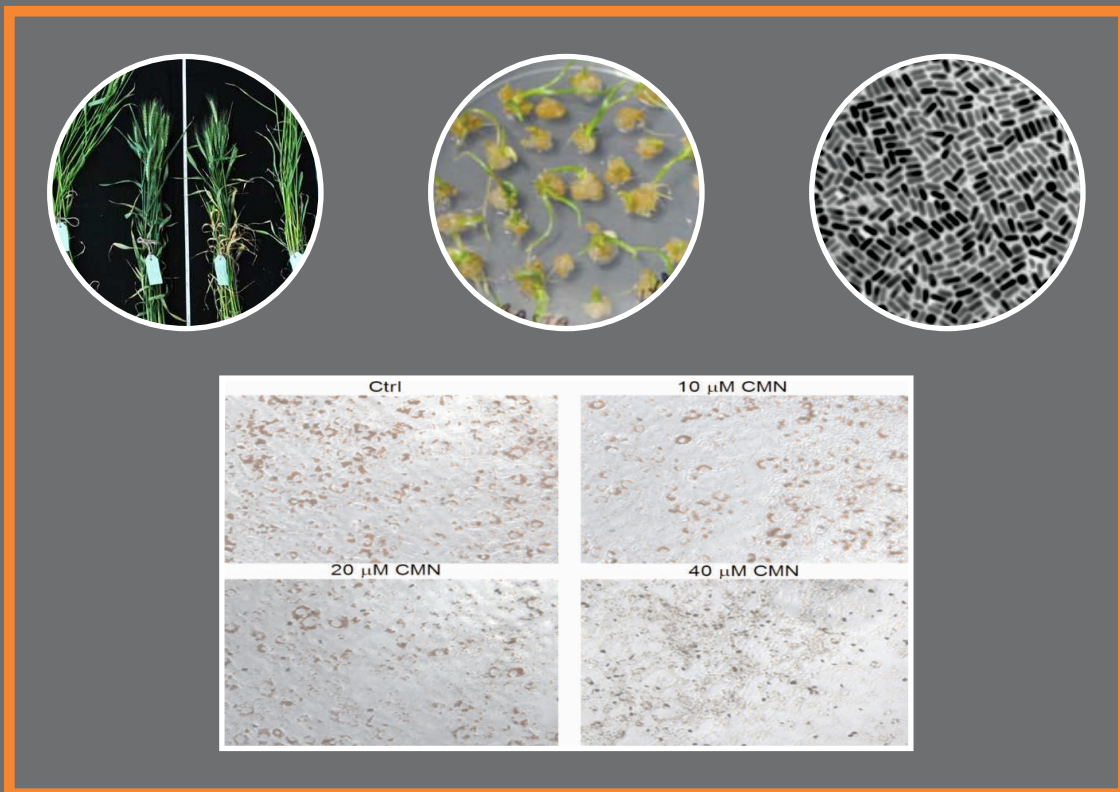


# वार्षिक प्रतिवेदन ANNUAL REPORT 2014-2015

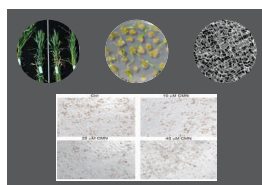


राष्ट्रीय कृषि-खाद्य जैव प्रौद्योगिक संस्थान  
**National Agri-Food Biotechnology Institute**

(An Autonomous Institute of Department of Biotechnology, Government of India)

Published by:  
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Punjab, India – 160071

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**Figure On Cover:**

**Upper Panel :** The cover page portrays wheat mutant population, wheat transformants and nano-biotechnology related research work. (Details on pages 10, 13 and 59)

**Lower Panel :** The picture portrays anti-adipogenic activity of cinnamaldehyde on 3T3-L1 adipocytes. (Details on page 47)



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## FROM THE DESK OF EXECUTIVE DIRECTOR

National Agri-Food Biotechnology Institute (NABI) was established with the objectives of promoting and coordinating research of high caliber in basic and translational aspects at the interface of Agriculture, Food and Nutrition. Research priorities of the institute encompass five core areas that include, a) *Improving Cereals for nutrition and processing quality*; b) *Improving fruits for post-harvest quality and nutrition*; c) *Basic Biology for crop improvement*; d) *Diet and health*, and e) *Computational biology approaches for marker and gene discovery*.

In the area of improving cereals for nutrition and processing quality, NABI is focusing on improving traits such as starch granule size and enhanced amylose content. Role of novel genes and their regulators that determines these parameters is in progress. In wheat, an ethyl methanesulfonate induced M<sub>4</sub> population has been developed for identification of causative genes for the processing and nutrition quality traits. Advanced breeding material for improvement of chapatti, biscuit and bread making quality has been generated. Additionally, a polymorphism in the genes related to the grain hardness and softness was explored and an updated database was created, which could be beneficial for developing breeding material with improved processing quality traits. This year, a new initiative related to the study of the diversity in celiac disease epitopes in Indian wheat cultivars has also been initiated.

In an approach to enhance the bioavailability of iron in wheat grain, comparative transcriptional profiling of two wheat genotypes with contrasting levels of minerals has been performed. Distinct expression pattern during grain filling was observed. Transgenic wheat targeted for gene silencing to modulate antinutrient like phytic acid has been generated. We are also developing multiple

resources for targeting the multiple genes for silencing as well as for genome editing especially in a crop like wheat. To address the issue of nutrition, NABI has collaborated with Queensland University of Technology, Australia and successfully transformed banana with multiple gene constructs so as to enhance the provitamin-A content in the fruit. Several transgenics were generated and detailed biochemical characterization is in progress. In the areas of basic biology for crop improvement, as a proof-of-concept, we are utilizing *Arabidopsis* b-ZIP proteins in developing a dominant negative approach in plant to access the functionality of the gene. In vitro data suggests a preferential interaction of certain b-ZIP transcription factors from *Arabidopsis*. First time, a transcriptome data has been generated for *Annona squamosa* that could be useful for investigations on the molecular mechanisms of fruit development. Dominant negative and root-scion approaches have been tested using marker genes and now expanded for controlling the seedlessness nature of given fruit crop. In the areas of improving fruits for post-harvest quality and nutrition, a dietary fiber based edible fruit coating material has been developed. Its physico-chemical properties and post harvest application is under investigation.

In the area of diet and health, NABI performs basic as well as translational research pertaining to both forms of malnutrition, i.e., over nutrition and undernutrition. In over nutrition, alternate and safe approaches for controlling the metabolic disorders such as obesity and diabetes, which is the need of the hour, is in progress. Dietary constituents of spices that could modulate various Transient Receptor Potential Channels and prevent obesity have been investigated. Cinnamaldehyde, a dietary constituent from cinnamon, reduced adipogenesis via promoting lipolysis. Oral administration of cinnamaldehyde in diet induced obese mice which initiates lipolysis and increased expression level of thermogenesis related genes in brown adipose tissue. Similarly, role of whole grain millets and

their constituents has been taken up. The combinatorial effect of millet, whole grain/bran, showed potential in alleviating symptoms of high-fat diet induced metabolic alterations such as serum cholesterol and glucose levels and promotes abundances of the selected beneficial gut bacteria. A new initiative on the development of synbiotics/cobiotics for the prevention of obesity has been initiated. Several acid and bile resistant potential probiotic lactic acid bacteria with an ability to metabolise prebiotics have been isolated and their role in prevention of obesity will be studied. In the area of undernutrition, iron-alginate formulations that could enhance the iron bioavailability under *in vivo* and *in vitro* conditions has been developed. A novel natural compound that could bind to human hepcidin and enhances iron bioavailability has been identified through computational biology and *in vitro* approaches. Its evaluation in iron homeostasis in mice is under progress. Further, functionalized gold nanorod based biosensors for the detection of food born pathogens has been initiated. Analysis of dietary fibers from millets showed considerable differences in their structure and significant variation in the antioxidant potential. Understanding the relationship between the variability of the fine structures and with their antioxidant potential in cell culture models is in progress.

In the area of computational biology, NABI has developed multiple genomics software resources that are helpful in genome annotation. These tools comprise of advanced algorithms, databases, and pipeline for data mining. In this regard, a tool WImpiBLAST has been developed that could help the biologists to perform large-scale annotation using high performance computing. Another tool

referred as SSFinder was developed that could predict CRISPR-Cas target sites in a high throughput manner.

Human resource development is one of the priorities of NABI. The institute currently has 47 students that include Ph.D. students, junior research fellows and project assistants. Besides, the institute also offers research training to young students in the diverse areas of Agriculture, Food and Nutrition Biology. The institute is currently hosting 14 extramural research grants from different national and international funding agencies. Department of Biotechnology has recommended a five year Nutritional Biology program (integrated Masters and Ph.D.) to NABI with Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh as a partnering institute. This proposal aims at developing a state-of-the-art teaching and research center by combining the resources, expertise and vision of both the institutes. This will open a broad horizon of training and knowledge development, spanning from the basic science of food and nutrition to clinical manifestations and validation of designer foods and nutritional interventions. On the scientific output, the institute has published more than 25 high impact research publications in various international peer reviewed journals. Also one patent application has been submitted.

Over the last few years, research advancements at NABI have shown promising results. In continuation, during last academic year, NABI scientists and staff have taken significant steps towards providing multiple biotechnological and inventive interventions in the field of agriculture, food and nutrition research, with wide outreach.

**Prof. Akhilesh Kumar Tyagi**  
Executive Director (Additional Charge) and Professor



## VISION & MISSION OF NABI

To be a nodal organization for knowledge generation and translational science leading to value added products based on agri- food biotech innovations.

- *To transform agri-food sector into globally rewarding and sustainable biotechnology-based enterprise through innovative solutions in primary and secondary agriculture including high-end food processing.*
- *To develop synergy among knowledge providers and investors in agri-food sector to carry innovations to marketplace.*







## RESEARCH PROGRESS



# IMPROVING CEREALS FOR NUTRITION AND PROCESSING QUALITY

## 1.1 Gene discovery for improvement of processing and nutrition quality in wheat

### Principal Investigator

Joy K Roy

### Research Fellows

Anuradha Singh

Monica Sharma

Pankaj Kumar

Diksha Sharma

Ankita Mishra

### Introduction

Wheat flour is processed into several end-use food products, whose complex quality depends mainly on biochemical composition of grains such as storage protein, starch, phenolics, lipid, etc. Among them, starch contributes about 50-80% of dry seed weight. Hence, it indirectly contributes to yield contributing seed-related traits as biosynthesis of starch affects seed growth. It affects the processing, cooking, and organoleptic qualities and digestibility of starch-based food products. It can be modified to increase amylose portion in starch which is considered as healthy starch, i.e. resistant starch. The present wheat varieties require improvement in nutrition and processing quality to meet the increasing demand of consumers and baking and processing industries. Knowledge of genetics and molecular basis of nutrition and processing related quality traits are important for their improvement. In this project, the genes identified through microarray, transcriptomics, and proteomics approaches are targeted to identify single nucleotide polymorphisms (SNPs) in the wheat germplasm, and undertake their functional validation to identify causative SNPs for wheat improvement through molecular breeding approaches.

### Objectives:

1. Creating repositories for germplasm, aneuploid stocks, and genomic resources for

gene discovery for the processing and nutrition quality traits in wheat.

2. Identification of candidate genes through microarray, transcriptome, and proteomics studies.
3. Phenotyping of processing and nutrition quality traits and genotyping using SNPs and microsatellites i.e. simple sequence repeats (SSRs) on a diverse wheat germplasm set to identify the gene and genomic regions controlling the traits through association studies.
4. Development of mutant lines with the improved level of nutritional and processing quality traits.

### Research Progress

#### Objective 1

1. A comprehensive set of wheat germplasm has been maintained at NABI. It comprises of about 500 indigenous and exotic wheat varieties and landraces, about 250 aneuploid stocks, and 1500 EMS treated M4 population. These germplasms were multiplied at NABI research farm in 2014-15. A subset of this germplasm is being used for the gene and genomic region identification for processing and nutrition quality related traits.
2. A large set of microarray data and transcriptome sequencing data were produced to identify candidate genes for processing and nutrition quality related traits. The available whole wheat genome sequences and wheat SNP array data were downloaded for SNP identification.

#### Objective 2

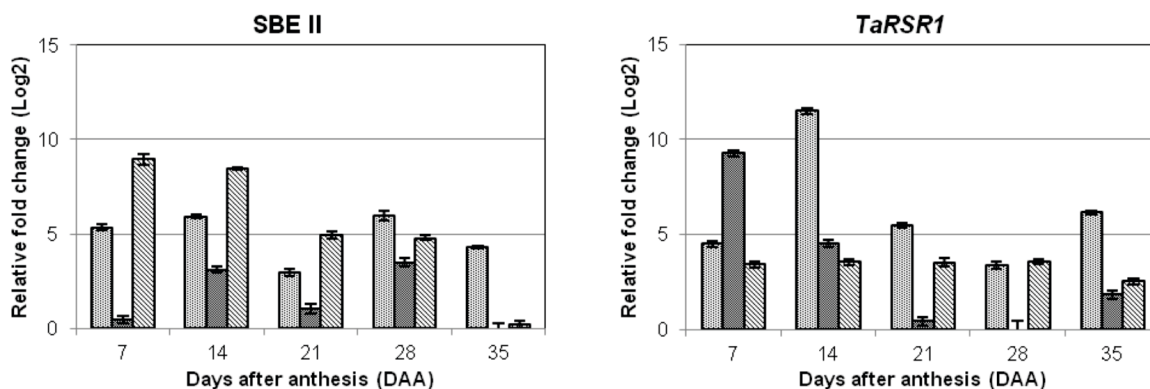
1. The quantitative gene expression analysis of 25 starch metabolic genes were conducted among the three bread wheat (*Triticum aestivum*) genotypes ('C306', 'K65', and 'Amylopectin') differing in starch related traits at five seed developmental stages, i.e. 7, 14, 21, 28, and 35 days after anthesis (DAA; Figure 1). Their sequences were physically mapped to the chromosomes using the wheat

genome sequence data. The starch metabolic genes with high expression level are being sequenced in a wheat germplasm set to identify SNPs for the improvement of starch related traits through association studies.

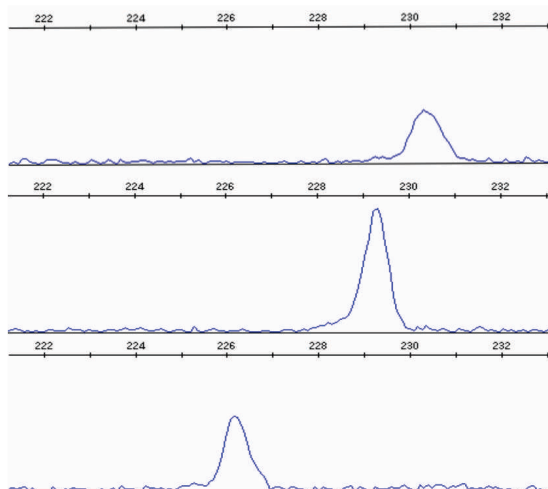
3. A comprehensive set of SNPs was identified in the majority of candidate genes (110 genes identified through microarray studies; 25 starch metabolic genes, and 10 phenyl propanoid pathway genes) using the SNP probe sequences on the 820K Axiom® Array probe set (<http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB>). An in-house

program was developed to identify the non-synonymous mutations. About 570 SNPs were identified for the 25 starch metabolic genes and 50% of them were non-synonymous SNPs. The non-synonymous SNPs of the candidate genes will be used for validation and genotyping for association mapping.

4. A set of about 200 SSR primers (about 10 SSRs per chromosome) are being used to genotype in the wheat germplasm set (Figure 2) on ABI 3730xl and LICOR systems for association mapping.



**Figure 1:** Relative expression (Y-axis) of starch branching enzyme, SBE II and transcription factor, *TaRSR1* at five seed developmental stages (X-axis) in the three wheat genotypes, 'Amylopectin' (dotted bar), 'K65' (crossed bar), and 'C306' (slanted bar).



**Figure 2:** The part of chromatogram showing polymorphism detected by the SSR marker, WMC 429 in the three wheat genotypes using ABI 3730xl sequencing/genotyping system.

### Objective 3

1. Processing quality related starch thermal properties such as onset, peak, conclusion temperature, and enthalpy were done on the second year subset of 50 wheat genotypes using Differential Scanning Calorimetry (DSC). A high level of variation was detected in the parameters. These data will be used in association mapping.
2. Processing quality related dough properties such as medline peak time, peak value, and peak width indicating gluten strength are being done on the second year set of 50 wheat genotypes using a mixograph.
3. A total of 39 phenolic compounds were detected on LC-QTOF (MS/MS), and their quantification (14 out of 39) is being done on the wheat germplasm sets using UPLC/QTRAP system.

### Objective 4

1. Two high amylose (65% amylose) and one low amylose (13% amylose) mutants were produced in background of the Indian wheat variety, 'C 306' (24% amylose). These mutant lines are stable and in M4 generation. Several mutants have been identified for semi-dwarf, long spike length, and lodging resistance in

the same genetic background (Figure 3).

2. Quantitative gene expression analysis of 25 starch metabolic genes were done in the high and low amylose mutants and wild type Indian wheat variety, 'C306'. The candidate genes such as granule bound starch synthase, GBSSI and pullanase were identified for high amylose and the genes, SBE II and RSR1 for low amylose (Figure 4).

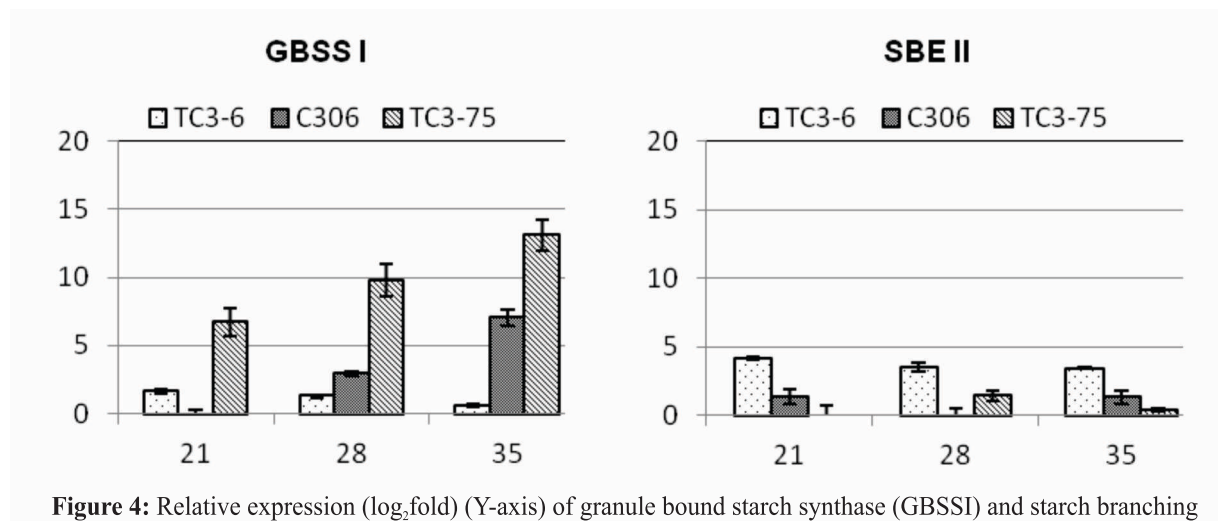
### Salient Achievements

1. The non-synonymous mutations have been identified for starch metabolic genes and the majority of the 110 candidate genes, that were identified using wheat microarrays with 55K wheat transcripts, using the available SNP probe sequences of the 820K wheat SNP Axion arrays (University of Bristol, UK).
2. Starch thermal properties and dough rheological properties were estimated on the subset of 50 wheat genotypes.
3. An EMS induced M4 population has been developed (2014-15) for identification of causative genes for the processing and nutrition quality traits.
4. The mutant lines have been developed for high and low amylose seed starch.



