CHAPTER 5

BOTANY

Doctoral Theses

01. AKRAM GHOLAMI

Taxonomy, Molecular Systematics and DNA Barcoding of Alysicarpus (Fabaceae).

Supervisor: Prof. Arun K. Pandey

Th 22735

Abstract
(Not Verified)

The genus Alysicarpus Necker ex Desvaux (tribe Desmodieae, Fabaceae) includes approximately 25-30 species distributed in tropical and subtropical regions of the old world. In India, the genus is represented by approximately 17 species, of which eight are endemic. A revision of the genus Alysicarpus in India is provided based on field studies, and examination of herbarium specimens. In India, 17 species of Alysicarpus are recognized: Alysicarpus bupleurifolius, A. gamblei, A. gautalensis, A. hamosus, A. heyneanus, A. ludens, A. luteovexillatus, A. monilifer, A. naikianus, A. ovalifolius, A. pubescens, A. poklianus, A. rugosus, A. scariosus, A. tetragonolobus, and A. vaginalis. Phylogenetic analyses based on ITS sequence data reveal that: (1) Alysicarpus is monophyletic, (2) molecular data suggest that the genus Alysicarpus is closely related to the members of subtribe Desmodinae, (3) taxa recognized under groups Microcalyx and Macrocalyx are non-monophyletic, (4) Macrocalyx and non-rugose pods are pleisomorphies for the genus, (6) Microcalyx and transversely rugose pods are apomorphies for the genus, (7) evolution of Macrocalyx and non-rugose pods are non-homologous and Microcalyx and rugose pods evolved from a single common ancestor and pertains to a homologous trait. The loci tested for DNA barcoding, ITS alone discriminated all the species on the basis of genetic distance as well as phylogenetic tree methods. During the systematic study of the genus Alysicarpus, the following new data have been generated: (1) Two news species namely Alysicarpus gautalensis and A. poklianus, have discovered, described and published, (2) Based on morphological characters, A. yunnanensis, A. vaginalis var. venosa, and A. monilifer var. mahbubnagarensis have been synonimized under A. monilifer, (3) A. saplianus, A. sanjappae and A. narimanii have been synonimized under A. rugosus and (4) A. heyneanus var. ludens has been reinstated as valid species.

Contents

1. Introduction 2. Review of literature 3. Material and methods 4. Morphology 5. Molecular systematic of alysicarpus 6. DNA barcoding of alysicarpus 7. Taxonomy 8. Summary. Literature cited. Appendix. List of publications.

02. ARORA (Dhara)

Mechanisms of Cellular Redox Homeostasis Maintenance under Salt Stess in Sunflower (Helianthus annuus L.) Seedlings.

Supervisor: Prof. Satish C. Bhatla

Th 22733

Abstract
(Verified)

Sunflower (Helianthus annuus L.) plants are moderately salt-tolerant. High salt concentrations result in an excessive generation of reactive oxygen species (ROS) in plant cells. An interaction between ROS and antioxidants acts as a metabolic interface in maintaining intracellular redox homeostasis. Modulation of the spatial distribution and activity of superoxide dismutase (SOD) is one of the major means to quench excess superoxide anion ($O_2^{\cdot-}$) in the plant cells. Association of different forms of SOD with various metal groups as a constituent of the active enzyme and their ability to quench superoxide anions necessitates their crosstalk with other signaling molecules. Extensive investigations on varied aspects of salt stress tolerance in sunflower have demonstrated significant roles of signaling molecules in relation with salt stress tolerance. Present work aims at investigating modulation of SOD activity in seedling roots and cotyledons in response to salt (120 mM) and varied NO-related pharmacological treatments in order to ascertain the impact of NO as an early and long distance-signaling molecule. Furthermore, possible role of melatonin, through its interaction with NO, is being analyzed in this work by investigating differential modulation of Cu/Zn SOD and Mn SOD in seedling cotyledons. The possible biochemical signaling routes of melatonin action through its interaction with calcium and calmodulin have also been examined. 2-D PAGE and LC-MS/MS analysis have been undertaken to investigate the differentially expressed cytosolic proteins in salt-stressed, melatonin-treated control and melatonin-treated salt-stressed seedling cotyledons. A significant number of proteins have been identified, which exhibit modulation in response to melatonin thereby highlighting the proteome plasticity and metabolic tendency of seedling cotyledons to adapt under saline conditions by altering varied biosynthetic routes. Thus, present work presents new information on the biochemical mechanisms and signaling routes in response to sensing of salt stress in seedling roots and across long distance in seedling cotyledons.

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03. CHONGTHAM (Rubina)

Development of RNAi-based Aphid Resistant Transgenic Plants in Brassica Juncea (Indian Mustard) and Identification of Novel Targets by Transcriptome Analysis of the Mustard Aphid (Lipaphis Erysimi).

Supervisor: Prof. Arun Jagannath

Th 23126

Abstract

(Verified)

Brassica juncea (Indian mustard) is an important edible oilseed crop of India and is severely affected by the aphid pest, Lipaphis erysimi (mustard aphid). With no known source of natural resistance in B. juncea against L. erysimi, its control is predominantly based on use of chemical insecticides. The present study evaluated efficacy of transgene-mediated RNAi as a control mechanism for mustard aphids. To maintain a continuous supply of aphid populations required for such studies, we developed and optimized protocols for maintenance and in vitro propagation of L. erysimi. Thirty nine potential target genes that are functionally important and/or expressed in the digestive tract were selected through BLAST analysis. In vitro feeding assays using long dsRNA and siRNA targeting seven genes in adults and N1 nymphs were performed to test the effects of these genes on L. erysimi. Though no statistically significant effects were observed on mortality, fecundity and nymphal mortality, a significant transient knock-down in expression was observed through qPCR. To test the efficacy of RNAi in planta, transgenic B. juncea plants were developed, targeting the salivary protein c002 gene of L. erysimi. A total of 289 Le-c002 RNAi T_0 plants were transplanted in containment net-houses and were scored for tolerance towards aphid infestation. Self-pollinated seeds of 27 lines, which showed promising tolerance to heavy L. erysimi infestation without any insecticide treatment, were collected for further studies. Additionally, de novo transcriptome sequencing of L. erysimi under feeding, non-feeding and different developmental stages were generated for the first time. We analyzed differential expression of genes between nymphs and adults and in response to feeding and non-feeding conditions. We identified transcripts involved in host selection, detoxification, defense and development and their differential regulation during feeding, starvation stress and/or developmental stages. These are potential RNAi targets and can be used for crop improvement.

