CHAPTER 15

BOTANY

Doctoral Theses

01. ANSHU

Interplay of Moss Cover, Seasons, and Forest Types on the Soil Ecology of Indian Central Himalayas and Heavy Metal Stress-Induced Changes on the Physiological Attributes of Mosses.

Supervisor: Prof. Ratul Baishya

Th 28184

Abstract

Mosses (Division – Bryophyta; Class – Bryopsida) are ubiquitous as a major group of bryophytes and form an essential component of ground-layer vegetation in different ecosystems. Mosses influence the global carbon, biogeochemical, and hydrological cycles due to their distinct ecology and physiology. They are one of the important plant communities in the Indian Central Himalayas (ICH), playing an essential role in ecosystem dynamics. Soil biochemistry is linked to seasonal and vegetation-related changes, but little is known about how moss cover, season, and forest types collectively affect soil properties. Considering this, the present study was undertaken in five forest types of the ICH under two ground cover types (moss-covered and bare soil) to assess their impacts on soil ecology during the rainy and winter seasons. This study also explored the role of mosses in the remediation of heavy metal pollution. The results showed that moss cover positively influenced soil physical, chemical, and biochemical properties, which were higher in the moss-covered soil during the rainy and in bare soil during the winter season. Most of the soil properties were higher in the Cupressus torulosa forest, and forests dominated by Quercus sp. as compared to the Pinus roxburghii forest. Mosses, due to their unique surface features, are widely used as biosensors of environmental contamination and models for heavy metal-induced physiological changes. Nickel (Ni) and lead (Pb) stress induced dose-dependent alterations in physiological parameters in six moss species. Atrichum undulatum and Thuidium glaucinum were found to be the most sensitive and resistant species, respectively. Also, the biochar prepared from moss Campylopus richardii Brid. was effective in the removal of heavy metals from the aqueous solutions. This study will improve our understanding of the ecological functions of mosses in temperate ecosystems and act as a foundation for the effective management and conservation of mosses.

Contents

1. General introduction and objectives of this research 2. Description of the Study area 3. Influence of moss cover and seasonal variations on soil physical and chemical properties in different forest types of the Indian Central Himalayas, Uttarakhand 4. Influence of moss cover and seasonal variations on soil bio-chemical properties in different forest types of the Indian Central Himalayas, Uttarakhand 5. Changes in physiological parameters under nickel and lead heavy metal stress in six different Himalayan moss species 6. Preparation and characterization of biochar from moss Campylopus richardii Brid. and batch adsorption study of nickel and lead heavy metal 7. Summary and Conclusion. Literature cited and List of Publications and conferences.

02. RAWAT (Sapna)

Evaluation of A Representative Global Safflower Germplasm Collection for Resistance to Uroleucon Compositae Theo. (Black Aphid), Transcriptome Profiling of Aphid Developmental Stages and Phenotyping of Mapping Populations for Various Agronomic Traits in Carthamus Tinctorius L. (Safflower).

Supervisor: Prof. Arun Jagannath

Th 28186

Abstract

Safflower (Carthamus tinctorius L.) is an annual oilseed crop valued for its high unsaturated fatty acid content. Aphid (Uroleucon compositae) infestation poses a significant threat to safflower cultivation. In this study, a representative global safflower germplasm collection of 327 accessions from 37 countries was evaluated for aphid resistance. Field-based natural infestation identified five tolerant lines of which, only one accession (PI 242419) was confirmed as tolerant in controlled infestation studies and demonstrated no significant difference in seed yield and hundred-seed weight. We also developed a hydroponics-based screening method which enabled rapid, robust, and high-throughput screening of accessions for aphid tolerance. Inheritance studies on F1 and F2 progeny of a cross between tolerant and susceptible lines indicated aphid tolerance to be a dominant trait governed by a single gene/locus. A mapping population was developed and is currently in the F6 generation. Transcriptome analysis of U. compositae adults and nymphs using Illumina technology identified 39,065 unigenes of which, 50.9% were annotated. Differential expression analysis revealed 146 upregulated and 257 downregulated transcripts in nymphs with reference to adults. Key transcripts involved in host recognition, detoxification, and reproduction were identified. The study identified 421 effector proteins and 1321 transcripts of the endosymbiont, Buchnera aphidicola. The study also reports the development and phenotyping of two independent RIL populations derived from parents contrasting for several traits. Phenotypic analysis of the populations was conducted across three locations (Delhi, Hyderabad and Phaltan) in randomized block design which indicated significant influence of environment on plant height, number of heads and spininess. Head diameter, hundred seed weight and number of primary branches demonstrated fewer variations between environments. This data would be used for genetic mapping following genotyping and construction of high-density linkage maps. Data generated in this study would allow understanding of the genetic and molecular mechanisms underlying aphid resistance and various traits of agronomic value in safflower.

