CHAPTER 6

BOTANY

Doctoral Theses

031. BARMAN (Paramananda)
Phylogenetic Biology of Dioscorea : Transcriptome, Biochemical Activities and Storage Protein Disocorin.
Supervisor : Prof. R Geeta
Th 22231

Abstract

Dioscorea is a large monocot genus of species with either underground tubers or rhizomes, many of which are used for food or medicine. The major storage protein of Dioscorea is dioscorin, a multifunctional protein identified as an alpha carbonic anhydrase. In the present study the aim was to address the biology of Dioscorea in a phylogenetic framework. The first objective of this study was phylogenetic analysis of Indian Dioscorea. In molecular phylogenetic analyses, using plastid markers, the tuberous and rhizomatous species formed separate clades with strong support and members of each section formed distinct clades supporting the traditional morphology based sectional classification of Dioscorea. Under the second objective, genome-wide expression profiles of tuberous and rhizomatous species and underground and aerial tissues of Dioscorea were compared for the first time. Transcript (mRNA) sequencing was done of shoot tips and underground parts (tuber or rhizome) of D. alata (edible tuberous species) and D. deltoidea (medicinal rhizomatous species). Large amounts of information on unigene sequences, differential and tissue specific expression profile, transcription factors and SSR marker sequences were obtained. The third objective was to study the secondary metabolite pathways and to perform in vitro antioxidant studies and estimate phenolic and flavonoid content in selected Dioscorea species. Secondary metabolite pathway intermediates (phenolic and flavonoid) are upregulated in rhizomatous species D. deltoidea compared to the tuberous D. alata. The phenolic and flavonoid content and antioxidant activity are higher in medicinal rhizomatous members of Dioscorea than the tuberous ones. The fourth objective was to study the expression and evolution of the alpha CA, dioscorin. The gene structure of dioscorin was characterized for the first time, revealing dioscorin genes with and without introns. Aerial and constitutive expression of different paralogs of dioscorin was established. The phylogenetic analyses suggested ancient duplications and independent rapid diversification of alpha CAs.

Contents

1. Introduction 2. Phylogenetic analysis of Indian dioscorea based on plastid markers. 3. Transcriptome analysis of species from tuberous (D. alata) and rhizomatous (D. deltoidea) clades of dioscorea 4. Secondary metabolite pathway mapping and in vitro antioxidant studies in dioscorea species 5. Expression and evolution of storage protein dioscorin (Alpha carbonic anhydrase) in some dioscoreaceae. Summary and appendices.

032. BISWAS (Shreyasee)
Effect of Elevated Levels of Arsenic in Soil on the Growth, Antioxidative Status and Secondary Metabolite of Plumbago *zey*lanica L.
Supervisors : Prof. S. B. Baddar and Prof. A. K. Bhatnagar
Th 22232

Contents

1. Introduction 2. Materials and methods 3. Results and observations 4. Discussion 5. Summary and Conclusions. References.

033. DWIVEDI (Anuj)

Analysis of Putative Candidate Genes Associated with Apomictic and Sexual Modes of Reproduction in Cenchrus Ciliaris L. using Transcriptomic, in Situ Hybridization and Phylogenetic Approaches.

Supervisor : Prof. Vishnu Bhat

Th 22491

Contents

1. Introduction. 2. Characterization of the developmental stages of megasporo and megagametogenesis in apomictic and sexual ovaries using ovule-clearing techniques. 3. Characterization of spatio-temporal pattern of expression of partial CcRLK & CcKIP1 genes during megasporo- & megagametogenesis using in situ hybridization. 4. Analysis of the pattern of expression of partial CcRLK and CcKIP1 genes using rice expression database. 5. Structural characterization of CcKIP1 and partial CcRLK genes through in silico analysis for understanding their putative function. 6. Recombinant expression of epitopic region of CcRLK & CcKIp1 genes for antibiody production. 7. Phylogenetic analysis of CcRLK and CcKIP1 gene familiies across green plants. 8. De novo flower transcriptome analysis of differentially expressed genes during apomictic and sexual reproduction. Summary, conclusions and references.

034. GAURAV KUMAR

Cultivation of Gracilaria Verrucosa (Hudson) Papenfuss at Chilika Lake and its Potential Utilization as a Source of Food, feed and fertilizer.

