CHAPTER 6

BOTANY

Doctoral Theses

01. BERRY (Eapsa)

Ecological, Evolutionary, and Developmental Studies of Corolla Pigment Patterns in Some Angiosperms; With Observations on Two Morphotypes in JusticiaAdhatodaL.

Supervisor:Prof. R. Geeta

Th 23248

Abstract

(Not Verified)

Angiosperms very commonly show patterns in corolla pigmentation in addition to the general flower colour. Such patterns on corolla can be stripes, spots, and blotches or combinations of these. This study is broadly a comparative ecological, evolutionary and developmental analysis of corolla pigment patterns (CPP) in angiosperm flowers basically using some case studies. The main objectives of the study were the following: Investigation of relationship of CPP variation in floral morphs of *Justiciaadhatoda*with other floral traits, pollinator preference, and fitness correlates. Since floral symmetry and pigment pattern symmetry are believed to go together, the hypothesis of evolutionary correlation between corolla monosymmetry and mere presence of CPP in *Rhododendron* was tested using comparative phylogenetic methods. Survey of morphological development of CPP as opposed to background pigmentation across angiosperms. An inter-population study to determine and investigate two probable morphotypes in *Justiciaadhatoda*across Indian populations. Based on the objectives and results of the study the conclusions arrived at can be summarized as follows: Variation of corolla vein pigmentation in *adhatoda*does not affect pollinator preference or any of the floral traits studied; but the different proxies for fitness show that balancing polymorphism exists in the studied population as an example of polymorphic adaptation Presence of CPP and floral monosymmetry in *Rhododendron* show evolutionary correlation. Most angiosperm flowers show that CPP appears before background pigmentation on the developing corolla. Two distinct morphotypes of *adhatoda*exist separately in the northern-western and southern-eastern parts of India. Moreover, these morphotypes suggest adaptation to drier and wetter regions of the country. This study underlines some still unaddressed general and particular aspects of CPP. It also raises questions related to the biology of CPP in angiosperms, and the ecology and evolution of *Justiciaadhatoda*.

Contents

02. BIDALIA (Ankita)
Performance of Tree Under Abiotic Stress In Keoladeo National Park Bharatpur, Rajasthan, (India)
Supervisor:Prof. K. S.Rao
Th 23736

Contents

1. Introduction 2. Review of literature 3. Study area 4. Diversity and structure of trees in keoladeo national park 5. Modeling tree diameter distribution of the important trees in keoladeo national park 6.Mitragynaparvifolia (Roxb).Korth.Seeding survival in keoladeo national park 7. Tolerance of naci induced salinity in mitragynaparvifolia (roxb.) korth. Seedings in keoladeo national park Tolerance to waterlogging by mitagynaparvifolia (roxb.) kort. And syzygiumcumini (L.) skeels.Seedings in keoladeo national park.Summary and conclusions.References.Plates Publications.Conference and seminar.

03. CHANDAN BARMAN
Reproductive Ecology of Two Threatened Tree Species: WrightiaTomentosa (Roxb.) Roem.&Schult.andSalvadoraOleoidesDecne.
Supervisor:Prof. Rajesh Tondon
Th 23259

Contents

1. Introduction 2.Results 3.Discussion 4.Summary and Conclusions. Literature cited. Annexures.Illustrations.

04. CHOUDHARY (Ashish Kumar)
Fatty Acid Profiling in Genus LeucasR. Br. (Lamiaceae).Characterization of Fatty Acid Desaturases and Comparative Trancriptome Analysis to Study Unusual Fatty Acid Biosynthesis in L. Cephalotes (Roth) Spreng.
Supervisor: Dr. Girish Mishra
Th 23256

Abstract
(Verified)

Biosynthesis of unusual allenic fatty acid, "laballenic acid", is an interesting biochemical process in lipid metabolism. Laballenic acid has anti-inflammatory activity and is uniquely synthesized bysome members of Lamiaceae family, including *Leucas*. This thesis comprises of: a comprehensive study of fatty acid profiling in the genus *Leucas*, and an investigation of unusual allenic fatty acid biosynthesis in *Leucascephalotes*. Seeds fatty acid profiling of 26 species and 5 varieties of *Leucas*revealed laballenic, oleic and linoleic acids as the major constituents. Three species namely, *L. hirta, L. ciliata*var. *vestita*and *L. helianthimifolia*contained ≥ 40% laballenic acid. Fatty acid profile of *Leucas*can be useful as a chemotaxonomic trait, and it bears great potential to be explored for pharmaceutical and nutritional purposes. Desaturation reaction in fatty acid biosynthesis is catalyzed by membrane and soluble desaturases. Therefore, membraneassociated *FAD2*, *FAD3* and three soluble acyl-ACP desaturases were isolated and characterized from *L. cephalotes*. *FAD2* expressing transgenic yeast accumulates palmitolinoleic (16:2Δ) and linoleic acid (18:2Δ) while FAD3 expression accumulates linolenic acid (18:3Δ) only. On the basis of sequence similarities, a divergent acyl-ACP desaturase was isolated. Expression of this divergent desaturase was ~146333 fold higher in developing seeds than roots and negligible in other tissues. Functionality of divergent acyl-ACP desaturase was confirmed via transgenic expression in *Arabidopsis* exhibiting its activity as Δ -acyl-ACP desaturase. Fatty acid profiles from various parts of *L. cephalotes*confirmed highest laballenic acid content in

developing seeds (25.91%) and showed its absence in petals. Transcriptomic analyses of seeds and petals tissues confirmed that Δ -acyl-ACP desaturase expressed only in developing seeds. Functional characterization, expression and transcriptomic analyses of Δ -acyl-ACP desaturase strongly suggest its crucial role in laballenic acid formation. Further, transcription factors and SSR markers were also identified from transcriptomic data that may be used for future analyses.

