

CHAPTER 6

BOTANY

Doctoral Theses

01. AGNIHOTRI (Ashish)

Effects of Exogenous Salicylic Acid and Jasmonic Acid on the Physiological Aspects of Brassica Juncea L. Grown Under Lead Stress.

Supervisor : Dr. Chandra Shekhar Seth

Th 24972

Abstract
(Not Verified)

Brassica juncea L. cv. Varuna plants were grown in soil spiked with lead nitrate having three different concentrations (500, 1000 and 2000 mg kg⁻¹ of soil) of Pb to study Pb-toxicity and effects of exogenous salicylic acid and jasmonic acid. Salicylic acid (0.5 mM) in first experiment and Jasmonic acid (0.1 mM) in second experiment was exogenously sprayed on 30-day-old plants, twice with a gap of 1 week to check for various parameters like Pb-accumulation and subcellular distribution, plants growth, photosynthesis, oxidative stress, AsA-GSH cycle, PCs content, and clastogenicity and aneuploidogenicity of Pb. Results of exogenous SA experiment revealed that notable amount of Pb was accumulated in root, which significantly ($P \leq 0.05$) posed toxicity on morphological and growth parameters, structural integrity of epidermal and guard cells, photosynthetic pigments, and increased malondialdehyde and H₂O₂. Decrease in leaf gas exchange parameters (E, G, H₂O, A), Fv/Fm, and poor AsA-GSH cycle was observed, and striking amount of DNA damage proved the clastogenicity of Pb. However, exogenous SA revoked Pb-toxicity by promoting plant growth, increasing chlorophyll content, improving A and Fv/Fm, boosting AsA-GSH cycle, and lessening DNA damage. Results of exogenous JA experiment revealed that Pb inhibited plant growth, disturbed epidermal and guard cells structures, and worsen leaf gas exchange parameters, Fv/Fm, and decreased photosynthetic pigments. Also, DNA damage proved clastogenicity of Pb, and analysis of total genome size showed differences between unstressed and Pb-stress conditions not significant ($P \leq 0.05$), but ploidy variations proved partial aneuploidogenicity of Pb. The Pb exposure affected AsA-GSH cycle negatively, but increased PC2 and PC3 contents. Exogenous JA inhibited plant growth under unstressed, but positively regulated growth, photosynthesis, AsA-GSH cycle, PC2 and PC3 contents, and DNA damage under Pb-stress, however, imparted no significant ($P \leq 0.05$) effect on genome size and ploidy.

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02. AKHTER (Tabasum)

Genetic Diversity in Populations of the Migratory Crucifer Pest Pieris Brassicae L. from Kashmir and Delhi Using ISSRs, mtCOI and nsp Genes as Molecular Markers.

Supervisor : Prof. Sudeshma Mazumdar-Leighton

Th 24973

Abstract
(Not Verified)

Pieris brassicae L. is a recurrent pest of brassicaceous crops. Early records indicate it to be a strong migratory Lepidoptera sighted intermittently between the Himalayas and northern plains. Not much is known about migration and/or residency habits of this butterfly in India. In this study, random and progeny populations were sampled from egg clusters on *Brassica oleracea* varieties in Kashmir and also from *Tropaeolum majus* in Delhi. Neutral, mitochondrial and nuclear markers were used to obtain estimates of genetic diversity. Low to modest genetic diversity, high gene flow, population expansions and slight genetic differentiation due to local adaptation to host plant types were indicated. Only two mtCOI haplotypes were evident. Further work is required to investigate if these shared global haplotypes represent migratory and/or insecticide-resistant genotypes. In contrast, high haplotype diversity was evident in the *nsp* gene locus. This sequence polymorphism may enable larvae to detoxify harmful products of glucosinolate-myrosinase interactions in diverse host plant types. Sequences from this study were submitted to NCBI as first reports from Kashmir and Delhi. Life history traits were similar for populations associated with major cultivated *B. oleracea* varieties in Kashmir and Delhi, but percent survival and development time were significantly different ($p \leq 0.05$) on local host plant types in Delhi. Digestive physiology and gut serine protease profiles in larvae showed plasticity in physiological adaptations to dietary changes. Such metabolic diversity may enable *P. brassicae* to utilize diverse host plants encountered across its geographic range for oviposition and/or larval food. This study suggests that pest management should include diversification of cropping patterns, decrease in duration of crucifer-growing seasons and reduced insecticide applications. Non-agricultural/forest habitats should be preserved as refugia to disrupt development of *P. brassicae* genotypes resistant to insecticides and/or break-down resistance in crop varieties and transgenic plants.