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03. SHARMA (Manvi)

Development of RNAi-Based, Aphid-Resistant Transgenic Plants in the Oilseed Crop, Brassica Juncea (L.) Czern & Coss (Indian Mustard) and Analysis of Differentiating Transcriptomic Attributes of a Specialist Aphid, Lipaphis Erysimi (Kaltenbach) and a Generalist Aphid, Myzus Persicae (Sulzer).

Supervisor: Prof. Arun Jagannath

Th 28187

Abstract

Brassica sp. is among the seven major oilseed crops globally. In India, mustard-rapeseed is the 4th largest edible oilseed crop. Aphids are phloem sap-sucking pests that lead to severe yield losses. The current study developed RNAi-based transgenic plants in B. juncea var. Varuna that confer resistance against two important aphid pests - Lipaphis erysimi and Myzus persicae. L. erysimi is a specialist aphid of the Brassicaceae family, with a narrow host range while M. persicae is a generalist, feeding on a broader range of host plants. Based on identification of important aphid genes involved in detoxification, digestion and development, four different RNAi constructs were developed and used for development of transgenic plants in B. juncea var. Varuna by Agrobacterium-mediated transformation. Effectiveness of the generated transgenic plants against aphid infestation was analyzed by controlled bioassays inside containment net-houses during the growing season (Oct – Mar). A significant reduction in aphid fecundity was observed in four single-locus and five homozygous transgenic lines across three constructs with respect to control (untransformed) plants against both aphid species. These lines could now be used for further studies under containment field conditions. In addition, a comparative transcriptomic study was conducted between the generalist and specialist aphids at adult and nymphal stages to explore molecular responses generated while feeding on the same host. Variations in the transcriptome profile between different developmental stages (adults and nymphs) of the generalist and specialist aphids were also studied. RNA-seq analysis revealed a distinct pattern of gene expression between the two aphid species in which the generalist aphid showed substantial levels of upregulation in feeding, detoxification and reproduction-related genes in comparison to the specialist aphid at both adult and nymphal stages. Both these transcriptomics studies provided valuable insights into aphid biology which could be used for development of improved strategies for crop protection

Contents

1 Introduction and review of literature 2. Development of RNAi-based constructs and transgenic plants in Brassica juncea for resistance against Lipaphis erysimi and Myzus persicae and screening of aphid resistant lines 3. Generation and analysis of transcriptome data from nymphs and adults of Lipaphis erysimi and Myzus persicae 3(A). Comparative transcriptomics of a generalist aphid, Myzus persicae and a specialist aphid, Lipaphis erysimi and identification of molecular signatures contributing to their feeding behaviour and other attributes 3(B). Comparative analysis of gene expression profiles between adult and nymph developmental stages in the generalist aphid, Myzus persicae and the specialist aphid, Lipaphis erysimi 4. Summary and conclusions 5. References and Appendices. List of publications and conferences. Reprints of Publication and Conference Certificates.

04. SHARMA (Megha)
Development of Genomic Resources and Strategies for Predictive Breeding Through Global Germplasm Evaluation, Pangenome Assembly, Genomic Prediction and Multi-Environment Assessment in Safflower (Carthamus Tinctorius L.).
Supervisors: Prof. Shailendra Goel and Prof. Ani A. Elias
Th 28188

Abstract

Safflower is a drought-resilient oilseed crop. Despite its wide utility, it remains underutilized due to its poor yield and low oil content. To enhance its competitiveness with major oilseed crops and establish its significance, it is crucial to delve into its genomics and develop its currently limited genomic resources. The

present research evaluated a global reference collection constituting 1497 accessions for their oil content and fatty acid profiles. A wide variability in the oil content (11.24%-59.16%), linoleic acid (8.22% -87.27%) and oleic acid (6.98%-85.84%) were detected. In total, eight accessions with very high oil content (<50%) coupled with desirable fatty acid composition were identified. A high-oil superior accession was sequenced using third-generation sequencing technologies resulting in a near complete highly annotated chromosome-level reference assembly (1.15 Gb; ~63000 genes). The generated reference assembly was used to analyze resequencing data of a global core-collection (123 accessions), to generate a pan-genome for safflower providing critical insights into the genome diversity and identifying an additional ~11000 genes. Further, genomic selection was implemented in the safflower breeding population in the four cultivations of India. Phenotypic variation was assessed using two seasons multi-environmental data which accounts for field variability in GS and observed significant effects of blocks, locations, seasons, and G×E interactions. Through robust modelling, 14 elite lines with high yield that are broadly adaptable to target environments were selected. Five of these lines showed high genomic estimated breeding values across multiple traits making them ideal candidates for future breeding cycles/commercialization. Finaly, high-resolution soil profile (14 physiochemical properties; 288 samples) was evaluated using machine and deep learning approaches for its application in safflower precision agriculture. It facilitated efficient resource management and designs that promotes sustainable production. This research work provides valuable resources for use in breeding and crop improvement programs for the global research community.