Contents

1. Introduction 2.Isolation, identification and characterization of fad2 and fad3 gene from cephalotes 3. Isolation, identification and characterization of plastidial acyl- acp desaturases from leucascephalotes 4. Comparative genome wide expression profiling of various fatty acid desaturases to understand unusual fatty acid biosynthesis 6. Summary 7.Appendices 8. List of publications and conference presentiations

05. MOHAN LAL

Isolation and Characterization of an Antagonistic Bacterium, Bacillus sp. JES1 and Molecular Characterization of its Antimicrobial Surfactin (sfp) Gene.

Supervisor: Prof. Ved Pal Singh and Dr. Rakesh Tuli

Th 23735

Abstract
(NotVerified)

Biocontrol is an emerging effective, safe and eco-friendly alternative to chemicals to control plant diseases. An antagonistic bacterium isolated from the surface of fresh litchi (*Litchi chinenesis*Sonn.) fruit collected from litchi garden of Haridwar (Uttarakhand). This bacterium showed antagonistic activity against the fungus isolated from infected litchi fruit, in dual culture assay. Based on phylogenetic analyse analysis of 16S rRNA gene sequences analysis, the antagonistic bacterium was identified and designed as *Bacillus* sp. JES1 and the fungus exhibited maximum (99%) similarity to *Fusarium* species, based on phylogenetic analyses of 18S rRNA sequences. The optimal growth conditions of the antagonistic bacterium were standardized. The growth optima of the bacterium were found to be 25°C, pH 6 and 1% NaCl concentration. Maximum growth was observed in basal medium using glucose (as carbon source) and ammonium chloride (as nitrogen source). Generally, in the antagonistic bacteria related to the genus *Bacillus,* the antimicrobial genes *sfp, itu, fen*D and *bam*C were frequently present which are responsible for lipopeptides production. Among them, *sfp*and *itu*genes were present in *Bacillus* sp. JES1, as revealed by PCR method. The gene *sfp*is responsible for the production of surfactinlipopeptide, which shows antimicrobial activity. For confirmations of surfactin bioactive compound, LCMS analysis was done. The surfactin (*sfp*) gene (675 bp) was cloned and sequenced, and in BLASTN analysis (NCBI), it matched 99% similarity with *sfp*gene of *Bacillus subtilis*. The molecular, phylogenetic and comparative phylogenetic analyses were done for amino acid sequence of deduced surfactin (*sfp*) gene of *Bacillus* sp. JES1. On the basic of gene sequencing and LC-MS analysis, the presence of the *sfp*gene in *Bacillus* sp. JES1 was confirmed. Because of its antagonistic properties, the isolated bacterium, *Bacillus* sp. JES1 could serve as a biocontrol agent for plant diseases suppression.

Contents

1. Introduction 2. Material and Methods 3.Results 4.Discussion 5.Summary 6.Conclusion.References. Publication and conferences

06. PATIAL (Vandna)

Studies on Biochemical and Molecular Characterization of Fatty Acid Biosynthetic Pathway in Safflower (CarthamusTinctoriusL.)For Oil Quality Improvement by Transgenic Approaches.

Supervisor:Prof. Amar Kumar

Th 23251

Abstract

(NotVerified)

To fulfill the increasing demand of quality edible oil, there is urgent need to exploit the potential of minor oilseed crops. Safflower (*Carthamustinctorius*L.), an important oilseed crop, contributes about 0.3% to the total oilseed production in India. Safflower cultivars under cultivation in India are low in oleic acid therefore; cultivars with high oleic acid and higher oil content are much desired. *In plants, oil biosynthesis mainly occurs in seeds and FAD2* is the main gene responsible for conversion of oleic into linoleic acid in seeds. Therefore, improved understanding of molecular aspects of complex seed developmental process and *FAD2* gene may provide novel opportunities to manipulate the safflower oil for improving its nutritional qualities and yield. The aim of present study was to determine *variability in fatty acid composition of* geographically diverse *safflower* germplasm*. Study also involve the* isolation and cloning of different *FAD2* genes, further, the transcript levels of different *FAD2* genes in different vegetative tissues, flower and seed developmental stages of high and low oleic acid safflower genotypes were also examined. Seed specific *FAD2* gene differentially expressed in high and low oleic acid safflower genotypes was identified. Furthermore, to elucidate the genes involved in seed development, genome-wide transcriptome analysis was performed at four different stages (2, 5, 8 and 10 DAP) of safflower seed development. This study provides the first comprehensive transcriptome resource data in seed development stages of safflower and will lay the foundation for further understanding of the underlying molecular mechanism involved in seed development and lipid biosynthesis. A prerequisite for genetic manipulation in a crop plant is availability of a regeneration and transformation protocol. Therefore, during the present study genotype independent, efficient plant regeneration and transformation protocol was also established.