Contents

1. Review of literature 2. Establishment of rearing protocols and feeding assays using double stranded RNA for RNAi-based target selection in lipaphis erysimi 3. Development of aphid resistant transgenic plants in brassica juncea using an RNAi construct targeting the c002 gene of lipaphis erysimi 4. De novo transcriptome of mustard aphid (lipaphis erysimi) and differential expression of transcripts associated with developmental stages, feeding and non-feeding conditions. Summary and conclusion. References. Appendices. Publication.

04. CHOUDHARY (Vivek Kumar))

Microbial Diversity at Iron Ore Mined-Out Sites in Odisha and its Significance in Ecological Restoration.

Supervisor: Prof. Sudeshna Mazumdar-Leighton

Th 23127

Abstract
(Verified)

Degraded mine dumps were selected for ecological restoration at Barsua and Kalta Iron ore mines in Odisha. Changes in aboveground plant communities, belowground microbial communities and soil physico-chemical attributes were studied at sites undergoing early restoration (2.5 years) with reference to un-restored mine dumps and forests. Sampling-based studies indicated recovery of vegetation cover based on increases in species richness (Sest) and Hill's diversity indices at sites undergoing restoration. Clonal libraries of ribosomal RNA genes of constituent soil archaea, bacteria, fungi and fauna were examined. In a novel approach, soil microbial eukaryotes/fauna were investigated using conserved domains of 28S rRNA genes. Amplicon restriction fragment length polymorphisms, Sanger-sequencing and bioinformatic analyses were used to detect shifts in culturable and un-culturable components of rhizospheric soil biota at 0.5 years and 2 years of restoration. Rhizospheric communities of metal tolerant and phytostabilizing grasses Thysanolæna latifolia and Eleusine indica from restoration sites sampled in the summer were distinct from those sampled in the monsoon season. Diverse members of ammonia oxidizing Thaumarchaeota, 40 bacterial classes, 13 fungal classes and soil fauna such as Ecdysozoa, Chlorophyta, Alveolata, Euglenozoa, Myxozoa and Cercozoa likely crucial for establishment of trophic levels were observed in this study. One way ANOVA and PCoA indicated amelioration of soil attributes such as pH, SOM, soil moisture, CHNS levels, cations (like Na, Mg, Ca and K), transition metals, metalloid and anions (extractable nitrates, phosphates and sulfates) in rhizospheric soils at sites undergoing restoration towards conditions at reference forest sites. Multivariate statistical analysis indicated that multiple soil attributes significantly influenced complex interactions between plant communities, and soil microbial communities at the study sites. Levels of phosphates can be used as indicator for ecological restoration at degraded iron ore mined-out sites.

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1. Introduction and review of literature 2. Materials and methods 3. Aboveground plant and belowground soil biodiversity at study sites 4. Soil attributes influencing communities of plants, soil bacteria and fungi at study sites 5. Summary and conclusions. References. Publications.

05. DAS (Kamal)

Isolation of Sex-Linked DNA Markers and their Validation on Populations of Hippophae rhamnoides ssp. Turkestanica Representing Geographically Isolated Three Valleys of Ladakh Region.

Supervisor: Prof. Shailendra Gowl

Th 22734

Abstract
(Verified)

Hippophae rhamnoides L. is known as for its medicinal, nutritional and environmental uses. In India, it is found in the hilly states of Himalaya's (Sikkim, Himachal and Ladakh region of Jammu and Kashmir). For mass cultivation of this plant requires establishment of orchard. Elimination of superfluous male plants can significantly increase the efficiency of orchards but identification of gender is possible after flowering, which takes a minimum of 3-4 years. Earlier efforts could not identify a robust marker, which were unable to predict sex in discrete populations from geographically isolated regions. The present study was aimed to develop and validate sex-specific markers in H. rhamnoides (unisexual taxon) growing at high altitudes of Ladakh region (Jammu and Kashmir). The samples were harvested from three different valleys viz. Indus, Nubra and Suru at the flowering stage. The study has used three different markers (RDA, AFLP and ISSR), which are known for their specific abilities in differentiating genomes. The markers identified were converted into SCAR markers. Representational Difference Analysis (RDA) was conducted to identify regions differing between male and female genomes of H. rhamnoides. RDA identified four population specific markers (RDAP-27, 33, 41 and 47), only one locus (RDAP-33) could be converted into a male specific SCAR marker (SCAR-33) differentiating sex in populations from two valleys (Indus and Suru) but was not suitable for populations of Nubra valley. AFLP screening resulted in identification of two markers, which were converted into SCAR markers (SCAR-P15 and SCAR-P32). These markers failed to identify sex reliably. 58 ISSR markers were used to different sex in genotypes from three valleys leading to identification of a marker, which was converted into a SCAR (SCAR-P45). This SCAR produced a robust reliable male specific marker, which could differentiate male and female genders of H. rhamnoides in populations from all three valleys.

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1. Introduction 2. Review of literature 3. Identification and validation of gender specific markers in H. Rhamnoides ssp. Turkestanica growing at high altitudes of Ladakh (Jammu and Kashmir) region with SCAR markers developed from: RDA (Representational difference analysis) 4. Identification and validation of gender specific markers in H. rhamnoides ssp tukestanica growing at high altitudes of Ladakh (Jammu and Kashmir) region with SCAR markers developed from: AFLP (Amplified fragment length polymorphism) 5. Identification and validation of gender specific markers in H. rhamnoides ssp. Turkestanica growing at high altitudes of Ladakh (Jammu and Kashmir) region with SCAR markers developed from: ISSR (Inter simple sequence repeat) 6. Conclusion. References. Publication.