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03. BHANDARI (Pradeep Kumar)
Assessment of Floristic Diversity of Basukedar Region in Garhwal Himalaya.
 Supervisor : Prof. P. L. Uniyal
Th 24974

Abstract
(Verified)

The present study mainly focussed on floristic study, vegetation analysis that includes diversity, dominance, distribution pattern, similarity and soil features in Basukedar, Rudraprayag region of Uttarakhand along an altitudinal gradient. Further, the different villages of Rudraprayag and Chamoli districts were surveyed to collect authentic information regarding traditionally used medicinal plants by local inhabitants for the treatment of various diseases. Regular field visits were carried out during 2015-2018, to document a significant data on the flora in the study area. Total of 348 plant species, 278 genera in 109 families of the vascular plants were recorded. Total 38 species of Pteridophytes were also recorded from the area. Total of 19 species of Orchids in 17 genera was observed in the study area. Most of the orchids were found epiphyte. The sampling of plant vegetation was done randomly from the lower tropics to the upper temperate region in 8 sites (S1-S8), along the elevation from 879 to 2536 m in the study area. Total of 226 releves or plots of different sizes (10*10m², 20*20m², 5*5m² and 1*1m²) was laid down at various locations of disturbed, moderately

disturbed and undisturbed sites, including 65 plots for tree species, 103 for shrubs and 58 for herbs. A total 179 species in 148 genera and 74 families were recorded. In the present study, the D-D curves attained log-normal distribution for all three strata of vegetation that indicates high diversity conditions. Among all surveyed sites, the maximum tree density was recorded between lower and middle diameter class. Random soil sampling was executed at each site of both undisturbed and disturbed zone. The ethnobotanical information was analyzed by using different quantitative indices like Use value (UV), Informant Consensus Factor (ICF), Family Use Value (FUV), Relative Frequency of Citation (RFC), Frequency of Citation, Relative Importance and Relative Important Index.

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1. General Introduction 2. To assess the floristic diversity in basukedar region of Rudraprayag district of (Uttarakhand) 3. Quantitative analysis of vegetation along altitudinal gradient of disturbed sites (DS), moderately disturbed sites and undisturbed sites (UDS) 4. To study the chemical properties of soil of different sites of forest stands 5. To document the traditionally significant medicinal plants used by local people against various ailments of both human and live stocks 6. Summary and conclusion. References. Publications.

04. BHATIA(Meenam)

Gametophyte Development and Reproductive Biology of some Homosporous Ferns.

Supervisor : Prof. P. L. Uniyal

Th 24975

Abstract (Not Verified)

The percentage of germination was found highest in *Acrostichum aureum* (95%) and least in *P. aureum* (23.03%). *Vittaria* type and polar type of spore germination pattern are observed in all the species. Semiaquatic species, *C. thalictroides*, and *A. aureum* showed *Ceratopteris*-type. The spore germinates, produces a germ filament, which led to spatulate and finally to cordate form. Semiaquatic species show rapid growth. Gametangial initiation pattern was found to be protandrous. Gametangia appeared much early in the semiaquatic species with earliest in *C. thalictroides* in 15 to 20 days. Following fertilization, sporophyte appears. The percentage of sporophyte production in composite culture was minimum in *P. bifurcatum* (20%) and the maximum was recorded in *M. torresiana* (96%). The majority of the studied species possessed mixed mating-type except for *P. aureum*, *M. scolopendria*, and *M. nigrescens* which showed intergametophytic mating system.

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1. Introduction 2. Study of reproductive biology of selected epiphytic, semiaquatic and terrestrial ferns 3. Materials and Methods 4. Results and discussion 5. Study of effects of nitric oxide on the growth and development of gametophyte and gametangial production in *Ceratopteris thalictroides* (L.) Brongn. 6. Summary. References. Publications.

05. GARIMA ANAND

Characterisation of Virulence, Biochemical and Genetic Diversity in *Alternaria Carthami* Chowdhury Isolates of India and Development of a PCR Based Assay for its Specific and Sensitive Detection.

Supervisor : Prof. Rupam Kapoor

Th 24984

Abstract
(Verified)

Safflower (*Carthamus tinctorius* L.) is one of the most important oilseed crops of semi-arid regions in the world. Alternaria leaf spot, caused by *Alternaria carthami* Chowdhury is one of the most destructive diseases of safflower. Field surveys were conducted in two major safflower growing states of India namely, Karnataka and Maharashtra for two consecutive cropping seasons (2015-16 and 2016-17). A total of 872 fields spanning 22 districts were examined to determine the occurrence and distribution of Alternaria leaf spot disease in safflower. Disease prevalence, incidence and severity varied among districts, cropping seasons, sowing periods and cropping patterns. The field survey during 2015-16 recorded higher disease in both states than the following year. Diversity among ninety-five isolates of *A. carthami* was determined using virulence assay, enzyme assays, dominant (ISSR) and co-dominant (SSR) markers. Collections and isolations were made from three major safflower producing states of India. The virulence assay categorized the population into four groups based on the level of virulence. Estimation of activities of cell wall degrading enzymes (CWDEs) yielded concurrent results to virulence assays with maximum CWDE activities in most virulent group. Eighteen ISSR primers were used and 23 polymorphic microsatellite markers were developed to assess the genetic diversity and determine the population structure of *A. carthami*. Unweighted Neighbour-joining analysis using both the marker systems differently arranged the isolates into three clusters. Microsatellite markers clustered the isolates in consonance with the virulence groups in the dendrogram. Fungicide application is considered as the most effective measure to control the disease. To shed light on the same, *A. carthami* isolates collected from safflower fields of India were assessed for sensitivity towards fungicides. Six widely used fungicides, three of each non-systemic and systemic, with different modes of action were included in the study. Mean inhibitory value (EC_{50}) values for both, mycelial growth and spore germination grouped *A. carthami* isolates in consonance with their geographical distribution. A PCR based diagnostic assay was developed for easy, quick and reliable detection of *A. carthami* in infected seeds and leaf samples of safflower. A primer set, AcSPF and AcSPR was designed using ribosomal internal transcribed spacer regions of *A. carthami* that consistently produced a distinct amplicon of 340 bp with DNA extracted from thirty *A. carthami* isolates. The sensitivity of detection was further enhanced from concentration of 100 pg by simple PCR to as low as 10 pg fungal DNA by a nested PCR assay using ITS and AcSPF and AcSPR primers. The primer pair also facilitated detection of *A. carthami* in infected seeds and leaf samples. In conclusion, the findings of the work highlight Alternaria leaf spot of safflower to be prevalent in states of Karnataka and Maharashtra in India. This necessitates research towards effective management of the disease. Late sowing and intercropping with chickpea and sorghum efficiently curtailed the occurrence of disease. High genetic and virulence diversity was observed in the collected *A. carthami* isolates from three states of India. The geographical distribution of the effective concentration values signifies the importance of site-specific management programs for Alternaria leaf spot in India. The work successfully developed a diagnostic assay for an accurate and sensitive detection of *A. carthami*.