Contents

07. PRITAM KAUR

Genome- Wide Discovery and Characterization of MirnasFromDelhi Both Tomato (Solanum Lycopersicum) Roots and Root – Knot Nematode (Meloidogyne Incognita) During Susceptible and Resistant Interations.

Supervisor:Prof. K. S. Rao

Th 23250

Abstract

(NotVerified)

Root-knot nematodes (RKNs, *Meloidogyne*spp.) are most damaging plant parasites causing severe losses to crop production. The present study reports genome-wide identification and characterization of tomato and RKN miRNAs simultaneously from RKN-infected susceptible tomato roots using highthroughput sequencing technique.RNAseq data from 11 small RNA libraries derived from five disease development stages identified 52 conserved, 4 variants of

conserved and 281 novel tomato miRNAs. The same set of RNAseq data identified 38 conserved and 290 novel RKN miRNAs. Both tomato and RKN miRNAs showed differential expression at different stages during susceptible and resistant interactions based on digital expression data. In tomato, majority of miRNAs validated through qRT-PCR were significantly upregulated across different stages during susceptible interaction. However, few conserved and a novel miRNAs were downregulated during resistant interaction. The predicted targets of 8 conserved and 1 novel miRNAs were validated through 5’RLM-RACE. Negative correlation between expression profiles of few conserved miRNAs including miR156, miR159, miR164, miR396 and their targets, *SBP*, *GAMYB-like*, *NAC* and *GRF*1 transcription factor, respectively was confirmed during susceptible interaction through qRT-PCR. Novel Sly_miRNA996 also showed negative correlation with its target*MYB-like* transcription factor. In RKN, few conserved miRNAs including miR-100_3, miR-58_1 and lin-4 showed notable differential expression at different stages during susceptible interaction. Further, targets of conserved miRNAs were predicted and few are known to be involved in nematode parasitism. Among conserved RKN miRNAs, miR-58_1 predicted to target FMRFamide-like peptide (neuropeptide, FLP). Based on digital expression analysis, negative correlation in expression of miR-58_1 and its target FLP gene was observed during susceptible interaction. To best of our knowledge, this is first comprehensive study on identification and characterization of miRNAs from both tomato and RKN.Further, role of tomato miRNAs during disease progression and RKN miRNAs during its development andparasitism in infected tomato roots have been discussed.

Contents

1. Introduction and review of literature 2. Material and Methods 3.Results 4. Discussion, Conclusion and future prospects References. Annexures. Supplementary Tables. List of PublicationsReprints publications.

08. RAESHWARI

Heavy Metal Induced Phytotoxicity,Oxdidative Stress and Cellular Damage in Senna Alexandrina Mill. (Syn: CassiaangustifoliaVHAL) and Their Reversal ThroughPiriformosphoraIndicaand Glutathione.

Supervisor:Prof. Veena Agrawal

<u>Th 23252</u>

Abstract
(Not Verified)

*Senna alexandrina*Mill.is a medicinally valuable shrub and widely used as a laxative. To evaluate the phytotoxic effects of Zn and Cu in *Senna alexandrina*, various parameters such as seedling growth, antioxidant enzymes and DNA damage were assessed. Seeds were cultured on Knop’s medium containing Zn and Cu individually in various concentrations (0, 1, 10, 50, 100 and 200 mg L). Maximum inhibition in seed germination, root and shoot length was seen at 200 mg L Zn and Cu. Atomic Absorption Spectroscopy (AAS) revealed that maximum Zn and Cu were stored in roots at 200 mg L . Antioxidant enzyme activities of superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase and glutathione reductase increased significantly under stress over control. Lipid peroxidation, H O , cell death and DNA damage increased significantly at higher concentrations of Zn and Cu over control. However, Cu proved more toxic than Zn and reversal of Cu toxicity was performed by individually employing *Piriformosporaindica,* a symbiotic fungus and glutathione, an antioxidant. Seeds were germinated on above mentioned Cu concentrations alone and in combination with *P. indica*and 10 mg L glutathione individually. *P. indica*and glutathione significantly reversed Cu phytotoxicity. Maximum increase in germination and seedling growth was achieved at 50 mg L with *P. indica*and glutathione. Similarly, maximum increase in antioxidant enzyme activity and proline content was observed at 50 mg L with *P. indica*and glutathione over Cu alone. Lipid peroxidation, H O , cell death and DNA damage were significantly decreased with *P. indica*and glutathione. *P. indica*and glutathione inhibited transport of Cu from root to shoot suggesting its potential role in phytoremediation. Sennoside bioactive content enhanced to 2292.9% over control in seedlings colonized with only *P. indica*. This study proved beneficial in heavy metal toxicity evaluation and its reversal through biotic and abiotic components.

