

CHAPTER 6

BOTANY

Doctoral Theses

01. DHARMENDRA KUMAR
Ecological Restoration and Sustainable Management of River Yamuna through Phycoremediation.
Supervisor: Prof. Dinabandhu Sahoo
Th 27867

Abstract

Rivers are essential to human life. However, pollution from domestic, industrial and agricultural sources is adversely impacting rivers, especially the Yamuna River, which faces significant water quality issues. This study investigates pollution levels in the River Yamuna and explores the potential of selected algae in bioremediation through their physiological and metabolic adaptations. Water samples were collected from Haryana, Uttar Pradesh, and Delhi. Water quality assessments were conducted from Winter 2020 to Monsoon 2022, analyzing parameters such as pH, Salinity, Conductivity, Total Dissolved Solids (TDS), Total Hardness, Ammonia, Heavy Metals, Nitrate, Phosphate, Chloride, Sulphate and Silicon. Significant findings include Biochemical Oxygen Demand (BOD) levels ranging from 0.2 ± 0.03 mg/L to 27.6 ± 1.18 mg/L (WHO Limit <5 mg/L), Chemical oxygen demand (COD) from 56 ± 3.47 mg/L to 368 ± 24.6 mg/L (WHO Limit 10 mg/L) and Ammonia from 0.84 ± 0.17 ppm to 21.23 ± 0.51 ppm (WHO Limit 1.5 mg/L) while Nitrate ranging from 23.6 ± 1.08 to 2542.26 ± 13.12 mg/L (WHO Limit 50 mg/L). Heavy metal analysis revealed alarming level of Chromium up to 132.064 ± 2.01 $\mu\text{g/L}$ (50 $\mu\text{g/L}$) and Lead concentrations reaching up to 93.03 ± 2.20 $\mu\text{g/L}$ (10 $\mu\text{g/L}$). This study distinctly identified high pollution levels in the Yamuna River across Haryana, Delhi and Uttar Pradesh with Delhi contributing the highest pollution. The present study demonstrated the phycoremediation potential of algal species such as *Hydrodictyon* sp., *Scenedesmus* sp., *Klebsormidium* sp., *Nephrocytium* sp., *Oocystis* sp., *Monoraphidium* sp. and *Chlorococcum* sp. in removing multiple contaminants. Further, the study highlighted the sustainable utilization of algal biomass for possible commercial utilization.

Contents

1. Introduction 2. Review of Literature 3. Materials and Methods 4. Observations
5. Discussion, Summary, Conclusion and References.

02. DHYANI (Anshul)
Biosystematic Studies on the Mosses of North-Western Himalaya.
Supervisor: Prof. Prem Lal Uniyal
Th 27357

Abstract

Being one of the biodiversity hotspots, Himalaya are known to possess diverse range of forest types. These forest types provide suitable habitat for variety of life forms. One such life form comprises mosses (Bryophyta), which play crucial role in ecosystem functioning and dynamics. These mosses are known to occupy different habitats. Adapted to extreme conditions such as cold temperatures,

mosses exhibit a diverse range of fatty acids, including very long-chain polyunsaturated fatty acids with notable ecological and therapeutic value. Therefore, the present study was conducted across four different forest types in the North-western Himalaya, documented 174 moss taxa, with 49 new records for the study site and three taxa new to the country. Highest moss species richness was found in moist temperate deciduous forest (n=87). The results also showed a bi-modal curve for optimum elevational peak and a highly significant effect of elevation on moss species richness ($\chi^2 = 67.44$, $p < 0.001$). Precipitation and temperature were also found as critical parameters affecting moss species richness patterns. Among all the substrata, phorophytes in moist temperate deciduous forests and rocks in other forest types were the most preferred substrates, with *Rhododendron arboreum*, being the most favoured phorophyte. Additionally, lectotypes were designated for 14 moss names. The compound analyses identified five taxa showing the presence of all the tested compounds and a wide range of fatty acids, including essential ones were as reported in Gas Chromatography-Mass Spectrometry analysis. Eicosapentaenoic acid and arachidonic acid, commonly found in mosses but absent in higher plants, were also detected. Pharmacokinetic analyses and molecular docking suggest therapeutic potential for some fatty acids and reveal new binding sites. These findings are helpful for improving forest conservation and management policies and highlight the potential of mosses in biotechnology for industrial and commercial applications.