**Figure 3:** The first plant in left snap and the first spike in right snap represent the Indian wheat variety, 'C306', as wild type and the others in both snaps are mutant types of 'C306' showing variation in plant height and spike height





**Figure 4:** Relative expression (log<sub>2</sub> fold) (Y-axis) of granule bound starch synthase (GBSSI) and starch branching enzyme, SBE II at three seed developmental stages (21, 28, and 35 days after anthesis; X-axis) in the wild type, 'C306' (crossed bar) and their two mutant lines, low amylose (dotted bar) and high amylose (slanted bar).

### Future Objectives

1. The diverse subset of wheat germplasm were grown at two locations (Mohali and Indore) are being harvested in 2014-15. The processing quality related traits such as starch thermal and function properties and dough rheological properties will be estimated on the wheat genotypes. Phenolic compounds profiling will be done to identify metabolites and their variation in the above germplasm sets.
2. Markers (SNPs and SSRs) genotyping will be done on the germplasm set.
3. SNP and SSR markers will be identified for processing and nutrition traits through association studies.
4. Transcriptome and genomic sequencing of the mutant lines for high and low amylose shall be done to identify the candidate genes.
5. The registration process of the genetic stocks is being developed.

### 1.2 Metabolic engineering of phytic acid pathway to enhance iron bioavailability in wheat

#### Principal Investigator

Ajay K Pandey

#### Co-Investigator

Siddharth Tiwari

#### Research Fellows

Kaushal K Bhati

Sipla Aggarwal

Vishnu Shukla

#### Introduction:

Approaches for reducing phytic acid (PA; anti nutrient) content in seed to enhance iron bioavailability has been employed in different crops like maize, soybeans and rice. Genes involved in PA pathway are not reported from wheat. In this project we want to utilize functional genomic tool/s to address the role of phytic acid synthesis genes. Our goal is to first identify genes contributing for PA pathway and subsequently generate low PA wheat lines by targeted gene silencing. We anticipate that wheat grains with reduced PA content might show an increase in iron bioavailability.

#### Objectives

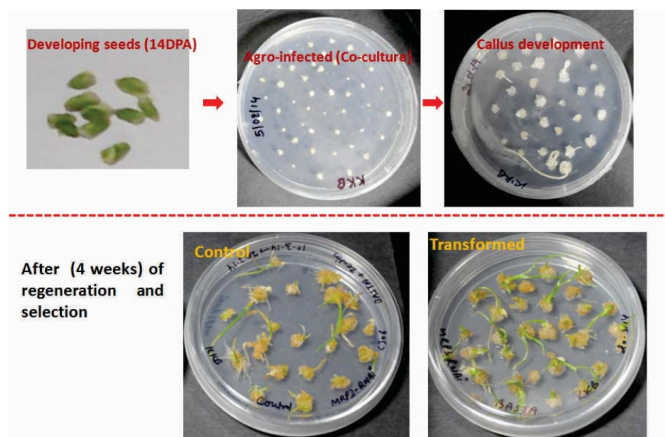
1. Identification and functional characterization of PA pathway genes from wheat.
2. Development of low phytate wheat crop by using RNAi approach.

## Research Progress

1. Our *in silico* analysis identified six wheat genes that might be involved in the biosynthesis of inositol phosphates.
2. Analyses of the mRNA expression levels of these seven genes showed that they are differentially expressed during seed development, and that some are preferentially expressed in aleurone tissue. These results suggest selective roles during PA biosynthesis, and that both lipid - independent and -dependent pathways are active in developing wheat grains.
3. *TaIPK1* and *TaMRP3* were able to complement the yeast *ScAipk1* and *ScAycf1* mutants, respectively, providing evidence that these wheat genes have the expected biochemical functions. This is the first comprehensive study of the wheat genes involved in the late phase of PA biosynthesis.
4. Monocot specific RNAi silencing vector (pMCG161) was selected for preparation of the silencing vectors (Figure 5). The final constructs were then transformed into AGL1 strains of *Agrobacterium tumefaciens*. The transformants colonies were subsequently screened on chloramphenicol (25 mg/l) and rifampicin (35 mg/l) and followed with plant transformation.
5. 12-14 DAA old seedlings (surface sterilized with NaClO: 0.4 % w/v) were used to dissect to separate the immature embryos. The embryo was transferred to callus induction media for 3 days. The calli were subjected for co-cultivation with AGL1-pMCG161 for 48 hrs in dark condition. The transformed calli were then washed with antibiotic and transferred to the callus induction medium plates [MS + 2-4 D (2 mg/L) + cefotaxime (300 mg/L)]. The plates were incubated for 30 days (callus induction, in dark) and further screening was done by sub-culturing onto selection shoot inducing media (MS + zeatin-1 mg/L + cefotaxime-300 mg/L + Basta-2 mg/L) and 15 days cycles repeated for 3 times (Figure 6). Survived shoots were subsequently transferred to root induction media (half strength MS + antibiotics) for 10 days.
6. The generated callus from the Figure 7, after undergoing through multiple selection screening cycles were subjected for rooting. The survived plants were then transferred to small pots containing vermiculite for hardening. The putative transgenic plants T<sub>0</sub>, were further grown. After maturation of the plants, the spikes were collected for subsequent study.



**Figure 5:** Schematic representation and orientation of the RNAi constructs used for wheat transformation for targeted gene silencing of *TaMRP3*. Vector backbone of pMCG161 was utilized to clone fragments of *TaMRP3* gene in sense and antisense orientations.

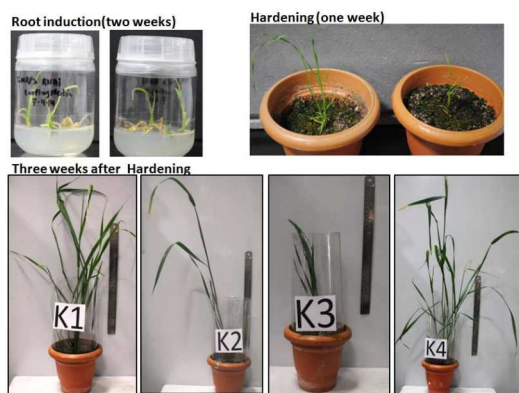


**Figure 6:** Different stages of wheat transformation used for silencing *TaMPR3*. Developing wheat seeds were used for embryo isolation and subsequently co-cultivated with the *Agrobacterium* bearing the construct for CaMV::TaMPR3. Representative pictures were used to explain the process of wheat transformation.

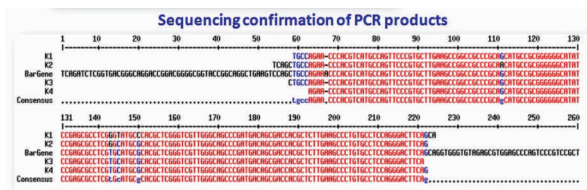
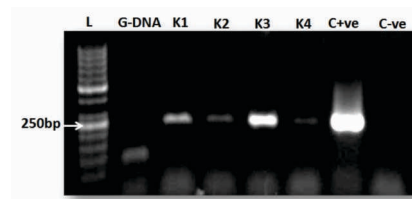
7. Initial confirmation of the integration in wheat genome ( $T_0$  plants): Genomic DNA of the above plantlets from Figure 7 was isolated (from the four different plantlets). PCR amplification was performed using the gene specific primers for BAR gene. The amplicon were cloned and eventually sequenced. Figures 8 showed the expected amplicon for the PCR reaction and further

the sequence alignment of the same. These results suggested an integration of the construct for gene silencing of TaMRP3 in the wheat genome.

8. Screening of  $T_1$  seeds and further selection of transgenics: The  $T_1$  seeds were subjected to multiple rounds of screening on the optimized BASTA selection under hydroponic conditions.

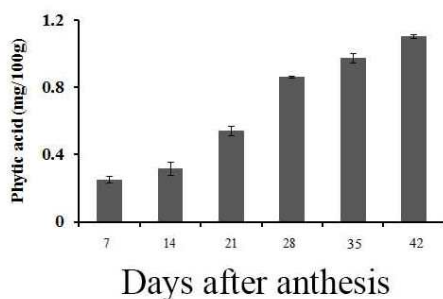


**Figure 7:** The putative transgenic were further screened and subjected for root induction and growth. The plantlets after the proper root induction were then transferred for hardening in the pots. Representative possible transgenic lines (K1, K2, K3 and K4) are shown in the picture.



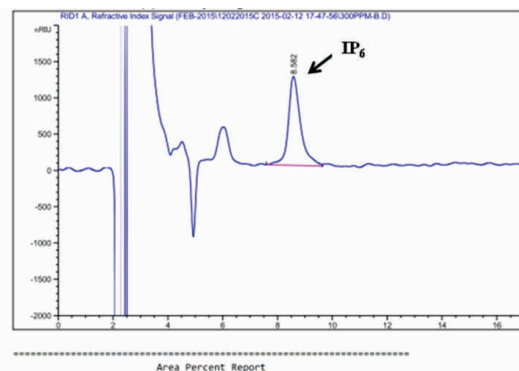
**Figure 8:** Screening and confirmation of genomic integration of the RNAi constructs in wheat genome. The genomic DNA of  $T_0$  plants were subjected for PCR amplification (Upper panel). The primers used in the study were directed for BAR gene. The amplicon were cloned and subjected to sequence alignment was performed (lower panel).

9. Estimation of PA by MEGAzyme kit and modified HPLC method: MEGAzyme kit: Total phytic acid was estimated from developing wheat grains using the K-PHYT kit (Megazyme, Inc). Standards were plotted as mentioned in the manufacturer instruction booklet (Figure 9).



**Figure 9:** Estimation of the PA content using the course of seed development. Each bar indicates the mean of four to five replicates with the indicated standard deviation of the mean.

10. Modified anion-exchange chromatography method for PA profiling: We are currently optimizing the protocol for the detection of IP<sub>6</sub> and other forms using anion-exchange chromatography. Initially, we are working



**Figure 10:** Estimation of the PA content using modified HPLC

with calibrating the standards. Standards were dissolved in water; mobile phase (56% methanol+44% 0.035M formic acid) was subjected for sonication before subjecting to HPLC. Standard samples were injected (20micorL) to a calibrated PLRP-S (Agilent) column with 40° C (Figure 10). A flow rate

0.9ml/min for 30 min was adjusted for each sample.

### Salient Achievements

1. Genes for targeted gene silencing has been identified and further constructs were successfully made.
2. The T3 plants for the RNAi transgenic wheat plants for TaMRP3 are generated and subsequent analysis will be performed.

### Future Perspectives

1. Screening of T0 plants for Cam35S::TaIPK1 RNAi lines.
2. Designing strategy to replace CaM35S with the promoter of lipid transfer protein from wheat.
3. Characterization of TaMRP3 silenced transgenics from wheat.

## 1.3 Functional characterization and implications of plant inositol pyrophosphate kinase

### Principal Investigator

Ajay K Pandey

### Co-investigator

Vikas Rishi

### Research Fellows

Mandeep Kaur Bedi

Shivani Sharma

### Introduction

The higher anionic forms (IP<sub>7</sub> and IP<sub>8</sub>; PPx-InsPs) of phytic acid (IP<sub>6</sub>) derived after hyper phosphorylation act as a strong reservoir of phosphate molecule. Genes responsible for the production of the pyrophosphate derivatives are referred as inositol phosphate-6 kinase (IP<sub>6</sub>K) and reported in yeast and human (kcs-1 and vip-1). Recently, two genes (*AtVIP1* and *AtVIP2*) were reported and characterized from *Arabidopsis* without offering any functional importance for them. Recently, we have cloned *TaVIP1* (yeast



homolog of VIP1) from wheat (*Triticum aestivum*). Previous studies in yeast have suggested the role of inositol pyrophosphates in regulating the phosphate homeostasis and IP<sub>6</sub> synthesis. Since wheat seeds are an important reservoir of bound and free phosphate we hypothesize that TaVIP1 may be one of the regulators for phosphate homeostasis and other cellular function especially in seeds. Based on this, the current research will test the hypothesis that in plants VIP1 could act as a sensor/regulatory molecular for cellular homeostasis in wheat grains. To test this hypothesis, we propose to work on the following objectives:

### Objectives

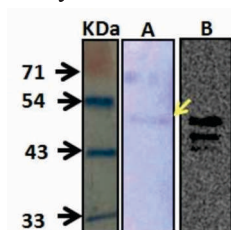
1. Identify and characterize wheat VIP1 gene/s that regulate the levels of IP<sub>7</sub> synthesis,
2. Functional characterization of TaVIP1 using other model system like yeast and Arabidopsis
3. Identifying the interacting components of TaVIP1 to understand its other functional role.
4. To achieve our objectives we would primarily use wheat, model plant Arabidopsis and yeast.

### Research Progress

1. Yeast Vip1 was used as a query to perform Blastx against TAIR database of *Arabidopsis*. We identified two predicted genes (AT5G15070.2 and AT3G01310.2) which have all the potential domains of yeast protein. *Arabidopsis* (AtVIPs1) sequences were subsequently used to identify the homolog/s from wheat genome database. IWGSC ([www.wheatgenome.org/](http://www.wheatgenome.org/)) and wheat EST ([www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)) were used to identify full length wheat IP<sub>6</sub>K. Specific primers were designed to amplify genes from cDNA prepared from different tissues.
2. Full length TaVIP (3.1kb) was cloned into pGEMT vector. Sequence analysis was

performed for the transcript cloned from C306. The sequences were translated and domain analysis was done. Our analysis suggested presence of conserved domains viz. ATP Grasp domain that ranges from 100-300 aa at the N-terminal end of the sequence. Similarly, Histidine Acid Phosphatase (HAPs) domain ranging from 350-950 aa was also present at the -C terminal of the sequence. HAPs domain is the catalytic domain of a functionally diverse set of proteins, most of which are phosphatases.

3. Protein purification and western analysis: *In vitro* transcription and translation was performed for the PCR purified fragment by using PURE-Express Kit (NEB). Purified protein was resolved on a 12% SDS-PAGE and subsequent western analysis was performed using His-tag antibody (Figure 11). Further, large scale expression of pure protein is underway and subsequent enzymatic characterizations to be followed.



**Figure 11:** Western analysis for kinase domain of TaVIP1 (A: Ni-NTA purified Protein, B: Western analysis using His tag antibody)

### Salient Achievements

Identification of the inositol pyrophosphate kinase gene from wheat was done and detection was performed by Western analysis

### Future Perspectives

1. Phenotypic complementation studies and Western Blot analysis of yeast mutants (*ScΔvip1*, *ScΔvip1Δkcs1* and *ScΔvip1Δlas17*).
2. Biochemical assays/studies of the phenotypic complemented yeast and *Arabidopsis* mutants under phosphate limiting conditions.

## 1.4 Identification, cloning and functional characterization of myo-inositol oxygenase (MIOX) from wheat

### Principal Investigator

Siddharth Tiwari

### Research Fellows

Anshu Alok

Harsimran Kaur

### Introduction

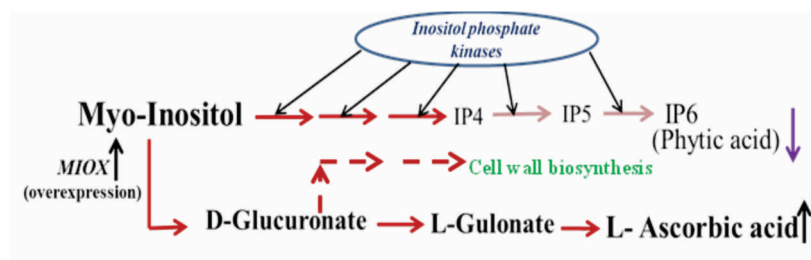
This project has been funded under Start Up Research Grant (Young Scientists - Life Sciences) by Science and Engineering Research Board (SERB), Department of Science and Technology (DST), Government of India in May 2014 for three years financial support. Myo-inositol is known as precursor for variety of low molecular weight compounds including cell wall, phytic acid and ascorbic acid biosynthesis. The whole process for biosynthesis of cell wall components, phytic acid and ascorbic acid is flux dependent. The targeted gene *myo*-inositol oxygenase (*MIOX*) is a key enzyme in L-ascorbic acid biosynthesis, associated with *MIOX* pathway. Upon completion of the project we anticipate transgenic wheat lines with low phytic acid and high ascorbic acid contents that might have an increase iron absorption and bioavailability.

### Objectives

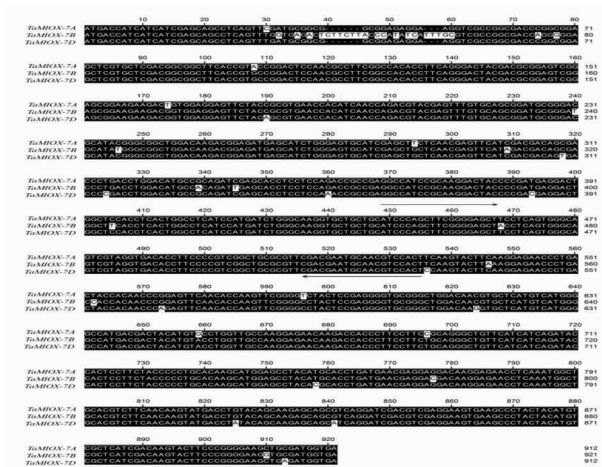
1. Identification and functional characterization of wheat *MIOX* (*TaMIOX*).
2. Over expression studies of *TaMIOX* in wheat for trait(s) development.