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1. Introduction 2. Review of literature 3. Assessment of alternaria leaf spot disease in safflower 4. Assessment of variations among alternaria carthami isolates of India 5. Assessing alternaria carthami population for sensitivity to fungicide 6. Pcr based diagnostic assay for accurate and sensitive detection of alternaria carthami. 7. Summary and conclusions 8. References.

06. GOGAN(Mansi)
Physiological and Biochemical Analyses of Salt Tolerance Strategies in Sunflower (*Helianthus Annuus* L.) and some other Oilseed Plants.
 Supervisors : Prof. Satish C. Bhatla and Prof. Rupam Kapoor
Th 24986

Abstract
(Verified)

The crop growth is heavily influenced by saline soil. Salinity results in a myriad of physiological consequences as well as responses that assist in decrypting various salt tolerance responses operating in sunflower as well as in some other important oilseed crops, such as safflower (*Carthamus tinctorius* L.), groundnut (*Arachis hypogaea* L.) and soybean (*Glycine max* L.). Present work focuses on differential sensitivities and biochemical and lipotoxic responses of three different sunflower varieties in response to 120 mM NaCl. Mobilization pattern of oil bodies, the major TAG reserves found in all oilseeds, along with major intrinsic membrane proteins (oleosins) have been analyzed. Actions of major cytosolic enzymes, such as thiol protease, phospholipase D, lipoxygenase and lipase, have been quantified in seedling cotyledons to determine their modulation with respect to the seedling's tolerance to salt stress. Differential accumulation of some novel fatty acids has been elucidated, particularly in salt-sensitive sunflower variety. To decipher the role of NO, application of NO donor (DETA) and NO scavenger (DETC) has been carried out. Consequent NO-mediated post translational modifications that take place, such as tyrosine nitration and S-nitrosylation in seedling roots as well as cotyledons, have been analyzed with reference to salt stress in sunflower seedlings. Thus, this work highlights essential differences in response of a salt-tolerant and a salt-sensitive variety of sunflower to saline growth conditions, both at seedling stage as well as at mature stage. Apart from sunflower, some other major oilseed crops, like safflower, groundnut and soybean also grow in salt affected regions of India. Thus, the present work attempts to demonstrate a comparative biochemical and physiological map, aimed at understanding the variations and factors responsible for salt-tolerance abilities in different oil seedlings, with respect to their enzymatic make up and fatty acid composition.

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07. GUPTA (Praveen)
Physiological and Biochemical Investigations on 24-Epibrassinolide and Nitric Oxide Induced Responses of Brassica Juncea L.
 Supervisor : Prof. Chandra Shekhar Seth
Th 24985

Abstract
(Not Verified)

The present study investigates physiological and biochemical mechanism(s) of 24-Epibrassinolide (EBL) and Nitric oxide (NO) mediated salinity stress tolerance in Brassica juncea L. Pilot experiments showed that salinity stress inhibited the seed germination in a dose dependent manner. Results revealed that salinity stress significantly ($P \leq 0.05$) inhibited the plant growth (root length, shoot length, fresh weight, dry weight, and leaf area index) by enhancing the H₂O₂, MDA,

protein carbonyls, and methylglyoxal accumulation. In addition, salinity stress also intensified cell death and DNA damage, which subsequently affected the growth and nutrient accumulation. However, EBL supplementation alleviated the toxic effects of salinity stress by triggering the compatible osmolytes and endogenous NO production along with increasing the efficiency of AsA-GSH cycle. In addition, EBL treatment also increased the γ -ECS activity to trigger the GSH biosynthesis that subsequently increased the activities of GST, GPX, Gly I, and Gly II enzymes, thus maintaining the H₂O₂, MDA, and methylglyoxal at low levels. Moreover, combined application of EBL (10⁻⁶ M) and SNP (100 μ M) alleviated the negative impacts of salinity stress on growth and photosynthesis by up-regulating the proline and nitrogen metabolisms. In addition, combined application of EBL and SNP improved the stomatal morphology by limiting the Na⁺ ions and ABA accumulation under salinity stress. Importantly, the present study provides evidence that EBL-induced NO production primarily occurred through the activation of nitrate reductase dependent pathway, and both EBL and endogenous NO participate together in the amelioration of salinity stress by maintaining the redox balance and ion homeostasis in the cells. Taking together, exogenous application of EBL and NO seems to be a suitable approach for alleviating the salinity stress in plants. Key words: Brassica juncea L., salinity stress, 24-Epibrassinolide, nitric oxide, nitrate reductase, proline metabolism, photosynthesis, oxidative damage, AsA-GSH cycle, nitrogen metabolism.