Contents

1. General Introduction 2. To study the floristic diversity of mosses across elevational gradients in selected forest types of North-western Himalaya 3. To provide the nomenclatural stability to a few moss names by Lectotypification 4. Assessment of substrata and phorophyte specificity of moss species in different forest types across elevational gradients 5. Qualitative estimation of compounds and quantitative estimation of fatty acid composition in selected moss species 6. To study the drug-like properties of fatty acids (FAs) from different moss species using bioinformatic approaches. Discussion, Summary and conclusions, Literature cited and Publications and Conferences.

03. GOYAL (Shipra)
Characterization of CcKIP1 Promoter in *Arabidopsis Thaliana*, and in-vitro Plant Regeneration through Somatic Embryogenesis in Cell Suspensions of *Cenchrus Ciliaris* L.
 Supervisors: Prof. Vishnu Bhat and Dr. Vishvas M. Kulkarni
Th 27358

Abstract

Identification and characterization of novel promoters help in targeting genetic manipulation of specific plant parts. Towards this, earlier CcKIP1 (*Cenchrus ciliaris* Kinase interacting protein 1) gene and its promoter were isolated from apomictic *Cenchrus ciliaris*. While the CcKIP1 gene showed expression in the mature ovule, its function is still to be unraveled. The 2477bp 5' upstream sequence of the CcKIP1 gene containing many regulatory elements was earlier cloned in a binary vector, pORE R1 vector with GUS as a reporter. *Arabidopsis thaliana* genotype Col-0 was transformed with pORER1::CcKIP1 promoter::GUS vector and the activity of GUS reporter was assayed in T1, T2 and T3 progenies. The activity of the CcKIP1 promoter was observed in shoot apical meristem (SAM), root apical meristem (RAM) of 2 to 5 days seedlings, rosette leaf, cauline leaf, flower (sepals, ovary wall, ovules (at different developmental stages), anther's connective tissue and pollen grains. To characterize CcKIP1 and Nucellin gene promoters in *Cenchrus ciliaris*, embryogenic calli were bombarded with pCAMBIA1391Z: CcKIP1 and pZP211: HvNuc constructs. GUS expression was observed in the bombarded calli but the transgenic plants could not be generated. Further, towards finetuning

the in vitro plant regeneration system in *Cenchrus ciliaris*, cell suspension cultures were initiated. Factors responsible for inducing somatic embryogenesis and plant regeneration were optimized. The inoculum size to initiate cell suspensions was optimized as 10g l⁻¹. Ultrastructural stages of somatic embryo development were recorded using a scanned electron microscope. Maximum shoots (16±3.78) could be regenerated on MS medium containing 1mg l⁻¹ 2,4-D, 1 mg l⁻¹ 6-BAP, 300 mg l⁻¹ casein hydrolysate, 400 mg l⁻¹ glutamine, and 400 mg l⁻¹ proline. Maximum roots (29±1.45) were observed on basal half-strength MS medium. The genetic fidelity test of the regenerated plants showed genetic similarity with their mother plant

Contents

1 General introduction 2. Characterization of CcKIP1 gene promoter in *Arabidopsis thaliana* 3. Assessment of CcKIP1 and Nucellin gene promoters activity in transgenic calli of *Cenchrus ciliaris* 4. Establishment of in-vitro plant regeneration using cell suspension cultures in *Cenchrus ciliaris* 5. Summary, References and List of Publications.

04. MANISHA

Investigations on Sexual Dimorphism and Gender Variation in *Hippophae rhamnoides L.* through Morphological, Molecular and Cytogenetic Approaches.