Contents

1. Introduction 2. Review of literature 3. Materials and Methods 4.Results 5.Discussion 6.Summary and Conclusions 7.Reeferences 8.Appendix.

09. ROY (Sudip Kumar)
Reproductive Biology of Ret Tree Species of Central and Western Himalaya: Acer Caesium Wall. ex Brandis and UlmasWallichianaPlanchon.
Supervisor:Prof. Arun Kumar Pandey
Th 23245

Abstract
(NotVerified)

Himalayan plant diversity faces grave threat due to overexploitation, habitat loss, climate change, and other anthropogenic interventions. Human interference has driven a large number of tree species of this region to rare, endangered and threatened category. In order to develop a sustainable conservation strategy, the present study was undertaken with an objective to study thereproductive biology of two important RET tree species in their native habitat *Acer caesium*(Sapindaceae) and *Ulmuswallichiana*(Ulmaceae). Both the tree species are ecologically and economically important to Central and Western Himalaya. *A. caesium*is a dioecious tree species with predominantly male biased sex ratio. Flowering in male and female trees is synchronous and begins in the first week of March. Species is ambophilous i.e., pollination is accomplished both by wind and insects. *U. wallichiana*is a hermaphrodite tree species with bisexual flowers arranged in lateral recemes. Flowering begins in early March and lasts till end of April. Species exhibits anemophily and facultative xenogamy. Breeding system experiments in both the species revealed that species experiences pollen limitation. Pollen limitation causes low seed set as effective pollen dispersal in the populations is low as compared to inter-tree distances. Fruit is a samara in both the species and anemochory is the characteristic diaspore dispersal mechanism. Autoratatory and gliding movement of samaras in *A. caesium*and *U. wallichiana*ensures maximum dispersal of 100 and 130 m respectively. Species experience low seed establishment rate at forest floor and seedling survival rate is also very low. Based on the present study it is recommended that to circumvent pollen limitation due to fragmented populations is important to maintain reproductive sustainable populations and measures should be taken to protect the natural habitat of both the species to reduce the anthropogenic pressure causing unsuccessful regeneration of these two RET species

Contents

1. Introduction 2. Review of literature 3. Material and Methods 4.Observations 5. Discussion 6. Summary and Conclusions 7. Literature Cited 8. Illustrations.

10. SALONI
DNA Barcoding of Selected Medicinal Plants For Identification, Authentication and Taxonomic Circumscription.
Supervisor:Prof. Shashi B. Babbar
Th 23253

Abstract
(Verified)

DNA barcoding is a molecular diagnostic tool, which can be used to identify a species with its little amount of tissue or DNA. This unique capability of DNA barcoding opens up numerous applications hitherto not possible with traditional taxonomic methods. The present thesis describes (i) the generation and application of DNA barcodes of selected medicinal plants of high trade volume or those which are rare or endangered and (ii) the use of DNA barcoding as a supplementary tool for taxonomic circumscription. DNA barcodes of 112 medicinal plant species were generated. For checking the adulteration or substitutions prevailing in Indian markets, the market samples of 13 endangered medicinal plants were authenticated. Of 22 samples, only three samples were found to be authentic. This indicated the extent of substitutions prevailing in Indian herbal markets. DNA barcoding of three taxa was done for taxonomic delimitation/ discrimination. The first taxon studied was *Hippophae*, an important nutraceutical plant. DNA barcoding of 80 tentatively identified accessions of *Hippophae*, collected from various geographical locations in India and some procured from other countries (i) helped in correcting the botanical identifications of some accessions, (ii) confirmed the existence of its three species in India and (iii) revealed interrelationships of the investigated species and subspecies. Second was to check whether DNA barcodes distinguish naturally growing *Withaniasomnifera*and its cultivated form, raised to the species level with the name being, *W. ashwagandha*. A comprehensive analysis based on four markers, ITS, ITS2, *matK* and*rbcL*revealed that *W. ashwagandha*possesses DNA barcodes different from *W. omnifera*, thus providing additional support for the delimitation of *W. ashwagandha*. DNA barcoding of a newly discovered species of *Oberonia*i.e., *O. bopannae*and its closely allied species provided unequivocal support for the circumscription of new species.

Contents

1. Introduction 2. Review of literature: DNA barcoding of medicinal plants 3. Materials and Methods 4. Developing dna barcodes of selected medicinal plants and demonstration of their use for authentication of their market samples 5. Dna barcoding for taxonomic circumscription 6. Summary and Conclusions. References. Appendix.

