

CHAPTER 7

BOTANY

Doctoral Theses

01. AGGARWAL (Aakrati)
Over-Expression of PgLEA2, PgeIF4A and Bar Genes in Transgenic Rice Plants for Abiotic Stress Tolerance and Weed Management.

Supervisor : Dr. Yashwant Mudgil

Th 23924

Abstract (Verified)

LEA (Late embryogenesis abundant) proteins and *eIFs* (eukaryotic initiation factors) provide abiotic stress tolerance in microbes, animals and plants (Xiao et al. 2007; Uang et al. 2016). ESTs encoding for *LEA2* and *eIF4A* were found in a cDNA stress library generated from *Pennisetum glaucum*, an arid crop which provided new candidate genes to validate their role in stress (Mishra et al. 2007). Transgenic rice plants expressing *PgLEA2* and *PgeIF4A* were generated to elucidate their role in drought and salinity stress. A third gene *bar* from *Streptomyces sps.* was chosen to provide herbicide tolerance. *in silico* analysis of *LEA2* and *eIF4A* genes revealed their genome wide distribution and expression in developmental stages of rice and salinity and drought. All three genes were cloned via Gateway Cloning in a destination vector, pMDC99, transformed into *Agrobacterium* EHA105 which was used for transformation in PB1 rice variety. The regeneration percentage was 70% and the transformation percentage was around 49%. Transgene integration was confirmed by PCR (T0) and southern blot analysis (T1) which led to 11 stably integrated lines. Number of multiple copy integrations was higher than single copy integrations. Two single copy and two double copy integration lines were chosen for subsequent analysis. Transgene expression was confirmed by semi quantitative RT-PCR and the transgenic plants performed better than wild type under stress during physiological and biochemical analysis with increased growth and activation of antioxidant systems. Seed set was phenomenally higher than control in transgenic plants after stress treatments and the transgenic plants were phosphinothricin resistant. Genome walking analysis in a single copy integration line confirmed integration of transgene construct in rice chromosome number 11. Summing up all these findings provides clear evidence on the function of *PgLEA2* and *PgeIF4A* in abiotic stress tolerance.

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1. Introduction 2. Review of literature 3. Materials and methods 4. Results and discussion. Summary and conclusion. References and appendix.

02. AGNIHOTRI (Pankaj Kumar)
Isolation of Promoters of Nuclellin Gene from Hordeum Vulgare L. and Kinase Interacting Protein Gene from Cenchrus Ciliaris L. And Characterization of Nuclellin Promoter Activity in Arabidopsis Thaliana (L.) Heynh.

Supervisor : Prof. Vishnu Bhat

Th 23929

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1. Introduction 2. Review of literature 3. Materials and methods 4. Results 5. Discussion 6. Summary. References.

03. AKANKSHA PRIYA
Employing DNA Barcode Markers for Identification and Authentication of Herbal Samples of Traded Indian Medicinal Plants.
Supervisor : Prof. S. B. Babbar
Th 24315

Abstract
(Verified)

A DNA barcode library was prepared by in silico analysis of the sequences of four barcode markers, viz. ITS, ITS2, matK and rbcL, available in GenBank, NCBI, of 960 traded medicinal plant species, including 178 species of high trade volumes, as per National Medicinal Plant Board of India (NMPB). The sequences of one or more locus/loci of 438 species and all the four loci of 144 species were available. Individually, ITS provided the maximum species-specificity to 83% of the 438 species. Three-locus combination of ITS+matK+rbcL was species-specific for 96.5% of the 144 species. As a result of this meta-analysis, DNA barcodes of 438 traded plants, including 115 highly traded plants, became available. In addition, DNA barcodes, again based on these four markers, were developed through wet lab research for 23 species, which included 13 species of high trade volume, seven traded species and three perceived to be of significant therapeutic importance. The barcode library developed was used for authenticating the botanical identities of 163 herbal samples, procured from different markets or online, supposed to be belong to 54 species, including 41 species of high trade volume, by genetic distance and phylogenetic tree methods. A total of 147 herbal samples, expectedly of 53 species, could be tested. Of these, 89 (60.5%) samples were found to be authentic. BLAST1 search of the sequences of the samples, not found to be authentic, revealed that some of the substitutions were as per Ayurvedic principle "Abhava Pratinidhi Dravyas" i.e. the herbal samples have been substituted with their known substitutes. While other samples were substituted with other unrelated medicinal plants, or even totally unrelated plant species, such as, 'Besan' (Cicer arietinum) in place of 'Vachhnag' (Aconitum ferox) and an obnoxious weed, Parthenium hysterophorus substituting for 'Pashanbheda' (Bergenia ligulata).