Supervisor: Professor Rajesh Tandon

Th 27360

Abstract

Hippophae rhamnoides, a subdioecious species inhabiting the western Himalayan region, is increasingly recognized for its nutritional, medicinal and ecological value. *Seabuckthorn* has three sexual morphs: male, female and polygamomonoecious (PGM) which makes it a suitable model system to study evolution and maintenance of dioecy. The present study was aimed to explore the extent of sexual dimorphism, novel gender-specific markers, genetic variability and cytological characteristics. Investigation on sexual dimorphism, resource allocation (RA) and sex-ratio in the species, revealed pronounced sexual dimorphism in both vegetative and reproductive traits. However, RA pattern was more pronounced in the reproductive traits, with higher allocation to males during flowering and to females during fruiting. Despite the 1:1 primary sex ratio, the prevalence of female-biased secondary sex-ratio in moist habitats, indicated influence of ecological conditions in proportion of male and female flowering individuals. PGMs representing the transitory state, exhibited overlapping vegetative traits with both genders but varied in reproductive traits. The limited genomic differences between the genders, was also reflected by lack of PGM-specific markers, despite testing thirty-two primer combinations, which emphasizes their overlapping genetic background with the other two genders. High genetic similarity between genders was also revealed across populations of Leh-Ladakh and Himachal Pradesh at a previously identified gender-linked region (HRMSSR). Genetic variations were also analyzed using SNPs among the populations across the regions investigated, identifying six genetic clusters corresponding to their geographical locations. The cytological investigation revealed predominance of metacentric chromosomes. The morphology of chromosomes as well as localization of HRMSSR on chromosomes via FISH, revealed females with homomorphic (two large XX) chromosomes while males with heteromorphic (one large X and small Y) chromosomes, indicating its advanced evolutionary stage. Overall, this study provides comprehensive insights into the evolutionary dynamics of *Seabuckthorn*, emphasizing the need for continued research to understand its complex genetic structure.

Contents

1. Introduction 2. Study of sexual dimorphism, resource allocation pattern and sex-ratio 3. Development of SCAR marker to distinguish Polygamomonoecious (PGMs) from males and females 4. Testing of previously characterized HRMSSR locus from Leh-Ladakh in natural populations of Himachal Pradesh 5. Study of genetic variation between the populations of the two geographical regions through SNPs (HP) 6. Cytological investigation in the species. Summary and Conclusions, Literature Cited, Illustrations and Appendices.

05. MEHRA (Aashima)

Interactions of *Millettia Pinnata* (L.) Panigrahi With Two Novel Gracillariid Leaf Miners: Ecological and Molecular Perspectives in Herbivory.

Supervisor: Prof. Sudeshna Mazumdar-Leighton

Th 27361

Abstract

Recurrent, extensive infestations by two foliar microlepidoptera that produce circular blotch and haphazard blotch mines respectively, were observed on *M. pinnata* (family Fabaceae), a tree legume used for urban plantations and as biofuel crop. Molecular provenances were obtained using *rbcL*, *mtCOI*, 28S rRNA, actin and Elongation factor 1- α genes (Accessions# MH020177, OR140583-OR140592, MN149606-MN149611), with the insects being first reports. Evaluation of leaf phenology and damage caused by the two insects over two years at the study site in Delhi indicated increasing infestation on mature leaves from April-November. The two insects differed in extents of leaf area infested; regions of canopy infested; time of infestation and voltinism. The leaf miners also differed in genetic diversity estimated using *mtCOI* haplotypes. Shared haplotypes with geographically and temporally distinct populations indicated wide distribution and gene flow. Monitoring of blotches in the field and lab revealed life cycle of the leaf miners, as well as diversity of predators/parasitoids associated with these insects. Furthermore, identification of proteases in the gracillariid leaf miners using *in vitro* assays and their susceptibility to foliar plant protease inhibitors (PPIs) indicated different levels of trypsin and chymotrypsin inhibitor activities in various leaf types of *M. pinnata*. Leaf miner-infested leaves showed significantly higher PPI levels, consistent with locally-induced plant defense response. The study also identified cDNAs encoding serine proteases (MG993089, OK490353, OR134255-OR134257) and cysteine proteases (OR123589-OR123592) from both leaf miners. *De novo* RNA-sequencing revealed differentially expressed genes from healthy (un-infested) versus leaf miner-infested leaves belonging to Jasmonic acid and Terpene synthase pathways. These results were validated by quantitative RT-PCR in leaves with different histories of herbivory by the two miners. This study sheds light on interactions between two novel leaf miners and *M. pinnata*, providing valuable gene resources for future applications.