11. SHABIR AHAMAD RATHER

Taxonomy, Molecular Phylogeny and Biogeography of the Genus Crotalaria L. (Fabaceae) in India

Supervisor:Prof. ArunKuamr Pandey

<u>Th 23255</u>

Abstract
(Not Verified)

The genus *Crotalaria* L. (Fabaceae, Crotalarieae) includes 702 species chiefly distributed in tropica and sub-tropical regions of the world. In India, the genus is represented by 89 species, of which 43 species are endemic. In the present study, a taxonomic study has been carried out on molecular phylogeny, biogeography, pod morphology and anatomy. Two new species, *Crotalaria suffruticosa*and *C. multibracteata*from Western Ghats have been described. *rotalariamedicagenia*var. *neglecta*and *C. sessiliflora*var. *sessiliflora*f. *garhwalensis*have been recorded for the first time. The phylogenetic tree derived from combined nuclear and chloroplast markers are well supported with a strong support for the monophyly of the genus. The DNA dataset for the four regions include ITS, *trnH-psbA*, *matK*, and *trnL-trnF*intergenic spacer. The analyses yielded ten major clades. The shift to grassland and cut slope, habitats with herbaceous and shrubby habit established independently, in a single radiation, coincides with higher rates of speciation and range expansion into the Indian sub-continent, with main diversification in the peninsular India mainly in the Western Ghats and the Eastern Ghats province, due to the tropical environment and also the connectivity of the land mass to the water currents indirectly coming from the India ocean. The pod morphology shows extreme diversity in terms of size, colour, persistence of calyx, presence of trichomes, and number of seeds per pod. Pod pericarp anatomy reveals three major groups in the genus. In these pods the role of sclerenchyma is taken over by the pod endocarp and hence it was observed that the seeds were not dormant and readily germinated. Adaptive features play an important role to acclimatize these species in their habitat and assist in their dispersal. The multivariate nalyses reveal that in taxa included in Section *Calycinae*there is two times evolution of pods.

Contents

1. Introduction 2. Discovery of two new endemic species of crotalaria (fabaceae, crotalarieae) from western ghats, India and two new records to the flora of

Himachal Pradesh (India) from sirmour district. 3. Molecular phylogeny and classification of the genus crotalaria in India inferred from sequence data of ITS, matk, trnH-psbA and trnL-trnF 4. To understand the biogeography and estimate the Indian crown and stem ages and to provide and explanation for the current day distribution of crotalaria by molecular dating 5. Systematic and evolutionary significance of the pod morphological and anatomical characters with special reconstruct the ancestral states of taxonomically important characters. Summary.References.List of publications.

12. SHARMA (Esha)

Characterization of Mutants to Identify The Roles of Three Genes in Virulence of Botrytis Cinerea Person ex. Fries

Supervisor:Prof. Rupam Kapoor

Th 23258

Abstract

(Verified)

*Botrytis cinerea*is one of the most scrupulously studied phytopathogenic fungi. It inflicts grey mould disease in over 500 plant species. The damage caused by this pathogen incurs annual losses of up to $100 billion worldwide. Due to its high plasticity and significant economic osses, the pathogen has captivated great attention in researchers to resolve its complexity. A large number of candidate genes have been decoded in past few years. However, only a few have been explicitly linked to virulence. In this study, *Agrobacterium tumefaciens*mediated transformation of *B. cinerea*was carried out to gain some novel insights into its virulence strategy. Three transformants that showed significant impairment in pathogenicity even after repeated rounds of screening were selected for further characterization. The transformants were identified by walking through the known flanking insert end of borders via Thermal Asymmetric-Interlaced PCR (TAIL-PCR). The tagged gene sequence in the transformants showed homology to a Diacylglycerol O-acyl transferase 2 (*BcDGAT2*) an enzyme that plays a vital role in triacylglycerol synthesis, nucleoporin complex protein-184 (*BcNup-184*) that plays an important role in transport of macromolecules across the nuclear envelope and a predicted protein that was unique to *B. cinerea*. The transformants were characterized on the basis of their phenotypic variations, pathogenic potential on different hosts, penetration and colonization ability and biochemical characteristics. Targeted deletion of *BcDGAT2* and *BcNup-184* was also carried out to ascertain their role in pathogenicity. Response of tomato plants in terms of magnitude of defense elicitation towards all the three mutants was also evaluated. In addition, molecular mechanism underlying defense in tomato plants against mutants was unveiled. The study has helped in better understanding of plant-fungus interactions and has provided important cues for development of bio control strategies against *B. cinerea*.

Contents

1. Introduction 2. Review of literature 3. Material and Methods 4. Characterization of dgat2 to identify its role in virulence of botrytis cinerea 5.Characterization of nup-184 to identify its role in virulence of botrytis cinerea 6.Characterization of a predicted protein to identify its role in virulence of botrytis cinerea. Summary. References. Research Publications. Posters/conferences.

13. SINGH (Deepak Kumar)

In Vitro Multiplication and Biochemical Profiling of Three Medicinal Plants, SatyriumNepalenseD. Don, HerminiumLanceum (Thumb.Ex Sw.) Vuijk and Anacyclus Pyrethrum Dc.