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04. ANAND (Saurabh)
Evolutionary and Functional Analysis of MIT159 and Selected MYBs in Mediating Stamen Development in Arabidopsis Thaliana and Brassica Species.
Supervisor : Dr. Sandeep Das
Th 23921

Abstract
(Verified)

Comparative genomics has emerged as a powerful approach for understanding evolutionary processes and to unravel impact of polyploidization. Morphological and adaptive novelties are driven by post-polyploidization events such as sequence and functional diversification. In India *Brassica juncea* (mustard) is a major source of edible oil. However the average yield is low and a large gap in supply exists. Understanding genomics of regulatory elements such as MiR159 and MYB33, MYB65, MYB101 involved in reproductive organ development is critical to increasing productivity. In the present study, synteny analysis of MIR159 containing genomic segment across Brassicaceae revealed extensive re-arrangements. A segmental duplication is responsible for origin of

MIR159A-MIR159B paralogy in Brassicaceae. Sequence analysis of the target MYBs revealed loss of *MYB33* in *Brassica* lineage. Comparative analysis of the mature miR159 and the miRNA binding site in *MYB33*, *MYB65* and *MYB101* shows the complexity of regulatory network that is dependent on strict sequence complementarity. Functional characterization of *Bj-miR159a* through reverse genetics showed altered vegetative and reproductive organ development. The major defects in anther development included shorter filament length, altered dehiscence zone, reduced pollen viability, altered central cell in embryo sac, and defect in pollen-pistil interaction, and lignin deposition. RNA-seq analysis indicated that several developmental and metabolic pathways are severely affected. Staging allowed us to correlate stages of male meiosis and floral buds. An improved RNA extraction procedure from anther, gynoecium, and seeds of *Brassica rapa* and *Brassica juncea* was standardised. Spatio-temporal expression analysis of several MYBs across anther developmental stages of *B. juncea* revealed expression diversification. Functional characterization of promoter of *MYB65* from *B. juncea* revealed reporter activity in leaves, root, anther filament, style and pedicel of silique. Functional characterization of *MYB65* using artificial miRNA is in progress and is expected to throw light on role of *MYB65* in reproductive organ development.

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1. Introduction 2. Review of literature 3. Material and methods 4. Comparative genomics and phylogeny of *MIR159* across brassicaceae 5. Functional characterization of *Bj-miR159* in *Arabidopsis thaliana* and *Brassica juncea* 6. Study of developmental stages of flower of *Brassica rapa* and *Brassica juncea* and analysis of expression pattern of MYBs during anther development 7. Discussion 8. Summary and conclusions. References. Appendices.

05. CHAUHAN (Samvedana)
Role of Nitric Oxide in In Vitro Flowering of Lemna gibba L.
Supervisor : Prof. S. B. Babbar
Th 24251

Abstract (Verified)

The results presented in the thesis besides convincingly demonstrating the positive role of NO in enhancement and induction of flowering in *L. gibba*, also reveal the deficiency of Bonner-Devirian (BD) nutrient medium that makes it incapable of supporting its flowering. The conclusions that emerge from the study are : (i) Flowering in *L. gibba*, along with other physical and chemical factors, is critically dependent on the threshold concentrations of KNO_3 and KH_2PO_4 , provided together. Therefore, for studies on its flowering, choice of nutrient medium need not to be restricted to the use of E or M media, rather a modified BD medium having KNO_3 and KH_2PO_4 at the concentrations included in E medium can be used, (ii) NO provided externally through its donors enhances flowering under inductive conditions, but fails to induce flowering under non-inductive conditions. The application of NO donors somehow results in production of fresh NO in the plants just prior to flowering, which in turn leads to higher stress level, (iii) Even the capability of E medium to support flowering is due to higher production of NO and stress levels on this medium than BD medium, (iv) Earlier known inductive and stimulatory effects of SA on flowering of this plant have been found to be because of stress generated by the combined effect of NO and ROS, (v) SHAM, an analogue but antagonist of SA for some physiological processes and inhibitor of alternative oxidase (AOX), simulated the all effects of SA on flowering of *L. gibba*, and (vi) Carbon monoxide, which in recent times has emerged as an important gaseous regulatory molecule in plant developmental processes, has been shown to promote flowering for the first time.

Its effect is also mediated through increased production of NO and consequent stress in the plants.

