# CHAPTER 6

# BOTANY

## Doctoral Theses

01. ADIT (Arjun)
**Orchids of Tripura: Taxonomic Diversity and Dynamics of Mutualistic Interactions.**
Supervisors: Dr. Rajesh Tandon and Dr. Monika Koul
Th 25715

*Abstract*

Orchidaceae, the largest family of angiosperms in India. is insufficiently investigated for its reproductive and survival strategies. The present work is based on an assessment of taxonomic diversity and mutualistic interaction patterns in some of the selected species of orchids in Tripura. Tripura is one of the species-rich north-eastern states of the country and unlike its neighbouring states, a fair assessment of orchid diversity in the region has been a longstanding issue. The work established that there are 51 species (27 genera) of orchids. Eleven genera and 25 species were reported as the new distributional records in the state flora. Reproductive ecological investigation of two of the epiphytic orchids namely. Cymbidium aloifolium and Aerides odorata revealed that despite being self-compatible, they rely on suitable pollinators for reproductive success. For the first time, it was shown that 4pis cerana indica, a non-oil bee is involved in pollination of oil-rewarding flowers of C. aloifolium. Further, mutualism in the species is driven by a prolonged flower-handling time, non-competitive pollinator environment, and phenotypic matching. On the other hand, pollination success in A. odorata is accomplished in a more complex environment, involving active interaction with floral antagonists such as ants and moths, besides carpenter bees as the pollinator. Slow rate of nectar replenishment, prolonged flowering duration and continuous engagement with the pollinator seem to ensure consistency of pollinator visits to the orchids in bloom. The net outcome on the reproductive fitness of C. aloifolium is attributed to overarching effect of its breeding system on the overall interacting network of the foragers. The objective to understand the extent of endophytic mutualism was investigated by employing community level approach. The findings suggest that the interacting partners (orchid/endophytes) have not co-evolved, although the generalised orchid taxa and their endophytes tend to associate in the nested clades

*Contents*

1. Taxonomic survey of Orchids in Tripura 2. Reproductive ecology of cymbidium aloifolium 3. Reproductive ecology of aerides odorata 4. Orchid endophyte association at the community level. Summary and Conclusions.

02. ARYA (Meenakshi)
**Analysis of Leaf Cuticle Proteome from Four Brassicaceae Members for Exploring their Translational Potential.**
Supervisor: Prof. Renu Deswal
Th 25717

*Abstract*

Plant cuticle being primary sensor of stress signals, is protective extraceliular barrier over pele the epidermis of above-ground parts Genetic studies and mutant analysis o Gu sent biosynthesis has been extensively done However, cuticle proteins and their abundance. are least studied in Brassicaceae species. Therefore, leaf cuticle proteome was examine agronomically important Brassicaceae members namely, B juncea, B nigra. B rapa four sativus using gel based (MALDI TOF/TOF) and quantitative shotgun pr sed (nLC MSMS) for understanding MS identification highlighted physiological relevance of cuticle proteins G MSMS only eight abundant analysis identified 181 secretory polypeptides Whereas d (22%). defense secretory proteins, majorly associated with catalytic a 4 related (16%). oxidoreductase activity esterase/lipase ESM1 (involved in cuticle structurina and defense) in leat c towards tne extracellular "mustard oil bomb", Besides. four abundant classica sec proteinsincluding GDSL-esterase/lipase ESM1. 6-alucanase (an antifreeze pro peroxidase and ascorbate peroxidase showed in vitro enzyme activity. Analysis o extract by Nanoliter osmometer- phase contrast microscopy detected antifreeze acivy to non-protein component, monosaccharides that may provide freezing tolerance aiong u cutins. Sef-assembly of cutin monomers into spherical nanostructures, cutinsomes Was done from the leaves of four species. Cutin being a more resilient biopolymer has Deen employed tor bioplastic formation from tomato pomace. Comparison of B. juncea leat and stem cutinsomes indicated stem cuticle as better candidate for bioplastics formation from agrowastes. This study not only identified leaf cuticle proteins, but also laid the foundation for exploring the extracellular glucosinolate- myrosinase system, PTM crosstalk, and antifreeze activity as stress adaptive strategies in Brassicaceae. The cutinsomes may be directed towards bioplastics formation from Brassica agrowastes, an underutilized Bioresources in India.