### Research Progress

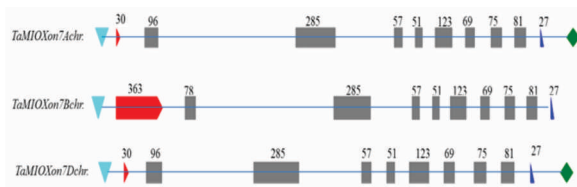
1. Proposed work hypothesis: We presume that if *myo*-inositol resources channelize towards ascorbic acid biosynthesis by the overexpression of *MIOX*, might be reduced total phytic acid and enhanced ascorbic acid biosynthesis in wheat (Figure 12).
2. Identification, cloning and sequence analysis of *TaMIOX*: Four reported Arabidopsis *MIOX* (*AtMIOX1*, NP\_001154337.1; *AtMIOX2*, NP\_565459.1; *AtMIOX4*, NP\_001190844.1; and *AtMIOX5*, NP\_200475.1) were used as a query to identify the homologous sequence of *MIOX* gene from *T. aestivum*. The alignment of the multiple EST obtained led to the identification only one Unigene ID (*Ta.53897*) which was referred as *TaMIOX* (*AK334637*). The highest identity of *TaMIOX* was found to be 77% with *AtMIOX1*. Gene specific primers were designed and ORF of 912 bp encoding 303 amino acids was amplified by PCR using cDNA as a template and confirmed by sequencing. The homologous genes of *TaMIOX* were located on A, B and D genome on large segment of chromosome 7 (Figure 13).



**Figure 12:** Proposed hypothesis where *MIOX* gene will be overexpressed and it could lead to biosynthesis of ascorbic acid. Since we anticipate more flux of *myo*-inositol towards glucuronic acid synthesis, this might lead to lowering phytic acid concentration.



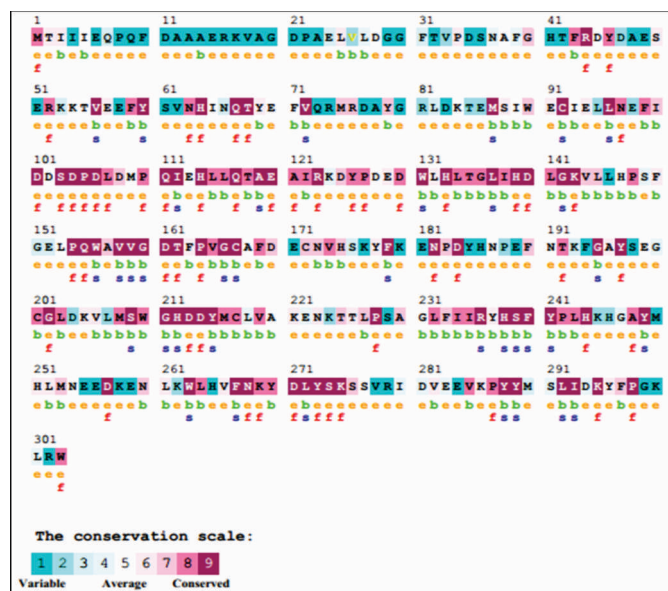
**Figure 13:** Alignment of *TaMIOX* located on A, B and D genome of chromosome 7 in wheat genome



**Figure 14:** Genomic structure of MIOX gene family in *Triticum aestivum*. Grey boxes and blue line indicate position of exons and introns, respectively. Nucleotide length of exons shows on the above of box. Red, sky blue, dark blue and green colors indicate the positions of start codon, TATA-box, stop codon and Poly A tail, respectively.

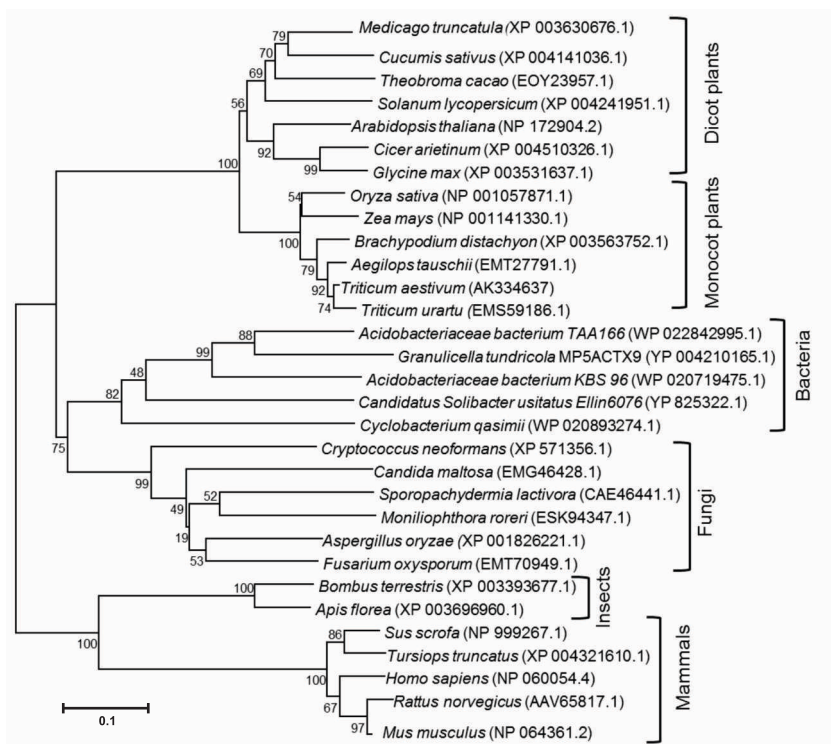
Genomic information at IWGS and FGENESH+ program revealed that *TaMIOX* is composed of nine introns for each homolog (Figure 14). ConSurf program analysis confirmed that the MIOX protein is conserved across all plants, animals, bacteria and fungi. The result suggested initial 100 amino acid sequence at N-terminal was variable, 100 to 250 amino acid region was highly conserved and the remaining 251 to 303 amino acid at C-terminal was less conserved (Figure 15).

3. Phylogenetic analysis: The phylogenetic tree displayed 3 groups and 6 clades (Figure 16). In the first group, plants MIOX sequences were aligned into monocot and dicot subgroups. The second group contained microorganism MIOX, separated into yeast and bacteria while the third group subdivided insect and mammal. The *TaMIOX* (AK334637) showed close relationship to MIOX from *Triticum urartu* (EMS59186.1) (the donor of A genome) and then followed by *Aegilops tauschii* (EMT27791.1) (the donor of D genome).



**Figure 15:** Conserved domain of MIOX across kingdoms. Purple and green color amino acid residues indicate conserved and variable regions, respectively.





**Figure 16:** A phylogenetic tree of wheat MIOX protein with other known MIOX. The tree was constructed using Neighbor-Joining method. Accession numbers are shown in parentheses.

### Salient Achievements

1. *TaMIOX* was identified and cloned from wheat genome.
2. In-silico analyses of *TaMIOX* have been performed.

### Future Perspectives

1. Biochemical and functional characterization of *TaMIOX* will be performed.
2. *TaMIOX* overexpressed wheat transgenic lines will be generated and evaluated for the desirable trait(s).

### 1.5 Mineral distribution and tissue-specific transcriptomics in grains of contrasting wheat genotypes

#### Principal Investigator

Sudhir P Singh

#### Co-Investigator

Shrikant Mantri

### Research Fellow

Raja Jeet

### Introduction

Mineral deficiency is widespread in the world, especially in cereal diet-based countries. Though cereals are a significant source of minerals, the level of mineral accumulation, their localization in tissues within the grain, their occurrence in different chemical forms and other food constituents present in the matrix, determine their dietary availability in cereal. In cereals like wheat, the bran layer of the grains is an important source of minerals. However, the dietary availability of mineral micronutrients in wheat flour is limited due to the loss of the mineral-rich bran during milling and processing and the presence of anti-nutrients like phytic acid that keep iron strongly chelated in the aleurone layer. The starchy endosperm is devoid of micronutrient minerals. It is desirable to develop an approach for the mobilization of minerals from bran to endosperm making it more bioavailable.

## Objectives

1. Investigation of mineral distribution pattern in grain tissues of wheat and related genotypes.
2. Differential transcriptomics in maternal and filial tissue of developing grains of wheat genotypes with contrasting levels of minerals.
3. Identification of candidate genes for higher level mineral accumulation in grains.
4. Identification of candidate genes for the mobilization of minerals from the outer bran to endosperm.
5. Designing strategies to enhance mineral bioavailability of wheat.

## Research Progress

1. We have reported distribution pattern of a number of trace minerals in filial and maternal tissues of grains of wheat and its related genotypes. Distinct distribution patterns were observed for mineral nutrients in grain tissues. The wild relative of wheat and the Landrace accumulated more iron and zinc in aleurone and scutellum than the cultivated wheat varieties. The endosperm showed very low accumulation of iron and zinc. However, the primitive genotypes accumulated relatively more micronutrients in endosperm than present day cultivar.
2. We have reported comparative transcriptomics in two wheat genotypes, with contrasting levels of minerals, during grain development. The study revealed differential regulation of transcripts related to metal homeostasis, metal tolerance, lignin and flavonoid biosynthesis, amino acid and protein transport, vacuolar-sorting receptor, aquaporins, and stress responses. The study identified the candidate genes which may facilitate the elevated levels of minerals in the grains.
3. Transcriptome sequencing was done from the RNA-seq libraries prepared from aleurone and endosperm of developing grain (14 days after anthesis) of two wheat

genotypes, showing contrast in grain mineral concentration. The average number of paired end reads produced for each library was 95 million. De novo assembly of the paired end reads generated a total of 205159 transcripts. Differential expression analysis is in progress.

4. Transcripts related to transcription factors (TFs) were determined in the transcriptome data by BLASTx search analysis using the *Arabidopsis* TF protein database. A total of 160 transcripts were identified as putative transcription factors, satisfying the criteria of 50% query coverage, 50% identity, 100 bitscore, and 1 log<sub>2</sub> fold expression (aleurone vs. endosperm). Further analysis is in progress.
5. Transcripts related to hormones were determined in the transcriptome data by BLASTx search analysis using the *Arabidopsis* hormone protein database. A total of 447 transcripts were identified as putative genes related to hormone, satisfying the criteria of 50% query coverage, 50% identity, 100 bitscore, and 1 log<sub>2</sub> fold expression (aleurone vs. endosperm). Further analysis is in progress.
6. The genes related to iron transportation and accumulation in wheat, such as Nicotianamine Synthase (NAS), Natural Resistance Associated Macrophage Protein (NRAMP), Vacuolar Iron Transporter (VIT), have been cloned in plant gene expression vector. Their transformation in the respective mutant lines of *Arabidopsis thaliana* is in progress, for the functional validation of wheat genes.

## Salient Achievements

1. We have reported the distribution pattern of nutritionally important minerals in filial and maternal grain tissues of *T. aestivum* and *A. kotschy* genotypes.
2. The comparative transcriptomic study during grain development revealed differential regulation of transcripts related

to metal homeostasis, metal tolerance, lignin and flavonoid biosynthesis, amino acid and protein transport, vacuolar-sorting receptor, aquaporins and stress responses.

3. The study is useful in determining candidate genes for the higher accumulation of micronutrients in wheat grains.

### Future Perspectives

1. Differential expression analysis in aleurone and endosperm of the contrasting wheat genotypes.
2. Identification of candidate genes for the mobilization of minerals from the outer bran to endosperm.
3. Functional validation of the candidate genes for mineral transport and accumulation.
4. Designing strategies to enhance mineral bioavailability of wheat.

## 1.6 Accelerated breeding for quality improvement in wheat

### Principal Investigator

Monika Garg

### Research Fellows

Rohit Kumar

Aman Kumar

Navneet kaur

### Introduction

In the developed countries, grain market is driven by wheat quality. A wheat class/grade is awarded to a product based on its processing and end-use quality. Validated Markers are available for each product type and are being routinely utilized. But in India cultivars are released based on agro climatic zones, time of sowing and soil fertility. Validated Markers are not available for the major product i.e. chapatti. Available validated markers are not being utilized. In India there is need of breeding cultivars based on processing quality (milling and baking characteristics), marker development and utilization of validated markers.

Processing quality of wheat depends on seeds harvested from field and its components like proteins, starch, non starch carbohydrates and lipids. Protein's contribution to processing quality is well known. The protein content and types determine the end product quality like bread, biscuit, cake, chapatti and noodles etc. Biscuit making requires soft wheat with low protein content and specific combination of different alleles (2+12 allele of high molecular weight glutenin subunit gene (HMW-GS) at chromosome 1D (locus *GluD1*), *Pina-D1a*, *Pinb-D1a* alleles of Puroindoline gene etc). Bread making requires hard wheat with high protein content specific combination of different alleles (5+10 allele of *GluD1*-HMWGS, *Pina-D1a/b*, *Pinb-D1a/b* etc). Chapatti making requires medium strength wheat with medium protein content. The contribution of different genes/alleles to chapatti making is poorly understood. It is important to understand structure, allelic variation and interaction pattern of different seed components and transfer them to high yielding, disease resistant and locally adapted cultivars.

### Objectives

1. Generation of breeding material with improved processing quality.
2. Identification of different genes/alleles related to processing quality.

### Research Progress

#### 1. Accelerated breeding for processing quality improvement

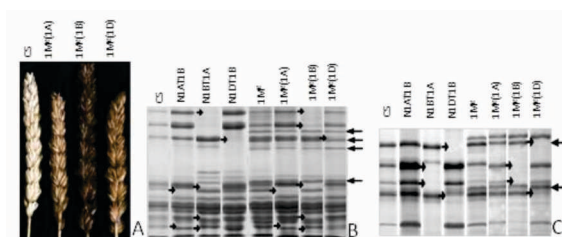
- a) For improvement of chapatti making quality, good chapatti making old cultivars (C306, Lok1) were crossed with high yielding recent cultivars (PBW343, PBW550 and PBW621, HD2967). F<sub>5</sub> seeds of BC<sub>3</sub> and other crosses (Table 1) were sown at NABI and around 120 morphologically selected lines were screened for absence of GBSS-1B. Seeds from selected lines/plants will be utilized for chapatti quality analysis and generation advancement.

b) For improvement of Biscuit making quality donor landraces were crossed with high yielding recent cultivars (PBW343, PBW550, PBW621 and HD2967).  $F_5$  seeds of  $BC_3$  and other crosses selected by the presence of *PinD1a* were tested for quality analysis. The biscuit spread factor of advanced crosses was compared with other soft wheat varieties, biscuit spread of advanced crosses ( $10.5 \pm 0.5$ ) relatively higher than soft wheat varieties ( $9.0 \pm 0.2$ ).  $F_5$  seeds of different crosses (Table 2) were sown at NABI and around 150 morphologically selected lines were screened based on presence of puroindoline gene *PinaD1a*. Positive plants were screened for morphologically superior plants. Seeds from selected lines/plants will be utilized to check biscuit quality and generation advancement.

c) For improvement of bread making quality, we are utilizing wild species *Ag. elongatum*, *Ae. longissima*, *Ae. searsii*, *Ae. geniculata* and *Ag. intermedium*. These genetic stocks are being crossed with high yielding cultivars PBW550, PBW621 and HD2967. We intend to transfer HMW-GS genes related to high grain strength from wild species to chromosome 1A of wheat. Chromosome specific substitution lines of *Ae. geniculata* 1Mg(1A), 1Mg(1B), 1Mg(1D) in wheat were generated and screened. 1Mg(1A) had higher gluten strength, polymeric proteins, bread loaf volume and loaf quality than rest of the lines, confirming our hypothesis that chromosome 1A possesses genes/alleles that contribute negatively towards bread making quality (Figure 17).

Table 2: Screening and selection of  $F_5$  plants/lines of  $BC_3$  and other crosses for good chapatti making quality

Cross	No. of Homozygous lines selected after screening	Yellow rust
C306/4*PBW343	6	S
C306/4*PBW550	14	MS
C306/2*PBW550/2*HD2967	4	R-MR
C306/4*PBW621	8	MR
LOK1/4*PBW343	1	S
LOK1/2*PBW343/2*HD2967	2	S
LOK1/4*PBW621	3	R-MR



**Figure 17:** Spikes of different created substitution lines (A), and their glutenin (B) and gliadin (C) profiles showing additional *Ae. geniculata* bands (arrows) and removal of corresponding wheat bands (arrow heads)

Chromosome 1A specific translocation line of *Ag. elongatum* [1EL(1AS)] with potential of bread making quality improvement was generated in the background of soft wheat cultivar Norin61 (Table 3). Transfer of this translocation to hard wheat cultivars PBW621 is in 1EL(1AS)/5\*PBW621-F3 stage. Crosses of other wild species were also screened and crossed/backcrossed for transfer of desired traits to Indian high yielding wheat cultivars from the exotic material.

**Table 2:** Screening and selection of  $F_5$  plants/lines of different crosses for good biscuit making quality

Cross	No. of Homozygous lines selected after screening	Yellow rust
IITR67/4*PBW343	15	S
IITR67/4*PBW550	10	S-MS
IITR67/2*PBW550/2*HD2967	10	MR
IITR67/4*PBW621	6	MR
IITR67/2*PBW621/2*HD2967	7	MR

## 2. Study of molecular structure and physiochemical properties of starch granules in soft and hard wheat cultivars



**Table 3.** Bread making quality characteristics of *Ae. geniculata* substitution lines

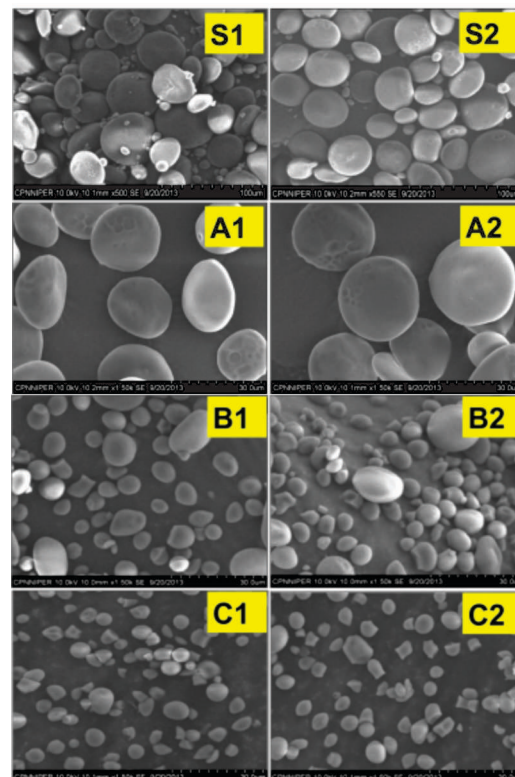
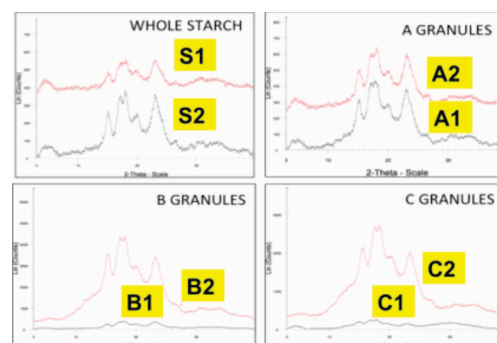
Cultivar	Sedimentation Value (ml)	Gluten Index	Bread Loaf Volume (ml)	Loaf Score	Gliadin/Glutenin ratio	HMWGS/LMWGS
1Mg(1A)	45.5 <sup>d</sup>	69.5 <sup>b</sup>	550 <sup>b</sup>	6.2 <sup>b</sup>	1.9 <sup>c</sup>	1.3 <sup>a</sup>
1Mg(1B)	42 <sup>c</sup>	49.5 <sup>a</sup>	535 <sup>a</sup>	5.5 <sup>a</sup>	2.6 <sup>a</sup>	1.3 <sup>a</sup>
1Mg(1D)	36.5 <sup>b</sup>	42.0 <sup>a</sup>	540 <sup>a</sup>	5.1 <sup>a</sup>	2.8 <sup>ab</sup>	1.08 <sup>b</sup>
C/S	39.7 <sup>a</sup>	47.7 <sup>a</sup>	543 <sup>a</sup>	5.2 <sup>a</sup>	2.4 <sup>a</sup>	0.74 <sup>c</sup>

Starch and proteins are major components in the wheat endosperm. Size and composition of wheat starch granules is of high significance for industrial usage and in imparting quality to various end products. Hard wheat starch granules are tightly bound with the lipids and proteins due to lower expression of interfering proteins namely puroindolines. In this work physiochemical properties and structure of the A, B and C-type granules of wheat starch from hard (C306) and soft (IITR67) wheat lines were studied.