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1. Introduction 2. Review of literature 3. Materials and methods 4. Results 5. Discussion 6. Summary and conclusions .References and Appendix.

06. CHOUDHARY (Anjali)
Reproductive Biology, Trap Diversity and Phylogenetic Analysis of Some Indian Utricularia Species.
Supervisor : Prof. Rajesh Tondon
Th 23928

Abstract
(Not Verified)

Utricularia L. (Lentibulariaceae) is the largest genus among the carnivorous plants of flowering plants known, and is represented by approximately 228 species. India harbours 39 species which constitutes about 16-17% of the total *Utricularia* species. Over 55% of the Indian species are known to be endemic. The study was carried out for five years between 2013 and 2018. The study established that both the species adopt different mating strategies for perpetuation. *Utricularia praeterita* is an obligate inbreeder while *U. babui* adopts a mixed mating strategy. The mode of selfing in both the species is delayed-selfing type. The mechanism in delayed-selfing is autonomous type in both the species and occurs by 'diminished herkogamy'. The stigma is thigmo sensitive and its lobes close within ~5 sec once touched. Among the two species, only *U. babui* produced floral nectar and occasionally pollinated by *Apis* spp. Plant density showed a significant effect on the insect visitation rate in *U. babui*. A direct positive correlation was observed between the density and the insect visitation rate. The trap morphological features correlated with the ecological conditions. Enormous structural diversity occurs among the glands of the bladderworts. The glandular features provide additional information that can be used for identification of species. Resource allocation pattern study revealed that aquatic species invested more in biomass, trap size and in trap production than terrestrial ones. Phylogenetic analysis revealed that the recently described species *U. babui*, *U. naiki* and *U. janarthanamii* should be placed in the Section Oligocista. *Utricularia purpurascens* should be treated as a separate species and not as a synonym of *U. graminifolia*. Based on the results of ancestral state reconstructions, terrestrial habit and quadrifid chamber glands + bifid threshold glands can be considered as the ancestral state.

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1. General introduction 2. Reproductive biology of two species of *Utricularia* from western ghats 3. Structural diversity of traps in some species of the *Utricularia* 4. Assessment of phylogenetic relationship among some of the species of *Utricularia*. Summary and conclusion. Literature cited. Illustrations. Annexures.

07. DANDA (Shagun)
Taxonomy, Floral Ontogeny and Fatty Acid Profiling of the Genus *Crotalaria* L. (Fabaceae) in India
Supervisor : Prof. Arun K. Pandey
Th 23925

Abstract
(Not Verified)

The genus *Crotalaria* L. (Fabaceae) includes 702 species, distributed in the tropics and sub-tropics. In India, the genus is represented by 89 species, of which 73 occur in Peninsular India. Despite a systematic revision known for the genus, several aspects

such as floral ontogeny, phytochemistry, leaf anatomy and morphology remain untouched. Hence, the present work was undertaken with the aim to address debatable systematic questions and add to our knowledge about the complexities in the genus *Crotalaria meghalayensis*, a new species collected from Meghalaya, has been illustrated and described. The functional dimorphism found in the androecium of *Crotalaria* reflects a division of labour between the two different stamen morphs as strategies to face the pollen dilemma. Studies on evolution of keel beak, callosities type and style curvature provided several insights. Our work reveals that ancestor of the genus *Crotalaria* had a rostrate-not-twisted keel beak and rostrate spirally twisted beak arose within the genus. Based on the flower development studies, it is concluded that the mode of sepal development is not stringently unidirectional but sometimes bidirectional. Fatty acid profiling in seeds of 38 species of Indian *Crotalaria* was carried out to explore the potential source of seed oil under the non-conventional plant resources. This is the first major inclusive study, which has reported the fatty acid profiles in the genus *Crotalaria*. Leaf venation patterns, stomatal types and trichomes in the genus *Crotalaria* have been studied. Venation pattern studied for 58 species of *Crotalaria* show pinnate type of primary venation pattern while secondary venation pattern is brochidromous. The trichomes are non-glandular, uniseriate across all the species studied. Present work is significant as this is the first detailed study on reproductive morphology, pollination syndrome, floral ontogeny, leaf venation patterns and fatty acid composition of *Crotalaria* species in India.