*Contents*

1. Introduction 2. Review of literature 3. Material and methods 4. Result and discussion 5. Summary and conclusion. References. Annexure. Publication.

03. BHARDWAJ (Ekta)
**Evolution and Characterization of Non-Canonically Organized MIR 395 & MIR 399 Regulated by Bi-Directional Promoters from Arabidopsis Thaliana and Brassica Juncea, and Natural Variation in Root and Sulfate-Stress Response.**
Supervisor: Prof. Sandip Das
Th 25723

*Abstract*

MIRNAs, encoded by specific genomic loci are a class of non-coding regulatory RNAs that play important role in development and adaptation by transcriptional, posttranscription and translational regulation of gene expression. MIRNAs have been categorized into families based on similarity or differences in mature miRNA. Tandem and segmental duplications responsible for expansion of MIRNA families cause these families to form non-canonically organized clusters. Studies on evolutionary history and complexity in transcription regulation of such non-canonically organized clusters are scanty. Therefore, in the current study we identified eight classes of non-canonical MIRNA clusters across 25 species spanning from algae to angiosperms. MIRNA395 and MIRNA399 were

identified as two families most frequently found in clusters across plant kingdom. A comparison of phylogeny and organizational patterns revealed recurrent independent events, some of which are species-specific, ancient or of recent origin, occurring in different lineages, responsible for non-canonical organization and expansion. Two genes positioned head-to-head with the intergenic sequence as a putative bidirectional promoter sharing transcription regulation is a major class of non-canonical organization. We thus examined the role of non-canonical organization in transcriptional regulation and functionally characterized bi-directional promoters associated with MIRNA395, MIRNA399 families from Arabidopsis thaliana and Brassica juncea. These promoters were found to be regulate reporter activity asymmetrically during development, and were induced by NaCl, hormones, and sulfate stress. Functional characterization of MIRNA395 through gain-of-function, and, loss-of-function mutants revealed developmental role, especially in roots and in S-nutrient uptake. Root architecture was a major traits found to be affected under sulfate-stress. We also surveyed and identified natural variation in root architecture and length under natural condition and sulfate stress conditions among varieties of B. juncea. The study paves way for modifying root trait employing MIR395, designing novel bi-directional promoters, and, exploiting natural variation for selecting optimal root architecture for efficient nutrient uptake

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04. CHAUDHARY (Komal)
**Cuticular Waxes: Variation in Composition and Wax Load in Selected Brassica Spcies, role in B. rapa-Albugo Candida Interaction, Origin and Evolution of Alkane Biosynthetic Genes CERI and CER3 in Land Plants.**
Supervisor: Dr. Priya Panjabi
Th 25718

*Abstract*

Cuticular waxes covering the plant surfaces provide protection against biotic and abiotic stress. Variations in the wax composition and morphology due to genetic and environmental factors have been reported in several plants, however little is known in Brassica crops, especially B. juncea (Indian mustard), The present study was undertaken to study: (a) variation in cuticular wax load, composition and micromorphology of leaf cuticular waxes across six Brassica species, (b) origin, phylogenetic relationships and variation in copy number of alkane (major wax constituent compound) biosynthetic genes, CERI (ECERIFERUMI) and CER3 (ECERIFERUM3), within and beyond Viridiplantae (green plants) and (c) the role of cuticular waxes in B. rapa- Albugo candida interactions using RNA-Seq. Variations in both cuticular wax load and composition were observed (using GC-MS-FID) among the 23 Brassica accessions and different developmental stages and organs. These variations were also reflected in the wax micromorphology observations using SEM. Phylogenetic analysis of CERI and CER3 protein sequences revealed that the genes originated by duplication of CERI/3 gene in the ancestral embryophyte and their evolution

within embryophytes involved differential divergence of their constituent domains. Evidences suggest that CERI/3 gene originated in Viridiplantae most probably by fusion of pre-existing domains. Mixed RNA-Seq analysis of A. candida infected tissue of resistant host (B. rapa) identified several differentially expressed genes involved in crucial pathways including cuticular wax biosynthesis/regulation. The study provides a resource for better understanding of cuticular wax composition, micromorphology in Brassica crops and gives insights into molecular mechanisms of host-pathogen interaction.