- Starch granules had different shape, size, relative number, gelatinization peak temperature and substitution pattern of amylopectin (Figure 18).
- All had symmetrical birefringence pattern and typical A-type crystallites with the different degrees of crystallinity.
- Positive correlation was observed between amylose content and crystallinity.

### Salient Achievements

- Advanced breeding material for improvement of chapatti and biscuit and bread making quality has been generated
- Variation in structure and properties were observed in A, B and C type of starch granules.
- Relatively poor contribution of chromosome 1A compared to 1B and 1D of wheat, towards bread making quality was identified by creation and analysis of *Ae. geniculata* homoeologous group-1 substitution lines.
- Translocation line of *Ag. elongatum* in hard wheat background 1EL(1AS)/5\*PBW621-F<sub>3</sub> has been created.


**Figure 18A** SEM images of starch granules

**Figure 18B** XRD pattern for starch granules S1, A1, B1 and C1- soft wheat whole starch, A granules, B granules and C granules respectively. S2, A2, B2 and C2- hard wheat whole starch, A granules, B granules and C granules respectively.

**Future Perspective**

1. Generation of breeding material with improved processing quality
2. Study of structure and interaction pattern of major seed components like starch, proteins and lipids affecting processing quality.

### ***1.7 Identification of celiac disease epitopes in Indian wheat cultivars and their modulation by RNAi and breeding approaches***

**Principal Investigator**

Monika Garg

**Research Fellow**

Nand Kishore

**Introduction**

CD is a T-cell mediated autoimmune enteropathy caused by permanent intolerance to gluten fraction of wheat or the homologous proteins from barley or rye. The only available treatment for this disease is the adherence to a strict life-long gluten free diet. This study was initiated with an objective of comprehensive mapping of CD epitopes in Indian wheat cultivars and their elimination by RNAi and breeding approaches.

**Objectives**

1. Immunogenic epitope screening in Indian germplasm.
2. Generation of wheat lines with lowered immunogenicity.

**Research Progress**

1. Transcriptome data screening of Indian wheat cultivars indicated differential expression of CD causing  $\alpha$ -gliadins at different stages of seed development.
2. Preliminary antibody based screening of the selected translocation lines of *Hynaldia villosa* (6VS-6DL) in wheat indicated that it has lower immunogenic proteins as it replaces chromosome arm 6DS that codes most immunogenic proteins.
3. Marker assisted backcross based breeding to transfer 6VS-6DL translocation from exotic lines to regionally adapted cultivars is in second to third backcross stage.

**Salient Achievements**

Transfer of *Hynaldia villosa* (6VS-6DL) translocation with reduced immunogenic epitopes responsible for celiac disease is in progress.

**Future Perspective**

Creation of breeding material with lowered number of immunogenic epitopes.

### ***1.8 Transfer and characterization of anthocyanins from blue, purple and black grain colored germplasm to high yielding Indian wheat cultivars***

**Principal Investigator**

Monika Garg

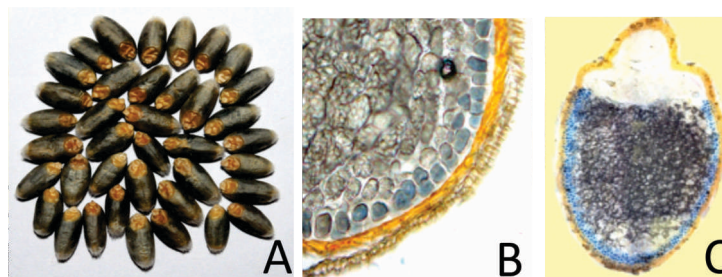
**Research Fellows:**

Venktesh Chunduri

Saloni Sharma

**Table 4:** Anthocyanins contents of selected coloured wheat crosses ( $F_4$  generation).

Cross es	Colour	Anthocyanin Content (mg/kg)
BW/2*PBW621	black	128.5 $\pm$ 3.4 to 198.8 $\pm$ 17.3
BW/2*PBW621	purple	16.0 $\pm$ 0.2 to 86.1 $\pm$ 2.0
BW/2*PBW621	blue	85.1 $\pm$ 2.4 to 130.5 $\pm$ 5.4



**Figure 19:** Seeds (A), transverse (B) and longitudinal (C) sections in blue wheat showing multilayered aleurone at specific locations in wheat seed

### Introduction

Color coded diet (fruits, vegetables, cereals, etc.) rich in phytochemicals e.g. anthocyanins and carotenoids confer innumerable health benefits. For improvement of anthocyanin content, exotic black and blue coloured wheat lines were crossed with high yielding recent cultivars (PBW550 and PBW621, HD2967). All the plants were differentiated according to their seed colour (black, purple and blue; Table 4). Seeds from BC<sub>2</sub> F<sub>4</sub> generation were selected for colour analysis and sectioning.

### Objectives

1. Creation of Indian colored wheat germplasm.
2. Assessment of health benefits associated with colored wheat.

### Research Progress

1. Segregation analysis indicated that two genes with enhancers and suppressors control blue aleurone color.
2. LC/QTOF-MS/MS could identify 26

different putative anthocyanins.

3. Microscopic visualization of transverse sections of blue and black donor as well as advanced lines showed blue color development in the aleurone layer (Figure 19). Aleurone layer was multilayered at specific locations in the seed.
4. Black derivative lines had highest anthocyanin content followed by blue and purple wheats.

### Salient Achievements

Colored germplasm was created and analytical studies of coloured wheat crosses were carried out.

### Future Perspective

1. Product development.
2. *In vitro* studies of anthocyanins extracts from different colored wheat crosses.





## IMPROVING FRUITS FOR POST HARVEST QUALITY AND NUTRITION

## 2.1 Genetic transformation of banana for quality improvement

### Principal Investigator

Siddharth Tiwari

### Project Scientist

Ashutosh Pandey

### Project Fellows

Shivani

Navneet Kaur

### Project Assistants

Vikrant Sharma

Prateek Kumar

### Introduction

The project entitled “Transfer and Evaluation of Indian Banana with Pro-Vitamin A ( $\beta$ -carotene) Constructs” has been funded by BIRAC since Nov. 2012. This project is a part of the Multi-Institutional International Core Project entitled “Development and Transfer of Technology from Queensland University of Technology (QUT), Australia to India for Biofortification and Disease Resistance in Banana”. The aim of proposal is to utilize the experience and achievements of QUT for the development, validation and transfer of specific traits in two Indian banana varieties cv. Grand Naine and Rasthali. At the first stage, QUT has provided the best four pro-vitamin A (PVA) Generation 2 gene constructs, containing Asupina banana derived *phytoene synthase* (*APsy2a*) gene under the control of Expansin1 (*Exp1*), 1-aminocyclopropane-1-carboxylate oxidase (*ACO*), Ubiquitin (*Ubi*) and Banana bushy top virus DNA-4 (*BT4*) promoters for the genetic transformation of Indian banana varieties. *Exp1* and *ACO* promoters have fruit specific activity while *Ubi* and *BT4* promoters have constitutive expression activity. The name of constructs are given as DC12 (*Exp1*>*APsy2a*), DC32 (*ACO*>*APsy2a*), DC34 (*Ubi*>*APsy2a*) and DC35 (*BT4*>*APsy2a*). The optimized protocols for embryogenic cell suspension (ECS) culture and

genetic transformation of selected cultivars are being utilized for genetic improvement with PVA gene constructs (QUT Generation 2).

### Objectives

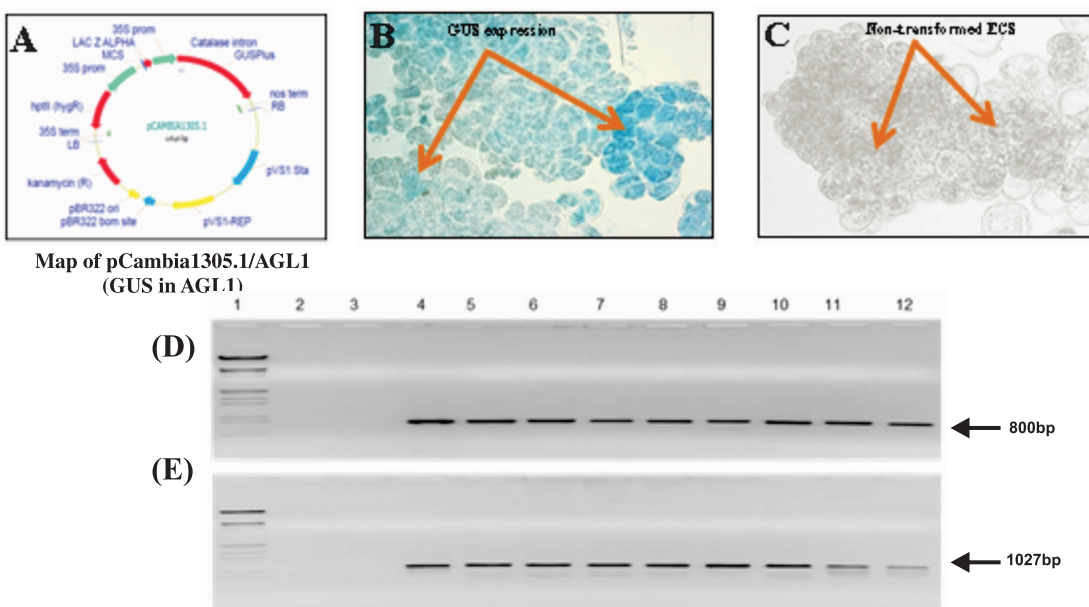
1. To utilize the experience and achievement of QUT for the development, validation and transfer of PVA gene constructs in two Indian banana varieties cv. Grand Naine and Rasthali.
2. To develop pro-vitamin A (PVA;  $\beta$  carotene) rich biofortified and agronomically improved transgenic varieties of Indian bananas.

### Research Progress

1. Banana plantation at NABI research field and establishment of Embryogenic Cell Suspension (ECS) culture for regeneration of somatic embryos: This is round the year process to develop and maintain efficient ECS by using different lots of immature male flower buds (IMFB) for regeneration of somatic embryos. The research progress on the development of ECS culture for regeneration and germination of somatic embryos has been shown in previous Annual Report (2013-14). The same work was followed on regular basis.
2. Establishment of genetic transformation of ECS by using reporter gene and confirmed by PCR: Efficient regeneration and genetic transformation protocols are pre-requisites for crop improvement through genetic engineering. The protocol for *Agrobacterium*-mediated genetic transformation of banana cultivars Rasthali and Grand Naine has been optimized using pCambia1305.1/AGL1 (GUS-Intron in AGL1 strain) (Figure 1A). The histochemical assay confirmed expression of reporter (GUS-Intron) gene as blue colour in the transformed ECS and no expression of GUS was noticed in non-transformed control ECS lines (Figures 1 B & C). Transgenic embryogenic cells (ECS) survived and looked healthy on several round of selections

on the regeneration medium containing 25 mg/l hygromycin while non-transformed cells did not survive on the selection medium. Transgenic embryos germinated and rooted on the germination and rooting medium containing 25 mg/l hygromycin. Genomic DNA was isolated from selected plants. Putative T<sub>0</sub> transgenic plants were screened and confirmed by PCR analysis (Figures 1 D & E). The PCR results showed amplification of the predicted 800 bp *gusA* as well as 1027 bp *hptII* fragments of genes in transgenic plants (lane 5-12). The positive control (plasmid PCAMBIA1305.1) also gave similar size amplicons (lane 4). No

genetic transformation of ECS of Rasthali and Grand Naine. Multiple transformation experiments with these four PVA gene constructs have been performed at different time intervals to generate several independent transgenic events. Transgenic embryogenic cells survived and looked healthy on several round (5-7 cycles, each 1 months) selection on kanamycin (100 mg/l) containing regeneration medium (Figure 2A). Non-transformed cells turned brown and did not survive on the selection medium (Figure 2B). Healthy transgenic embryos (approx. 50-100 numbers/plate) germinated on the germination medium containing 100 mg/l



**Figure 1:** Optimization of *Agrobacterium*-mediated genetic transformation using GUS as reporter gene. **(A)** Map of pCambia1305.1 vector. **(B)** Transformed (Arrows indicate blue color expression of GUS) and **(C)** non-transformed (Arrows indicate no GUS expression) ECS lines. **(D & E)** PCR analysis of genomic DNA from T<sub>0</sub> transgenic plants.

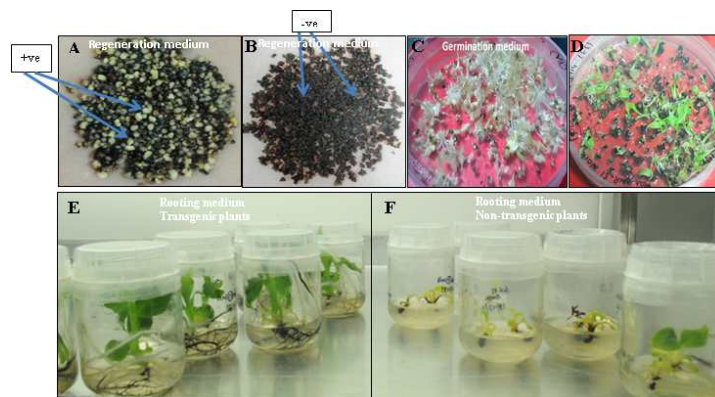
amplification was observed with two negative controls, one was reaction mixture without template (lane no. 2) and other was non-transformed plants (lane no. 3).

- Genetic transformation of ECS with PVA gene constructs (Gen 2) received from QUT:** Four QUT gene constructs named as DC12 (Exp1>*APsy2a*), DC32 (ACO>*APsy2a*), DC34 (Ubi>*APsy2a*) and DC35 (BT4>*APsy2a*) are being used for

kanamycin (Figure 2C & D). Putative independent transgenic events were selected and transferred on to rooting medium containing 200 mg/l kanamycin. After 15-20 days, germinated transgenic embryos have shown healthy root formation (Figure 2E) while the non-transformed (control) germinated embryos revealed growth retardation and eventually died in next 30-45 days on the rooting media containing 200

mg/l kanamycin (Figure 2F). Total 503 and 213 transgenic plants of Rasthali and Grand Naine, respectively developed with all four

without template (Lane: 2) and other was non-transformed (Lane: 3) plants (Figure 3). There were no



**Figure 2:** Visual observation of *in vitro* regeneration, germination and root formation of transgenic (Gen2) plants on kanamycin selection medium. (A) Transformed white and healthy globular embryogenic cells (Arrows indicated) with brown colored non-transformed cells on 100 mg/l kanamycin. (B) Non-transformed (control) embryogenic cells on 100 mg/l kanamycin. (C) Germination of transformed embryos on 100 mg/l kanamycin after 1 month and (D) After 2 months. (E) Healthy rooted transformed plants on 200 mg/l kanamycin. (F) Poor growth with no root formation of non-transformed plants on 200 mg/l kanamycin.

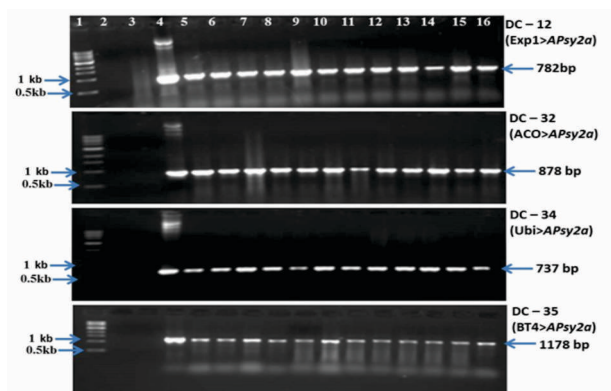
constructs and are available in rooting and germination medium. More than 5 individually transformed ECS lines of each cultivar (Rasthali and Grand Naine) are available in regeneration (selection) medium and soon will be transferred on to germination and root formation medium which will again provide several new independent transgenic events.

4. **Putative transgenic plants (Gen2) confirmation and screening by PCR:** We randomly selected 20 transgenic events developed from each of four constructs. Hence, total 80 transgenic plants of each Rasthali and Grand Naine were screened by PCR using promoter-gene specific primers (Figure 3). Total genomic DNA was isolated from leaves of transgenic and non-transgenic banana plants using DNeasy Plant Maxi kit (Qiagen). The results showed amplification of the predicted fragments of promoter-gene region in positive plasmid control (Lane: 4) and transgenic plants (Lanes: 5-16) while no amplification was observed with two negative controls, one was containing reaction mixture

chimeras/false positives noticed during the PCR analysis. The possible reason behind it may be the several round continuous exposure of kanamycin selection during *in vitro* embryo regeneration (5-7 cycles), germination (1-2 cycles) and rooting (1 cycle) stages which eliminated the possibility of development of transgenic chimeras.