Supervisor : Prof. Dinabandhu Sahoo

Th 22494

Contents

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

035. HEIKRUJAM (Monika)

Development of Sex-Linked Markers and Genetic Diversity Analysis Among Different Genotypes of Simmondsia Chinensis (Link) Schneider (Jojoba) Employing DNA Fingerprinting.

Supervisor : Prof. Veena Agrawal

Th 22498

Abstract

Simmondsia chinensis (Jojoba), a dioecious crop is of immense agricultural importance where only the female plants are preferred for commerce. Molecular markers such as;ISSR, STS, SRAP, SCoT and CBDP analysis along with bulk segregant analysis have been carried out with 17 male and 22 female to identify sex specific markers. Of 80 ISSR primers, only three primers UBC807; ISSR848 and VIS11 generated male sex specific bands of ~1200bp, ~1500bp and ~1300bp respectively. STS primers “STS807F3/R3” and “STSVIS11F1/R1” which were converted from the male specific ISSR markers, amplified ~800bp band and ~584bp respectively specific to males. These two markers were further validated on 200 plants. SRAP primer “Em-14/Me-10” generated male specific bands of 400bp which were validated on 200 plants. To detect genetic diversity among the 39 Jojoba genotypes, 15 SCoT and 17 CBDP markers were compared. CBDP

markers proved more effective over SCoT. The Polymorphic information content (PIC) and marker index (MI) of CBPD were 0.62 and 1.66, respectively which were higher than those of SCoT where the respective value of PIC and MI were 0.38 and 1.000. UPGMA dendrograms clustered the 39 jojoba genotypes into male groups and female groups. FT-IR spectroscopy was conducted among 8 male and 8 female genotypes. In hexane extract, three female genotypes, viz. 92F, 32F and MSF displayed the presence of three specific functional groups, viz. α, β-unsaturated aldehyde, α, β-unsaturated ketone and carbonyl. The functional group aromatic amine was present in only three female genotypes, viz. MSF, Q106F and 17-21F while the functional group α, β-unsaturated ester was present only in a Q106F. In methanolic extract, the functional group 'Alkyl halide' was found to be present in only MS M while 'aromatic' was present in only Q104M and 17-21M. Thus, three sex-linked markers expressed only in male and absent in female were developed.

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

036. JOSHI (Bharat)

Identification, Characterization and Validation of Potential Candidate Genes Influencing Seed Size in the Oilseed Crop, *Brassica Juncea* (Indian Mustard)

Supervisor : Dr. Arun Jagannath

Th 22230

Abstract

Brassica juncea (Indian mustard) is the second most important oilseed crop of the Indian subcontinent after groundnut. The present study was initiated to identify candidate genes regulating seed size in B. juncea. Seven potential candidate genes (DEF2, GASA4, RGL3, GA3OX4, GA3OX1, GA2OX1 and GA2OX3) were selected based on their roles in influencing seed size in Arabidopsis and other crop plants and their putative co-localization with QTL for seed size in B. juncea. These genes were analysed for their potential contribution to seed size in B. juncea using a combination of different approaches viz., genetic mapping, analysis of phenotypic traits (seed size, seeds per siliqua and siliqua length), anatomical variations (embryo development) and expression level variations during various stages of seed development in two bold-seeded varieties and two small-seeded varieties of the crop. For functional validation of selected candidate genes, nine different transformation vectors based on over-expression and/or antisense (AS)-mediated suppression of candidate genes were developed. The genes were cloned under transcriptional control of the seed-specific Napin promoter. Transgenic plants were raised in bold-seeded and/or small-seeded varieties of B. juncea by Agrobacterium-mediated transformation and grown in a containment net-house during the growing season (Oct-Mar). T_1seeds obtained by self-pollination of T_0 plants were analyzed for hundred seed weight (as a measure of seed size) and subjected to segregation analysis for the selection marker gene (bar) for identification of single locus events. We identified several single-locus transgenic lines across different constructs that showed a promising increase in seed size of T_1 seeds. These lines would be subjected to further molecular and phenotypic studies to validate the above observations. Information generated in this study could be used to design strategies based on transgenic technology and/or breeding approaches for increasing seed size of B. juncea.