06. DWIVEDI (Mayank Dhar)
Taxonomy and Phylogeny of Zehneria ENDL. (Cucurbitaceae).
Supervisor: Prof. Arun K. Pandey
Th 23128

Abstract
(Not Verified)

Zehneria is a genus of small creepers and climbers from the Old-World Tropics andSubtropics. In its broadest circumscription, it comprises up to 94 species and its centre of diversityis Southeast Asia. Zehneria has been split into several smaller genera but this remainedcontroversial mainly due to morphological plasticity of the species groups and lack ofcomprehensive molecular data. Here, we use one nuclear DNA (ITS1-5.8S-ITS2) and three plastid DNA regions (trnL-trnF, rpl20-rps12, and matK) sequenced for about half of the species in Zehneria and its segregate genera Anangia, Neoachmandra, and Pilogyne to test the monophylyof the genus and analyse the evolutionary history of the group. The study found that Zehneria s.l. ismonophyletic and splitting is not supported by molecular, morphological or biogeographicalevidence. Within Zehneria, we recover two strongly supported clades, one consisting mainly of species placed in Pilogyne, the other including Anangia, Neoachmandra and the generic type of Zehneria. Both clades comprise African, Asian, and Australian/Pacific Island species, which isevidence for high dispersal capacity in this young bird-dispersed group. For India, we accept thefive species Z. bodinieri, Z. hookeriana, Z. maysorensis, Z. odorata, and Z. thwaitesii. ForMyanmar, we increase the number of known species from four to eight, including three stillunnamed species. The diversity hotspots for the genus, however, remain Indonesia and Thailandwith together 25 species. Keywords Cucurbitaceae; India; molecular phylogeny; Myanmar; Neoachmandra; Pilogyne; Zehneria

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1. Introduction 2. Taxonomic history 3. Morphological study of zehneria and allied genera 4. Phylogeny of the genus zehneria 5. Biogeography of the genus zehneria 6. Taxonomic novelties, new records and new combinations 7. Taxonomy 8. Notes on the identity and typification of zehneria thwaitesii and Z. Tridactyla. Summary. Literature cited. Appendix. List of publications.

07. HARI RAM
Identification and Characterization of Thermophilic and Mesophilic Bacteria and Comparative Study of Evolutionary Divergence with Respect to PolIIIα Subunit Peptide and 16S rRNA Gene Sequences of Bacteria Thriving under Different Temperature Regimes.
Supervisor: Prof. Ved Pal Singh
Th 22736

Abstract
(Not Verified)

Prokaryotic organisms surviving under different environmental conditions make themselves fit through alteration in nucleotide compositions of genomic DNA that responds to the existing challenge. The genomic DNA of bacteria consisted of coding and non-coding regions that encodes for proteins and non-protein genetic elements, respectively. Two thermophilic (Anoxybacillus sp. M103A and Anoxybacillus sp. M103B) and mesophilic (Myroides sp. UKS3 and Kocuria sp. UKS5) bacterial strains were isolated and characterized with respect to morphological, biochemical and molecular parameters. A mesophilic novel bacterial species Myroides indicus of genus Myroides was identified using polyphasic approach of taxonomy. The 16S rRNA and PolIIIα subunit gene was amplified using universal as well gene specific primers and sequenced. The amplified PolIIIα subunit gene from Anoxybacillus sp. M103A and Anoxybacillus sp. M103B was cloned using pGEMT easy vector and sequenced. Comparative In silico study on adaptive divergence was carried out on 16S rRNA gene sequences from bacteria thriving under psychrophilic, mesophilic and thermophilic habitats that revealed habitat specific biasness in nucleotide composition of the gene. The effect of habitat specific selection pressure on PolIIIα peptide and 16S rRNA gene sequences was studied that provided the relevant evidences for the similar type of selection pressure on the coding and non-coding regions of genomic DNA of bacterial species thriving under different habitats. Both PolIIIα peptide and 16S rRNA gene were able to decipher evolutionary relationships among bacterial species with comparable phylogenetic resolutions. During blast for homology search, both PolIIIα peptide and 16S rRNA gene sequences retrieved phylogenetic neighbours on the basis of sequence similarity that included species of genera thriving under different temperature regimes. These findings support the hypothesis that both coding and non-coding genes of organisms experience the similar type of habitat-specific selection pressures during the course of evolution.

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1. Introduction 2. Review of literature 3. Identification and characterization of thermophilic and mesophilic bacteria with respect of morphological biochemical and molecular parameters 4. Characterization of a novel mesophilic bacterium myroides indicus sp. nov. 5. In silico studies on adaptive divergence in nucleotide composition of the 16S rRNA gene among bacteria thriving under different temperature regimes 6. Amplification, cloning and sequencing of PolIIIα subunit gene from the genomic DNA from thermophilic (Anoxybacillus sp. M103A and anoxybacillus sp. M103B) and mesophilic (Kocuria sp. UKS5) Bacteria 7. Investigation on DNA polymerase III alpha (PolIIIα) subnit peptide sequence as potential genetic marker for bacterial taxonomy. Summary. Conclusion. References. Appendix. List of publications.