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08. KANCHAN KUMARI

Photosynthetic Potential and Lipid Production by the Green Alga *Chlorella Vulgaris* in Response to Elevated Carbon Dioxide and Nutrient Deficiency.

Supervisors : Prof. Dinabandhu Sahoo and Prof. B. C. Tripathy

Th 24982

Abstract (Not Verified)

Limiting or starving the availability of essential nutrients such as nitrogen (N), phosphorus (P) or sulphur (S) induced triacylglycerols (TAGs) accumulation in *C. vulgaris*. Most significant enhancement in lipid yield up to 66.0 % (dcw) was obtained when the cells were cultured in N (10) deficient medium under 5% CO₂ for 12 days, followed by 56.0 and 52.3 % (dcw) under S (10) and P (10) deficient medium, respectively. However, inhibition of cell growth, sharp degradation of chlorophyll and decrease in photosynthetic rates, PSI, PSII and whole chain electron transport activity in *C. vulgaris* were observed under stress conditions. The measurements of chlorophyll a fluorescence, a non-invasive signature of photosynthesis, demonstrated that the photosynthetic efficiency is higher in high CO₂. The I to P rise was faster in high CO₂ demonstrating that the photosynthetic rate is higher in high CO₂. TEM analysis revealed that the number and volume of oil bodies were enlarged concomitantly in the stressed cells compared to control, which was further confirmed with BODIPY staining. Cultures grown under stress conditions was predominated with a mixture of saturated and monounsaturated fatty acid components, with a minor proportion of polyunsaturated fatty acid, which is desired for a good quality biodiesel. This study revealed that rapid lipid accumulation under nutrition pattern alternation, several critical genes encoding ACC and DGAT are highly related, which provides a clue for prospective metabolic engineering.

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09. KAIN (Dolly)

Isolation and Characterization of Bioactive Compounds of Achillea Millefolium L.

Supervisor : Prof. Suresh Kumar

Th 24981

Abstract
(Not Verified)

Achillea millefolium L. has been collected from poonch, Jammu & Kashmir at immature/ pre- flowering (vegetative, V) and mature/ post flowering (stem and leaves (S+L) and inflorescence (I)) stages and extracted by soxhlet and maceration. Antibacterial activity has been done by disc- diffusion method against *E. coli*, *S. aureus*, *B. subtilis* and *P. aeruginosa* with maximum activity in plant sample "V" with MIC 50µg/ml and maximum ZOI against *B. subtilis* of 19± 0.5 mm. Antioxidant potential has been determined by TPC, TFC, FRAP and phosphomolybdate assay with maximum activity in plant sample "I" i.e. 76.57667±0.52667 mg of GAE/g of extract, 18.82333±0.84667 mg of RE/g of extract, 199.799±4.48545 mg of AAE/g of extract and 327.9 467±0.534208 mg of AAE/g of extract respectively. Maximum DPPH and ABTS radical scavenging activity was also observed in plant sample "I" with 86.3± 0.00 % and 69.655± 0.245 % inhibition at 1000µg/ml concentration respectively. In-vitro antiurolithiatic activity has been determined by turbidity changes in artificial urine method, nucleation and aggregation assay with maximum activity in plant sample "I" i.e. 80 ± 0.034641 %, 41.84% and 63.41% inhibition at 1000µg/ml concentration respectively. Metabolic profiling has been done by phytochemical screening, UV- Vis spectroscopy, FT- IR and GC- MS. "Bioassay guided fractionation" of plant sample "V" confirms the derivatives of vitamin D3, presqualene diphosphate and methoxy derivative of luteolin as antibacterial compounds of *A. millefolium*. Chitosan nanoparticles of inflorescence of *A. millefolium* have been prepared by ionic gelation method and characterized by UV spectroscopy, FT- IR, DLS and TEM. Antibacterial screening has been done by well- diffusion method with ZOI of 30± 0.5 mm against *B. subtilis* and *P. aeruginosa*. Antiurolithiatic screening has been done by nucleation and aggregation assay with maximum 68 % inhibition at aggregation stage. Current study justifies the excellent therapeutic potential of *A. millefolium*.

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10. KATIYAR (Arpana)

Comparative Analysis of Arabidopsis Thaliana N-myc Down Regulated Like (NDL) Gene Family and Detailed Molecular and Functional Characterization of Selected Candidates.

Supervisor : Dr. Yashwanti Mudgil

Th 25541

Abstract
(Verified)

Overall objective of the present study was to find-out the molecular mechanism of action of AtNDL1 protein in Arabidopsis. We started with comparative analysis of