Contents

1. Introduction and review of literature 2. Materials and methods 3. Results and Discussion. Summary and Conclusion. References and appendix.

037. KADAMBINI

Reproductive Biology and Dispersal Ecology of Two Threatened Himalayan Tree Species - Pittosporum Eriocarpum Royle and Engelbardia Spicata Leschen. ex Blume.

Supervisors : Prof. Rajesh Tandon and Prof. A. K. Bhatnagar

Th 22499

Abstract

Detailed investigations have been carried out at multiple sites on the reproductive and pollination biology, breeding system, fruit-set, diaspore dispersal, seed germination, seedling recruitment and survival of two endemic and threatened tree species of central and western Himalaya. Pittosporum eriocarpum is subdioecious tree. The trees are pollinated by bees in Uttarakhand, and by butterflies in Himachal Pradesh. Natural pollination results in very low amount of fruit set. Enhanced fruit set by supplemental pollination indicated that the species is pollen limited. Seed dispersal occurs primarily through gravity (79%), and to a limited extent through ornithochory. In nature, seed germination requires scarification treatment in the bird's gut for germination. Underlying morpho-physiological dormancy of seed which requires 7-8 months to overcome reduces the regeneration. This time-period was successfully reduced in laboratory conditions to two weeks using hormonal treatment and temperature stratification. Populations of Engelhardia spicata are trioecious with male, female and monoecious individuals. Trees are obligate out-crossers and show heterodichogamy. Natural fruit-set is low and species is partially incompatible as geitonogamy yields only 9% fruit-set. Diaspore dispersal occurs through wind up to a distance of 80 m away from the source tree. Pollen from distant populations yields more vigorous seeds and seedlings. Habitat fragmentation is having highly negative impact on the reproductive success. Seed germination and seedling survival are affected due to low viability, poor rate of germination. Habitat fragmentation, inbreeding depression due to curtailed gene flow, and poor seed germination because of reduced moisture content in the soil are the major factors affecting the regeneration. The data generated will help in conservation of the two species in their native habitat. It will also serve as precise database for comparison in future, to assess the impact of climate change on trees in the Himalaya.

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1. Introduction. 2. Material and methods. 3. Results. 4. Discussion. 5. Summary and conclusions.

038. KAUSHIK (Sandeep)

Delineating the Host Plant-lac Insect Interaction Using Histological and Biochemical Analysis in Selected Plant Taxa and Molecular Characterization of Endosymbionts of *Kerria Lacca* (Kerr)

Supervisor : Prof. Suman Lakhanpaul

Th 22224

Contents

1. Introduction 2. Materials and methods 3. Anatomical investigations on the lac hosts with reference to feeding behavior 4. Analysis of the selected biochemical parameters of the feeding source 5. Molecular detection and phylogenetic analysis of endosymbionts in lac insect 6.Summary and Conclusions. References, annexure and publications.

039. LAKHOTIA (Nisha)

Genome-Wide Discovery and Characterization of miRNAs and Their Targets Involved in Tuber Initiation and Development in Potato (Solanum Tuberosum L.).

Supervisor : Prof. Amar Kumar

<u>Th 22495</u>

Abstract

Potato (Solanum tuberosum L.) is the fourth most important food crop due to the high carbohydrate and nutritional content of its tubers. Potato tubers are modified underground stem and their initiation and development is a complex process involving integrated signaling pathways and regulatory elements controlling expression of a specific set of genes. Although extensive research in this field has provided important insights into complex regulatory networks controlling tuber induction, the cross talk between different regulatory pathways is not understood yet. Significant roles of miRNAs in the regulation of plant development have been well established. Therefore, it was expected that miRNAs are also involved in tuber initiation and development. The aim of this research work was to characterize both miRNAs and their targets that are likely to be involved in tuber initiation and development in potato. A population of potato miRNAs was identified at genome-wide level using three different vegetative tissues (leaf, stem and root) and four early stages of tuber development by high-throughput sequencing and bioinformatics tools. Expression profiles of identified miRNAs in the above developmental tissues were also studied by qRT-PCR. Additionally, degradome sequencing was performed for genome-wide discovery of targets of identified miRNAs in potato. Several conserved miRNA targets were experimentally validated by RLM-5ᾳRACE. Furthermore, inverse correlation in expression profile of miRNAs and corresponding target genes was investigated by qRT-PCR to gain a better understanding of their regulatory role in developmental processes. Since photoperiod is critical to promote tuber induction, this study also involved the identification of miRNAs that are involved in photoperiodic control of tuber development in potato. In this study, identification and characterization of potato miRNAs have given new insights into molecular basis of tuberization process in potato.