### Salient Achievements

1. Tissue culture raised several plants of Grand Naine and Rasthali were generated and grown at NABI research field for the collection of explant for ECS development.
2. Protocol for development of ECS culture of Grand Naine and Rasthali cultivars was optimized.
3. ECS of Rasthali and Grand Naine are maintained and multiplied for genetic transformation experiments.
4. Genetic transformation protocol was optimized by using reporter (*gusA*) gene.
5. Several round of genetic transformation experiments with four QUT gene constructs (Gen 2) have been performed for the



**Figure 3:** PCR analysis of genomic DNA from T<sub>0</sub> Rasthali transgenic plants developed from four QUT gene constructs named as DC12 (Exp1>APsy2a), DC32 (ACO>APsy2a), DC34 (Ubi>APsy2a) and DC35 (BT4>APsy2a). Lane 1: 1kb ladder (NEB); Lane 2: Control; Lane 3: Non-transformed plant; Lane 4: +ve plasmid; Lane 5-16: Transgenic lines.

generation of several independent transgenic events for further analysis.

6. Putative transformants were generated by four constructs and screened by PCR analysis.
7. Several transgenic plants are ready to transfer in soil pots under containment facility for fruit development and analysis.

#### Future Perspectives

1. Development of pro-vitamin A ( $\beta$ -carotene) rich biofortified Indian bananas.
2. Bioavailability study, nutritional analysis and agronomical contained field trials of transgenics.

## 2.2 Metabolic engineering for enhanced biosynthesis of provitamin-A in Indian banana fruit

#### Principal Investigator

Siddharth Tiwari

#### Project Scientist

Ashutosh Pandey

#### Project Fellow

Navneet Kaur

#### Introduction

Under the DBT-BIRAC funded multi-institutional project, NABI is undertaking

research on genetic transformation of two commercial cultivars of Indian banana with pro-vitamin A (PVA) gene constructs, provided by the QUT, Australia. However, the scope of research work is limited to the gene constructs provided by the QUT while no leads are currently available on the prospective results in terms of enhanced expression level of PVA and ultimately the bioavailability in Indian population. The knowledge about the regulatory mechanism of the carotenoid biosynthesis is largely unknown. The cytosolic mevalonate (MVA) and plastidial 2-C-methyl-D-erythritol 4-phosphate (MEP) are the two independent pathways that synthesise isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) precursors for the biosynthesis of carotenoids in plants. Multiple enzymatic steps are known to regulate carotenoid metabolic flux in the downstream enzymatic steps for the biosynthesis of lycopene,  $\alpha$ -carotene and  $\beta$ -carotene. Thus, the identification of limiting step(s) is the bottleneck for the biosynthesis of carotenoids in specific plant tissues. The proposed study will lead to identify and characterize genes and promoters involved in high carotenoid biosynthesis in banana. The identification of such regulators for carotenoid biosynthesis would pave the way to genetically engineer of Indian banana with enhanced content of carotenoids.

#### Objectives

1. Genome-wide identification, isolation



- and characterization of genes involved in key enzymatic steps in MVA-, MEP- and carotenoid-biosynthetic pathways in banana.
2. Biochemical and transcriptomic analyses of germplasm with contrasting levels of PVA in target tissues to understand the regulation of metabolic pathways and limiting steps.
3. Evaluation of candidate genes-promoters and combinations thereof after transformation.
4. Development of nutritionally enriched varieties of Indian bananas, providing  $\beta$ -carotene, essential for human health.

### Research Progress

#### 1. Germplasm collection and plantation at NABI research field:

- a) Suckers of around thirty established banana cultivars have been collected from different places of India and grown at NABI research field for establishing germplasm (Figure 4 A).
- b) Several tissue culture raised banana plants have been generated and grown at NABI research field (Figure 4 B). The in-vitro grown cultures are being maintained

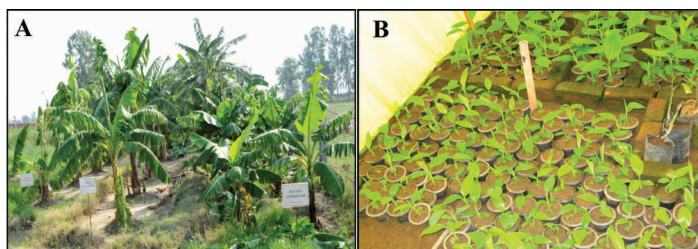
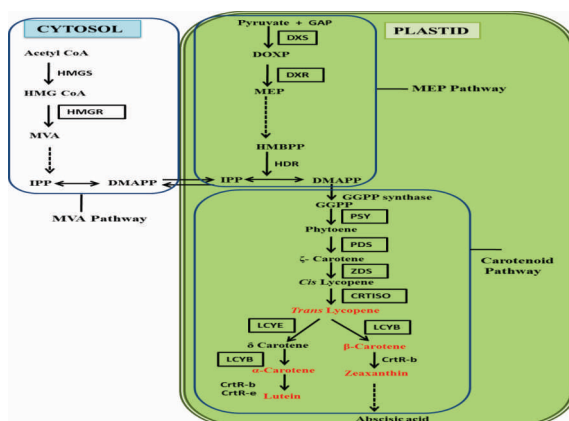
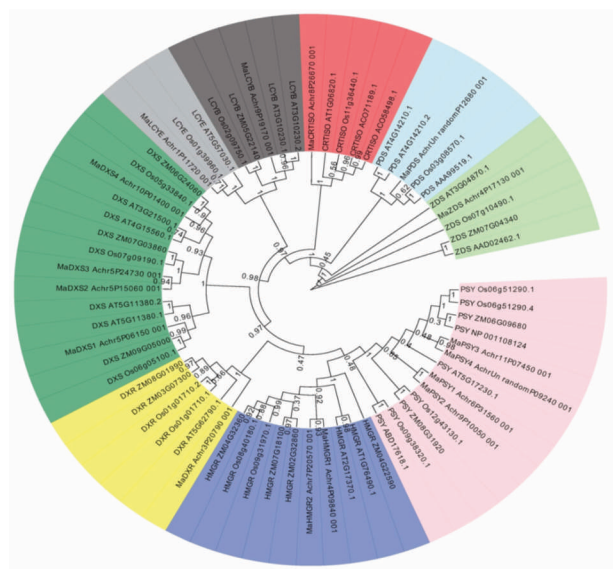


Figure 4: (A) Banana germplasm at NABI research field, (B) Tissue culture raised banana plants.



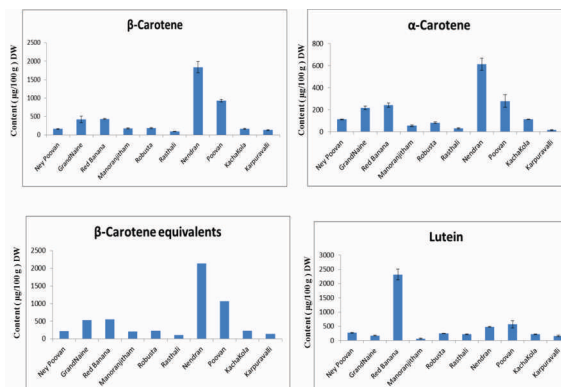
- at NABI.
2. **Genome-wide in-silico analysis of MVA, MEP and carotenoid pathways genes in banana:** Total sixteen putative genes were identified by systematic screening from the database of the Banana Genome Hub (<http://banana-genome.cirad.fr/>). These genes are the members of nine gene families which encode key enzymes involved in the MVA-, MEP- and carotenoid-biosynthetic pathways (Figure 5). The multiple sequence alignment was carried out using these genes of banana and their corresponding orthologues in rice, maize and *Arabidopsis* to generate molecular phylogenetic tree (Figure 6). The sequences similarity was used to assign the putative functions of the identified genes sequences of banana. Phylogenetic analysis shows that banana proteins are similar with the proteins involved in the carotenoid and related biosynthesis pathways in maize, rice and *Arabidopsis*.



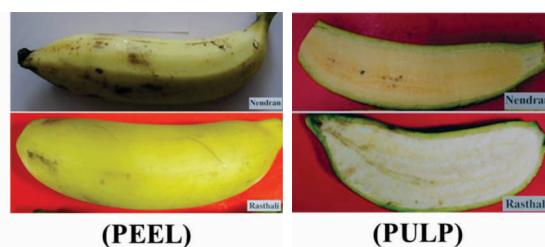
**Figure 6:** A Maximum Parsimony based phylogenetic tree of the banana, *Arabidopsis*, rice, and maize MVA-, MEP- and carotenoid-biosynthetic pathways genes was generated. Different colours represent nine gene families which encode key enzymes involved in the MVA-, MEP- and carotenoid-biosynthetic pathways.

### 3. Carotenoid profiling during banana fruit development:

Ten banana accessions were selected from the field germplasm plot at the NABI, Mohali for the analysis. Carotenoid content were measured by HPLC in the ripe pulp tissue (dry weight) of selected cultivars. The analysis suggested that Nendran content highest (2143  $\mu\text{g}/100\text{g}$ ) and Rasthali content lowest (112  $\mu\text{g}/100\text{g}$ ) amount of  $\beta$ -carotene equivalents (Figure 7). The analysis showed that lutein,  $\alpha$ -carotene and  $\beta$ -carotene were presented in detectable amount, whereas lycopene and zeaxanthin were not detectable in the tissues. The highest content of all the carotenoids was noticed at the ripening stage of Nendran. Nearly 19-fold higher  $\beta$ -carotene was observed in the Nendran ( $1836.81 \pm 152.76 \mu\text{g}/100\text{g}$ ), compared to Rasthali ( $97.05 \pm 6.75 \mu\text{g}/100\text{g}$ ) (Figure 7). Similarly, lutein and  $\alpha$ -carotene were nearly 2- and 20-folds higher in Nendran, compared to Rasthali. The visual observation showed that the ripe pulp of Rasthali appeared creamy-white and Nendran was deep-yellow



**Figure 7:** HPLC based qualitative analysis of carotenoids in ripe fruit-pulp of ten Indian banana cultivars



**Figure 8:** Visual observation of ripe fruit-pulp and -peel of Nendran and Rasthali cultivars.

in color (Figure 8). It established positive correlation between pulp colour intensity of banana with  $\beta$ -carotene accumulation.

#### Salient Achievements

1. Banana cultivars have been collected from different places and grown at NABI research field for establishing germplasm.
2. Tissue culture raised several plants were generated and grown at NABI research field.
3. Genome-wide identification and in-silico analysis of genes involved in key enzymatic steps in MVA-, MEP- and carotenoid-biosynthetic pathways in banana have been performed.
4. Biochemical analyses of selected germplasm in ripe fruit-pulp were performed.

#### Future Perspectives

1. Development of pro-vitamin A ( $\beta$ -carotene) rich biofortified Indian bananas.
2. Bioavailability study, nutritional analysis and agronomical field trials of transgenics.





## BASIC BIOLOGY FOR CROP IMPROVEMENT

### 3.1 A designed A-ZIP53 dominant-negative protein heterodimerizes with B-ZIP53, B-ZIP10 and B-ZIP25 transcription factors and inhibits their DNA binding activity.

#### Principal Investigator

Vikas Rishi

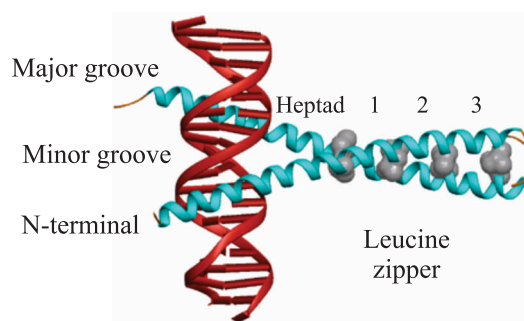
#### Research Fellow

Prateek Jain

Koushik Shah

#### Introduction

Basic leucine zipper (B-ZIP) are eukaryotic specific transcription factors that binds to promoter of a gene as a homodimer or as heterodimer. Figure 1 shows the X-ray structure of



**Figure 1:** X-ray structure of the b-ZIP dimer bound to DNA. DNA is in red, the alpha-helices are in blue. The d or leucine position amino acids are shown in grey.

the b-ZIP dimer bound to DNA. DNA is in red, the alpha-helices are in blue. The d or leucine position amino acids are shown in grey. In Arabidopsis, in vitro and in vivo studies have described the role of B-ZIP10, B-ZIP25, and B-ZIP53 in seed development. Furthermore, studies involving B-ZIP53 defined this transcription factor to be a key regulator of genes involved in seed maturation. B-ZIP53 expression increases during seed development and localizes to the embryo and endosperm during maturation phase. B-ZIP53 binds to G-Box (CACGTG) as a homodimer or a heterodimer partnering with B-ZIP10 or B-ZIP25 (Figure 1). When compared to homodimer,

heterodimers are proficient and impart a synergistic effect on gene expression. Surprisingly, B-ZIP53 knockout plant sets viable seeds, suggesting biological redundancy. In absence or sub-optimal expression of B-ZIP53 other B-ZIP TFs like B-ZIP10, B-ZIP25 or some unknown protein(s) hitherto may regulate seed-specific gene expression. In order to address the issue of overlapping functions, we have designed a dominant-negative protein A-ZIP53 that heterodimerizes with B-ZIP53, B-ZIP10, and B-ZIP25 and inhibits their DNA binding activity. Such heterodimers cannot bind to the DNA and can be used to study gene regulation. Previously this strategy has been used successfully in animal model systems.

#### Objectives

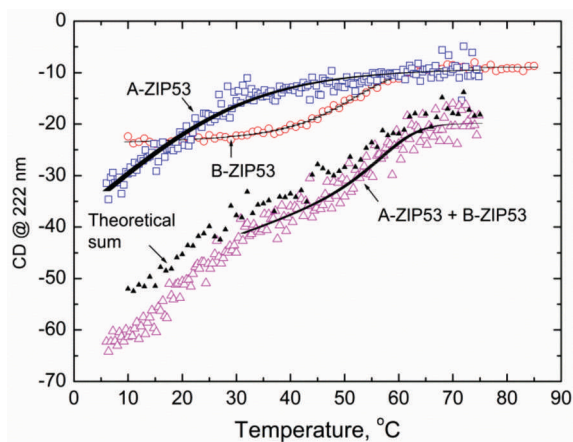
Characterization of A-ZIP53, a dominant-negative protein that heterodimerizes with wild type B-ZIP53, B-ZIP10, and B-ZIP25 and inhibits their DNA binding.

#### Research Progress

A-ZIP53 was designed by replacing the DNA binding domain of B-ZIP53 by a rationally designed acidic extension. Cues for such design have come from our understanding of coiled-coil structure and forces that are responsible for the stability of this extra ordinary domain. A-ZIP53 protein's ability to heterodimerize with these three wild type transcription factors was tested using circular dichroism spectroscopy (Figure 2).

#### Salient Achievements:

A-ZIP53 was designed, cloned and expressed in *E.coli* with high homogeneity. In vitro experiments have shown that A-ZIP53 preferentially heterodimerizes with B-ZIP53, B-ZIP10, and B-ZIP25 and inhibits their DNA binding. Such reagents may be used to study dimerization properties of any B-ZIP transcription factor *in vitro* and *in vivo*.



**Figure 2:** Stability studies of B-ZIP53, A-ZIP53 and their equimolar mixture were performed by thermally denaturing the protein and observing changes in circular dichroic signals. Protein samples were heated either from 10-85 °C or 6-75 °C. Thermodynamic parameters  $T_m$ ,  $\Delta H_m$  and  $\Delta G_D$  were obtained considering denaturation to be a two-state process.

### Future Perspectives

More mutants of A-ZIP53 will be designed. The most effective A-ZIP *in vitro* will be used to generate transgenic *Arabidopsis* and efficacy of this protein will be studied *in vivo*.

## 3.2 Biology of seed development in custard apple and litchi

### Principal Investigator

Sudhir P Singh

### Co-Investigator

Shrikant Mantri

### Research Fellows

Yogesh Gupta,  
Ashish K Pathak

### Introduction

Seeds in many fruit crops like custard apple, litchi, guava, orange, mango and grape are a hindrance to fruit processing and fresh fruit consumption, especially when the seeds are hard and/or have a bad taste. Therefore, less number of seeds or small size of the seed or seedlessness in fruit is appreciated by consumers. Furthermore, the

absence of seed/s can enhance the shelf life of the fruits. In case of custard apple (*Annona squamosa*), the fruit is a syncarpium i.e. formed by amalgamation of many ripened pistils and the fleshy receptacle. The fruit is many seeded (60-80 seeds in a fruit). We have identified accessions with a significantly reduced number of seeds as compared to the common custard apple- Sitaphal. Likewise, in litchi (*Litchi chinensis*), accessions have been reported with contrast in seed size. The fruit genotypes with contrasting fruit-seed number and seed size are models for identifying candidate pathways or genes for seedlessness.

### Objectives

1. Understanding of the molecular basis of seed development in fruit crops .
2. Differential transcriptomics in the developing fruits and ovules of fruit crops with contrast in fruit seed number and/or seed size .
3. Identification of candidate genes for seedlessness in fruit crops.
4. Strategies for inducing seedlessness in fruit crops.

### Research progress

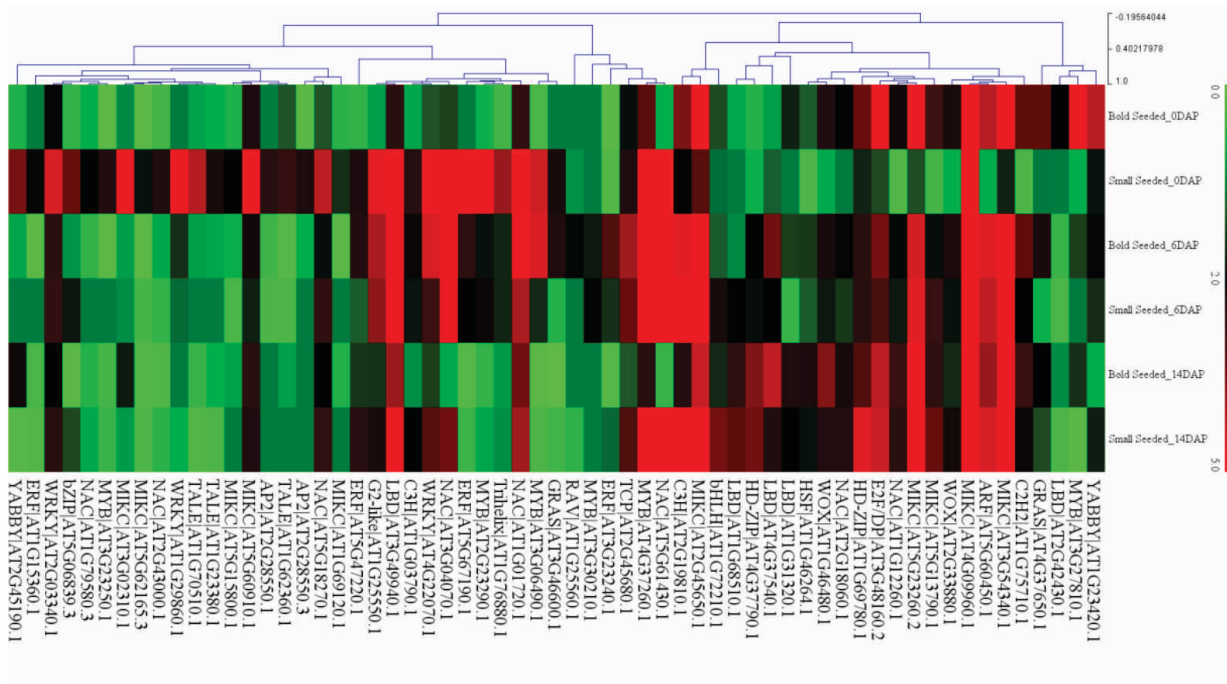
1. We have reported *de novo* assembly and analysis of transcriptomes of early-stage fruits from two genotypes of custard apple (*Annona squamosa*) with contrast in seed number. Orthologous genes related to hormone pathways, transcription factors and seed development were determined in the early-stage fruit transcriptome. Several of such unigenes were related to seed and fruit related traits, and expressed at a higher level in the densely seeded genotype, Sitaphal. Additionally, a large number of SSRs were identified, which will be a useful resource in marker development for future genetic studies in *Annona* sp. This repository will serve as a useful resource for investigating the molecular mechanisms of fruit development, and improvement of fruit related traits in *A. squamosa* and related species.
2. We have reported AFLP fingerprinting and

genetic relatedness among 23 popular commercial cultivars of lychee (*Litchi chinensis*), with contrasting seed size, and identified the markers associated with small-seeded cultivars. The marker system resolved the instances of synonymies and homonymies among the Indian cultivars. At present, we are examining the transcriptome in developing ovules of bold and small seeded lychee cultivars.