Contents

1. Literature review and introduction 2. Identification, life cycles, parasitism and relation of two gracillariid leaf miners with phenology of *Millettia pinnata* (L.) Panigrahi host trees 3. Detection of serine proteases in gracillariid leaf miners and foliar protease inhibitors in *Millettia pinnata* (L.) Panigrahi host trees 4. *De novo* transcriptome analysis of *Millettia pinnata* (L.) Panigrahi leaves infested by a gracillariid leaf-miner, identification of plant defense response genes and genes encoding larval proteases 5. List of Conclusions. References, Annexures and NCBI GenBank accessions.

06. ORAON (Praveen Kumar)
Consequences of Autopolyploidy Studied Through Morphological and Transcriptomic Analysis in *Vigna mungo* (L.) Hepper and *Vigna radiata* (L.) R. Wilczek.

Supervisor: Prof. Shailendra Goel
Th 27362

Abstract

This thesis presents a comprehensive study involving the development and characterization of synthetic autotetraploids in two species of genus *Vigna* viz. *V. mungo* and *V. radiata*, both of which are economically important pulse crops. The study also generated genomic resources for the diploid accession used for generating polyploids in the study. The first objective of the present study was to successfully develop synthetic autotetraploids in both species through colchicine treatment. The resulting autotetraploid lines were carefully evaluated using flow cytometry and validated through mitotic chromosome count. The second objective involved a detailed morphophysiological profiling of the generated autotetraploids and their diploid progenitors. Extensive phenotypic assessments were conducted, examining traits such as plant height, leaf size, flower morphology, cell density, and seed size etc. Physiological parameters, including photosynthetic rates, were also investigated. The third and fourth objectives focused on whole genome sequencing of *Vigna mungo* and *Vigna radiata* aiming to generate genomic resources which will serve as a foundation for subsequent analysis. For both the species, high-quality reference genomes were assembled, annotated, and made available as valuable resources for the research community. Building upon the genomic data, the fifth objective explored transcriptomic changes between the diploid and synthetic autotetraploid lines. We have tested various ways of normalization of the transcriptome analysis to identify the differences between diploid and autotetraploid of two species. We could identify a number of transcripts upregulated through novel ways of normalization. The findings from this thesis contribute to our understanding of autopolyploidization. The generated autotetraploid lines, diploid genomic resources, and insights into the transcriptional landscape also provide a foundation for future crop improvement efforts targeting these important grain legumes.

Contents

1. General Introduction 2. Morpho-physiological characterization of autotetraploids and their diploid progenitors 3. Whole genome sequencing of *Vigna mungo* 4. Whole genome sequencing of *Vigna radiata* 5. Transcriptome analysis to identify differentially expressed genes and transposable elements between diploids and autotetraploids. Appendix and List of Publications and Conferences.

07. PRIYANKA
Adaptation Along the Altitudinal Gradient as Deciphered by Genetic Diversity, Transcriptome, and Reproductive Studies in *Primula denticulata* Sm.