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1. Comparative morphological account of the genus *Crotalaria* 2. A new species of *Crotalaria* (Fabaceae, Crotalariaeae) from Meghalaya, India 3. Floral ontogeny, pollination syndrome and functional significance of flower type with focus on stamens and to trace the evolution of significant characters for assessing the trends across the genus *Crotalaria* 4. Fatty acid profiling, multivariate analysis and ancestral state reconstruction in the genus *Crotalaria* reveals evolutionary trends 5. Leaf venation patterns, stomatal types and trichomes explain ecological and evolutionary patterns in the genus *Crotalaria* L. Summary. References.

08. DINESH KUMAR
Biotechnological and Biochemical Investigations on Two Medicinal Plants, *Holarrhena pubescens* Wall. ex G. Don and *Nerium Oleander* L. and Bioefficacy of Green Synthesized Nanoparticles against Malaria, Filariasis and Dengue Vectors.

Supervisor : Prof. Veena Agarwal
 Th 23923

Abstract (Verified)

Holarrhena pubescens an important medicinal plant which synthesizes a major alkaloid, conessine with wide biomedical applications. Quantitative analysis of conessine was done from its different parts through HPLC and green bark having optimum quantity was chosen for raising callus. Elicitation of conessine was done employing organic and inorganic elicitors, isolated and characterized using CC, TLC, FT-IR, ¹H-NMR and HR-MS. Phytotoxicity of different metals was evaluated on the *in vitro* nodal cultures of *H. pubescens*. The toxic effect of metals was dose dependent and concentration specific. Among the different metals tried, maximum inhibition in shoot number and length was observed in 25 mg/L As³⁺. Maximum conessine content was achieved at 100 mg/L CoCl₂. With increase in concentration of heavy metals, antioxidant enzyme activities also increased. Silver nanoparticles (AgNPs) were synthesized using aqueous bark extract of *H. pubescens* and were characterized by UV-vis, XRD, FE-SEM, HR-TEM, FT-IR, HR-TEM and FE-SEM. Such nanoparticles were assayed against different mosquito vectors causing malaria, filariasis and dengue. Maximum larvicidal activity was observed with AgNPs having LC and LC 2.672 ppm and 4.482 ppm, respectively for *A. stephensi* (24 h); LC and LC values being 9.3 and 19.24 ppm, respectively, for *C. quinquefasciatus* (72 h) and LC and LC values were 5.53 and 12.01 ppm, respectively, for *A. aegypti* (72 h). *Nerium oleander* was selected to understand biochemical and physiological

mechanisms related to drought and salt stress. One-year-old seedlings were exposed to 15 and 30 days treatment with increasing salt concentrations, up to 800 mMNaCl, and complete withholding of irrigation. Strong water deficit and salt stress both caused growth inhibition, photosynthetic pigments degradation, slight increase in osmolytes, accumulation of malondialdehyde. Stress tolerance mechanism is based on the constitutive accumulation of high soluble carbohydrates and glycine betaine and activation of the antioxidant systems.

Contents

1. Introduction 2. Review of literature 3. Evaluation, elicitation, isolation and characterization of conessine bioactive molecule using green bark derived callus culture of *holarrhennapubescens* wall. Ex G. Don and its bioefficacy against malaria mosquito vector. 4. Effect of heavy metals on in vitro morphogenesis, antioxidant system and conessine content in the nodal cultures of *H. Pubescens* 5. Green synthesis and characterization of silver nanoparticles (AgNPs) using bark extract of *H. Pubescens* antifungal activity against *Anopheles stephensi* Liston, *Culex quinquefasciatus* Say and *Aedes aegypti* L. mosquito vectors 6. Impact of salt and drought stress on plant growth, antioxidant systems and on accumulation of compatible solutes in *nerium oleander* L. 7. Summary and conclusion. References. Appendix.

09. JOSHI (Gopal)

Transcriptome Profiling of Vegetative and Reproductive Tissues of *Carthamus Tinctorius* L. (Safflower) and Analysis of Global Transcriptome Changes in *Fusarium Oxysporum* sp. *Carthami* and Safflower roots During Susceptible and Resistant Interactions Between Host and Pathogen.

Supervisor : Prof. Manu Agarwal

Th 23931

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1. Introduction 2. Review of literature 3. Material and Methods 4. Results. Discussion. Summary and Conclusion. References. Annexures.

10. MAMGAIN (Akshay)

Morphological Variation and Distribution Pattern of *Rhododendron Arboreum* Sm. In Uttarakhand, India.