Contents

1. Advances in the genetic, genomic, and agronomic aspects of safflower – A comprehensive literature review 2. Exploring natural variation in seed oil traits across the global germplasm of safflower 3. Construction of reference genome and pan-genome assembly to facilitate exploration of important agronomical traits for the safflower improvement 4. Genomic selection of broadly adaptable high-yielding multipurpose lines from safflower breeding population 5. Predictive modelling of soil profiles for precision agriculture in safflower cultivation fields. Appendices and List of Publications and Conferences.

05. SHUKLA (Richa)
Transcriptome-based insights, analysis of natural anti-sense long non-coding RNA (NAT-lncRNA) and SAUR homologs from Arabidopsis thaliana and Brassica juncea involved in stamen development.
Supervisor: Prof. Sandip Das
Th 28664

Abstract

Brassicaceae members hold significant importance not only as model plants for various developmental and adaptive traits. The complex evolutionary history of Brassicaceae is marked by multiple whole genome duplications and allopolyploidization events making it an excellent model family to explore the evolutionary processes. Inspite of their importance, major lacunae remain in understanding the molecular basis of a Brassicaceae-specific trait, the tetradynamous stamens. The molecular mechanisms that differentiate and control filament length are also unknown. The present thesis was therefore undertaken to identify molecular regulators of long and short filaments in Arabidopsis thaliana Col-0 and Brassica juncea var. Varuna through transcriptome profiling. Based on the results of differentially expressed transcripts between short and long filaments, a long non-coding RNA (lncRNA), and members of SAUR63 sub-clade were characterized. LncRNAs have been identified as critical regulators of developmental and adaptive traits. A candidate lncRNA, NAT-SAUR61, was functionally characterized by analysing transcriptional regulation mediated by associated cis-elements or promoters, and through reverse genetic mutants. SAURs represent the largest group of early auxin-responsive genes, yet their evolutionary trajectory and biological significance remain poorly understood in crop plants. A genome-wide identification and characterization of SAUR genes was performed across representative Brassicaceae members, including A. thaliana and B. juncea. Although considerable progress has been made in understanding functional roles of SAUR genes in auxin signalling, much less is known about their regulation mediated via non-coding RNAs. Recognizing this gap, the present study also focused on investigating the potential regulatory interactions between the candidate lncRNAs and select SAUR genes in A. thaliana by analysing the expression of SAUR63 sub-clade members in NAT-SAUR61 overexpression and knockdown lines. Finally, the transcriptional regulation of homologs of SAUR63 sub-clade from B. juncea was analysed by employing promoter::reporter transcriptional fusion lines to investigate regulatory diversification.

Contents

1. Introduction 2. Review of Literature 3. Methodology 4. To gain insights into molecular basis of tetradynamous condition of stamen in Arabidopsis thaliana and Brassica juncea through transcript profiling 5. Functional characterisation of a natural antisense lncRNA against SAUR63-subclade (NAT-lncRNA, NATSAUR61) 6. Evolutionary and organisation complexity of SAUR gene family across Brassicaceae employing computational analysis 7. Regulatory diversification among members of SAUR63 sub-clade of Brassica juncea 8. Discussion 9. Summary, conclusion and future prospects. References, Appendices and List of publications and conferences.

06. YADAV (Poonam)

Molecular and Functional Characterization of the Stress Interactome of AGB1 and NDL1 in Arabidopsis Thaliana.

Supervisor: Prof. Yashwanti Mudgil

Th 28189

Abstract

Plants adapt to a variety of stressors, such as extreme temperatures, drought and salinity, through complex molecular, physiological and biochemical mechanisms. G-protein signaling, plays a pivotal role in stress tolerance, G-proteins receptors sense the signals and transduce them to the heterotrimeric G-proteins for further transmission through various suitable downstream effectors depending upon signals resulting in activation of various components of plant stress signaling. In Arabidopsis G-protein subunit AGB1 regulates cell division, growth regulation, ion channel activity, hormonal responses and biotic as well as abiotic stress responses like salt, drought and heat stress. AGB1 well characterized mutant, agb1 has established phenotypes under salt, heat and cold stress. AGB1 interacts with various effector molecules to mediate signaling pathways that regulates plant development and various stress responses. In Arabidopsis N-myc downregulated like-1 (NDL1) is an interactor of AGB1 and this interaction is known to regulate root growth and shoot development. AGB1-NDL1 module have been established to play role in salt stress and drought response however the molecular mechanism of action of AGB1-NDL1 module in development and stress signaling pathways is not well understood and this vital information is missing. Therefore, primary objectives of this thesis were designed to explore the molecular mechanisms of action of Arabidopsis AGB1 and NDL1 interactome through following specific goals: molecular characterization of AGB1 mutant under salt stress, characterization of the selected candidate proteins modulated by salt stress in agb1 mutant, isolation and characterization of T-DNA insertion transcript null mutants of selected downstream effectors of the NDL1 (CAD9 and ANN1), their in-planta interaction analysis with AGB1 and NDL1 followed by further detailed molecular and

functional characterization of both the interactors. Our study suggests that AGB1 and NDL1 play various roles in plant growth, development and abiotic stresses through protein-protein interaction with specific interactors like GAPC1, ANN1 and CAD9.

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