Supervisors: Prof. Shailendra Goel and Prof. Rajesh Tandon
Th 27363

Abstract

Altitudinal gradients have gained prominence in studying plant adaptations since they provide a continuum of environmental conditions over relatively short distances. This provides an opportunity to study the interplay between genetic variation, phenological traits, and ecological conditions and helps in elucidating the adaptive strategies adopted by plants in response to changing environmental conditions. In this study, 13 populations of *Primula denticulata* Sm., the most prevalent Primrose species from the Indian Himalayan region, present between an

altitudinal range of 2286m to 3612m were investigated. The study used in-house generated microsatellite and SNP markers using Illumina platform. 31584 microsatellite (SSR) loci were identified, out of which 64 polymorphic loci were used in the present study. Similarly, GBS (Genotyping by sequencing) was used to identify 6172 SNP (Single Nucleotide Polymorphism) markers. Both SSR and SNP markers were used to study population structure and the clustering pattern along the altitude. The two sets of markers generated comparable results, although SNP markers provided a better resolution of population structure. Analysis of this data has shown a strong altitudinal correlation with the genetic structure. The genetic clusters following the altitudinal gradient were found to correlate with the phenological shifts. Also, the decrease in the percentage of polymorphic loci at higher elevations suggests a decline in genetic diversity, pointing to reduced pollinator activity at this altitude. To further investigate, RNA sequencing was performed along the altitude and DEGs were identified. Annotation of DEGs revealed their association with genes involved in abiotic stress management. The Ecological Niche Modelling (ENM) of the species indicates an upward shift in response to changing climatic conditions. This upward shift corroborates with the genetic and phenological findings, suggesting an adaptation process driven by changing environmental conditions along the altitudinal gradient.

Contents

1. General Introduction 2. Reproductive behavior of *Primula denticulata* along the altitudinal gradient 3. Development of genome-wide microsatellites from *Primula denticulata* and their cross-transferability in different species of the genus 4. Genetic diversity and population structure analysis in *Primula denticulata*, using SSR and SNP markers and digital expression analysis of genes expressed along the altitudinal gradient 5. Assessment of current and future habitat distribution pattern of *Primula denticulata* in the Indian Himalayan Region. Appendices and List of Publications and Conferences.

08. SHARMA (Prachi)
A Sustainable Approach for Enhancing Morpho-Physiological, Biochemical and Anti-Cancerous Alkaloids in *Catharanthus roseus* and *Withania Somnifera* Plants Growing in Salinity Stress Conditions Using Plant Growth Promoting Bacteria and Arbuscular Mycorrhizal Fungi.
 Supervisor: Dr. Ratul Baishya
Th 27364

Abstract

Salinity stress is a major abiotic factor affecting agricultural productivity by damaging several vital mechanisms in plants, viz., photosynthesis, osmotic balance, and enzymatic activity. *Catharanthus roseus* and *Withania somnifera* (L.) Dunal are known for their medicinal value in Ayurveda. These plants are in high demand in the pharmaceutical industry due to their anti-carcinogenic alkaloids viz. vinblastine and withaferin A. However, soil salinity negatively reduces the economic yield of these plants under commercial cultivation in India. We need an environmentally friendly approach to reduce salt stress and boost economic yield, as conventional approaches are unsustainable for long-term productivity. We hypothesize that plant growth-promoting bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF) will be a cleaner and more sustainable tool to combat salinity stress and enhance alkaloid production in both the plants. The study investigates the efficacy of nitrogen-fixing bacteria (*Azotobacter chroococcum*), phosphate-solubilizing bacteria (*Bacillus amyloliquefaciens*), potassium-solubilizing bacteria (*Enterobacter esburiae*) and mycorrhizal consortium under saline (4.5 dS m⁻¹) and non-saline treatments on the growth and production of secondary metabolites. When compared to the control treatment, the salinity

significantly reduced the growth attributes while increasing malondialdehyde content (MDA), electrolytic leakage (EL), and sodium-potassium ratio in both the studied plants. Inoculation with AMF augmented the growth parameters while decreasing the MDA content, EL, and sodium-to-potassium ratio. AMF inoculation in salinity stress conditions resulted in a significant increase in alkaloid content in both plants. The results indicate that arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPB) alleviate salinity stress by decreasing lipid peroxidation, electrolyte leakage, regulating sodium-to-potassium ratio, and enhancing secondary metabolite production in both the medicinal plants. The study suggests that microbial inoculation is a sustainable and eco-friendly approach to enhance the growth and yield of secondary metabolites in *C. roseus* and *W. somnifera* in salt-affected regions.