Arabidopsis thaliana N-myc Down Regulated Like (NDL) gene family members AtNDL1, AtNDL2 and AtNDL3 in AGB1 dependent manner. Our analysis revealed specific localization/expression during different stages of development for all three AtNDL family members. Stress analysis after treatment with various hormonal and abiotic stresses showed stress and tissue specific differential expression patterns for all three AtNDL members and role in dehydration stress along with specific responses to various treatments. Their specific expression patterns were affected by the presence of interacting partner AGB1. Detailed molecular and functional characterization of the twelve selected candidates, putative NDL interactors in research (PNIR) from AtNDL interactome has been performed to find AtNDL mediated protein-protein interaction signaling networks. Interactions between AtNDL1 and eight PNIRs were reconfirmed by Yeast two hybrid assays, and in vivo interactions between AtNDL1 and four PNIRs were confirmed using BiFC analysis. Two T-DNA insertion homozygous alleles in PNIR were successfully isolated, vq32-1 and cks2-1 both showed altered root phenotypes and were characterized in detail. Phenotypic characterization of vq32-1 mutant revealed a reduced number of stomata and delayed bolting. The endo-duplication was not affected in both mutants. vq32-1 mutant displayed enhanced root growth at inhibitory concentration of sucrose and was susceptible to *B. Cinerea* infection. AtVQ32 showed nuclear localized protein present in all parts of the plant, except for petals and seeds. Site-directed mutagenesis of VQ motif didn't affect nuclear interaction with AtNDL1. Increased levels of the AtVQ32 protein during drought stress indicated role of AtVQ32 during drought stress. Yeast and pRD29A-LUC stress assays indicated that both AtNDL1 and AtVQ32 work together during salt stress response. Phylogenetic analysis of the AtVQ gene family revealed expansion of gene family due to whole/segmental duplication events of Brassicaceae. AtCKS2 second PNIR subjected to detailed analysis also revealed nuclear localization of the protein. AtCKS2 along with AtNDL1 indicated functioning together during salt stress response while AtCKS2 alone did not show any response to salt stress in yeast. Overall, our analysis proves that AtNDL proteins play important roles during different stages of plant growth and stress responses. Various functions of AtNDL proteins in plant during development and stress responses specifically to salt and drought stress are mediated by protein-protein interaction with specific interactors like AtVQ32 and AtCKS2 under different conditions.

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1. Introduction 2. Review of literature 3. Comparative analysis of *Arabidopsis thaliana* n-myc down regulated like (NDL) gene family in agbi dependent manner 4. Selection, cloning, knockout mutant isolation and in vivo interaction studies of the selected putative NDL integrators (PNIRs) from NDLI interactome 5. Detailed molecular and functional characterization of the PNIRs in NDLI regulated developmental processes 6. Material and methods 7. Summary and conclusion. Tables. References. Appendix.

11. LAISHRAM SUNDARI DEVI
Silico, Phylogenetic and Differential Expression Analyses of CcEZI in Apomictically and Sexually Reproducing Plants of *Cenchrus Ciliaris* L. and Optimization of Gene Gun Mediated Genetic Transformation of *Cenchrus Ciliaris* L.

Supervisor : Prof. Vishnu Bhat
Th 24983

Abstract
(Not Verified)

Endosperm development in sexually reproducing angiosperms occurs through double fertilization while in apomicts, it occurs autonomously or through pseudogamy. In *Cenchrus ciliaris* endosperm develops through pseudogamy which often cause an imbalance in endosperm balance number resulting in poor seed-set. In order to induce autonomous endosperm development in *C. ciliaris*, a Polycomb gene *CcEZ1* isolated earlier has been further characterized. Similarly, another gene, *CcKIP1* isolated and characterized earlier, indicated its possible role in seed development. In order to understand the functions of these two genes, functional genomic approaches are adopted using CRISPR/Cas9 technology. Also, an attempt was made to transform *C. ciliaris* using both *Agrobacterium* and gene gun approaches. Different stages of endosperm development of *C. ciliaris* using methyl salicylate clearing method were identified and in silico, phylogenetic, and expression analyses of *CcEZ1* were carried out. We compared two methods, *Agrobacterium* and gene gun mediated genetic transformation using pCambia1301, which revealed that the frequency of transient GUS expression was significantly higher in the case of gene gun mediated approach with 56.33 ± 2.36 as compared to 11.17 ± 1.41 from the *Agrobacterium*-mediated transformation method. For the tissue culture mediated genetic transformation, four different developmental stages of immature inflorescences were used for calli induction, and the III and IV stages gave better quality embryogenic calli. CRISPR/Cas9 vectors of *CcEZ1* and *CcKIP1* were also developed using Gateway® LR Clonase™ reaction and were validated in *Nicotiana benthamiana* and *Arabidopsis thaliana*, respectively. Shoot induction from the gene gun transformed calli could be accomplished with pCambia1301, but CRISPR/Cas9 constructs (*CcEZ1* and *CcKIP1*) showed the only progression in response to the regeneration media.

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1. General Introduction 2. Study of endosperm development, and in silico, Phylogenetic, and expression analyses of a polycomb gene *CcEZ1* in *Cenchrus ciliaris* L. 3. Development of CRISPR/Cas 9 constructs of *CcEZ1* and *CcKIP1* genes, and their validation in *Nicotiana benthamiana* and *Arabidopsis thaliana*, respectively 4. Assessment of genetic transformation methods and optimization of gene gun mediated genetic transformation of *Cenchrus ciliaris* L. 5. Summary. Conclusion. References. List of Publications.