Supervisors : Prof. P. L. Uniyal and Prof. R. Geeta

Th 23922

Abstract (Verified)

Rhododendron arboreum Sm. is an important component of higher regions of Himalayan forests. In this study *R. arboreum* is used to measure the effect of environmental change. Total 43 populations were assessed and mapped in Uttarakhand using land survey. Due to anthropogenic pressure, low (1400 – 1700 m) and lower – middle (1700 – 2000 m) altitude areas are experiencing reduced and disturbed populations. Leaf physiognomy was studied in relation to environmental variables (altitude, temperature and precipitation). Leaf samples (2400) were analyzed in 40 locations (60 leaves/location). Lamina area displayed an inverse relationship with altitude and precipitation, but a direct relationship with temperature. Petiole area showed direct relationship with altitude and precipitation, whereas an inverse relation with temperature. The leaves exhibited morphological variation in lamina shape, apex, base, margin and venation was pinnate brochidodromous type. Different flower colour morphs and floral attraction pattern in form of nectar guides (spots) were studied. Seven different colour morphs and five different types of nectar guide patterns were observed. The flower colour and attraction pattern vary according to environment and plant genetics. Species distribution modelling was performed,

utilizing the Uttarakhand species location data, in India and adjoining region, where the species is reported. It is predicted that in future at low altitude (<3000 m) the species will exhibit range contraction, population localization and finally extinction, whereas, areas with higher altitude (>4000 m) will show range expansion. Habitat fragmentation will cause the species to survive in refugia habitats. Leaves and flower of the species demonstrate variation in relation to different environmental variables (sunlight, temperature, altitude, precipitation). In future environment the species will show reduced distribution. Coordinated conservation plans should be implemented so that plant could be able to resist environmental change and maintain its viable population.

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1. Introduction 2. Review of literature 3. Study area 4. Population assessment, mapping and flowering response of rhododendron arboreum sm. – A keystone in Central Himalayan region of Uttarakhand, India. 5. Leaf physiognomy of rhododendron arboreum sm. In relation to some environmental variables of Central Himalayan region of Uttarakhand, India. 6. Flower colour variation and floral attraction pattern of rhododendron 7. Species distribution modelling of rhododendron arboreum s. In India and adjoining region 8. Summary. 9. Appendices.

11. MANN (Neha)
Reproductive Attributes, Population Genetic Studies and Detection of Cyclotides in Selected Species of Viola L. from Uttarakhand.
Supervisor : Prof. P. L. Uniyal
Th 23930

Abstract (Verified)

Viola L. belongs to the family of Violaceae, it is a perennial herb occurs in cool and humid climate. There are 525 – 600 species of *Viola* worldwide and 35 to 41 species in India, which occur in Himalayan regions and hills of Western Ghats at an altitudinal range of 900 – 3000m. *Viola* is known as “Banafsha”, has medicinal importance in Ayurvedic and Unani medicinal systems. Present study was conducted on selected species of *Viola* namely, *V. canescens* Wall and *V. biflora* L. and a few other species with five objectives. *Viola* show mixed breeding system, as it consists of two types of flowers within the same plant that are chasmogamous flowers (open) and cleistogamous flowers (closed). Both the species namely, *V. canescens* Wall. and *V. biflora* L. were found as self-compatible and self-pollinated that was highly supported by unusual phenomenon of *in-situ pollen* germination within anther. The roots of *V. canescens* and *V. biflora* were found associated with twenty one different arbuscular mycorrhizal fungal species i.e. fourteen from *V. biflora* and seven species from *V. canescens* were isolated from the rhizospheric soil. *Glomus* sp. was found as a dominant colonizer in both *Viola* species. Total of ten metals (Na, Ca, Cd, Cu, Fe, K, Mg, Ni and Zn) were analyzed in substratum as well plant tissue (Leaves) of both selected species of *Viola* i.e. *Viola canescens* and *Viola biflora* from different regions of Uttarakhand. *Viola* species seem to occupy the substratum high in Fe content. Population genetic diversity of *V. canescens* collected from Uttarakhand was assessed using SSR markers. Population genetic structure revealed the significant differentiation between the subpopulation of the species of *V. canescens* Wall. occurring in Garhwal and Kumaon regions of Uttarakhand. Relatively low genetic differentiation in Kumaon populations and high differentiation in Garhwal population. *Viola* species possess medicinally important class of stable cyclotides that are peptide in nature. *V. canescens* showed the presence of cyclotide sequences that depicted the mobius type of structure with 83 % homology with previously known cyclotides i.e. circulin A. Cyclotides from *V. canescens* revealed unique mass ranges and potent antimalarial activity against *Plasmodium falciparum* strain Pf3D7.