*Contents*

1. Introduction and review of literature 2. Analysis of chemical composition and morphology of cuticular waxes across six brassica species 3. Origin and evolution of CERI and CER3 genes in land plants 4. Exploring the role of cuticular waxes in brassica rapa-albugo candida interactions using RNA-Seq. Bibliography. Annexures.

05. GARIMA

**Leaf Morphometry, Seed Fatty Acid Composition and Content, Development of Novel SSR Markers and Genetic Diversity Analyses in Moringa Oleifera Species Complex in India.**

Supervisors: Dr. Priya Panjabi and Prof. R. Geeta

Th 26516

*Contents*

1. Introduction and review of literature 2. Field visits and acquisition of Moringa study material and germplasm 3. Linear morphometric analysis and allometric scaling studies in leaves of Indian Moringa species 4. Development of genomic SSR markers in Moringa oleifera Lam. using next generation sequencing of PKM-1 variwry and use in analysis of genetic variation and diversity 5. Study of inter and intra-specific variation in fatty acid composition, in M. olefera, M. concanensis and M. stenopetala, collected in India, using Gas Chromatography Mass Spectrometry (GC-MS). Appendices and Publication and Participation in conference.

06. MALIK (Sonal)

**Systematics and Molecular Phylogeny of Selected Indian Barleria Species & De Novel Genome Wide SSR Markers in Assessment of Genetic Diversity in Barleria Cristata L. A Medicinally Important Species.**

Supervisor: Prof. Suman Lakhanpaul

Th 25719

*Abstract*

Barleria L. (Family- Acanthaceae) is the 3rd largest polymorphic and widespread genus of herbs and shrubs, comprising more than 300 species. It is predominately an old world genus having major diversity spots in tropical Africa followed by Indian subcontinent. Among Asiatic countries, India is one of the major diversity centre having more than 30 taxa. Due to presence of various secondary metabolites and their active constituents many Barleria species show potential for pharmacological applications thus, making it a valuable taxon both medicinally and economically. However, due to complex morphology, there are ambiguities and doubts regarding the infra-generic classification of the genus. Ironically, many Barleria species have become endangered due to high level of endemism whereas there are others that are widespread and even show invasive tendencies. However, despite being highly valuable taxon and intriguing distribution pattern, studies

are nearly lacking within genus Barleria. Moreover, negligible work has been done to understand its diversity both at inter- and intra-population level. In-depth studies aimed at resolving taxonomic ambiguities, ascertaining phylogenetic relationship and assessment of genetic diversity using morphological and molecular markers are highly desirable in genus Barleria to fill the vital knowledge gaps thereby, enabling its judicious conservation and utilization. Thus, with this background the present study was undertaken. The "Introduction' part of the thesis provides a compendium of previous research and sets the background for the current research work. It highlights the importance of genus Barleria and need to investigate its systematics and development of markers to understand its phylogenetic as well as genetic diversity at population level. The Chapter 1 deals with the analysis of infra-generic classification of genus Barleria using seed micro- and macro-morphological characters. The Chapter 2 deals with the study of phylogenetic relationship among Indian Barleria using selected chloroplast DNA based markers. A detailed account of De novo discovery and characterization of novel genome-wide SSR markers in selected Barleria species i.e. B. cristata and B. prionitis and assessing the extent of cross-species transferability of the novel SSR markers from B. cristata to other species of genus Barleria has been dealt in Chapter 2 3. Chapter 4 deals with analysis and comparison of genetic diversity in selected populations of B. cristata using novel SSR markers. The results have been discussed separately for each chapter. The salient findings from each study have been summarised in the 'Summary and Conclusions' part. The scientific literature referred has been provided at the end under the title 'References'. The study aimed at resolving the taxonomic disputes in Barleria will help in better understanding of the genus and enable us in elucidating its phylogenetic relationship with other taxa. Further, identification of novel SSR markers transferable across other Barleria species and effective in detecting genetic variation will be significantly useful in understanding the partitioning of genetic diversity among and between populations. Overall, the present study provides the insightful platform for forthcoming research aimed to resolve taxonomic ambiguities and also to develop conservation strategies in this highly valuable and unexplored genus