08. HARMEET KAUR
Interaction of Glutathione Reductase and Catalase with Melatonin and Nitric Oxide During Long Distance Sensing of Salt Stress in Sunflower Seedlings (Helianthus annuus L.).
Supervisor: Prof. Satish C. Bhatla
Th 22741

Abstract
(Verified)

Nitric oxide is known to counter oxidative stress in biological systems by scavenging ROS and/or by modulating gene expression. It stimulates glutathione (GSH) biosynthesis by modulating gene expression, leading to greater tolerance against oxidative stress by plants. NO is a competitive inhibitor of catalase. Four NO molecules form a complex with each catalase tetramer. Modualtion of intracellular GSH levels due to the increased expression of γ-glutamylcysteine synthetase by NO, is a powerful mechanism for activating the antioxidant machinery of the cell. Melatonin can also detoxify the harmful effects of ROS by directly scavenging free radicals, and by the attenuation of radical formation. It helps in the detoxification of several ROS and reactive nitrogen species (RNS) such as peroxynitrite ($ONOO^-$), nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3), etc. Up to four or more reactive species can be scavenged by a single melatonin molecule making it an efficient antioxidant. Endogenous levels of melatonin have been reported to increase in response to various environmental stresses. Melatonin also upregulates the transcript levels and stimulates the activities of several

antioxidant enzymes. Based on the above-stated background information present investigations have been undertaken. Broadly they aim at examining 1. modulation of glutathione content and glutathione reductase activity by melatonin and nitric oxide accompanying salt stress, 2. assessing H_2O_2 accumulation and catalase activity modulation by melatonin and nitric oxide in salt-stressed seedlings and 3. evaluating the impact of salt stress on nitric oxide interaction with melatonin in regulating peroxynitrite-mediated long distance signaling and protein phosphorylation. The significant findings have discussed in light of the related information from the earlier literature.

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1. Introduction 2. Review of Literature 3. Material and methods 4. Results 5. Discussion 6. Summary 7. References. Research publications.

09. HEENA AMBREEN

Survey of Repeat Components of Carthamus tinctorius L. Genome, Development of Molecular Markers and their Utilization for Assessment of Core Collection, Association Mapping and Phylogenetic Analysis.

Supervisor :Prof. Shailendra Goel

Th 22737

Abstract

(Verified)

Carthamus tinctorius L. is an underutilized oilseed crop producing high quality oil. Genomic resources are largely underdeveloped in this crop. The present study was undertaken to generate high throughput molecular markers and utilize them for crop improvement programs. Genome sequencing identified 23,067 microsatellites in safflower, which revealed high heterogeneity in repeat classes, motif types and length in microsatellites. Primers were designed for 5,716 loci and a sub set of 325 loci was experimentally validated. A high percentage of tested primer pairs (90.4%) produced robust amplification with 93 primers being polymorphic. These markers were utilized to access the suitability of a safflower panel "CartAP" for association mapping. The safflower panel showed high genetic diversity (Shannon information index; H = 0.7537 and Nei's expected heterozygosity; I = 0.4432), weak population structure and low kinship values at the population and individual level. Both General Linear Model (GLM) and Mixed Linear Model (MLM) were used to identify candidate markers showing association with eight agronomic traits. Next phylogenetic relationships among thirty accessions representing 10 species of genus Carthamus was studied using SSR markers, which showed high genetic diversity (H = 0.9684; I = 0.5315). Neighbor-joining, Principal coordinate and population structure analysis divulged important information regarding the tested species. We also performed graph-based clustering to study repetitive sequence in safflower genome, which grouped ~68% sequences into 96,116 repeat clusters. Within these clusters, a wide range of repetitive elements was identified and RNA transposons constituted 18.535% of the total genomic proportion. Higher heterogeneity was observed in Ty1-copia elements than Ty3-gypsy retrotransposons. Further, attempts were made to isolate full length Ty1-copia retrotransposons, which can then be utilized for marker development in the crop. The present work will contribute in addressing challenges faced by safflower.

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1. Introduction and review of literature 2. A survey of microsatellite component of safflower genome and development of microsatellite (SSR) markers using next generation sequencing 3. Genetic diversity, population structure and association mapping for important agronomic traits in safflower core collections using SSR markers 4. Phylogenetic analysis of carthamus sp. Using SSR markers 5. In silico analysis of repetitive elements, assessment of heterogeneity of LTR retrotransposons and isolation of full length LTR copia retrotransposons in safflower. Summary and conclusions. References. Appendices. Reprints of publications.

10. JAIN (Aditi)

Analysis of Synteny and Regulatory Diversification in miR164 and its Targets from Polyploid Brassica Species.

Supervisor: Dr. Sandip Das

Th 22738

Abstract
(Verified)

Abstract Polyploidization is a major force in evolution providing opportunities for organism to diversify and innovate in form and function. Study of duplicated genes resulting from a genome duplication event is central to understanding how polyploidy can act as a driver for phenotypic and adaptive novelties. In the present thesis, we studied impact of polyploidization on functioning of both miRNA and protein-coding genes using Brassica as a polyploid plant system. Identification and comparative analysis of miRNAs using synteny based approach allowed us to categorize them as species-, lineage-, sub-genome and karyotype-specific manner. MiRNA, like protein coding genes were found to follow the principle of genome fractionation in polyploid Brassica. Study of regulatory module miR164-NAC in Brassica genomes showed conservation of functional regions such as necessary domains, mature region and miRNA binding sites. Spatio-temporal expression profile of miR164 and its target genes reveals that this regulatory module is widely expressed in all plant parts and at all developmental stages. Phylogenetic analysis of miR164, miR164-target genes and promoter region of miR164 grouped sequences as per their lineage, sub-genome and parental affiliations. Phylogenetic footprinting analysis of promoter regions of miR164 from Brassicaceae showed conserved regions corresponding to functionally important transcription factor binding sites. Further, many transcription factor binding sites were found to be lineage-, karyotype and sub-genome specific. Functional analysis of paralogous promoter regions from Brassica juncea showed both regulatory conservation (miR164a, miR164b) and divergence (miR164c). Functional analysis of promoter regions by making 5' deleted fragments showed variation in spatio-temporal activity. Absolute requirement of SITEIIACYTC motif, a TCP binding site, in transcriptional activity of miR164a was demonstrated through site-directed deletion. Hormonal treatment suggest that miR164a is regulated by auxin and ethylene in a complex manner.

