterization for insights into disease biology. Generation of patient/healthy individual derived induced pluripotent stem cell (hiPSC) based cellular model of disease combined with CRISPR/Cas9 gene editing are powerful new strategies. This study aimed at RIC3 characterization using three approaches namely Cre/lox-P; piggyBac and ssDNA along with CRISPR/Cas9 gene editing for generating isogenic RIC3 knock-out and variant(s) hiPSC lines. Plasmid co-transfections and stable/transient antibiotic selection, yielded multiple lines with transgene insertion and/or indel(s). Of these, a 'del' line with homozygous 25bp deletion in exon 2 of RIC3, possibly generated by microhomology-mediated end joining double strand break repair mechanism (JB#22-00247, in press) was used for gene characterization in astrocytes and dopaminergic (DA) neurons. Altered RIC3 transcript ratio due to deletion induced splicing and an unexpected gain of α7 nicotinic acetylcholine receptor (nAChR) expression was observed in 'del' cell-types. Transcriptome analysis in astrocytes showed higher expression of neurotransmitter/G-protein coupled receptor mediated by cAMP and calcium/calmodulin-dependent kinase signaling. Tunicamycin induced ER stress in both 'del' cell-types manifesting reduced expression of stress markers CHOP, phospho-PERK and lowered XBP1 splicing in western blot and qPCR, validated by proteome-based pathway analysis (GLIA#00266-2022, under revision), but not in DA neurons suggests cell-type specific neuroprotective role of

RIC3. Findings indicate i) a complex RNA regulatory mechanism via exonic deletion induced splicing; and ii) RIC-3 as a disordered protein having gain of function effect on α7nAChRs with implications for peptidomimetic-based drug discovery in nicotine related brain disorders. Furthermore, cellular rescue mechanism through deletion induced exon skipping possibly opens up ASO based therapies for tauopathies.

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