3. Transcriptome sequencing was done from the RNA-seq libraries, prepared from developing ovules at 0, 6, and 14 days after anthesis (DAA) in the two genotypes of *L. chinensis*, with contrasting seed size. The average number of reads produced for each library was 50 million paired end reads. *De novo* assembly of the paired end reads generated a total of 1,19,939 transcripts. About 7917 transcripts were found differentially expressed with at least two folds at  $p$  value  $\leq 0.0001$  (Table 1).
4. Transcripts related to transcription factors (TFs) were determined in the litchi transcriptome data by BLASTx search analysis using the *Arabidopsis* TF protein database. A total of 61 transcripts were identified as putative transcription factors, satisfying the criteria of at least 0.00001 evalue, 50% query coverage, 50% identity and 100 bitscore. The expression pattern of the TFs was examined in the developing ovules of bold and small seeded litchi cultivars (Fig. 1). Further analysis is in progress.
5. Seed-preferentially expressed transcripts were identified in the transcriptome data by BLASTx search analysis using the *Arabidopsis seed gene database*. A total of 48 contigs were identified as putative seed-preferentially expressed transcripts, satisfying the criteria of at least 0.00001 evalue, 50% query coverage, 50% identity and 100 bitscore. Their expression pattern was examined in the developing ovules of bold and small seeded litchi cultivars (Figure 3). Further analysis is in progress.
6. The transcripts were examined for the presence of simple sequence repeats (SSRs) with differences in length. Polymorphic SSRs were identified with variable number of tandem repeat loci between the two genotypes (Table 2). The SSR motifs could be potential candidates for transcript based microsatellite marker development in *Litchi chinensis*. Further analysis is in progress.

**Table 1.** Number of differentially expressed transcripts in developing ovules (0, 6, 14 DAA) of bold (HK) and small (HS) seeded litchi cultivars

	HK0	HK06	HK14	HS0	HS06	HS14
HK0	0	1001	235	4938	934	1376
HK06	1001	0	879	2282	221	1098
HK14	235	879	0	5088	784	1238
HS0	4938	2282	5088	0	1311	3170
HS06	934	221	784	1311	0	190
HS14	1376	1098	1238	3170	190	0



**Figure 3:** Heat map showing hierarchical clustered Pearson correlation matrix obtained from transcript expression values (TMM-normalized FPKM) of putative transcription factors (left), and seed-preferentially expressed transcripts (right) in Litchi. Expression values are log<sub>2</sub> transformed.

**Table 2.** Polymorphic SSRs with variable number of tandem repeat loci in the transcripts of bold (HK) and small (HS) seeded litchi cultivars.

Unigene id	Annotation	SSR Motif	Number of repeats	
			HK	HS
Contig18064	Histidine--tRNA ligase-like	Tri (AAG)	6	7
Contig 5845	Transcript variant X5, misc_RNA	Tri (AAT)	11	7
Contig14915	Transcription initiation factor TFIID subunit 12b-like	Tri (GCT)	7	5
Contig2060	RNA recognition motif protein 1 (RRMP1)	Tri (ACC)	6	8
Contig42776	Hypothetical protein (CICLE_v10022275mg) mRNA	Tri (AAC)	11	6
Contig43616	Basic helix-loop-helix DNA-binding superfamily protein	Tri (GGT)	6	8
Contig33157	Probable mitochondrial chaperone BCS1-B-like	Di (AG)	17	8
Contig36569	Not annotated	Di (AG)	9	7



### Salient Achievements

1. The first transcriptome information has been generated on *A. squamosa*. This repository will serve as a useful resource for investigating the molecular mechanisms of fruit development, and improvement of fruit related traits in *A. squamosa* and related species.
2. A large number of SSRs were identified, which will be a useful resource in marker development for future genetic studies in *Annona* sp.

### Future Perspectives

1. Differential expression analysis in early-stage developing fruits from two genotypes of *A. squamosa* with contrast in seed number.
2. Differential expression analysis in early-stage developing fruits from two genotypes of *L. chinensis* with contrast in seed size.
3. Identification and characterization of candidate genes related with seedlessness in *A. squamosa* and *L. chinensis*.

## 3.3 Development of approaches for the modulation of trait through long distance signalling

### Principal Investigator

Sudhir P Singh

### Research Fellow

Anita Kumari

### Introduction

Grafting is a well-established practice to facilitate asexual propagation in horticultural and agricultural crops. It has become a method for studying molecular aspects of root-to-shoot and/or shoot-to-root signalling events. The research project anticipates establishing long-distance transmission of mobile signals in the form of siRNAs to achieve gene silencing in flowering tissues. As an alternative strategy, we intend to modify the viral genome to achieve targeted silencing of the gene for seed

development.

### Objectives

To develop an approach for inducing seedlessness by long distance signaling through improved rootstock or modified viral vector.

### Research Progress

1. We investigated differences in gene expression between the organs of the scion and rootstock of a homograft in *Arabidopsis thaliana*. Grafting triggers differential expression of numerous genes related to stress, biotic and abiotic stimuli, hormonal pathway, and flowering etc. in flower buds and leaves of the scion and rootstock. The flower buds of scion showed over-representation of the transcription factor genes, such as Homeobox, NAC, MYB, bHLH, B3, C3HC4, PLATZ etc. The scion leaves exhibited higher accumulation of the regulatory genes for flower development, such as SEPALLATA 1–4, Jumonji C and AHL16. Differential transcription of genes related to ethylene, gibberellic acid and other stimuli was observed between scion and rootstock. The study is useful in understanding the molecular basis of grafting and acclimation of scion on rootstock.
2. We have established silencing of reporter gene (*uidA*) in flowering buds of scion by siRNAs delivered through rootstock. Small RNAs (sRNAs) were extracted from leaves and flower buds of *uidA* expressing scion grafted onto siRNA deliverable root-stock. Sequencing of small RNAs revealed that a considerable amount of small RNAs (21-24 bp) were generated (Figure 4A) in rootstock from the 801-1300bp region of *uidA* gene (Figure 4B), which was used as inverted repeats (IR) in the RNAi construct. In the scion, several folds less small RNAs were detected in the leaf and flowering buds of the scion (Fig. 4C). However, the small RNAs extracted from the scion were mapped on the

entire region of *uidA* gene (Figure 4D). Further analysis is in progress.

3. The wild scion was grafted on rootstock expressing inverted repeats of a gene which is important for proper ovule development *INO*. Seed setting was severely affected in the scion plants, as compared to the wild/wild grafted control. Further analysis is in progress.

### Salient Achievements

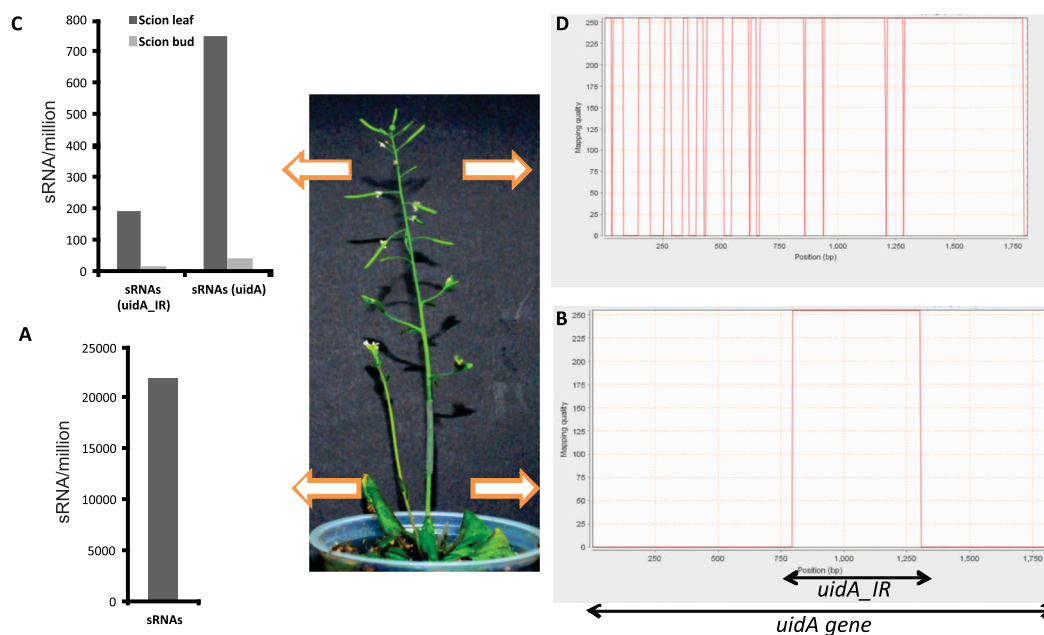
1. We have reported grafting triggered differential expression in flower buds and leaves of the scion and rootstock. The study is useful in understanding the molecular basis of grafting, acclimation of scions on rootstock, and crosstalk between scion and

rootstock.

2. Silencing of the reporter gene (*uidA*) has been established in flowering tissues by siRNAs delivered from the rootstock. The siRNAs have been detected and quantified in rootstock and scion tissues by small RNA sequencing.

### Future Perspectives

1. Comparison between transcriptional and post-transcriptional gene silencing transmission from rootstock to scion.
2. Establishment of silencing of gene related to seed development by the siRNAs transmitted from the modified root-stock.



**Figure 4:** *uidA* gene specific small RNAs (21-24 bp) detected in rootstock and scion. **(A)** The approximate amount of sRNAs produced in rootstock. **(B)** The sRNAs of rootstock were mapped on 800 to 1300 bp region of *uidA* gene. **(C)** The approximate amount of sRNAs detected in scion leaf and buds from IR region and entire *uidA* region. **(D)** The sRNAs of scion were mapped on entire *uidA* gene.





## DIET AND HEALTH

## 4.1 Effect of millet consumption on high fat diet induced changes in mice

### Principal Investigator

Kanthi Kiran

### Co-investigator

Mahendra Bishnoi

### Research fellow

Siddhartha M Sarma

### Introduction

Obesity is a major health crisis around the world. Sedentary lifestyle and excess calorie intake are the leading causes, which result in low grade inflammation, oxidative stress, dysbiosis of beneficial gut microbiota. This leads to abnormalities like atherosclerosis, diabetes and some forms of cancer. Current drugs are known to have serious side-effects highlighting the need for alternate approaches. Millet whole grain consumption, non-starch dietary fibres and prebiotics are shown to have ameliorating effects in diet induced obesity. Inflammation is a complex process that occurs in response to cell damage and vascularization of tissues. Inhibiting pro-inflammatory mediators is a plausible target for treatment of various inflammatory diseases including obesity.

### Objectives

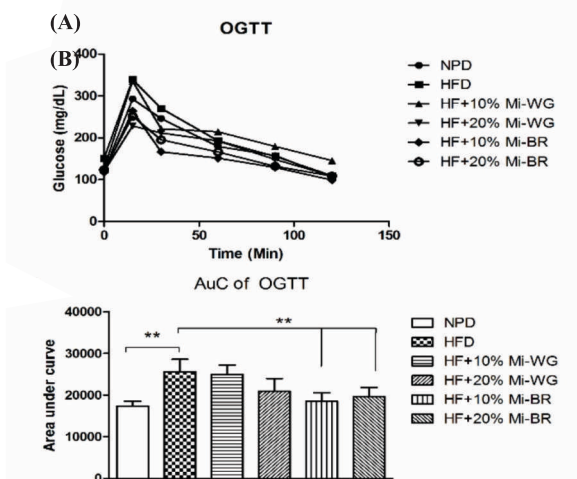
1. Nutrigenomic alterations associated with millet whole grain/bran (Mi-WG/BR) consumption in high fat diet fed mice.
2. To understand the role of non-starch dietary fibres (NSDF) in regulating inflammation *in vitro* using RAW 264.7 murine macrophages.

### Research Progress

Previously, we have reported (Annual report-2013-14) about the effect of finger millet whole grain/bran on high fat fed mice. In continuation to the study, this year we have initiated experiments with another millet.

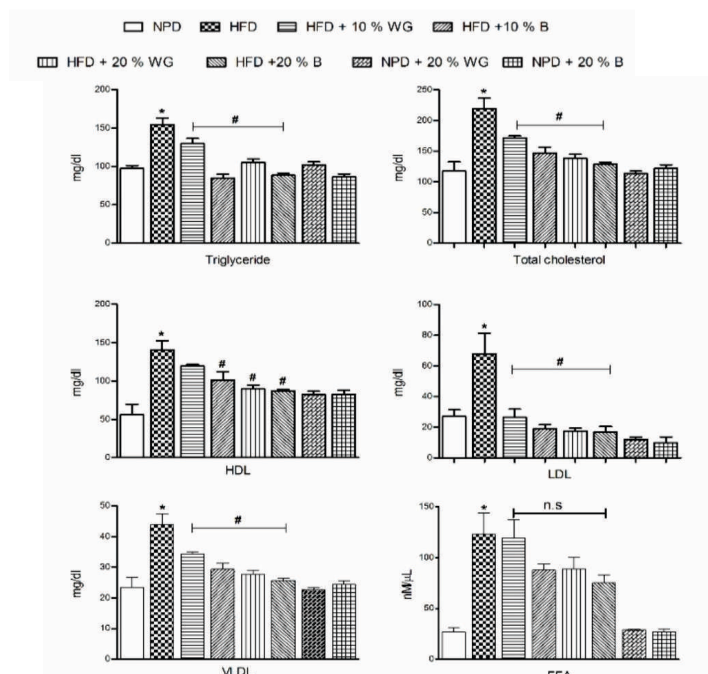
## 1. Study of effect of millet whole grain/bran supplementation on high fat diet fed LACA mice

- a) **Body weight, Oral Glucose Tolerance Test (OGTT) and glucose clearance:** Millet whole grain/bran (Mi-WG/BR) was supplemented with high fat diet (45% energy from fat) for 16 weeks to mice for studying the effect of millet on diet-induced obesity model animals. There was no change in the weight gain of groups fed with normal diet and HFD-fed millet groups. OGTT revealed significant change in glucose disappearance rate in animals treated with Mi-BR compared to their HFD counter parts while no significant change was observed in HFD+WG fed groups (Figure 1).
- b) **Serum biochemical parameters:** Mi-WG/BR showed significant improvement in serum cholesterol parameters such a total



**Figure 1:** Effect of millet WG/BR supplementation on (A) OGTT at different time point's upto 120 minutes (B) Glucose disappearance rate.

cholesterol (TC), triglycerids (TG), low density lipoproteins (LDL-c) and very-low density lipoproteins (VLDL-c), with 20% Mi-BR showing the most change. Serum free fatty acids (FFA) showed no significant change while high density lipoproteins (HDL-c) were significantly reduced (Figure2).



**Figure 2:** Effect of millet WG/BR supplementation on serum lipid profiles. NPD = Control, HFD = High fat diet, B = Bran, WG = Whole grain. Data is represented as mean with SEM. \* = Significant vs NPD, # = significant Vs HFD, ns = non-significant.

Supplementation with Mi-WG and Mi-BR caused various changes in serum cholesterol parameters when compared with HFD fed control group. Although, oxidative stress parameters assessed in liver, pancreas, muscle visceral and subcutaneous white adipose tissue showed no significant changes (Data not included).

- c) Gut microbial expression and SCFA production:** Millet WG/BR administration significantly altered certain bacterial groups by promoting growth of *Lactobacillus sp.*, *Rosburia sp.* and *Akkermensia sp.* when compared with HFD alone fed mice. Groups such as *Bacteroidetes*, *Firmicutes* and *Enterobacter* showed treatment induced variations in gene expression. Total short chain fatty acids was increased in caecal contents of HFD-fed groups treated with Mi-WG/BR. Acetate production was high in treatment groups, when compared to HFD and NPD fed groups.

## 2. Estimation of anti-inflammatory potential on RAW 264.7 murine macrophages

### a) Extraction of non-starch dietary fibres:

Powdered millet flour (100 g) was washed to remove starch and bran was collected. This was sequentially treated with  $\alpha$ -amylase, amyloglucosidase, and protease; delipidified using n-hexane followed by 2:1 (v/v) methanol and chloroform. The delipidified bran (~10 g) was extracted with 20% KOH containing 1% NaBH<sub>4</sub> at room temperature, overnight. Supernatant was collected after centrifugation and neutralized with glacial acetic acid. Neutralized extract was dialyzed and lyophilized to get soluble non-starch dietary fibres (NSDF).

- b) Anti-inflammatory potential on RAW 264.7 cell line:** Murine macrophage cell line RAW 264.7 (NCCS, Pune, India) was cultured as per standard procedures. Various doses of NSDF (0.125, 0.25, 0.5, 0.75 and 1 mg/ml) treatment showed that cells retained viability at these doses. Inflammation was

induced in the cells using bacterial lipopolysaccharide (*E. Coli*, Sigma, USA) using an optimum dose (1µg/ml). NSDF concentrations of 0.5, 0.75 and 1 mg/ml was administered to the cells with and without LPS dissolved in DMEM. The quantity of nitrite accumulated in the culture medium was measured by Griess reagent and used as an indicator for NO production. Millet NSDF at different doses reduced the nitrite levels to those of control levels, with highest being NSDF at 1 mg/ml. This was seen even after prolonged exposure to LPS (up to 48 hours) (Figure 3).