12. NINDAWAT (Shruti)
Arnebia Hispidissima (LEHM.) A. DC. Mediated Synthesis of Silver Nanoparticles: Elucidation of their Phytotoxicity on Vigna Mungo (L.) Hepper and Potential Biotechnological Applications.
 Supervisor : Prof. Veena Agrawal
Th 24980

Abstract
(Not Verified)

In *Arnebia hispidissima*, phytochemical analysis was performed using the root and leaf extracts prepared in chloroform, ethyl acetate and methanol. Maximum total phenolic content was observed in methanol extract (275.53 ± 18.72 GAE mg g⁻¹ of root extract; 221.50 ± 15.20 GAE mg g⁻¹ of leaf extract). Maximum total flavonoid content was obtained in ethyl acetate extract (398.36 ± 5.79 QE mg/g of root extract; 328.64 ± 6.69 QE mg/g of leaf extract). GC-MS and LC-MS analyses of the plant extract revealed the presence of many pharmacologically important compounds. *A. hispidissima* plant extract exhibited strong anti-oxidant activity. Silver nanoparticles (AgNPs) were synthesized using aqueous root (RE-AgNPs)

and leaf extract (LE-AgNPs) and characterized using UV-Vis spectroscopy, XRD, FESEM, EDAX, TEM, DLS and FTIR. The AgNPs were anisotropic, polydisperse, stable and face centred cubic crystals. FT-IR analysis showed presence of phenolics, flavonoids and proteins in the plant extract. AgNPs showed high antioxidant activity against DPPH and H₂O₂ radicals. The AgNPs exhibited strong anti-cancer activity against cervical cancer cells, HeLa (RE-AgNPs, IC₅₀ 4.44 µg/mL and LE-AgNPs, IC₅₀ 7.18 µg/mL) and were non-toxic towards non malignant L20 B cells. AgNPs also exhibited effective antimicrobial activity against seven human pathogenic strains tested. The AgNPs showed strong catalytic dye degradation activity against structurally different organic dyes. AgNPs were highly sensitive and selective for the detection of Hg²⁺, Fe³⁺ ions and NH₃. The AgNPs possessed strong larvicidal activity against mosquito vectors causing dengue (*Aedes aegypti*) and malaria (*Anopheles stephensi*). Phytotoxic effect of AgNPs on the growth and physiology of *Vigna mungo* was studied and further alleviation of nanoparticle induced stress through glutathione supplementation was done. Antioxidant enzyme activity (SOD, CAT, APX, GPX, DHAR and GR) increased with increasing AgNPs concentration. Glutathione supplementation significantly enhanced plant growth and reversed toxicity symptoms (cell death and DNA damage) caused by high AgNPs concentration (100 and 200 ppm).

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13. RAJPUT (Shubhra)

Biotechnological and Biochemical Investigations on *Atropa Acuminata* Royle Exlindl : A Critically Endangered Medicinal Herb of Northwest Himalayas.

Supervisor : Prof. Veena Agrawal

Th 24979

Abstract (Not Verified)

In the current investigation, an efficient micropropagation protocol has been developed via indirect organogenesis using root-derived callus in *Atropa acuminata* Royle ex Lindl., a critically endangered medicinal herb of Northwest Himalayas. The micropropagated plants were successfully acclimatized under greenhouse conditions, and their genetic fidelity was evaluated using sequence related amplified polymorphism (SRAP) and start codon targeted (SCoT) markers, which revealed 96% similarity. These plants showed higher yield of phenols, flavonoids and exhibited better antioxidant (DPPH and phosphomolybdenum assay) activities in comparison with mother plant. Higher levels of photosynthetic pigments and antioxidant enzymes were also observed in these plants as compared to the mother plant. In addition, the effect of heavy metals (lead and cadmium) have also been studied on in vitro regeneration of *A. acuminata* in order to check their impact on morphogenesis and antioxidant levels. Increased proline content and enhanced activities of SOD, CAT, APX, GPX and GR in response to stress as compared to control clearly indicated the tolerance mechanism used by *A. acuminata* to minimize the oxidative stress. Qualitative analysis of micropropagated plants using FT-IR and GC-MS analysis revealed the presence of various functional groups and compounds present in crude leaf extracts of *A. acuminata*. Quantitative analysis of atropine and scopolamine using HPLC in different plant parts of micropropagated plantlets revealed higher amount of both the alkaloids in leaves. Besides, elicitation of atropine and scopolamine biomolecules has been done in root-derived callus of *A. acuminata* using different elicitors, where yeast extract and caesin hydrolysate yielded higher amounts of atropine and scopolamine, respectively. In addition, silver

nanoparticles were synthesized using aqueous leaf extract of *A. acuminata*. The synthesized AgNPs exhibited excellent antioxidant, anti-inflammatory, anticancer and larvicidal activities which proves the utility of biosynthesized AgNPs as a potential 'nanodrug' in the field of pharmaceuticals and medicine in the future.

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

14. RATHORE (Priyanka)
Identification and Characterization of Different Families of Retroelements, and Study of Epigenetic Regulation of a Pomixes Associated Retroelements in *Cenchrus Ciliaris* L.
 Supervisor : Prof. Vishnu Bhat
Th 24978