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11. JAIN (Prachi)
Mechanisms of Salt Stress Tolerance in Sunflower Seedlings (Helianthus annuus L.): Role of Peroxidase-Nitric Oxide Crosstalk and S-Nitrosylation of Proteins.
Supervisor: Prof. Satish C. Bhatla
Th 22739

Abstract
(Verified)

Peroxidases (POD; EC 1.11.1.7) are heme-containing oxidoreductases which catalyze the reduction of H_2O_2 by oxidizing a wide variety of electron donor substrates. Peroxidases can be broadly classified as heme-containing and non-heme containing, which are further categorized into thiol-based peroxidases and alkylhydro peroxidases. Thiol-based peroxidases consist of peroxiredoxins (PRX; EC 1.11.1.15) and phospholipid hydroperoxide glutathione peroxidases (PHGPX; EC 1.11.1.12). Glutathione peroxidases constitute many isoenzymes involved in the reduction of lipid hydroperoxides or hydrogen peroxide to water and alcohol with the help of a reductant, such as reduced glutathione (GSH). Peroxiredoxins are low-efficiency peroxidases which are involved in the scavenging of H_2O_2 and peroxynitrite. Nitric oxide may directly scavenge ROS, such as superoxide, to form peroxynitrite ($ONOO^-$), thus acting as an antioxidant itself. It might function as a signaling molecule in the cascade of events thereby altering gene expression. NO reacts with thiol or heme-containing proteins either directly or indirectly and may bind to cysteine residues, iron or heme centres or tyrosine residues in proteins, thus altering their structure and function. NO-induced signaling is mediated through generation of a variety of reactive nitrogen species (RNS). S-nitrosylation and tyrosine nitration of proteins constitute two major NO signaling routes for evoking biochemical responses. Based on the above-stated significant roles of ROS and RNS in plants, present investigations are aimed at examining the mechanisms of their action through detailed analysis of 1. correlation between NaCl stress sensing in sunflower seedlings and the activities of heme- and thiol-based peroxidases, 2. nitric

oxide-dependent modulation of cytosolic peroxidase activity in sunflower seedling roots and cotyledons in response to salt stress, and 3. proteomic analysis of S-nitrosylated cytosolic proteins in salt-stressed seedling cotyledons and roots as a mechanism for nitric oxide-mediated signaling under salinity stress.

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12. KAPOOR (Himanshi)

In Vitro and in Silico Evaluation of Anti-Cancerous Potential of Two Important Medicinal Herbs, Nardostachys Jatamansi (D. Don) DC. and Cullen Corylifolium (L.) Medik. (SYN: Psoralea Corylifolia L.) Against Human Glioblastoma Cell Lines.

Supervisors: Prof. Veena Agrawal and Prof. Sushil Chandra Mahapatra

Th 22740

Abstract
(Not Verified)

Glioblastoma is the most common and lethal brain tumour. Despite multimodal therapies, the median survival of patients is 12 months. Therefore, development of new more effective treatment methods is important. Herbal drugs are potential candidates due to fewer side effects and multi-targeted and synergistic action. The present investigation highlighted the anti-cancerous potential of two important medicinal herbs Nardostachys jatamansi (D.Don) DC. and Cullen corylifolium (L.) Medik. against human glioblastoma cell lines using various in vitro and in silico bio-analytical tools. The results revealed that Nardostachys jatamansi rhizome extract (NJRE) had a strong dose and time dependent anti-tumorous action on the U87 MG and U373 MG cells. Moreover, NJRE exhibited low toxicity to the normal cell line HEK. The compounds of NJRE had drug like properties, CNS activity, blood barrier crossing ability and exhibited molecular interactions with, EGFR, VEGFR2, CDK2 and BCL2. Biochemical fingerprinting revealed the presence of various anti-cancerous compounds in NJRE. In Cullen corylifolium, a comparative evaluation of the anti-tumorous effect of its green seed extract (GSE), brown seed extract (BSE) and marker compounds psoralen, genistein and diadzein were seen in glioblastoma cells. GSE was the most effective in inhibiting the proliferation of cells at low doses. GSE was also least cytotoxic to the normal cell line HEK. The content of genistein, diadzein and psoralen in the GSE was more than BSE as quantified by HPLC. Further in vitro studies with GSE revealed cytotoxicity, DNA damage and apoptosis through intrinsic pathway in glioblastoma cells. Psoralen, genistein and diadzein had drug like properties, CNS activity, blood barrier crossing ability and molecular interactions with EGFR, VEGFR2, CDK2 and BCL2. The biochemical characterization of GSE exhibited the presence of antioxidant and anti-cancerous compounds. The present investigation for the first time reports that NJRE and GSE both had a multi-targeted anti-tumerous effect on glioblastoma cells. They can be useful for the development of novel, safe and cheap herbal drugs for glioblastoma in future.

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13. KASHYAP (Prakriti)

Analysing CBF Dependent Cold Stress Signaling in Solanum Lycopersicum, Brassica Juncea and Hippophae Rhamnoides.

Supervisor: Prof. Renu Deswal

Th 23057

Abstract
(Not Verified)

CBF dependent cold stress signaling pathway is the most investigated pathway till date. However, the studies are limited in crop plants like Indian mustard (Brassica juncea). The CBF dependent cold

stress signaling pathway was analyzed in Brassica juncea. Two differentially expressed novel isoforms (BjICE53 and BjICE46) of an upstream transcription factor ICE were isolated,BjICE53 activating with cold stress and BjICE46 with salinity stress. BjICE46 showed upregulation with MeJA and ABA whereas, BjICE53 was constitutive. The downstream transcription factor, BjCBF was isolated and the promoter region analyzed after genome walking revealed the presence of three E-boxes, the binding region of ICE. For functional characterization, BjCBF was expressed in bacteria and purified. The gel shift assay showed that BjCBF binds to the DRE elements in the promoter of cold responsive genes and thus might be involved in providing cold tolerance to Brassica juncea. The CBFs from cold sensitive tomato, cold tolerant brassica and freeze tolerant seabuckthorn were analyzed for S-nitrosylation sites in silico. One cysteine in AP2 domain (DNA binding domain) as a potential nitrosylation site was observed in all three systems. Nitrosylation might affect binding of DRE elements in cold responsive genes with CBF and thus affecting cold stress signaling. LeCBF1 was analyzed for transcriptional and post-translational regulation by NO. LeCBF1 was expressed and purified from bacteria. The purified protein was analyzed for in vitro and in planta S-nitrosylation using BST (Biotin Switch technique). Moreover, a potential cold responsive gene, a class I chitinase (HrCHI1) was isolated from seabuckthorn. Bacteria expressing recombinant HrCHI1 showed better rate of growth in cold stress. Transcript profiling and genome walking revealed that it was a methyl jasmonate dependent cold/freeze responsive gene. The study also indicated chitinase to be a putative downstream target of CBF.