Contents

1. General Introduction 2. Assessment of PGPB and AMF consortium on plant morphological attributes under salinity stress conditions 3. Photosynthetic efficacy under salinity stress conditions in few medicinal plants mitigated through PGPB and AMF 4. Effect of PGPB and AMF application on the osmolytes, lipid peroxidation, nutrients under salinity stress conditions 5. Evaluation of PGPB and AMF application on enzymatic antioxidants, non-enzymatic antioxidants and alkaloid content under salinity stress 6. Conclusions and Future Prospective. Literature Cited and List of Publications and Research Conferences.

09. SINGH (Sorokhaibam Sachin)
Elevational Influence on the Genetic Diversity, Eco-Physiology and Reproductive Biology of *Rhododendron arboreum* sm. In Western Himalaya.
 Supervisor: Prof. Rajesh Tandon
Th 27366

Abstract

Rhododendron arboreum Sm., the most widely distributed *Rhododendron* in India, ranges from 1200-4000 m in the Himalaya, holding significant ecological, economic, and cultural value. Over the past few years, climate change has advanced its blooming periods, prompting research into population responses, assessment of genetic diversity, and adaptation. Despite its importance, gaps remain in understanding eco-physiology and reproductive biology. Among the 140 SSR primer pairs designed, only 55 were polymorphic. Analysis with these polymorphic SSRs revealed low genetic diversity at extreme elevations and higher diversity at mid-elevations. Four genetic clusters were identified, one with three sub-clusters, influenced by geographic proximity. NJ, PCoA, and AMOVA confirmed significant genetic differentiation among populations and clusters, and substantial variation within them. Although leaf dimensions were similar across elevations, thickness increased with elevation due to anatomical changes. Higher elevations had thicker cuticles, higher fresh/dry weights, lower moisture, and reduced SLA, with increased leaf density and mass per area. Stomatal traits varied with elevation. Chlorophyll content was highest at low elevations, while carotenoids peaked at high elevations. Soil N content significantly affected leaf N and pigment contents, lowest at mid-elevation. Soil moisture increased with elevation, but pH decreased, with no trend in EC or salinity. Red and pink morphs of *R. arboreum* occupy lower and higher elevations, respectively, significantly differing in floral traits and pollinators—red morphs are bird-pollinated, while pink ones are entomophilous. Both morphs are self-compatible, but selfing yields lower number of fruits. Outcrossing rate is higher among the red morphs ($t_m=0.82$) than that in the pink ($t_m=0.76$), indicating a mixed-mating shift in the latter. These differences are linked to floral traits and pollinator interactions. This study gives new insights into the species' adaptive responses to elevations and highlights the importance of understanding these variations for conservation efforts amid climate change.

Contents

1. General Introduction 2. Variation in eco-physiological parameters of *Rhododendron arboreum* along the elevational gradient 3. Reproductive ecology of two elevational morphs of *Rhododendron arboreum*. Summary and Conclusions, Literature Cited, Illustrations and Publication.

10. YADAV (Himani)
Biosystematic Studies on Mosses and Properties of Moss Associated Substrata of Some Himalayan Sites.
 Supervisor: Prof. P.L. Uniyal
Th 27868