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1. Introduction and review of literature. 2. Materials and methods. 3. Results. 4. Discussion. Summary, conclusions and references. Annexures.

040. MANDAL (Shantanu)

Unravelling the Mechanisms Involved in Arbuscular Mycorrhizal Fungi Mediated Increase in Secondary Metabolite Production in Artemisia Annua L. and Stevia Rebaudiana Bertoni.

Supervisors : Prof. Ved Pal Singh and Dr. Rupam Kapoor

<u>Th 22496</u>

Abstract

Arbuscular mycorrhiza (AM) affects secondary metabolism and the production of active ingredients of medicinal plants. However, the underlying mechanisms that are responsible for these changes in secondary metabolite production are not yet clear. Therefore, the study aimed to investigate various mechanisms underlying enhanced production of artemisinin and steviol glycosides (SGs) (stevioside and rebaudioside-A) in Artemisia annua and Stevia rebaudiana plants colonized by AMF. The study reports that mycorrhizal colonization showed higher endogenous jasmonic acid (JA) concentration than control treatment and the concentration was

statistically similar to the treatment that were sprayed with 200 µM MEJA. These two treatments also closely matched in terms of trichome density, artemisinin concentration and transcript profile of artemisinin biosynthesis genes suggesting that altered trichome density as well as, transcriptional patterns in M plants is mediated via enhanced jasmonic acid (JA). Transcriptional analysis of some rate-limiting enzymes of mevalonate and methyl erythritol phosphate (MEP) pathways revealed that AM contributes more isoprenoids by induction of MEP pathway. A decline in artemisinin concentration in NM and M plants treated with ibuprofen (an inhibitor of JA biosynthesis) further confirmed contribution of JA. In case of Stevia rebaudiana, the higher concentration of SGs in M plants is due to enhanced biomass of the shoots. The increase in biomass is directly due to the improved uptake of nutrients, chlorophyll and carbohydrate concentrations in M plants. Transcript analysis 11 SGs biosynthesis genes grouped under three stages of the SGs biosynthesis pathway showed upregulation by mycorrhizal inoculation. Field validation of the performance of both R. fasciculatus and R. intraradices on Artemisia annua and Stevia rebaudiana in collaboration with IPCA, Ratlam and CSIR-IHBT Palampur revealed that the tested AMF have significant effect on the herbage and increase the concentration of active principles resulting in significant increase in total yield per plant.

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1. General introduction and review of literature. 2. Arbuscular mycorrihizal fungi mediated secondary metabolite production in artemisia annua. 3. Arbuscular mycorrhizal fungi mediated secondary metabolite production in stevia rebaudiana. 4. Summary. 5. References. 6. Research publications.

041. MATHUR (Piyush)

Effect of elevated CO_2 on Plant-Pathogen Interactions in Brassica Juncea L. (Czern. & Coss.).