Contents

1. Reproductive attributes of selected species of Viola viz. Viola canescens Wall. and V. biflora L. of Western Himalayan region (Uttarakhand) 2. Detection and identification of mycorrhizal fungal association in the roots of V. canescens Wall and V. biflora L. using ultrastructural studies from different locations of Western Himalaya 3. Analysis of various metals in substrate as well as in plant tissue (leaves) of Viola canescens and V. biflora collected from the different sites of Uttarakhand 4. Population genetic studies in V. canescens using SSR markers 5. Detection of sycloptides from different species of Viola 6. Summary and conclusions. References.

12. MEENA (Devender Singh)

Taxonomy and Phylogeny of the Genus Indigofera L. (Fabaceae) in India.

Supervisor : Prof. Arun K. Pandey

Th 23926

Abstract (Not Verified)

The genus *Indigofera* (tribe Indigoferae, Fabaceae) includes approximately 775 species distributed throughout the tropical and subtropical regions of the world. In India, the genus is represented by 60 species of which 16 species are endemic. In the present study, a detailed taxonomic and molecular systematic study of the genus *Indigofera* in India is provided. Two new species, *Indigoferacurvicarpa* and *I. pandeyana* have been described. *Indigoferaamblyantha*, has been reported as first record from India. *Indigoferazollingeriana*, *I. arrecta* and *I. bracteata* were collected and reported for the first time from Meghalaya. *Indigoferaheterantha* and *I. tinctoria* were reported for the first time from Arunachal Pradesh. Molecular phylogenetic analysis, based on ITS and *matK* sequence data reveals that the *Indigofera* species in India form a monophyletic group. Taxonomy of the *dosua* complex which includes *Indigoferaheterantha*, *I. dosua*, *I. dosuavar. simlensis* and *I. himachalensis* has been resolved. All the unifoliolate *Indigofera* has Brochidodromous type of leaf venation pattern except *I. nummulariifolia* which has Fестоoned brochidodromous. Evolution of leaf forms (unifoliolate, trifoliolate and multifoliolate) was examined by ancestral state reconstruction. Our results reveal that immediate last common ancestor for the ingroup were either trifoliolate or multifoliolate. Unifoliolate condition within the group is showing synapomorphy, this condition has evolved at least four times. Revision of unifoliolate *Indigofera* from India has been done. Seeds of *Indigofera* are generally cylindrical in shape, but seeds may be spherical (*I. glandulosa*), discoid (*I. hochstetteri*), boat-shaped (*I. nummulariifolia*) or bilateral (*I. prostrata*). Spermoderm pattern varies from tuberculate (*Indigoferalinifolia*), retico-rugulate (*I. nummularifolia*), retico-tuberculate (*I. cordifolia*), stellate (*I. dalzellii*), psilate (*I. mysorensis*) to matted type (*I. arrecta*, *I. himalayensis*). The seed coat consists of an outermost layer of macroscleried, followed by osteosclerieds and innermost parenchymatous layers.

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1. Introduction 2. Taxonomy history and classification 3. Molecular phylogenetic analysis 4. Taxonomic revision of unifoliolate *Indigofera* 6. Seed morphology and anatomy. Summary .Literature cited .Appendices.

13. PANDIT (Shatakshi)

Characterization of Phospholipase D α 1, Identification of Novel Phosphatidic Acid (PA) Binding Proteins and Motif Essential for PA-SPHK1 Interaction.

Supervisor : Dr. Girish Mishra

Th 23920

Abstract (Verified)

Phospholipase D (PLD) and its product Phosphatidic Acid (PA) has been implicated in various cellular processes such as programmed cell death, cell growth, vesicular trafficking, cytoskeletal changes, root growth, a variety of stresses especially osmotic stress and

wounding. This study was conducted to investigate signaling networks mediated by PLD α 1, PA and their interacting components using model plant *A. thaliana*. Studies were initiated by analyzing PLD promoters and the results show various *cis*-acting elements involved in biotic, abiotic stresses and developmental processes. A minimal functional promoter at -1134 nt 5' upstream region was identified and validated. Further, experiments were designed to identify role of PLD α 1 for expression of downstream effector proteins during drought stress. 2D-PAGE followed by Mass spectrometry analysis led to the identification of 4 proteins which were absent in *plda1* KO while abundant in control plant. Quantitative expression studies revealed negligible expression of these four genes in *plda1* KO while enhanced expression was observed in Col-0 plants under ABA treatment. These proteins are involved in biotic stress response suggesting a critical role for PLD α 1 in biotic stresses. PA acts via binding and translocating target proteins to membrane. Owing to the importance of PA-protein interactions, a proteomic approach through mass spectrometry revealed 23 novel PA binding proteins indicating their involvement in various cellular processes. Validation of lipid binding property was also examined for two selected proteins. Further to identify PA binding region on AtSPHK1, a known PA interactor, various deletion fragments were recombinantly expressed, proteins were purified and analyzed through lipid overlay assay. Results suggested the involvement of N-terminal region, between 110-205 amino acids, in binding with PA. *In-silico* SPHK1-PA docking study indicated involvement of VSGDGI in the hydrophobic region. Deletion of amino acids VSGDGI perturbed PA-AtSPHK1 binding, suggesting its essential role in PA-AtSPHK1 binding.