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1. Introduction 2. Review of literature 3. Materials and methods 4. Results and discussion 5. Summary and conclusions 6. Literature cited. Tables. Publications.

14. KOHLI (Isha)
Investigations on the Alkaline and Acid α-Amylases of Soil Bacterial Isolates and their Industrial Applications.
Supervisor: Prof. Ved Pal Singh and Dr. Rekesh Tuli
<u>Th 23130</u>

Abstract
(Not Verified)

Starch is the naturally available substrate that is hydrolyzed by amylase. Direct hydrolysis of starch is economical as the complete step of starch hydrolysis would take place in just one single step. Till to date, very few bacteria are reported that are able to hydrolyze high levels of raw starch. Therefore, the present research was focused on finding new bacteria that were able to hydrolyze raw starches, thus simplifying the starch conversion process. Besides thermostability and capability to digest raw starch, other important criteria required by industries are pH stability (at alkaline and acidic range of pH for diverse industrial purposes) and calcium ion independency. Currently, the amylases used in industry show maximum activity at neutral pH and are calcium ion dependent, so the pH of the enzyme is changed time and again as per the requirement of the industrial process and the product that is to be formed. Also, the calcium ions needs to be removed at the end of the process, thus increasing the cost of the process. Thus, to reduce the cost, time and energy of hydrolysis, the search of novel raw starch digesting, Ca^{2+} independent, pH stable (alkaline and acid stable) and thermostable amylases find promising research area and this lays the foundation of the present investigation.

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1. Introduction 2. Materials and methods 3. Results 4. Discussion 5. Summary 6. Conclusion. References. Annexures.

15. MADAN (Akanksha)
Floral Rewards and Pollination Studies in Cascabela thevetia (L.) Lippold (Apocynaceae) with an Assessment of its Potential as a food Source for Bees.
Supervisor: Dr. P. L. Uniyal and Dr. A. K. Bhatnagar
<u>Th 23058</u>

Abstract
(Verified)

Anthropogenic interference, global-climate change and resulting changes in adaptive traits of plants and their interaction webs are posing threats on these plant-animal interaction. The decreasing trend of pollinator distribution, abundance and richness, attributed to perturbations of anthropogenic origin and climate change, is considered a major reason behind 'global pollinator decline'. Therefore, along with studying plant-pollinator adaptive strategies towards changing environment, conservation and restoration measures aimed at promoting the establishment of pollen and nectar-rich communities need to be undertaken. Cascabela thevetia was chosen for study owing to its global, round-the-year presence, in addition to production of copious nectar. The study summarises that the flower structure in C. thevetia is elaborate and allows a highly precise pollination mechanism. Floral architecture precludes self-pollen to reach receptive stigma head region. Pollination has to be mediated by insects that insert proboscis through one of the five access points delimited by filaments, anthers and trichomes, in order to reach the nectar concealed at the base of the corolla tube. The plant has high potential as food-source especially for the bees because nectar and pollen grains are rich in sugars (sucrose, glucose and fructose); amino acids (valine and proline), fatty acids (erucic acid); organic acids (citrate and acetate); phenolics (shikimic acid); and minerals (potassium and sodium). The rewards are secreted in large quantities and are highly nutritious in terms of biochemical composition. Nutritional quotient of rewards is high when compared to other plants. In spite of the fact that the plant is poisonous, no harmful or detrimental effects have been observed on insects and other animals visiting for pollination and feeding (nectar, pollens, floral tissue and fruits) and on birds that visit as seed dispersers and feed on the fruits. This study has an indirect economic extension which would help elevate the economic gains achieved through bee-keeping.

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1. Introduction 2. Collection of phonological data in selected study site: Delhi NCR 3. Study of detailed floral biology 4. Reward structure: Ultrastructure of reward producing organs 5. Reward structure: Biochemical profile of rewards. 6. Pollination biology. Conclusions and summary.

16. MEENA (Archana)
Soil Carbon Dynamics in Different Terrestrial Ecosystems of Delhi, India.
Supervisor: Prof. K. S. Rao
<u>Th 23131</u>

Abstract
(Not Verified)

The effect of land use change on terrestrial ecosystems constituting vegetation and soil is significant as these ecosystems can act either as a source or as a sink for atmospheric CO_2 and plays important role in global carbon (C) cycle. The study area Delhi, represents a semi-arid ecosystem with varied terrestrial ecosystem diversity due to rapid urbanization and industrialization, population growth, soil erosion and toxicity, reduction in cultivated area. The vegetation ecology of the forest showed loss of indigenous trees and dominance of exotic species (P. juliflora) leading to potential threat to biodiversity. The biomass and carbon stock contribution was higher for young individuals, however the old trees despite of having low tree density contribute significantly to the total carbon stock. The effect of different land uses on soil respiration showed higher emission of CO_2 from cultivated land uses than forest analogues. The strong influence of soil moisture rather than soil temperature on soil respiration, hence suggesting soil moisture as the single and best controlling factor in these ecosystems. The microbial and enzymatic activity were found to be high in forests then arable lands and their significant correlation with soil organic matter in all land uses suggested these as sensitive indicators. The heavy metal pollution in Yamuna river sediments which is the local area problem was also studied. The sediments showed seasonal and spatial variation of metal concentration on the basis of land use activity along the river. Further, positive correlation of metal concentration with sediment respiration suggested their effect on carbon decomposition processes. The results of present investigation will be useful for the understanding the distribution of carbon stock in different terrestrial ecosystems components and modifications in these stocks due to anthropogenic activities related to land use change.