Abstract

Bryophytes constitute a major part of Himalayan flora. The north-western Himalaya comprises of enormous bryophyte diversity and composition. Bryodiversity in some of many Himalayan pockets is not known and their relationship with various types of substrata is completely unexplored. Bryophytes have a longer elevation gradient range and broader distribution, but their influence on a particular landscape is poorly understood. They are known to possess a variety of compounds which can be exploited for medicinal, cosmetic and bio-pesticidal uses. Diversity analysis of Manila forest range revealed 9 new records to Western Himalayas and 8 taxa are endemic to India. Diversity analysis of Lachung-Yumthang Valley revealed 4 new records to Northern Sikkim. This study demonstrated significant regulation of soil moisture, total carbon, and enzyme activities in the presence of mosses. They create a microenvironment for faster decomposition, thereby promoting nutrient availability, enabling further growth of mosses and vascular plants. This also suggests that soils with well-developed moss cover can be used as indicators of better soil quality and soil functioning and can be further used to improve soil quality. According to the study majority of microbiological traits exhibited a seasonal pattern, with highest activity occurring in soil with bryophytic substrate during spring and without bryophytic substrate during winters. The present study highlights the importance of mosses in influencing the biochemical properties of soil in the Kumaon Himalaya. The findings of this study also suggested that of *Hydrogonium consanguineum*, *Brachythecium kamounense* and *Hepetioneuron toccoae* are rich sources of secondary metabolites and revealed high levels of valuable compounds (Hexadecanoic acid, Octadecadiynoic acid, Palmitic acid, Stigmasterol) which can be further used in cosmetic, medicinal, pharmaceutical, forensic, environmental and food industry. Key words: Bryophytes, *Brachythecium kamounense*, *Hydrogonium consanguineum*, *Hepetioneuron toccoae*, Lachung-Yumthang Valley, Manila Forest range.

Contents

1. Assessment of species diversity of mosses in Manila ForestRange in Uttarakhand and Lachung-Yumthang Valley of North Sikkim 2. Evaluation of the physical, chemical and biological properties of bryophyte associated substrata 3. Study of the chemistry of selected moss species from Manila Forest Range. References and Publications and Conferences.

11. YADAV (Manisha)
Molecular and Biochemical Analysis of Defencerelated Proteins of Glycine Max (L.) Merr.During Spodoptera Litura Infestation.
 Supervisor: Dr. Archana Singh
Th 27368

Abstract

Glycine max L. (Soybean) is an oil-seed cash crop and protein-rich legume with great therapeutic and nutritional potential, cultivated worldwide for human food and raw material for industries. The polyphagous herbivore, *Spodoptera litura* (common cutworm) can cause significant loss (25-30 %) to soybean production; making it mandatory to understand the underlying defence mechanisms. To decipher the induced plant defence orchestration of soybean in response to *S. litura* infestation, a comparative proteomics of *S. litura* infested and mechanical wounded soybean (Pusa-9712) was performed. Functional studies identified that most of differentially abundant proteins were involved in secondary metabolite biosynthesis, calcium signalling, biotic and abiotic stresses, cell wall organisation, amino acid metabolism, transcription and translational activities. CYP450s are versatile enzymes, which thwart plants against insect pests by ubiquitous biosynthesis of phytohormones, antioxidants, and secondary metabolites, utilizing them as feeding deterrents and direct toxins. A comprehensive analysis of biotic stress-responsive CYPs from *G. max* ascertained GmCYP78A5like, GmCYP76C1, and GmCYP78A5 as potential candidates involved in *G. max* -*S. litura* interaction. Additionally, a sudden change in cytosolic Ca^{2+} concentration was observed during *S. litura*-feeding indicating their role in plant defence signalling. Ca^{2+} -signalling is a crucial event during plant-herbivore interaction, which is sensed by Ca^{2+} -sensors, and the received message is transduced to downstream target proteins leading to appropriate defence response. The study on the characterization of GmCMLs provided insights into their roles in Ca^{2+} signalling as Ca^{2+} sensors proteins during *S. litura*-*G. max* interaction. Differential gene expression patterns, structural and biochemical characterization of GmCML77, recognized GmCML77 as a Ca^{2+} sensor protein, which regulate defence-responsive Ca^{2+} signalling on *S. litura* infestation.

Contents

1. Introduction and review of literature
2. Investigation of *G. max* shoot proteome on *S. litura*- infestation
3. Study of *G. max* shoot proteome upon mechanical wounding
4. Comprehensive and evolutionary investigation of *S. litura*-inducible Cytochrome P450 monooxygenase gene family in *G. max*
5. Comprehensive and evolutionary investigation of CML proteins of *G. max* to deduce their role in Ca^{2+} signalling and plant defence against herbivory
6. Structural and biochemical characterisation of *S. litura* inducible GmCML77
7. References, Appendices and List of workshops and Conference presentations.