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07. MONIKA

**Comparative Study of miRNA and 21-Nucleotide Reproductive Phase siRNA between Developmental Stages of Female Gametohyte in Sexual and Apomictic Addition Lines of Pearl Millet [Cenchrus Americanus (L.) Morrone syn. Pennisetum glaucum (L.) R. Br.]**

Supervisor: Prof. Shailendra Goel

Th 25722

*Abstract*

Apomixis is a plant reproductive pathway where seeds are produced without reductional division and gametic fusion. Apomix has been extensively studied into the continuous production of elite germplasm. Apomixis has been extensively studied in the genus cenchrus which include a wild species cenchrus squamulatus, an oblige aposporous apomict. Apomixis in the cenchrus

squamulatus is known to be associated with a transposable element rich chromosomal block termed as apospory specific genomic region. An apomictic pearl millet through an elaborated breeding scheme by Dujardin and Hanna is available to us and the same was used to study the role of small RNA in the regulation of apomixis.

*Contents*

1. Introduction of apomixes and a comprehensive review of literature in respect to the various aspects of apomictic reproductive pathway 2. Maintenance and screening of experimental plant material, tissue harvesting, and post sequencing analysis of small RNA, and transcriptome sequencing resource for the downstream small RNA analysis 3. Analysis of known and novel microRNAs from apomictic and sexual female and degradome assisted miRNA target gene prediction 4. Prediction and characterization of reproductive phaseloci form apomictic and sexual female and degradome assisted 21-nt phasi RNAs target gene prediction 5. Summary and conclusion. References

08. MUKUND LAL

**Insights into Evolutionary Trends and Comparative Genomics of MYBs, Structural and Functional Genomics of AtMYB32 and AtMYB96, and, Analysis of Variation in Brassica species.**

Supervisor: Prof. Sandip Das

Th 25724

*Abstract*

Myeloblastosis (MYB) family, the largest plant transcription factor family, has been subcategorised based on the number and type of repeats in the MYB domain. In spite of several reports, evolution of MYB genes and repeats, and, impact of genome-segmentaland local-duplication on this family remains enigmatic. The present study revealed a complex evolutionary history supporting repeat-gain model. Sequence and structure analyses unravelled ancient as well as novel intron phases, and, conserved 3D-structure of MYB domain albeit with variation in positioning among members of MYB-family. Comparative genomics identified lineage-, and genome-specific losses and gains, and synteny among Brassicaceae members. Paralogs of MYB family were under positive and negative selection. MYB genes are involved in various developmental pathways, and information about their role/s in gynoecium development is scanty. Two 2R-MYB genes - MYB32 and MYB96 from Arabidopsis and Brassica were therefore functionally characterized. Promoter sequence analysis revealed variation in TFBS motifs; differential transcriptional regulation, and expression level diversification among paralogs and homeologs were found to during development, under stress and hormonal treatments. Functional characterization using constitutive gain-of-function of full-length, deletion series, and, amiRNA-mediated loss-of-function revealed pleiotropic effects on plant development including gynoecium development. global transcriptome-profiling of gynoecium from transgenic lines of MYB96 show “regulation of pollen-germination”, and, “zygote/endosperm formation” are the most affected processes. We also performed cataloguing of phenotypic variations among Brassica species/varieties to investigate natural variation in vegetative and reproductive traits. The understanding of ontogeny of floral structures was achieved by categorizing B. juncea var. varuna floral-buds into 20 stages, based on bud length, of which stage-3 buds correspond to megasporogenesis. This study thus lays the foundation for characterization of the domains and motifs in MYB32 and MYB96 protein, dissecting role of MYB32 and MYB96 in gynoecium development, and investigating natural variation in developmental processes in Brassica