Supervisor:Prof. Shashi B. Babbar

Th 23257

Abstract
(Verified)

The investigations presented in the thesis have resulted in standardization of simple-to-use reproducible protocols for large scale *in vitro* multiplication of three medicinal plants, viz. *Satyriumnepalense*D. Don, *Herminiumlanceum*(Thunb. ex Sw.) Vuijk.and*acyclus pyrethrum* DC. The first two belong to the family Orchidaceae, while the third is aember of the family Asteraceae. Other related aspects investigated are: (i) use of alternative gelling agents for reducing the cost of multiplication, (ii) analysis of therapeutically important phenolic acids in orchids and pellitorine in*A. pyrethrum* in *in vitro* and *in vivo* grown plants, (iii) assessment of antimicrobial and antioxidantactivities of roots/ tubers of micropropagated and *in vivo* plants, and (iii) isolation and identificationof endophytic ungi from roots of *S. nepalense*and *H. lanceum.*For both the orchids, the protocols involved their asymbiotic germination, multiplication of theprotocorm like bodies (PLBs), velopment of shoots from PLBs, followed by rooting of shoots ondefined media. For some of these steps alternative less expensive gelling agents proved to bebetter than traditionally used agar. High performance liquid chromatographic (HPLC) analysesrevealed the presence of medicinally important phenolic acids in leaves and tubers of *in vitro* and*in vivo* plants of both species. Eight and three fungal species were isolated from *in vivo* tubers of*S. nepalense*and *H. lanceum,* respectively. The standardized protocol for in vitro multiplication of*A. pyrethrum* involved *in vitro* seed germination followed by regeneration of shoots from thecotyledonary nodal explants. The regenerated shoots could be rooted to get plantlets. HPLCanalyses of different plant samples revealed the presence of pellitorine in all, with the contentbeing the highest in the in *vivo* root. Eugenol was detected in market root sample and *in vivo* rootsonly.

Contents

14. SINGH (NeerKomal)
Genomic Organisation and Evolution AcrossBrassicaceae, and Functional Characterization of Fatty Acid Elongase 1 From Brassica Juncea.
Supervisor:Prof.Sandip Das
Th 23249

Abstract
(Verified)

An important area of research is improvement in oilseed production and their nutritional value by enhancing essential components such as Poly-unsaturated fatty acid (PUFA), and by reducing the harmful compounds. Rapeseed-Mustard oil stands third after groundnut and soybean oil in terms of production in India but its use is still debated due to the presence of a Very Long Chain Fatty Acid (VLCFA), erucic acid. Erucic acid is synthesized by Fatty Acid Elongase 1 (FAE1) and down-regulation or complete silencing of *FAE1* can be used as a strategy to improve oil quality. The present thesis deals with understanding the organization and evolution of KCS18 (*FAE1*), study fatty acid profile across Brassicaceae and functionally characterize *FAE1*. We performed comparative genomic analysis of *KCS18/ (FAE1)* between the nine Brassicaceae members and found that KCS18 is arranged in a tandem manner with KCS17 across all members. This cluster was present in only one sub-genome, out of LF, MF1 and MF2, each in *B. rapa, B. oleracea*and *B. napus*but it is present in all the three sub-genomes of *C. sativa,* an outcome of genome fractionation. Phylogenetic analysis revealed an ancient duplication to be the likely cause of expansion of KCS gene family. We generated knock-down mutants of *FAE1* using artificial miRNA and transgenic lines were functionally characterized where erucic acid levels were found to be reduced by 50%; presence of cleavage product confirmed amiRNA mediated silencing of *FAE1*. Sequence characterization of promoter region of *KCS18* across Brassicaceae identified speciesspecificpolymorphsims along with differences in transcription factor binding site (TFBS) motifs. Fatty acid profiling of seeds of different members of Brassicaceae members was performed that revealed diverse germplasm base. Finally, data on steady-state levels of *FAE1* transcript and fatty acid profile during different stages of seed development was generated.

Contents

1. Introduction 2. Review of literature 3. Materials and Methods 4.Comaparative genomics of fatty acid elongase 1 locus 5.Functional characterization of fatty acid elongase 1 6.Fatty acid profile and expression dynamics 7. Summary and Conclusions. References. Appendix. List of Publications

15. SRIVASTAVA (Sikha)

Acclimatization of in Vitro Raised DecalepisArayalpathraKMA 05 Clones and Assessment of Antibacterial Properties of Secondary Metabolites Produced.
Supervisor:Prof. Ved Pal Singh
Th 23254

Abstract
(NotVerified)

Decalepisarayalpathra(J. Joseph & V. Chandrasekaran) Venter, belonging to the family Apocynaceae isa critically endangered medicinal plant chosen for the study. Since its root latex is used for treatment ofpeptic ulcer, its clonal propagation, acclimatization and ssessment of antibacterial properties of thesecondary metabolites produced were studied. A secondary metabolite, 2-hydroxy, 4-methoxybenzaldehyde was produced by its root and internodalcalli. The acclimatization was done usingsilver nitrate (SN) and thidiazuron (TDZ). TDZ treatment was effective for the survival of plantlets underfield conditions. A phyllospheric bacterium, *Methylobacterium*sp. VP103 was isolated, which producedmany secondary metabolites, including Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-,3-(2-methylpropyl)(PPDHMP). Two fungal strains, *Cladosporium*sp. SS5 and *Epicoccum*sp. SS6 were isolated from therhizoplane of field established *D. arayalpathra*KMA 05 clones. Strain SS6, which inhibited the growth ofstrain SS5, also produced PPDHMP, thus exhibiting its biocontrol potential. Bio-priming studies on *D.arayalpathra*KMA 05 clones using *Methylobacterium*sp. VP103 suggested that this bacterium can serveas a plant growth promoting bacterium (PGPB). The lyophilized bacterium powder (LBP) of*Methylobacterium*sp. VP103 has been implicated in the elicitation of secondary metabolites in *D.arayalpathra*KMA 05 clones. GC-MS profiling showed the presence of α-amyrin and 2-methoxy, 4-vinylphenol. The amount of α-amyrin was enhanced in root extracts of LBP treated plantlets. The rootextracts of plantlets showed antibacterial activities against all five bacteria tested, with maximumantibacterial activity against *Rhodococcus*sp. UKS7. Thus, *D. arayalpathra*KMA 05 clones can beexploited for the production of α-amyrin from its *in vitro* grown roots using *Methylobacterium*sp. VP103as an elicitor. The molecular docking studies suggest that a secondary metabolite, α-amyrin of plantorigin can be used in the near future for making potential drugs against *Helicobacter pylori* which causespeptic ulcers in human beings.