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1. General introduction 2. Review of literature 3. Study area 4. Structure, composition and diversity of trees in a semi-arid forest community 5. Biomass and Carbon stock estimation in a semi-arid forest 6. Soil respiration in different land uses in a semi-arid ecosystem 7. Effect of different land use on basal respiration, microbial biomass carbon and enzyme activity in rhizosphere zone of a semi-arid ecosystem 8. Assessment of heavy metal contamination is sediments along the Yamuna river stretch in Delhi. Summary and conclusion. References. Publications. Conferences and seminar.

17. MOHD HANIEF

Land Use/Land Cover Change and Associated Ecosystem Soil Carbon Dynamics in a Mountainous Watershed in North Western (Kashmir) Himalaya, India.

Supervisor: Prof. K. S. Rao

Th 23129

Abstract

(Not Verified)

The present study investigated the LULC dynamics, regeneration status of dominant tree species, distribution of total SOC, SOC stable and labile fractions and other microbiological soil variables under six different LULC types in Darhal watershed. LULC dynamics analysis showed that throughout the study period, there has been significant increase in the area under agriculture mainly RAG at the cost of GL and different forest covers. Most of the tree species showed poor regeneration which warrants the immediate attention of forest managers and local forest dependent communities. The distribution of SOC and TN concentration and stocks differed significantly among different LULC types due to differences in vegetation cover, land use management history and micro-climatic conditions. The higher SOC and TN stock observed in different forests covers (MF, FF and OF) and GL as compared to croplands suggests that these forest cover types and GL have enormous potential for enhanced SOC and TN sequestration in mountainous watersheds. The progressives increase in the amount of stable SOC pool and enrichment of $\delta^{13}C$ with soil depths indicate that the deeper soil profiles constitute more old carbon. The stable SOC pool was comparatively higher under MF, FF, OF and GL than those under RAG and IAG indicating the prime importance these forest cover types for long term stabilization of SOC in deeper soil depths. This study also revealed that the significant decrease in labile SOC fractions and enzyme activities in soils under croplands as compared to forest covers and grassland should be considered as early indicators of soil quality and biological activity. This study results will also help the policy makers, forest and land managers for their land management decisions.

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1. General introduction 2. Review of literature 3. Study area 4. Land use/land cover dynamics in darhal watershed in Pir Panjal mountain region of Kashmir Himalaya, Jammu and Kashmir, India 5. Natural regeneration dynamics of dominant tree species along an altitudinal gradient in three different forest covers of darhal watershed in north western (Kashmir) Himalaya, India 6. Soil organic carbon and total nitrogen concentrations and stocks under different land use/land cover types in a mountainous watershed of Jammu and Kashmir Himalaya, India 7. Isolation and quantification of biochemically stabilized soil organic carbon pool and natural ^{13}C abundance signatures under different land use/land cover types 8. Labile organic carbon pools, soil respiration and enzyme activities in soils under different land use/land cover types in Darhal watershed. Summary and conclusions. References. Publications.

18. SAGAR (Anjana)

Effect of Arsenic Stress on Growth, Structural and Biochemical Parameters of Cicer arietinum L. And their Mitigation by 24-Epibrassinolide.

Supervisor :Prof. A. K. Pandey and Dr. Inderdeep Kaur

Th 23059

Abstract
(Not Verified)

The present work is a detailed account of ameliorate effect of 24-Epibrassinolide (EBL) on adaptive strategies exhibited by Cicer arietinum L. (chickpea) under As toxicity. Cicer arietinum var. Pusa 362 plants were raised in pot culture on soils spiked with As (As series). A parallel hormone series was also maintained where plants under As stress were supplemented with 10^{-8}M EBL. Increase in As concentration reduced chlorophyll, carotenoid and leghaemoglobin content significantly ($p<0.01$) while hormone application enhanced these parameters. LC-ICP/MS results show a higher concentration of As^{III} (arsenite) in both shoot and root as compared to As^{V} (arsenate). Once As^{V} is taken up by roots it gets reduced to As^{III}. Chickpea plants at elevated levels of As showed increased levels of PC_3 as compared to PC_2 in both the series. This demonstrated that PC_3 shows preferential binding with As^{III} as compared to GSH and PC_2 and forms As^{III}-PC_3 complex. The formation of As^{III}-PC_3 complex and its subsequent compartmentalized can be considered as a defence mechanism adopted by chickpea under As stress. Other significant observations were lowering the As accumulation in plants by EBL and higher accumulation in roots compared to shoots. EBL and/or As treatment showed additive effect on the activity of SOD, APX, GPX and CAT strengthening the defence strategy against ROS. The in planta As speciation showed presence of lesser toxic oAs species i.e DMA as compared to more toxic iAs species at elevated levels of As in soil. The mean As accumulation in different tissues was of the order root > shoot > pods. The investigation suggests an eco-friendly means to combat As stress and enhance adaptive mechanisms through EBL foliar application. The efficacy of detoxification mechanisms operating in chickpea help us categorize it as an As excluder.

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1. Introduction 2. Material and methods 3. Observations and results 4. Discussion 5. Summary and conclusions. References. Publications and conferences. Illustrations.

19. SHIVENDRA KUMAR
Assessment of Genetic and Phenotypic Diversity in the Oilseed Crop, Safflower (Carthamus tinctorius L.), Development of Core Collections and Genetic Resources for Linkage Mapping.
Supervisor: Prof. Arun Jagannath
Th 22743

Abstract
(Verified)

Carthamus tinctorius L. (safflower) is an oilseed crop yielding high quality edible oil rich in poly- and mono-unsaturated fatty acids. Most studies on assessment of genetic diversity in safflower are characterized by limited sample size and poor geographical representation. The present study investigated genetic diversity and population structure of safflower by evaluating a global reference collection of 531 accessions using AFLP markers. Ten informative AFLP primer combinations identified 157 polymorphic markers. High values of Shannon's (0.4536) and Nei's (0.2955) diversity indices indicated that the panel captured high molecular variability. Based on distance-based and Bayesian model-based analysis, several important genetic clusters corresponding to regional pools of safflower were identified. Phenotyping of 531 accessions for 12 agronomically important traits for two growing seasons (2011-12 and 2012-13) revealed significant phenotypic variability. Further, core collections were developed based on molecular data derived from AFLP analysis, phenotypic data of two growing seasons and geographical information of 531 accessions. Initially, six independent core collections were developed using POWERCORE and MSTRAT based on maximization (M) strategy. These independent core collections were merged to develop composite core collections, CartC1 and CartC2 which captured the entire range of phenotypic variability along with genetic variability of safflower represented in BAPS analysis. The phenotypic analysis of safflower germplasm led to the identification of six elite lines with significantly contrasting phenotypes in traits of agronomic value. These were used as parental lines for development of four different mapping populations (A - D). F1 progeny of each population was tested for hybridity using microsatellite markers polymorphic between the parents and true hybrids were propagated to the next generation. Additionally, polymorphic AFLP and microsatellite markers were identified for parental lines of population A. Information generated in the present study would be useful for designing effective breeding programs for crop improvement in safflower.