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1. Introduction 2. Review of Literature 3. Materials and methods 4. Comprehensive analysis of IR- and 2R-MYBs reveals novel genic and protein features, complex organisation, selective expansion, and insights into evolutionary tendencies 5. Sequence analysis and transcriptional regulation of cis-elements of MYB32 and MYB96 in Arabidopsis thaliana and brassica juncea 6. Functional characterization of homologs of MYB32 and MYB96 from Arabidopsis thaliana and brassica jancea 7. Analysis of phenotypic variation among brassica species, ontogeny and expression dynamics of BjMYB32 and BjMYB96 homeologs in gynoecium of brassica juncea var varuna 8. Discussion 9. Summary, Conusion and Future perspective. References.

09. PRITI

**Nutritional Composition, Phytochemistry and Antioxidant Potential of Some Ethnomedicinal Ferns.**

Supervisor: Prof. Prem L. Uniyal

Th 25716

*Abstract*

Pteridophytes are first non-flowering vascular plants on land that reproduce and dispersed through spores. The present study was carried out to evaluate the medicinal and nutritional potential of fronds of Nephrolepis cordifolia, Nephrolepis biserrata ,coniogramme intermedia, polypodiodes microrhizoma, Equisetum diffusum and diplazium esculentum. N. Cordifolia is being used to treat skin disorders, asthma, biliousness and liver disorders. N.biserrata has been used to treat boils,abscesses and fronds used in the preparation of vegetable salad. Young fronds of C. intermedia consumed as vegetable and used in the preparation of noodles in China. Fronds of P. microrhizoma are dried and powered, and a small amount of the powder is mixed with milk to reduce joints pain and rheumatic disorders. The study revealed that ferns could be useful as herbal goods in the nutritional and pharmaceutical industries.

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1. Introduction 2. Review of literatures 3. Material and methods 4. Results 5. Discussions 6. Summary and conclusions. References.

10. SEEMA PARVEEN

**An Analysis of Genetic Diversity, Niche Modelling and Transcriptome of Endosperm Developmental Stages in Genus Nymphaea: An Important Water Lily from ANA Grade with Diploid Endosperm.**

Supervisor: Prof. Shailendra Goel

Th 25714

*Abstract*

Nymphaea, commonly known as water lily, is the largest and most widely distributed genus in the order Nymphaeales. Apart from its ornamental, nutritional, and medicinal uses, Nymphaea also appear to have ecological and historical importance. The mportance of Nymphaea in wetland ecosystems and their increased vulnerability make them a great choice for conservation and management. In this work, we first studied the genetic diversity and population structure of two Nymphaea species, N. micrantha and N. nouchali using SSR markers. The study revealed extensive polymorphism in N. nouchali, while in N. micrantha, no apparent genetic divergence was detected. The study also revealed that N. micrantha predominantly reproduces asexually which might has impacted the genetic diversity of the species to a great extent. In the next study, we used

MaxEnt model to predict the current and future (2050) potential distribution of Nymphaea species in India and highlight climate change impact on their natural habitats over time. Nymphaea belongs to the ANAgrade of angiosperms. The presence of diploid endosperm and the unique phylogenetic position of Nymphaea suggest that its diploid endosperm might be the remnant of an ancestral state. Therefore, Nymphaea might serve as a model plant system for understanding the origin and early evolutionary events within the flowering plants. In this work, we explored the RNAseq data and compared the expression of genes expressed during different endosperm developmental stages (0DAP to 8DAP) of Nymphaea caerulea seeds with emphasis on imprinting related genes. The expression of some chromatin methylation and imprinting related gene homologs appeared to be conserved in Nymphaea. The information generated in the study is of consideration for genetic resources conservation in Nymphaea species, especially those with limited distribution. Further, this study would serve the groundwork for the characterization of candidate genes, which would provide new insights into seed development.

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