Abstract *(Not Verified)*

Retroelements are the class I mobile elements which move within the genome of virtually all organisms and play important role in various pathways related to stress, development and reproduction. REs are further classified into LTR-REs and non LTR-REs based on presence/absence of terminal repeats at the ends. *Cenchrus ciliaris* is a perennial grass belonging to family Poaceae which reproduce predominantly through apomixis with chromosome no. $2n=4x=36$. Apomixis Specific Genomic Region controls the apomictic trait in *C. ciliaris* that is rich in retroelements which are regulated through epigenetic mechanism. In silico transcriptome analyses helped in identify different lineages of Ty1-copia and Ty3-gypsy retroelements in *C. ciliaris* and detect their expression between apomictic and sexual inflorescence transcriptomes at MES. Ty3-gypsy lineages showed higher expression in apomictic plants while sexual plants showed similar expression of Ty1-copia and Ty3-gypsy retroelements. Physical distribution of Ty1-copia and Ty3-gypsy retroelements and their global methylation of different families of REs in the genome suggested that Ty1-copia REs were distributed mainly in centromeric regions and some in the pericentromeric regions while Ty3-gypsy elements were localized mainly in pericentromeric regions and some at centromeric regions of chromosomes. Immunostaining showed that some Ty3-gypsy RE signals were not overlapping with anti-5mC suggesting higher transcriptional activation of Ty3-gypsy elements in apomictic *C. ciliaris*. Retroelements (Gy163, Gy105 and Gy4) which are inserted into the ASGR were analysed in detail for their structure, methylation and expression patterns which revealed that these REs encode for different protein domains and showed differential methylation and expression between reproductive tissues of apomictic and sexual plants. CHH context of methylation was the predominant methylation observed implicating the involvement of RdDM pathway in regulation of REs. This study indicated involvement of epigenetic regulation of apomictic mode of reproduction in *C. ciliaris*.

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1. General Introduction 2. Classification and the expression analysis of different retroelements in the transcriptomes of apomictic and sexual *cenchrus ciliaris* through in silico and phylogenetic analyses 3. Physical mapping of different families of retroelements in apomictic *cenchrus ciliaris* and their epigenetic analysis through cytogenetic approaches 4. Characterization of polymorphic retroelements and identification of their conserved regions in apomictic and sexual plant of *cenchrus ciliaris* 5. Analysis of differential methylation and expression patterns of identified inserted retroelements using bisulfite sequencing and real time PCR methods 6. Summary. Appendix. References.

15. SHIKHA PRASAD

Effect of Seasons and Tree Species on Vital Ecosystem Processes and Functions in Semi-Arid Forest of Delhi.

Supervisor : Dr. Ratul Baishya

Th 24976*Abstract**(Verified)*

In arid and semi-arid regions, the ecosystem processes and functions are greatly influenced by soil moisture, temperature and tree species composition. Hence, it becomes important to understand the effect of seasonal variations in vital ecosystem processes and functions under seven native and non-native tree canopies for sustainable management of Delhi-ridge forests. We observed the seasonal changes in soil nutrients, nitrogen mineralization, respiration, microbial biomass carbon and decomposition rates. All the soil nutrients were significantly highest in winter and lowest in monsoon seasons. In the surface layer, soil total nitrogen ranged from 0.39 Mg ha⁻¹ (*Ficus religiosa*) to 3.54 Mg ha⁻¹ (*Albizia lebbeck*) whereas available phosphorus ranged from 12.26 Kg ha⁻¹ (*Azadirachta indica*) to 25.24 Kg ha⁻¹ (*Ficus religiosa*) and exchangeable potassium ranged from 105.78 Kg ha⁻¹ (*Azadirachta indica*) to 202.34 Kg ha⁻¹ (*Ficus religiosa*) during the study. Litter decomposition was observed to be highest under heterogenous litter set with a decay rate maximum in *Ficus religiosa* (0.79) and minimum in *Azadirachta indica* (0.45). The mineralization process was observed to be maximum during monsoon season under *Ficus religiosa* and lowest during winter season under *Cassia fistula*. Mineralization ranged from 0.7 µg g⁻¹ season⁻¹ to 43.50 µg g⁻¹ season⁻¹ during the study period. Among all the 7 tree species selected, *Ficus religiosa* showed the highest rate of soil respiration along with microbial biomass carbon (MBC) and lowest rate was observed under *Azadirachta indica*. Annual soil respiration ranged from 4.11 µmol CO₂ m⁻² s⁻¹ yr⁻¹ to 21.20 µmol CO₂ m⁻² s⁻¹ yr⁻¹. MBC ranged from 5.67 µg g⁻¹ to 156.85 µg g⁻¹ during the study. The findings of this research promise to enhance the ecosystem processes and functions of the semi-arid ridge forest ecosystem. Therefore, proper selection of tree species during plantations would aid in sustainable management and help in mitigating climate change.

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1. General Introduction 2. Review of Literature 3. Site description and vegetative analysis 4. Variation in soil macro-nutrients under different tree species 5. Litter production, decomposition, nutrient and carbon release pattern in mixed and individual species 6. Impact of different tree species on soil nitrogen dynamics and mineralization rate 7. Effect of tree species and seasons on microbial biomass carbon and total carbon 8. Seasonal variation in in-situ soil respiration under different tree species 9. General discussion and conclusions. References . publications.

16. SINGH (Tikkam)

Biotechnological and Phytochemical Investigations of Three Traditional Medicinal Plants : *Rumex Hastatus* D. Don, *Plumbago Zeylanica* L. and *Cullen Corylifolium* (L.) Medik.