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1. Introduction and review of literature 2. Assessment of genetic diversity and population structure of safflower germplasm using aflp markers 3. Evaluation of phenotypic diversity of safflower germplasm and development of compact composite core collections using molecular markers, phenotypic data and geographical information 4. Development of mapping populations in safflower and identification of polymorphic aflp and ssr markers for parental lines of mapping population 'A'. Summary and conclusions. References. Appendices. Reprints of publications.

20. SHUKLA (Neha)
Transcriptome Analysis of Root-Knot Nematode (Meloidogyne incognita) infected tomato (Solanum lycopersicum) Roots Reveals Complex Gene Expression Profiles and Metabolic Networks of Both Plant and Nematode During Susceptible and resistance responses.
Supervisor: Prof. Amar Kumar
Th 22744

Abstract
(Not Verified)

Root-knot nematodes (Meloidogyne incognita) are obligate sedentary endoparasites that cause severe crop losses worldwide. At the molecular level, RKN take over the host cellular machinery to induce nematode feeding sites, called as giant cells (GCs). The formation of GCs involves a highly complex cross-talk between the nematode effector molecules and the plant cellular machinery. To understand the molecular basis of plant-RKN interactions, simultaneous global gene expression profiling of tomato and RKN genes during both susceptible and resistance responses from in vivo RKN-infected tomato roots, under soil-grown conditions, was done. The present work provides a genome-wide perspective of transcriptome from five stages of disease development in susceptible tomato line and two stages in resistant tomato line by high-throughput sequencing and bioinformatics analysis. Additionally, the RNA-Seq based expression profiles of tomato genes were validated by qRT-PCR in different tissues from both susceptible and resistant lines. Furthermore, expression profiles of selected RKN genes were determined by qRT-PCR in enriched RKN-infected root knots at early (5-7 days post infection) and late (26-28 days post infection) stages during disease development. The principal findings of the current study include simultaneous identification of differentially expressed genes during susceptible (1827-tomato, 462-RKN) and resistance (25-tomato, 160-RKN) interactions. In conclusion, this study provides a much deeper and novel insights into the molecular mechanisms involved in plant-nematode interactions. The study has led to identification of a large number of novel and known differentially expressed genes simultaneously from tomato and RKN and their specific modulation during susceptible and/or resistance responses in in vivo infected tomato roots under soil-grown conditions.

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1. Introduction and review of literature 2. Materials and methods 3. Study on RKN disease development, construction and high-throughput sequencing of mRNA-Seq (transcriptome) libraries from RKN-infected and corresponding uninfected tomato roots from both susceptible and resistant lines 4. Identification and expression profiles of tomato genes using transcriptome analysis of RKN-infected roots during susceptible and resistance responses 5. Identification and expression profiles of RKN genes using transcriptome analysis of RKN-infected roots during susceptible and resistance responses 6. Discussion and future prospects. References. Annexures. Supplementary tables. List of publications. Re-print of publications.

21. SINGH (Neha)
Nitric Oxide–Heme Oxygenase Crosstalk and Modulation of Redox Homeostasis During Seedling Growth in Sunflower (Helianthus annuus L.).
Supervisor: Prof. Satish C. Bhatla
Th 22742

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1. Introduction 2. Review of literature 3. Material and methods 4. Results 5. Discussion. Summary. References. Research publications. Supplemetary data

22. YADAV (Neha)
Reproductive Biology of Acer oblongum Wall. Ex DC. (Sapindaceae): A Threatened Tree Species of Central Himalaya.
Supervisor: Prof. A. K. Pandy and A. K. Bhatnagar
Th 22745

Abstract
(Not Verified)

Sexual system and reproductive biology of the endangered tree Acer oblongum Wall. ex DC. has been investigated. Three different flowering morphs: Staminate type I, hermaphrodite and Staminate type II were present on each individual. A. oblongum shows andromonoecious type of sexual system bearing three types of floral morphs. Inception of flowering is marked by anthesis of Staminate type I flowers followed by hermaphrodite and Staminate type II flowers. The proportion of Staminate type I flowers is highest among the three floral morphs. The anthers of hermaphrodite flowers serve a relict function of attracting pollinators, but autogamy is avoided because of their pollen sterility and lack of dehiscence. The pollination is primarily by wind. In experimental pollination at DDN, geitonogamy yielded 43.14 % fruits, and xenogamy 54.83 % fruits indicating that cross pollination results in higher fruit set because of different gene pool of donor. But mature fruits (samaras) with wings do not invariably indicate presence of mature seeds. Because unpollinated hermaphrodite flowers develop parthenocarpically and may fall before actual time of dispersal. Insects (Apis dorsata and syrphid flies) facilitate cross pollination by a small percentage (3-5 %). Nearly 30 % of the diaspores fall beneath the parent tree, but the maximum distance to which the diaspores are dispersed by wind when aided by other biotic factor is 100 m. Viability of seed remains for six months. Some diaspores persist on the trees till next flowering season, but these lose their ability to germinate. The rate of seed germination in natural habitats is >30 %, but increasing temperature and inadequate soil moisture retention in summer months leads to poor seedling survival. The seedling survival is higher at Dehradun where the trees are growing in a protected area with minimal human disturbance and where the fields are maintained by watering and manuring.

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