Supervisors : Prof. Ved Pal Singh and Dr. Rupam Kapoor

Th 22500

Abstract

Increased global CO_2 concentration significantly affects the productivity of crops, but very few studies have addressed the role of pathogens in conjunction with productivity. In this study, effect of elevated CO_2 on plant-pathogen interaction was evaluated in an important oilseed crop (Brassica juncea L. Czern & Coss.) Indian mustard cv. pusa tarak. The work was carried out both in free-air CO_2enrichment (FACE) at Indian Agricultural Research Institute (IARI) and in open top chambers (OTCs) at Department of Botany, University of Delhi. The two concentration of carbon dioxide used for the study were elevated (550 ppm) and ambient (390 ppm). The studies from FACE revealed that there is increased incidence and severity of White rust caused by Albugo candida while decreased incidence and severity of Alternaria blight caused by Alternaria brassicae and Downy mildew caused by Hyaloperonospora brassicae in mustard plants grown at elevated CO_2. Disease severity was correlated with various structural and biochemical parameters. The acquisition of resources such as carbon and nitrogen under elevated CO_2 also affected resource allocation to seeds thereby affecting seed quality. Higher incidence of pathogens was observed in seeds harvested from plants grown under elevated CO_2. The results obtained from FACE were reconfirmed in OTCs by artificially inoculating mustard plants with equal amount of inoculum and it was seen that elevated CO_2 decreased the severity of Alternaria blight in OTCs also. Elevated CO_2 reconfigured the plant's phytohormone (salicylic and jasmonic acid) signaling networks in a manner that might have modulated the expression of some Pathogenesis related (PR) proteins genes. Significant interaction existed between CO_2 concentration, nutrient treatment in amending disease severity of Alternaria blight. The aggressiveness of A. brassicae was studied

by estimating the activity of pectinmethylesterases and polygalacturonases and it was observed that overall aggressiveness of pathogen reduced with successive generation.

Contents

1. Introduction. 2. Material and methods. 3. Objective I- To study the effect of elevated carbon dioxide on leaf quality of mustard plants that affects the natural incidence of foliar diseases. 4. Objective II- To compare the storage stability of mustard seeds harvested from plants grown under elevated carbon dioxide with that of seeds harvested from plants grown under ambient carbon dioxide. 5. Objective III- To study the effect of elevated carbon dioxide on development of alternaria blight in mustard and to elucidate the effect on molecular mechanism of defense. 6. Objective IV- To study the interactive effect of elevated carbon dioxide, nitrogen and sulphur on the physiological and biochemical properties of mustard plants that affects the alternaria blight development. 7. Objective V- To study the response of alternaria brassicae to elevated levels of carbon dioxide. 8. Summary. 9. Bibliography. Publication.

042. MOHANTY (Divya)
Genetic Manipulation via Male Germline in Pigeonpea and Evaluating the Potential of BABY BOOM Gene in Inducing Androgenesis.
Supervisors : Prof. Rajesh Tandon and Dr. Atika Chandra
Th 22493

Abstract

Pigeonpea [Cajanus cajan (L.) Millspaugh]/ 'arhar' is an important grain legume. There is an urgent need to enhance the production of the crop, as the yield has stagnated and is believed to have already reached its performance plateau. This may be achieved in two ways - either by exploiting heterotic breeding or by transgenic technology. Male germline transformation involves the introduction of desired genes into the male gametophyte and can prove to be a useful approach. However, there are several challenges to make it work in refractory systems like pigeonpea. Transgenic plants via male germline transformation can be produced only when the target cells (male gametophytes and/or gametes) are capable of regenerating into complete plants. The haploids can then be converted into doubled haploids. The main objectives of the present study were to develop a protocol for genetic manipulation of male germline in pigeonpea and investigate the various aspects that enhance/govern the androgenic response. The selection of genotype and identification of the developmental stage was essential for both in vitro haploid regeneration and transformation. The standardization of the culture media and conditions formed part of the preliminary and much needed investigations pertaining to regeneration. Thereafter, the role of BABY BOOM gene in inducing androgenesis was evaluated. The study established the amenability of male germline to genetic manipulation. Of the tested methods for gene delivery, particle bombardment emerged as the most effective. The role of 'BABY BOOM' transcription factor as a marker of embryogenesis led us to test if it had the potential to overcome recalcitrance and induce androgenesis. Our results are proof of this concept, as ectopic expression of this gene improved embryogenesis. Isolation and characterisation of the endogenous BABY BOOM gene (CcBBM) opens avenues for further evaluating its function and role in morphogenesis and genetic transformation in the crop.

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1. Introduction. 2. Material and methods. 3. Results. 4. Discussion. 5. Summary and conclusions. Annexures.

043. NIHARIKA BHARTI

Strigolactone Biosynthesis, its Photomodulation and Crosstalk with Other Biomolecules in Regulating Sunflower Seedling Growth and Development.