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1. Characterization of *cis*-acting elements and identification of minimal functional promoter of PLD α 1 in *Arabidopsis thaliana* 2. Comparative expression profiling of Col-0 WT and *plda1* KO plants of *Arabidopsis thaliana* under ABA treatment 3. Identification of phosphatidic acid binding proteins present in *Arabidopsis thaliana* 4. Identification of phosphatidic acid binding domain on *Arabidopsis thaliana* sphingosine kinase. Summary. Appendices.

14. PURI (Renu)
Phylogenetic Biology of *Impatiens* L. : New Species, Unusual Fatty Acids, Fruit Form and Seed Dispersal, Time-Tree and History of Disjunctive Distribution.
Supervisor : Prof. R. Geeta
Th 24252

Abstract (Verified)

Balsaminaceae family consists of two genera : the monotypic genus *Hydrocera* (L.) Wight & Arn and one of the largest genera *Impatiens* L. In India *Impatiens* is disjunctively distributed in Western Ghats and north-eastern Indian region. An extensive survey of the literature revealed nine genera with disjunct endemic species in the Western Ghats and north-eastern India whose global phylogenetic study included at least one Indian species. These phylogenetic studies show that Indian species in each genus are non-monophyletic and the Indian flora is influenced by the Chinese, South East Asian and African flora. *Impatiens* species were collected from Goa, Bangalore, Kerala, Sikkim and Meghalaya. A new species of *Impatiens* Sikkim was collected. There are two fruit forms in *Impatiens*. The variation in dispersal distances across species was significant. There were no trade-offs between seed number and seed weight. All *Impatiens* species (19) in this study contained parinaric acid (all four forms), but the closely related *H. triflora*, and *A. deliciosa* (Actinidiaceae) and *M. zapota* (Sapotaceae) did not contain any. Phylogenetic analysis revealed that Indian *Impatiens* is non-monophyletic. 9 major clades (Clades A- I) and 2 species lineages *I. circaeoides* and *Impatiens* sp. 2 were recognised. Ancestral state reconstructions suggest that the ancestral Balsaminaceae

had an indehiscent fruit. The ancestral fruit of Impatiens was dehiscent fusiform. The linear fruit form evolved unequivocally 15 times in different clades. The ancestors of the Balsaminaceae and Impatiens had 3-colpate pollen. There was a shift from 3-colpate pollen to 4-colpate pollen within Impatiens. Biogeographic analysis revealed Impatiens originated in China from where it into to north-eastern India (24 ma) and South East Asia, while diversifying in China.

Contents

1. A phylogenetic approach towards systematic and biogeography if Indian flora 2. Descriptions of five common species and one new species collected in Sikkim 3. Seed dispersal in some species of Impatiens L. 4. Parinaric acid, an unusual conjugated fatty acid, occurs in several Indian species of Impatiens L., but not in its closed relative Hydrocera triflora (L.) wight & Arn. (Balsaminaceae) 5. Molecular phylogeny and biography of Impatiens L. (Balsaminaceae). Perspective. Appendices.

15. SHARMA (Upasana)
Biotechnological and Biochemical Investigations of Two Traditional Medicinal Plants : Chitrak (PLUMBAGO ZEYLANICAL.) and Karu (GENTIANA KURROO ROYLE) and Evaluation of their Anticancerous Potential Against Human Cancer Cell Lines.
Supervisor : Prof. Veena Agrawal
Th 24314

Abstract (Verified)