Contents

1. Introduction 2. Review of literature 3. Standardization of protocols for in vitro propagation and ex establishment of decalepisarayalpathra KMA 05 clones and secondary metabolite production 4. Isolation and identification of microorganisms from the phyllospheric and rhizoplane regions of decalepisarayalpathra KMA 05 clones and their screening for secondary metabolites production 5. Bio – priming of tissue culture raised decalepisarayalpathra KMA 05 clones with phyllospheric bacterium, methylobacterium, sp.vp 103 6. In vitro secondary metabolite production from the roots of decalepisarayalpathra KMA 05 clones and their antibacterial potential using methylobacterium sp. Vp 103 as an elicitor 7. Comparative in silico analysis for assessing antibacterial (pyrrolo[1,2- a] pyrazine -1,4-dione,

hexahydro -3-(2-methylpropyl) and plant (alpha – amyrin, 2-methoxy-4-vinylphenol) origins against helicobacter pylori 8. General Discussion 9.Summary.References.Appendix.

16. THAKUR (Julie)
Studies on the Biology of two Threatened Species of the Himalayan Region-Pittosporum EriocarpumRoyle and CrepidiumAcuminatum (D. Don) Szlach.- for Developing their Conservation Strategies.
Supervisor:Prof. Prm L. Uniyal
Th 23737

Contents

1. Habit, habitat and distribution of pittosporum eriocarpum 2.To establish and efficient and reproducible method for in – vitro propagation pittosporum eriocarpum via nodal explants for large scale production 3. Assessment of the genetic homogeneity amongst in – vitro raised plantlets of pittosporum eriocarpum with mother plant using three molecular markers: start codon targeted (SCoT), Inter simple sequence repeat (ISSR) and random amplified pllymorphic DNA (RAPD) 4. Habit, Habitat and distribution of crepidiumacuminatum 5.Ultrastructural studies and molecular characterization of root-associated fungi of crepidiumacuminatum using fungal specific internal transcribed spacer regions 6.Study of phenology, reproductive potential and factors that influence the population structure of crepidiumacuminatum 7. Genetic diversity assessment of crepidiumacuminatum using start codon targetd (Scot) and inter simple sequence repeats (ISSR) 8. Prediction of suitable habitats for ex-situ conservation of crepidiumacuminatum and pittospurmeriocarpum ecological niche modelling programme (ENM).Summary.References.List of publication.

17. THOMAS (Lebin)
Production Optimization, Partial Purification and Characterization of CellulasesFrom an Isolated Actinobacterium, Promicromonospora Sp. Vp111, and Assessment of the Evolutionary Relationships and Conserved Signature Indels of Cellulases From Different Organisms
Supervisor:Prof. Ved Pal Singh
Th 23260

Abstract
(Not Verified)

Microbial cellulases are in high demand due to their various prospective industrial, agricultural andbiotechnological applications. Accordingly, three cellulolytic bacteria having non-hemolytic behaviorwere isolated from soil, characterized and identified as *Bacillus* sp. SV1, *Promicromonospora*sp.VP111 and *Pseudoxanthomonas*sp. SV2. The nutritionally varying media and physiologicalconditions studied had different effects on growth and cellulase production from these bacterialstrains. Cellulase production was induced by cellulose, though nitrogen sources were also essentialfor strains SV1 and VP111. In strain VP111, based on one-factor-at-a-time approach and responsesurface methodology, the factors such as (NH) SO , MnCl .4H O, glycine and Na-CMC were foundimportant for production of cellulases. Strain VP111 utilized commercial cellulose (Na-CMC andavicel) and untreated lignocellulosic substrates (wheat straw and sugarcane bagasse) in submergedfermentation for production of plant cell wall hydrolytic enzymes cellulases, xylanase and pectinase.The cellulases of strain VP111 showed industrially suitable characteristics, as they weremetalloproteins (requiring Co), had broad cellulosic substrate specificity, retained activities at wideranges of temperatures (20-60 °C) and pH (6.0-9.0), with organic solvents (log *P* ≥ 1.24),surfactants, denaturants and NaCl. Cellulases of strain VP111 were partially purified, usingammonium sulphate precipitation (80 % saturation) and dialysis. For strain VP111, activation energy(kJ/mol) decreased for CMCase and β-glucosidase but increased for FPase; whereas, Vmax (μMglucose/min) of CMCase

increased, for cellulases, subsequent to their partial purification. Furthermore, an *in silico* analysis revealed the presence of a convergently and divergently evolvedconserved cellulase domain along with diverse evolutionary relationships among cellulases fromArchaea, Bacteria and Eukarya. Catalytic aspartic acid and glutamic acid were found to have regularexpression patterns, although the former had higher propensity than the later. Characteristic groupand multigroup-specific conserved signature indels that were observed, could be distinctive moleculartools for the phylogeny of evolutionary related cellulolytic organsms.