Supervisor : Prof. Satish C. Bhatla

Th 22492

Abstract

Qualitative analysis and characterization by HPLC, ESI-MS and FT-IR revealed the presence of more than one type of strigolactones (SLs). Three major SLs identified in light-grown seedling roots of sunflower are 5-deoxystrigol, sorgolactone and orobanchol. Orobanchyl acetate and orobanchol have been identified in dark-grown seedling roots. First pair of leaves showed the presence of orobanchyl acetate and sorgomol. The HPLC eluates from seedling roots and first pair of leaves have been used to demonstrate presence of SLs using germination bioassay. Light-induced expression of carotenoid cleavage dioxygenase (CCD) activity in seedling roots and cotyledons has been observed in the present work suggesting light as a positive regulator of SL biosynthesis. SLs are likely to adjust the balance between auxin and ethylene signaling pathways to activate various developmental programmes responsible for root development. Confocal laser scanning microscopic (CLSM) imaging has further indicated a critical role of $[Ca^{2+}]_{cyt}$ in LR development in response to the coordinated action of auxins, ethylene and strigolactones. A negative modulation of CCD activity by endogenous nitric oxide (NO), since NO depletion by cPTIO (a well known NO quencher) treatment significantly enhanced CCD activity has been established. Present observations also highlight the regulatory role of melatonin in lateral root development. Diffusive distribution of PIN1 (auxin efflux transporter protein) in the differentiating zone of the roots in presence of 1-naphthylphthalamic acid (NPA; a blocker of auxin efflux) correlates with a reduction in the spatial distribution of ACT8 (a major vegetative actin isoform) abundance in seedling roots indicating its role in this altering cellular trafficking of biomolecules. Significant reduction in NO and $[Ca^{2+}]_{cyt}$ contents and enhancement in the abundance of Ca^{2+}-calmodulin (CaM) complex in fluridone (SL biosynthesis inhibitor)-treated hypocotyl explants indicates the critical role of NO, $[Ca^{2+}]_{cyt}$ and Ca^{2+}-CaM in adventitious root formation.

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044. PRATIBHA KUMARI

Studies on Endophytic Fungal Association in Bryophytes of Some Selected Sites of Uttarkhand.

Supervisor : Dr. P. L. Uniyal

Th 22225

Abstract

The study is based on the 25 species of liverworts belongs to 11 families and 59 species of mosses belongs to 22 families, collected from different localities of the Uttarakhand states. Mosses and liverworts are collected from seven different sites of Uttarakhand having varied forest types and different humus content. Present study revealed that the all collected 25 species of liverworts are found to be AM associated, whereas among 59 collected mosses only 23 species are observed to be fungal-endophyte associated. In case of liverworts, maximum number of AM associated species is observed in the family Aytoniaceae followed by Marchantiaceae, Cleveaceae and Ricciaceae, Conocephalaceae, Targioniaceae, Wiesnerellaceae, Calyculariaceae, Fossombroniaceae, Dumortieraceae and

Pelliaceae. In mosses eight species are observed to have fungal hyphae inside the rhizoids and show formation of vesicles. Various factors also studied which play an imported role in plant-fungus association. Habitat- Bryophytes collected from moist condition and temperate forest with high humus content show maximum degree of colonization of fungal association. pH- Recorded pH of fungal endophyte associated liverworts and mosses vary from slight acidic to alkaline. It seems that pH alone does not influence the association. Light: Those liverworts species, which grow in exposed condition show high degree of colonization. In mosses more arbuscules-like structure and vesicle formation is observed in the plants which are growing in shady places. Temperature: Present study shows that habitat conditions along with the temperature influence the association. Organic matter: High humus content in substratum influence high degree of colonization. Associated fungal spore: Fungal spores of 18 taxa are islolated from the rhizosphere soil of presently studied liverworts and mosses. Maximum bryophyte species are found to have fungal spores of Glomus sp.