Supervisor : Prof. Veena Agrawal

Th 24977*Abstract**(Not Verified)*

In the current investigation, efficient regeneration protocols have been developed via direct and indirect organogenesis using nodal and in vitro root explants,

respectively, for *Rumex hastatus* D. Don. The node-derived regenerants (NDR) and root callus-derived regenerants (RDR) were successfully acclimatized under greenhouse conditions, and their genetic fidelity was analyzed using start codon targeted (SCoT) and sequence related amplified polymorphism (SRAP) markers. UPGMA dendrograms of NDR and RDR revealed 98% and 95% similarity, respectively, with the mother plant. The RDR showed maximum contents of phenols, flavonoids as well as quantity of luteolin and rutin over NDR and mother plant. Besides, RDR had maximum total antioxidant capacity and DPPH free radical scavenging activity followed by NDR and mother plant. Photosynthetic pigments and enzymatic antioxidants such as SOD, CAT, APX and GPX were also observed higher in regenerants compared to mother plant. Phytochemical analysis of *R. hastatus* through FT-IR, GC-MS and LC-MS revealed the presence of various functional groups and several biologically active compounds. In elicitation, the highest luteolin and rutin contents were achieved with phenylalanine and chitosan, respectively, in root callus cultures of *R. hastatus*. In *Plumbago zeylanica*, a total of 65 fractions were collected through column chromatography and pooled into 8 fractions (F1-F8) on the basis of their similar R_f values using TLC. Isolated F2 was characterized and identified as plumbagin through TLC, FT-IR and ¹H-NMR. Besides, tremendous enhancement of plumbagin was obtained with combination of yeast extract and chitosan over control in root-derived callus cultures of *P. zeylanica*. In case of *Cullen corylifolium*, significantly higher amount of psoralen, daidzein and genistein was observed in green seeds compared to cotyledon callus. Remarkable enhancement in psoralen content was recorded in salicylic acid, whereas maximum quantities of daidzein and genistein were achieved in chitosan treated cotyledon callus cultures of *C. Corylifolium*.

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17. SURYAVANSHI (Amrita)

Phyto-Mediated Synthesis of Silver Nanoparticles of *Ajuga Parviflora* Benth. And their Therapeutic Studies.

Supervisor : Dr. Suresh Kumar

Th 25542

Abstract (Not Verified)

Ajuga parviflora Benth. is an annual and short-living medicinal herb in the Himalayas' foothills of India. The present investigation highlights the phytochemical screening of shoot, root and leaf parts in three different solvents namely hydroalcoholic, water and hexane. Among all these samples, leaf extracts showed the maximum presence of phytochemicals. FTIR and GCMS analysis confirmed the existence of active components present in the leaf extract. Subsequently, aqueous leaf extract was used for the green synthesis of silver nanoparticles (APAgNPs). The optimum conditions for the synthesis of APAgNPs were achieved by adding 5 ml aqueous leaf extract and 45 ml AgNO₃ solution (1 mM) at 60°C (pH 7.5). Characterization of APAgNPs was done using UV-Vis spectroscopy, TEM, FE-SEM, DLS, EDAX, XRD and FTIR. APAgNPs were crystalline exhibiting a spherical shape with a range of 4-20 nm. FTIR analysis revealed different functional groups in leaf extract for Ag⁺ ion reduction, capping and stabilization. Furthermore, APAgNPs and leaf extracts were assessed for their bio-efficacies. Among all the samples, APAgNPs showed the highest bio-application studies. The TPC, TFC and TTC content were found significantly higher in APAgNPs. The IC₅₀ values of APAgNPs exhibited the significant and enhanced potency for DPPH (65.81±0.21 µg/ml), ABTS (96.06±1.64 µg/ml) and

H₂O₂ (81.73±0.38 µg/ml) as well as for α-amylase (55.71±0.29 µg/ml) and α-glucosidase (45.37±0.36 µg/ml) respectively. APAgNPs and leaf extract showed a remarkably improved rate of glucose uptake in the yeast cells. Afterward, APAgNPs and leaf extract exhibited promising antibacterial activity against both gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains. These findings indicated that silver nanoparticles and leaf extracts of *A. parviflora* possessed antidiabetic, antioxidant properties and antibacterial potential. Hence, there is a scope for further study to understand the exact mechanism of silver nanoparticles and leaf extracts and their significance.

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18. YADAV (Renuka)
Vitro Screening, Isolation and Elicitation of Anti-Cancerous Compounds of Piper Longum L. and Cullen Corylifolium (L.) Medic and Therapeutic Potential of Bio-Nanoparticles.
 Supervisor : Prof. Veena Agrawal
Th 24987

Abstract (Not Verified)

Besides, silver nanoparticles (AgNPs) were synthesized using aqueous leaf extract of *P. longum* and characterized through UV-Vis, FE-SEM, HR-TEM, XRD, DLS, FTIR and AAS. Such nanoparticles were evaluated against different human cancer cell lines (HeLa, NCI-H23 and KB) and mosquito vectors causing malaria, dengue and filariasis. AgNPs exhibited strong anticancer activity against HeLa cells followed by oral and lung cancer cells with IC₅₀ value being 5.27±0.05, 10.47±0.94 and 19.93±0.32 µg/mL, respectively. Maximum larvicidal activity was observed with AgNPs having LC₅₀ and LC₉₀ as 8.969 and 16.102ppm, respectively for *Anopheles stephensi*; LC₅₀;14.791 and LC₉₀;28.526ppm, for *Aedes aegypti* and LC₅₀;18.662 and LC₉₀;40.903ppm, for *Culex quinquefasciatus*, after 72h of treatment. It was also proved harmless to the non-targeted cell line and microorganism.

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M. Phil Dissertations

19. KANOJIA (Abhishek)
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Supervisor : Dr. C.S. Seth