Contents

18. VERMA (Priyanka)

Nutraceutical Potential of a Few Micro and MacroalgaeFrom Different Agroclimatic Zone of India

Supervisor:Prof. Girish Mishra and Prof. DinabandhuSahoo

<u>Th 23247</u>

Abstract
(Verified)

The unique morphology, biochemistry and physiology of algae have led to its usage for multifunctional properties. The presence of high protein content, essential amino acid, fatty acid, lipids,carbohydrates, vitamins, minerals etc. makes it suitable for consumption as food/feed or fornutritional benefits. On the other hand, the presence of components with antioxidant, antimicrobialactivity and other bioactive compounds makes it an important source for nutraceutical andpharmaceutical products. In the present study bioprospecting of three microalgae and thirtyseaweeds from various agroclimatic zones of India were analysed for their biochemical componentsincluding fatty acids, pigments, antioxidant properties (i.e. DPPH radical scavenging activity,Hydrogen peroxide radical scavenging activity, Ferrous Ion Chelating Ability), ash content andnutrient contents. *Eicosatetraenoic acid* (ETA) was found in *Chlorella* sp. and *Dunaliella*sp. Despitebeing low in lipid content, seaweeds are rich in essential fatty acid and Eicosapentaenoic acid(EPA) was found in *Ulva lactuca, Ulva reticulata, BoodleaComposita, Caulerpavervelansis,Dictyopterisaustralis, Dictyotadicotoma, Lobophora variegate, Iyengaria stellate,Grateloupiaindica*and *Hypnea valentine*. The utritional quality of the lipids was assessed byconsidering three indexes: atherogenicity (AI), thrombogenicity (TI) and the ratio betweenthe hypocholesteronic and hypercholesteronic. Multivariate analysis of fatty acids, pigments,antioxidant properties and ash content was carried out using Principal Component Analysis (PCA).While Agglomerative hierarchical clustering (AHC) was done for biochemical components and fattyacids of thirty seaweeds to manifest chemotaxonomic relationship among various seaweeds. Theoverall analysis suggests possibilities as natural sources of functional ingredients. These algaehave multi-functional properties and can be utilised as promising bioresource for proteins, lipids,pigments and carbohydrates for the food/feed and biofuel industry.

Contents

19. ZEESHAN UR RAHMAN

StudeiesOn Heavy Metal – Microbe Interations For Arsenic (As), Chromium (Cr) (VI), Mercury (HG), Lead (Pb) And Cadmium (Cd) With Different Bacterial Isolates

Supervisor:Prof. Ved Pal Singh

<u>Th 23246</u>

Abstract
(NotVerified)

The heavy metal contamination is a global issue of health and environmental concern. Some heavy metals including arsenic (As), chromium (Cr)(VI), mercury (Hg), lead (Pb) and cadmium (Cd) are nonthreshold toxins and referred to as toxic heavy metals (THMs). Microbial community plays imperative role for the heavy metal setting in the environment. The heavy metal-microbe interactions vary with different heavy metals, their speciation, microbial community and structure, habitats and several environmental factors. This interplay is highly complex and deserves better insight of their coupled biological and geochemical processes. Therefore, the present study involves heavy metal-microbe interactions with a view to understand bioremediation, assessment of heavy metal contamination and mechanisms of heavy metal resistance for different THMs. The first research investigation deals with characterization of three highly As-resistant bacteria, *Bacillus aryabhattai*strain VPS1, *Bacillus licheniformis*strain VPS6 and *Sporosarcinathermotolerans*strain VPS7 for heavy metal resistance and biomineralization. Further, the diversity of proteins, ArsM and ArsB and the diversity and distribution of different taxa were studied. The second investigation was carried out for bioremediation of Cr(VI) using *Enterobacter* sp. DU17. Cr(VI) reduction process was optimized with respect to physical and chemical factors. Further, molecular studies, AAS, SEM-EDX TEM and FT-IR analyses were performed to identify the Cr(VI) reduction mechanism. The third investigation embodies an assessment of heavy metal contamination and Hg-resistant bacteria in surface waters from different regions of Delhi, India. Water samples were collected from six different sites for physiochemical and heavy metal analyses. A total of 88 Hg-resistant bacteria were characterized for heavy metal and antibiotic resistance. The fourth investigation deals with an attempt to isolate and characterize the Pb-resistant bacterium, *Staphylococcus* sp. AMB-2 for the biosorption of Pb and Cd using living and dead biomasses. Further, the biosorption mechanism is identified using SEM-EDX, FT-IR andXRD analyses.

Contents