Preliminary studies on RAW 264.7 cells suggest an increase in inflammatory genes after administration of bacterial LPS. Expression of these inflammatory genes such as *TNFα*, *IL-6*, *NFκβ* and *iNOS* are seen to be reduced upon treatment of NSDF at various doses. *IL-6* and *iNOS* expression was dose dependently

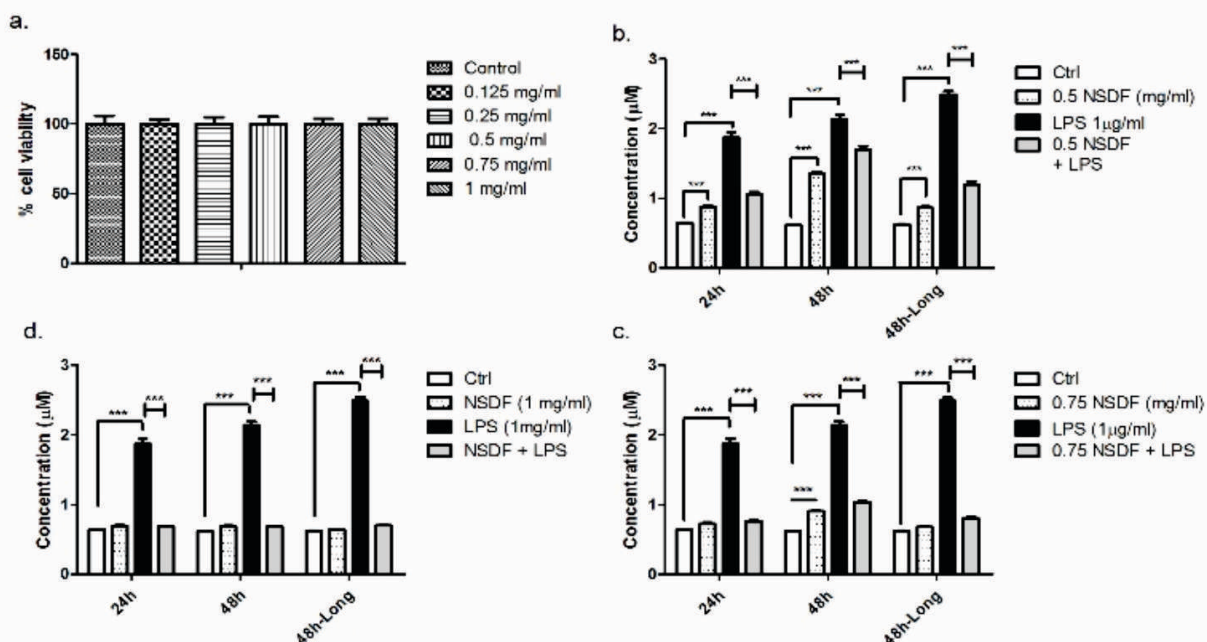
decreased, with highest change seen at 1 mg/ml dose (Figure 4).

### Salient Achievements

1. Millet WG/BR show potential in alleviating symptoms of high fat diet induced metabolic alterations such as serum cholesterol and glucose levels, modulating beneficial gut microflora and SCFA production.
2. Millet NSDF alleviated LPS induced pro-inflammatory stress in macrophages cell line model.

### Future Perspectives

1. Millet partially purified NSDF to be studied for their effect on high fat diet induced changes in mice.
2. Mechanism of action of NSDF at the cellular level and their interaction with host tissues, gut microbes and short chain fatty acid production.



**Figure 3:** Effect of NSDF on nitrite production at different doses (a) 0.5 mg/ml, (b) 0.75 mg/ml and (c) 1 mg/ml for (i) 24 hour exposure, (ii) 48 hour exposure and (iii) 48 hour continuous exposure.

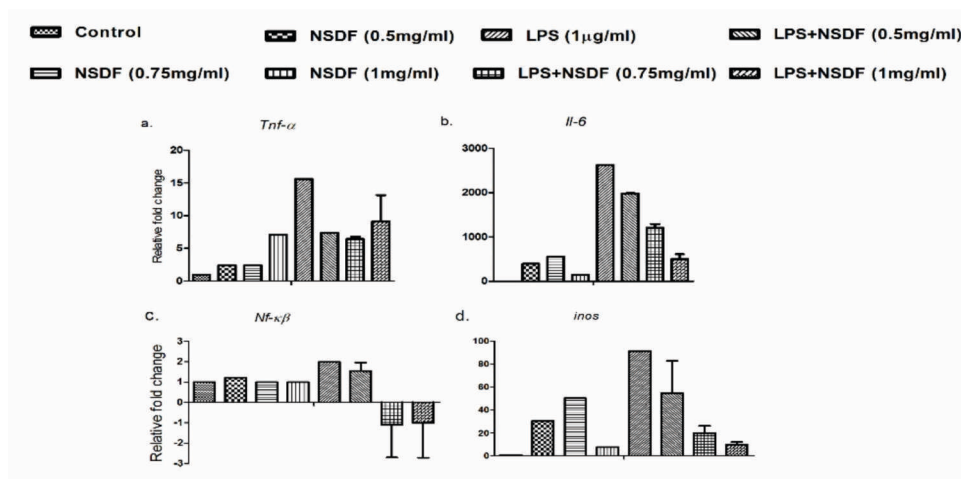


Figure 4: Effect of NSDF on Inflammatory gene expression profile in RAW 264.7 cells.

## 4.2 Development of synbiotics/cobiotics for the prevention of obesity

### Principal Investigator

Kanthi Kiran

### Co-Investigators

Mahendra Bishnoi

### Research Fellow

Shashank Singh

### Introduction

Human gastrointestinal tract (GIT) harbours trillions of bacteria that play an important role in maintaining host's health. There are up to  $10^{13}$ – $10^{14}$  bacteria in the human GIT, which is 10- to 20-fold more than the total number of tissue cells in the entire body. Dysbiosis of GIT microbiota results in inflammation which is associated with pathophysiology of multiple gastrointestinal and non-gastrointestinal disorders including obesity. Replenishing the altered gut microflora by probiotics, prebiotics and their combination as synbiotics seem to be a viable option to protect from such diseases. Probiotics are live microorganisms, which when administered in appropriate amounts; confer health beneficial effects. Lactic acid bacteria (LAB) are a group of Gram-positive bacteria that excrete lactic acid as their main fermentation product. Predominantly

bacteria from *Lactobacillus* and *Bifidobacterium* genera are considered as safe probiotics. On the other hand, prebiotics are non-digestible carbohydrate (NDC) food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or limited number of bacteria in the colon. Our literature survey showed that very few studies reported on the use of Indian origin probiotic strains with reference to metabolic disorders. Therefore, the present study has been taken up to isolate potential probiotic strains from various food sources, stools from healthy infants and adults and to characterize their probiotic attributes as per DBT-ICMR guidelines. Further, we will use these strains for functional food applications for the prevention of obesity using rodent models.

### Objectives

1. Isolation, identification and characterization potential probiotic strains of Indian origin.
2. Metabolism of prebiotics and dietary fibres by the isolated strains, short chain fatty acid production and hydrolytic enzymes.
3. Anti-obesity and hypoglycemic potential of potential probiotic strains (*in vitro* and *in vivo* models), novel prebiotics and to develop synbiotic and cobiotic formulations for functional food applications.



## Research Progress

More than 45 Gram positive and catalase negative lactic acid bacteria (LAB) were isolated. Some of the strains showed high auto-aggregation capacity, acid tolerance (pH 2.5 for 30 min) and grow in presence of 0.1-0.2% ox bile. Prebiotic profiling showed 22 strains could ferment isomaltooligosaccharides (IMOS); 17 strains could ferment fructooligosaccharides (FOS); 3 strains could ferment inulin; 5 strains were able to utilize soluble starch and resistant starch. Further 6 strains were able to produced cell associated  $\alpha$ -galactosidase in presence of glucose and lactose while 3 strains produced  $\alpha$ -galactosidase in presence of lactose and 12 strains were able to produce  $\beta$ -galactosidase in presence of glucose and lactose.

## Salient Achievement

Lactic acid bacteria with an ability to ferment various prebiotics, inulin and resistant starch has been isolated.

## Future Perspectives

Assessment of biological activity of selected strains using *in vitro* and *in vivo* model systems.

## 4.3 Transient Receptor Potential (TRP) channel mediated dietary modulation of adipogenesis, obesity and its associated complications

### Principal Investigator

Mahendra Bishnoi

### Co-Investigator

Kanthi Kiran

### Research Fellows

Ritesh K Baboota

Dhirendra Pratap Singh

Pragyanshu Khare

## Introduction

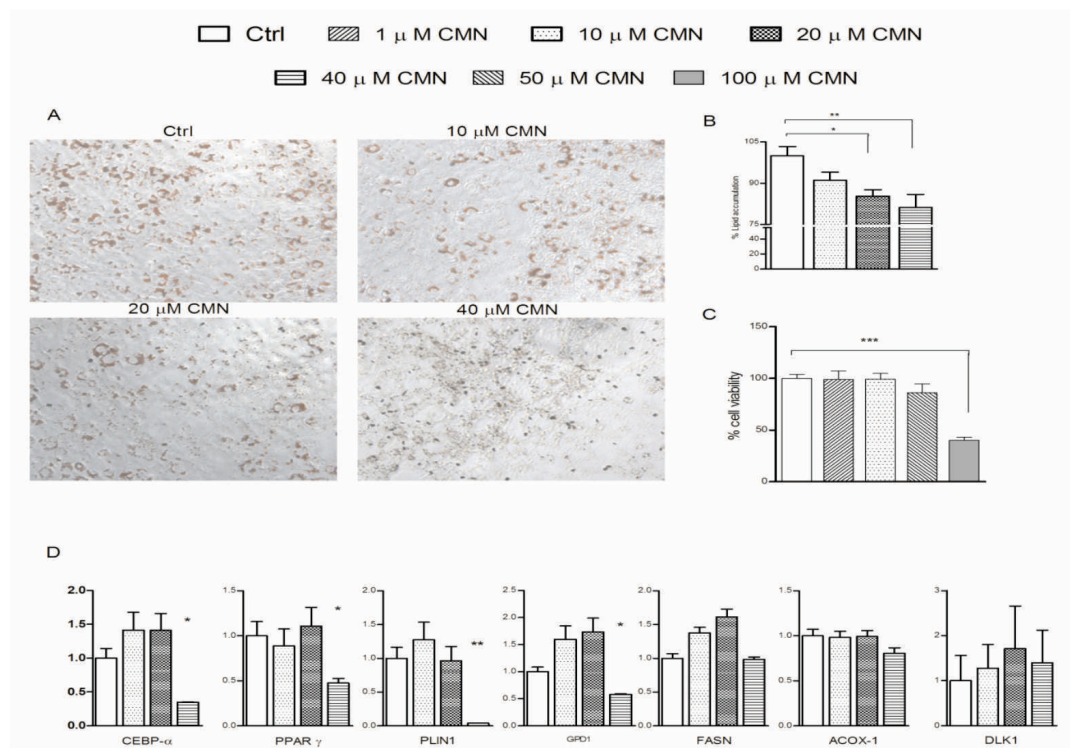
Current anti-obesity medications are pharmacological agents which can reduce or

control weight by affecting one of the fundamental processes of the weight regulation in human body i.e. altering appetite, metabolism, or consumption of calories. All these medications, including orlistat, rimonabant and sibutramine, have severe side effects including depression, oily bowel movements, cardiovascular concerns and steatorrhoea. The potential side effects profile of these drugs is much more than their beneficial effects, suggesting the urgent need for alternatives. Over the years it has been seen that best and most effective options for overweight and obese individuals remain diet and physical exercise. It is important to have dietary regulations to prevent life style problems rather than to search for the treatment. Available literature suggests that sensory ion channel receptor system, Transient Receptor Potential (TRP) channels, are possible candidates to regulate energy metabolism and thermogenesis, which can lead to calorie consumption and prevention of obesity via different mechanisms. Common dietary constituents like chilli pepper, black pepper, clove, garlic, cinnamon, mint and their constituents (capsaicin, piperine, eugenol, allicin, cinnamaldehyde, omega fatty acids, menthol etc) can modulate TRP channels. In this project, we will understand the role of TRP channels in adipogenesis, obesity and related complications using *in vitro* and *in vivo* model systems. Further, using the TRP channel receptor system we propose to come up with dietary constituents that can modulate the molecular mechanism associated with the process of adipogenesis.

## Objectives

1. Determination of expression, function and significance of TRP channels in commercially available mouse preadipocytes cell lines (3T3-L1), human preadipocytes (HPAd) and adipocytes (HAd) cells.
2. *In vitro* characterization of the molecular basis of adipogenesis and determination of effect of TRP channel modulation on





**Figure 5:** Effect of cinnamaldehyde (CMN) on 3T3-L1 preadipocyte. Effect of (A) CMN on differentiation of 3T3-L1 preadipocytes. Black spots in images represents area stained by ORO dye, (B) CMN on lipid accumulation in 3T3-L1 adipocytes, (C) 72 h treatment of CMN on cell viability in pre-adipocytes. (D) CMN on expression of genes related to adipogenesis and lipolysis. All values are expressed as mean  $\pm$  S.E.M. (n = 5). One way ANOVA followed by Tukey's multiple comparison was applied. \*\*\* p < 0.001, \*\* p < 0.01 and \* p < 0.05 compared to control.

adipogenesis and its associated changes.

- To study the effect of dietary modulations of TRP channels (TRPV1: capsaicin, piperine; TRPA1: garlic, cinnamon; TRPM8: menthol; TRPC1: omega-3 fatty acids and others) on weight gain, serum biochemistry, and adipose tissue genotype in a diet (high fat) based *in vivo* mouse model of obesity.
- Developing diets/ special dietary formulations constituted of modulating food components and study their effect on adipogenesis, obesity and related complications in human trials.

### Research Progress

Previously we found out that many TRP channel genes are expressed in mouse 3T3-L1 preadipocytes, differentiated adipocytes, murine white adipose tissue (WAT), brown adipose tissue (BAT) and human preadipocytes and adipocytes.

Critical analysis of TRP channel expression data in 3T3-L1 preadipocytes and differentiated adipocytes suggested the potential involvement of TRPV1, TRPV2, TRPA1 and TRPM8 in adipogenesis, obesity and related complications (Annual report 2012-2013). Taking lead into the role of TRPV1, we initiated and completed in-vitro (3T3L1 preadipocyte cell lines) and in-vivo (high fat diet (HFD)-induced weight gain model) studies for capsaicin, a TRPV1 agonist. In summary, capsaicin prevented adipogenesis by inducing "browning" like phenotype in differentiating 3T3L1 preadipocytes. Further, our in-vivo studies suggest that in addition to its well known effects, oral administration of capsaicin (a) modulates hypothalamic satiety associated genotype, (b) alters gut-microbial composition, (c) induces "browning" genotype (BAT associated genes) in subcutaneous WAT and (d) increases expression of thermogenesis and mitochondrial biogenesis genes in BAT (Annual report 2013-

2014). During the last year, we focused on the role of TRPA1 and TRPM8 and dietary constituents modulating these channels in adipogenesis, high fat diet (HFD) induced obesity and related complications.

### ***In vitro* studies for cinnamaldehyde (CMN), TRPA1 agonist, and menthol, a TRPM8 agonist**

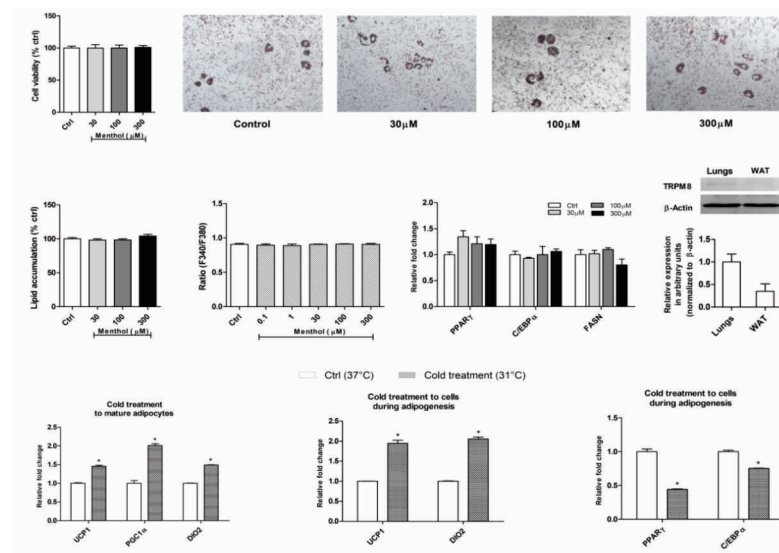
CMN dose dependently prevented lipid accumulation as compared to control as evident in Oil-Red O (ORO) staining (Figure 1A, B). CMN at 40  $\mu$ M significantly reduced the expression level of PPAR $\gamma$  and CEBP- $\alpha$ , major regulators of adipogenesis whereas no significant difference was observed at 10 and 20  $\mu$ M in comparison to control (Figure 5D). Furthermore, CMN at 40  $\mu$ M significantly reduced the expression level of PLIN 1 and GPD in comparison to control whereas no significant difference was observed at lower doses i.e. 10 and 20  $\mu$ M (Figure 5D). Expression level of DLK1, ACOX1 and FASN not altered significantly at any of three dose of CMN. However, CMN at lower doses showed a little increase in FASN expression level (Figure 5D). Further, CMN up to 40  $\mu$ M did not affect cell viability whereas at 100  $\mu$ M significant inhibitory effect on cell viability was observed (Figure 5C).

Menthol did not show significant change in adipogenesis as evident by ORO staining and expression levels of marker genes related to the process of adipogenesis. Also, we were not able to functionally show the presence of TRPM8 in adipose tissues. However, as TRPM8 is a cold receptor, we studied the changes induced by cold in undifferentiated and differentiated 3T3L1 cells and found significant gene expression changes linked to antiadipogenic and “browning” phenotype (Figure 6).

### ***In vivo* studies for cinnamaldehyde (CMN), TRPA1 agonist**

#### ***CMN prevented HFD induced weight gain and obesity like anthropometric changes***

Mice fed with HFD showed significant increase in body weight as compared to age matched NPD fed mice in 14 weeks of study (Figure 7A-C). HFD induced weight gain was significantly and dose dependently prevented by CMN 5 and CMN 10 (Figure 7A-C). Lower abdominal circumference and naso-anal length were measured where difference was not observed among any group (Figure 7D and Figure 7E). Despite higher values in HFD group as compared to NPD group in calculated parameters such as Lee's index (Figure 7G), and BMI (Figure 7F), difference was not statistically significant.