Contents

045. RAM KRISHNA

Isolation and Expression Analysis of Fatty Acid Biosynthetic Pathway Genes in Developing Seeds of Jatropha Curcas L. and Development of Transgenic Plants Using Antisense FAD2 Construct

Supervisor : Dr. Shailendra Goel

Th 22229

Abstract

Biodiesel obtained from Jatropha curcas has been considered as a potential alternate of petro-diesel. However, Jatropha curcas seed oil is rich in linoleic acid content, rendering the derived biodiesel susceptible to oxidation. Therefore, modification of fatty acids compositions of Jatropha curcas seed oil is required for making it suitable as biodiesel. In the present study, we have isolated full length cDNA, genomic and promoter sequences of some important genes which are involved in biosynthesis of fatty acids. Oil content, fatty acids compositions and expression level variation of fatty acid synthetic genes were also evaluated in developing seeds of Jatropha curcas. The oil content increased from 15 DAP to 25 DAP, remained almost constant at 30 DAP and again increased to reach maximum level of approximately 35% in mature seed. The fatty acid compositions of seed oil was found to vary at different developmental stages, however, oleic and linoleic acids were found to be the main constituent in mature seed accounting for approximately 44% and 35% respectively. The transcript levels of KASI, KASII and SAD genes were found to be maintained at low levels from 5 DAP to 25 DAP then up-regulated and reached to a maximum level at 35 DAP. FAD2 transcript level decreased between 5 DAP to 25 DAP then recovered to initial level at 30 DAP and 35 DAP. The FAD3 transcript increased at 10 DAP but declined continuously from 15 DAP to 35 DAP. For genetic manipulation of Jatropha curcas, both regeneration and transformation protocol were established using cotyledonary leaves explants. Based on the optimized protocol, transgenic plants were raised with FAD2 antisense construct driven by seed specific napin promoter for improving oxidative stability of biodiesel.

Contents

1. Introduction 2. Review of literature 3. Isolation and sequence analysis of candidate genes for fatty acid synthesis pathway 4. Expression analysis of fatty acid synthesis pathway genes during development of seed 5. Optimization of regeneration and transformation protocols and development of transgenic plants using antisense FAD2 construct 6.Summary and Conclusion. References and appendix.

046. SINGH (Heisnam Dinesh)

Understanding the Control of Apomixis Through Comparative Profiling of Transcriptome and Small RNAs in Apomictic and Sexual Lines of Pennisetum Glaucum (L.) R. Br.

Supervisor : Dr. Shailendra Goel

Th 22497

Contents

1. Introduction. 2. Review of literature. 3. Screening and maintenance of germplasm lines, establishment of correlation between pistil morphology and gametophyte developmental stages and collection of tissues. 4. Identification of apospory specific ovular transcripts through deep sequencing and comparative profiling between apomictic and sexual pennisetum glaucum lines. 5. Investigation of roles of small RNA in control of apospory. 6. Summary and conclusion. References and annexures.

047. SINHA (Somya)

Development of Frost-Tolerant Transgenic Lines and Identification of Cold-Responsive Genes in Developing Siliques of Indian Mustard (Brassica Juncea L.)

Supervisor : Dr. Manu Agarwal

Th 22227

Abstract

Plants face various abiotic and biotic challenges during their life cycle. One of the major abiotic stresses affecting crop productivity and distribution is low temperature stress. Frost injury significantly affects the crop yield of Indian mustard, thus incurring financial losses to the farmers. Though frost tolerant B. juncea germplasm has been identified, the information about the performance of these varieties in field conditions is not available. In the current work, genes that have been previously shown to provide freezing tolerance were used to raise frost tolerant B. juncea lines. Expression of transgenes was regulated with the help of stage-specific and conditional promoters such as Napin and RD29A. These transgenic lines were checked for frost tolerance in a frost chamber, under conditions optimized to induce lethality in untransformed plants. Two single copy homozygous lines, showing promising frost tolerant phenotype, have been obtained. A genomic methodology was employed to understand the gene expression changes in developing siliques that were exposed to cold stress. Manually self-pollinated siliques (5DAP-30DAP) of B. juncea were subjected to either short (6h) or longer (12h) durations of cold stress. De novo assembly of B. juncea transcriptome resulted in 1,33,641 transcripts. Hierarchal clustering along with Spearman correlation analysis identified gene expression patterns which grouped either in early (5-15DAP) or late stages (20-30DAP) of silique development. Approximately, 13,000 cold-responsive transcripts were differentially regulated by a factor of 2-fold on $\log_2$ scale. Expression profiling performed by QPCR was largely in consonance with the digital expression profiles. An embryo-specific promoter (Bnms4D-113), which is also inducible by low temperatures, was isolated with the help of genome walking methodology. Multiple motifs were identified in the

promoter of Bnms4D-113 gene. This promoter could be used to express genes for frost tolerance in subsequent studies.