For current investigation, two medicinal plants were selected : Plumbago zeylanica L. and Gentiana kurroo Royle. In P. zeylanica, aseptic cultures were established using nodal explants of elite (Hamirpur) chemotype and roots obtained from these cultures were used further to regenerate complete plantlets. Regenerants were successfully hardened and found to be genetically fidel, using ISSR and SCoT markers. For abiotic elicitation of plumbagin, root callus was treated with different concentrations of yeast extract, salicylic acid, L-tyrosine or sodium acetate for 30-d, resulting in maximum enhancement of 6.5, 3.4, 2.63 and 2.07-fold, respectively. For biotic elicitation, eight hairy root lines were obtained after transforming leaf explants with Rhizobium rhizogenes (A4). R5 root line showed maximum plumbagin (8.92-fold) and maximum growth index (0.86±0.002). Shoots co-cultivated with Piriformospora indica, exhibited marked increase in shoot biomass (1.07-fold), root biomass (9.11-fold) and plumbagin content (1.41-fold). Methanolic root extract showed significant cytotoxicity against Mia-pa-ca-2 (IC₅₀=55.96 µg/mL) and Colo320DM (IC₅₀=48.25 µg/mL) cancer cells. G. kurroo roots were extracted in chloroform, ethyl acetate and methanol. FT-IR analysis revealed the presence of phenols and flavonoids in all the extracts. Maximum phenolic and flavonoid content was recorded in methanol and chloroform extract, respectively. GC-MS and LC-MS exhibited many pharmacologically important compounds. Methanolic extract was found to be the most efficient scavenger of DPPH (IC₅₀=114 µg/mL), H₂O₂ (IC₅₀=109.9 µg/mL) and O₂- (IC₅₀=74.63 µg/mL). Gentiopicroside was isolated from methanolic root extract using silica gel column chromatography and collected fractions were characterized using TLC, HR-LCMS, FT-IR and ¹H-NMR. Silver nanoparticles were synthesized from G. kurroo aqueous root extracts (GkAgNPs) and characterized using UV-visible spectroscopy, TEM, FE-SEM, DLS, EDAX, XRD and FT-IR. GkAgNPs were anisotropic, polydisperse crystalline, stable, face centered cubic and exhibited variable size (50-300 nm). HeLa cells treated with GkAgNPs exhibited strong cytotoxicity (IC₅₀=1.964 µg/mL), reduced cell viability and survival efficiency.

Contents

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16. SINGH (Swati)
An Analysis of Evolution, Function and Regulation of KCS6 and KCS5, key Cuticular Wax Biosynthesis Genes in Arabidopsis Thaliana and Brassica Juncea.
Supervisor : Prof. R. Geeta
Th 23927

Abstract (Not Verified)

Cuticular wax is a major novel morphological adaptation that evolved during the transition of plants from aquatic to terrestrial habitats. The chemical composition of waxes is highly diverse, ranging from Very Long Chain Fatty Acid (VLCFA) to alkanes, esters, alcohol, and secondary metabolites. The condensation step of wax biosynthetic pathway is catalysed by the enzyme, β -ketoacylCoenzymeA synthase (KCS). Members of the KCS gene family are known to be expressed in a substrate- and tissue- specific manner and encode rate limiting enzymes essential for fatty acid biosynthesis. The present study investigates the evolutionary history of KCS6 and KCS5 (key genes involved in cuticular wax biosynthesis), through phylogenetic reconstruction across green plant lineage and synteny analysis across Brassicaceae. A large segmental duplication that occurred at base of Brassicaceae was found to be responsible for the KCS6-KCS5 paralogy. Functional characterization of KCS6 and KCS5 from *Arabidopsis thaliana* and *Brassica juncea* revealed sequence similarity and functional equivalence in enzymatic activity. Analysis of over-expression and gene silencing lines of KCS6 of *A. thaliana* revealed altered cuticular wax load and involvement in abiotic stress tolerance. Comparative sequence analysis of *cis*-regulatory sequence of KCS6 and KCS5 across Brassicaceae showed presence of several conserved and variable Transcription Factor Binding Sites (TFBS). Comparative functional analysis of the promoters of paralogous gene pairs KCS6 and KCS5 from *B. juncea* and *A. thaliana* using promoter : :reporter transcriptional fusion lines revealed regulatory diversification between homologs of KCS6 and KCS5 of *A. thaliana* and *B. juncea*. A 87 bp intron of KCS6 in *A. thaliana* was demonstrated to be a transcriptional repressor.

Contents

1. Introduction 2. Review of literature 3. Material and Methods 4. To identify and estimate evolutionary relationship among KCS6 and KCS5 – key genes of cuticular was biosynthetic pathway 5. To identify, isolate and perform sequence and functional characterization of KCS6 and KCS5 during development and stress 6. Summary and conclusions. References. Appendices.