Contents

048. SINGH (Vineet Kumar)

Reproductive Ecology of Two Threatened Tree Species, *Tecomella Undulata* and *Anogeissus Sericea*, from Arid Zone of India.

Supervisor : Dr. Rajesh Tandon

Th 22226

Abstract

Trees are the important forest forming components of forests. A great majority of trees are data deficient and many of these are threatened due a variety of reasons. In order to properly conserve them, it is important to understand their reproductive strategy. The main objective of the presented work was to study the reproductive ecology of two threatened trees viz. Tecomella undulata and Anogeissus sericea of arid zone of India. Tecomella undulata: The species exhibits mass synchronized flowering and the three colour morphs, lacked the compatibility barriers. The stigma is touch sensitive and required ~500 pollen grains to terminate the movement of the stigmatic lobes. Tree exhibits xenogamy and shows late acting self-incompatibility which is of post-zygotic type. Flowers produce copious amount of nectar which gets replenished after consumption. Two species of bulbul (Pycnonotus cafer and P. leucotis) were the pollinators while purple sunbird (Nectarinia asiatica) was the nectar robber. Experimental nectar robbing showed that pollinators covered lower number of flowers per tree and more trees per bout than the unrobbed. The robbed trees set a significantly greater amount of fruits than the unrobbed ones; the average number of seeds in a fruit did not differ. Anogeissus sericea: The two varieties of the species were geographically and reproductively isolated. Flowering is cornucopia and the key floral features were protogyny and cup shaped stigma. Both the varieties were autogamous. In var. nummularia, fruit-set is density dependent and exhibits inbreeding depression while var. sericea exhibited pollinator limitation. Flowers of both the varieties are pollinated by the dipteran flies which ensure targeted pollen deposition. Most importantly, the study established that plant-pollinator-robber interaction benefits the self-incompatible trees in T. undulata. Pollinator limitation, inbreeding depression, short distance dispersal and low natural regeneration are the major reproductive constrains among the varieties of A. sericea.

Contents

049. WAIKHOM (Sonia)

Development of Transgenic Plants Using Lectin Genes from Onion and Garlic for Introduction of aphid Resistance in the Oilseed Crop, *Brassica Juncea* (Indian Mustard)

Supervisor : Dr. Arun Jagannath

Th 22228

Abstract

Brassica juncea is the second most important oilseed crop in India. Mustard aphid (Lipaphis erysimi) is the most devastating pest of B. juncea and natural

resistance against aphids is not reported in cultivated mustard so far. Monocot mannose binding lectins have been reported to affect survival and fecundity of aphids. The present study was initiated to introduce aphid resistance in B. juncea using mature peptide sequences of lectin genes from onion and garlic. In vitro feeding assays on L. erysimi using crude protein extracts from different tissues of onion and garlic showed that leaf extracts of onion and garlic were the most potent inhibitors of aphids. Sequences coding for mature peptide regions of onion (ACA) and garlic (ASAL) leaf lectin genes were amplified by PCR from leaf tissues. Based on nucleotide and deduced amino acid sequence analysis, we identified five paralogs for onion and six for garlic leaf lectin genes. Secondary structure analysis and 3D structure prediction of deduced amino acid sequences showed that they are similar to each other and to the structures reported for onion and garlic lectins. All the identified paralogs of garlic and onion leaf lectin genes were used for development of transformation vectors and genetic transformation of B. juncea by Agrobacterium-mediated transformation. Transgenic B. juncea plants were transferred to containment net-houses and maintained through the growing season (Oct-Mar). T_0 plants were analysed for aphid resistance following natural infestation in net house conditions in two different growing seasons (2012-2013 and 2013-2014). Transgene expression profiles, copy number, thousand seed weight and seed yield per plant were analyzed for selected promising events. Based on the above analysis, six independent lines were identified and selected for further field-level studies. Protocols for over-expression and purification of lectins from E. coli were also optimized for toxicity and allergenicity studies.

Contents

1. Introduction and review of literature 2. Materials and methods 3. Results 4. Discussion 5. Summary and Conclusions. References and appendix.

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