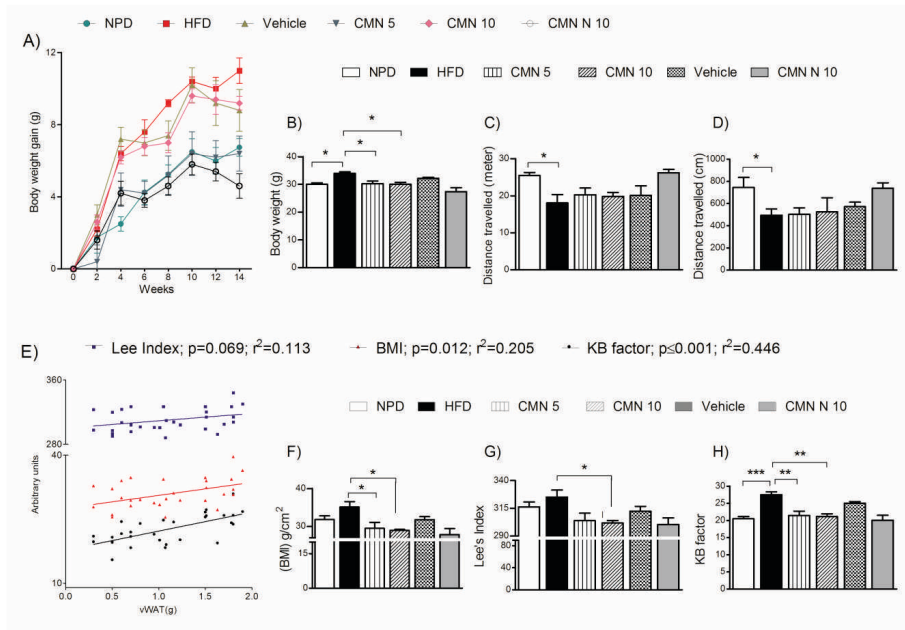
**Figure 6:** Effect of TRPM8, cold exposure and menthol on cell viability, differentiation of 3T3-L1 preadipocytes, marker gene expression using 3T3-L1 cell lines. All values are expressed as mean  $\pm$  S.E.M. (n = 5). One way ANOVA followed by Tukey's multiple comparison was applied. \*\*\*p < 0.001, \*\*p < 0.01 and \*p < 0.05 compared to control.

However, we noticed a significant difference between NPD and HFD group in “KB” factor (Figure 7H). Improved anthropometric parameters have been observed in CMN 5 and CMN 10 group (Lee's index, BMI and KB factor-Figure 3F-H) as compared to HFD group. Total distance travelled ( $p \leq 0.05$ ) and overall average speed ( $p \leq 0.001$ ) in open field apparatus was significantly lower in HFD group as compared to NPD group. Decrease in total distance travelled was also observed between NPD and HFD in opto-varimics animal activity meter. CMN treatment did not show any effect on locomotor activity in these experiments.

*CMN effectively reversed HFD-induced hyperphagia via hypothalamic orexigenic and anorectic gene modulation*

Feeding behaviour in mice was accessed *via* two different ways i.e. average feed intake and hyperphagic feed intake. No significant difference in average feed intake was observed among

different groups, however, a slight anorectic behaviour in CMN treated groups was observed (Figure 8A). Therefore, we also evaluated the hyperphagic feed intake, where mice fed on HFD showed higher feed intake as compared to NPD group (Figure 8B). CMN showed a dose dependent anorectic effect in CMN 5, and CMN 10 group as compared to vehicle group. Anorectic effect was also observed in CMN N 10 as compared to NPD group (Figure 8B). We assessed serum level of leptin and ghrelin, chemokines having active role in anorectic and orexigenic activity respectively. Serum level of leptin was 3 folds higher in HFD fed mice as compared to NPD fed mice ( $p \leq 0.001$ ). CMN at both the doses significantly reduced serum leptin level, however no effect was observed in CMN N 10 group as compared to NPD fed mice (Figure 8C). We observed dose dependent decrease in serum ghrelin level however difference was not statistically significantly different among groups (Figure 8D).

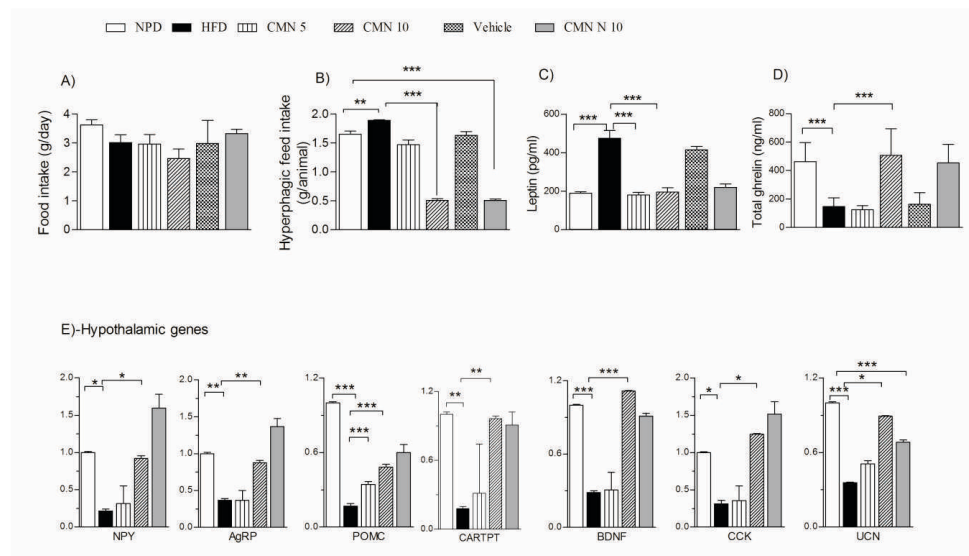


**Figure 7:** Anthropometric parameters related to obesity. Effect of different dose of Cinnamaldehyde (CMN) (5 and 10 mg/kg, per oral) on (A) Weekly change in body weight; (B) Weekly gain in body weight; (C) weight at the time of sacrifice; (D) Circumference of lower abdomen; (E) Naso-anal length; (F) Body mass index; (G) Lee's index; (H) KB factor; (I) Distance travelled in optovarimics; (J) Distance travelled in open field apparatus (K) Average speed in open field apparatus. Numerical values are expressed as mean  $\pm$  SEM; N=6; One way ANOVA followed by Tukey's multiple comparison was applied; \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$  and \*  $p < 0.05$ .



Feeding behaviour is controlled mainly by two type of neuronal population in hypothalamus i.e. neuropeptide Y (NPY)/agouti related protein (AGRP) neurons and pro-opiomelanocortin (POMC)/ cocaine and amphetamine related transcript (CART) neurons. Neuronal axis of NPY/AGRP and POMC/CART are related to feeding and satiety signal, respectively. HFD group decreased the expression of multiple anorectic and orexigenic genes in hypothalamus, which was reversed by CMN administration (Figure 8E).

show significant effect on serum triglyceride and serum NEFA. However, CMN 5 reversed the lower serum lipase of HFD and also increased the level of serum glycerol. CMN 10 significantly increased the serum NEFA, and glycerol (Figure 9B,C,D,E). Above observation supported our interest in genomic alteration into tissues those are mainly involved in lipid metabolism i.e. liver, brown adipose tissue (BAT) and visceral white adipose tissue (vWAT). Further, we assessed different genes involved in the regulation of lipid metabolism that can play role in above mentioned



**Figure 8:** Hunger and satiety related changes. Effect of different dose of Cinnamaldehyde (CMN) (5 and 10 mg/kg, per oral) on (A) Average feed intake; (B) Hyperphagic feed intake; (C) Serum leptin level; (D) Serum ghrelin level (E) Anorectic and orexigenic gene in hypothalamus. Numerical values are expressed as mean  $\pm$  SEM; N=3-6; One way ANOVA followed by Tukey's multiple comparison was applied; \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$  and \*  $p < 0.05$ .

Restricted feeding and increased capacity of brown adipose tissue associated increase in lipolysis and decrease in fat pad via chronic CMN administration

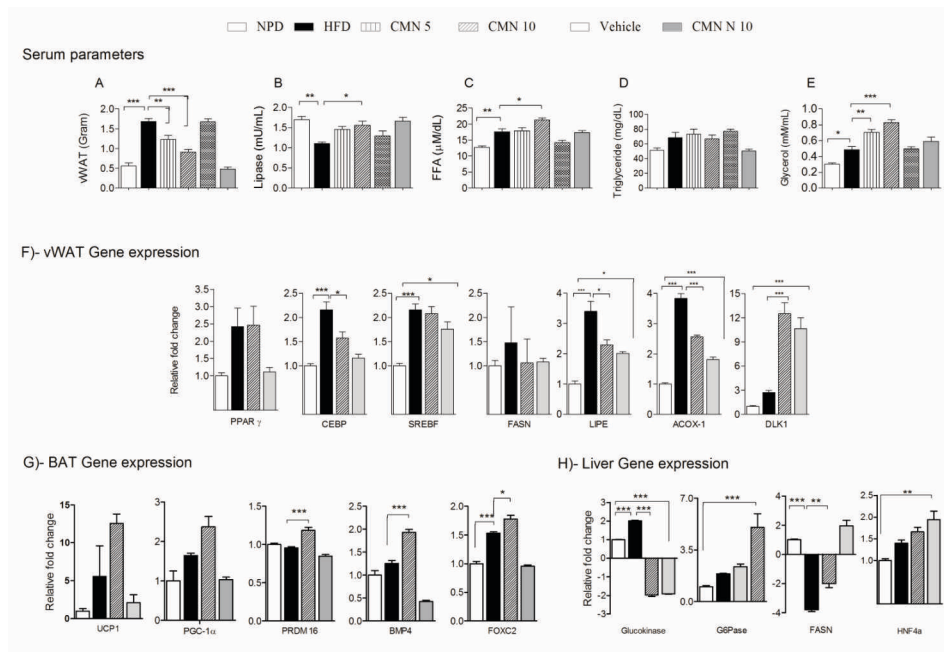
Epididymal and peri-renal fat pad were carefully isolated and weighed. The cumulative amount of both fat pads was 2.5 to 3 fold higher in HFD fed mice in comparison to NPD mice. CMN dose dependently decreased both fat pads (Figure 5A). Serum biochemistry revealed the higher NEFA and glycerol in serum of HFD fed mice in comparison to NPD fed mice. On the other hand, serum triglyceride and serum lipase were significantly lower in HFD mice. CMN 5 did not

phenomenon, in vWAT gene expression level of *CEBP- $\alpha$* , *ACOX1*, *LIPE* and *SREBF* significantly up regulated while *PPAR $\gamma$* , *DLK1* and *FASN* expression remain same in HFD fed mice comparison to NPD fed mice. Expression level of *C/EBP $\alpha$* , *ACOX1* and *LIPE* were down regulated upon 10 mg/kg CMN chronic per oral treatment while the gene expression level of *DLK1* was significantly up regulated. Expression level of *PPAR $\gamma$* , *SREBF*, and *FSN* remain unchanged in vWAT upon administration of 10 mg/kg CMN. In BAT, we performed RT-PCR analysis of *UCP1*, *PGC-1 $\alpha$* , *PRDM16*, *BMP4*, *FOXC3*, and *TBX1* as these genes are mainly involved in enhancing

energy metabolism. Expression level of *FOXC3*, and *TBX1* significantly increased while *UCP1*, *PGC-1 $\alpha$* , *PRDM16*, and *BMP4* did not express differentially in HFD fed mice compared to NPD fed mice. CMN at a dose of 10 mg/kg significantly increased the expression of *BMP4* while the expression level of *TBX1* decreased significantly. Glucokinase gene expression level was significantly high while *FASN* level was significantly down regulated in liver of HFD fed mice. We did not observe any difference in *G6Pase* and *FASN* expression level in liver of HFD fed mice. CMN at a dose of 10 mg/kg

### Salient Achievements

1. Our *in vitro* findings suggest modulatory role of cinnamaldehyde in adipogenesis via promoting lipolysis. Further, in-vitro studies did not show functional TRPM8 in adipose tissues but we understood that mechanistic link between cold and adipogenesis via TRPM8 can be established.
2. Our *in vivo* findings suggest that oral administration of cinnamaldehyde initiates lipolysis, increases expression level of thermogenesis related genes in BAT and decreases feed intake. All these changes are



**Figure 9:** Decrease in fat pad associated with lipolysis and browning. Effect of different dose of CMN (5 and 10 mg/kg, per oral) on (A) Visceral Fat pad weight; (B) Serum lipase level; (C) Serum NEFA; (D) Serum Triglyceride; (E) Serum glycerol; (F) gene expression pattern in vWAT; (G) gene expression pattern in BAT; and (H) gene expression pattern in Liver. Numerical values are expressed as mean  $\pm$  SEM; N=3-6; One way ANOVA followed by Tukey's multiple comparison was applied; \*\*\* p < 0.001, \*\* p < 0.01 and \* p < 0.05.

significantly down regulated the elevated expression of glucokinase gene at the same time CMN significantly up regulated the reduced expression of *FASN* in liver. CMN up regulated the expression level of *G6Pase* and *HNF4a* in *per se* group (Figure 8F-H).

positively correlated with phenotypic changes like reduction in body weight gain and other anthropometric parameters like BMI, Lee's index and 'KB' factor. Further, CMN also modulated multiple serum and tissue biochemical parameters to suggest it's lipolytic and feed intake modulatory behaviour.

### Future Perspectives

1. *In vitro* studies to understand the role of other TRP channels (i.e. TRPV2 and TRPC1/C5) in adipogenesis and its associated changes.
2. Mechanisms based *In vivo* studies of menthol, TRPM8 activator in weight gain and related changes using diet (high fat) based *in vivo* mouse model of obesity.
3. Mechanism based study the effect of dietary modulations of TRP (V1, A1 and M8) channels on obesity induced comorbidities like insulin resistance and inflammation in a diet (high fat) based *in vivo* mouse model of obesity.
4. Preclinical and clinical follow-up of dietary constituent's based studies.

### 4.4 Variability in the fine structures of phenolic acids bound arabinoxylans from Indian millet varieties and their consequence on anti-oxidant activity

#### Principal Investigator

Koushik Mazumder

#### Research Fellow

Vandana Bijalwan

#### Introduction

Millets are small seeded cereal crops belonging to the family poaceae. In Africa, east-asia and Indian sub-continent millets are considered as staple diet for large low income population. Several epidemiological studies have clearly demonstrated that increased consumption of soluble dietary fibers has been associated with a reduced risk of cardiovascular diseases, cancer and diabetes. Many of the life style disorders and chronic diseases are associated with oxidative stress which is combined with free radical formations such as superoxide anions, hydroxyl radicals and nitric oxide radicals.

Dietary fibers like hydroxy-cinnamic acid (HCA) bound arabinoxylans are the major non-starchy polysaccharides in millets which constitute the cell wall residues and exhibit stronger antioxidant

activities than free acids. Hence in the present study, the variability in the fine structures of the hydroxy-cinnamic acid bound arabinoxylans (HCA-AXs) from five Indian millet varieties namely finger (FM), proso (PM), foxtail (FOXM), kodo (KM) and barnyard millet (BM) and their antioxidant activity will be evaluated using *in vitro* model. The present study can be exploited in preparing nutraceutical health foods based on dietary fibers enriched with HCA-AXs.

#### Objectives

1. Isolation, purification and structural characterization of the HCA bound arabinoxylans from the cell walls of various Indian millets.
2. Comparative *in vitro* studies of the HCA bound arabinoxylans from various Indian millets to understand the structure-function relationship with respect to their antioxidant potential.

#### Research Progress

In our studies, we have standardized the protocol of mild alkali extraction for isolation of HCA bound arabinoxylans from five Indian millets and compositional analysis of the extracted materials was carried out using GC and GC-MS as alditol acetate derivatives. The analysis showed the presence of low branched HCA-AX structure (xylose: arabinose ration of 2.23:1.0) in kodo millet (KM) compared to other four millet HCA-AXs. The percentage of uronic acid in the millet HCA-AXs was estimated by carbazole method. The uronic acid content was found to be relatively higher (~9-10%) in HCA-AXs extracted from finger and kodo millet compared to other millet HCA-AXs (~4-5%). Further linkage analysis of the millet HCA-AXs as partially methylated alditol acetate derivatives indicated KM-HCA-AX comprised mainly relatively low-branched arabinoxylans; the xylan backbone having unsubstituted Xylp to mono substituted O-3/O-2 Xylp residues of 2.60: 1.0 whereas the ratio of unsubstituted to mono substituted Xylp residues in other four millet HCA-AXs varied in the range of



~1.50:1.0 to 1.70:1.0.

Further, the *in vitro* antioxidant potential of millet HCA-AXs were evaluated by Ferric reducing antioxidant power (FRAP) assay. FRAP is one of the most rapid and routine assay for determining antioxidant activity, the antioxidant activity is measured as increased in absorbance at 593 nm due to reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  and formation of colored ferrous-tri pyridyl triazine complex from FRAP reagent. *In vitro* antioxidant study, the KM-HCA-AX exhibited highest FRAP value ( $\mu$  mole of  $\text{Fe}^{2+}$ /gm sample) of  $347.92 \pm 6.19$  whereas FM-HCA-AX showed comparatively good FRAP value ( $125.46 \pm 1.54$ ). PM-HCA-AX showed FRAP value of  $87.63 \pm 1.98$  next to FM-HCA-AX and other millet HCA-AXs from BM and FOXM exhibited almost equal FRAP of  $74.64 \pm 1.41$  and  $71.37 \pm 1.50$  respectively. The detail of the FRAP was summarized in Figure 10.

In further, the role of millet HCA-AXs regulating oxidative stress were extended to evaluate their hepato-protective effects against tertiary butyl hydro-peroxide induced oxidative damage. HepG-2 cells were stressed with the different concentration of tertiary butyl hydro-peroxide

(100 to 1000  $\mu\text{M}$ ) and cellular viability was measured by MTT assay which revealed significant cell death of 50% at concentration of 400  $\mu\text{M}$ . The detailed studies on hepato-protective effects of millet HCA-AXs against oxidative damage of HepG-2 cells are in progress.

### Salient Achievements

1. The structural and *in vitro* studies showed considerable differences in the structure and antioxidant potential of millet HCA-AXs. These results suggested HCA-AX from kodo millet exhibited highest antioxidant potential compared to other four millet HCA-AXs,
2. The variation in individual phenolic acid content (caffeic acid, ferulic and *p*- coumaric acid) together with comparatively low branched AX structure and higher uronic acid content (~9-10%) might be responsible for highest antioxidant activity of KM-HCA-AX.
3. The detailed *in vitro* studies on the antioxidant potential of the HCA bound arabinoxylan poly and oligosaccharides using HepG-2 cell lines is in progress.

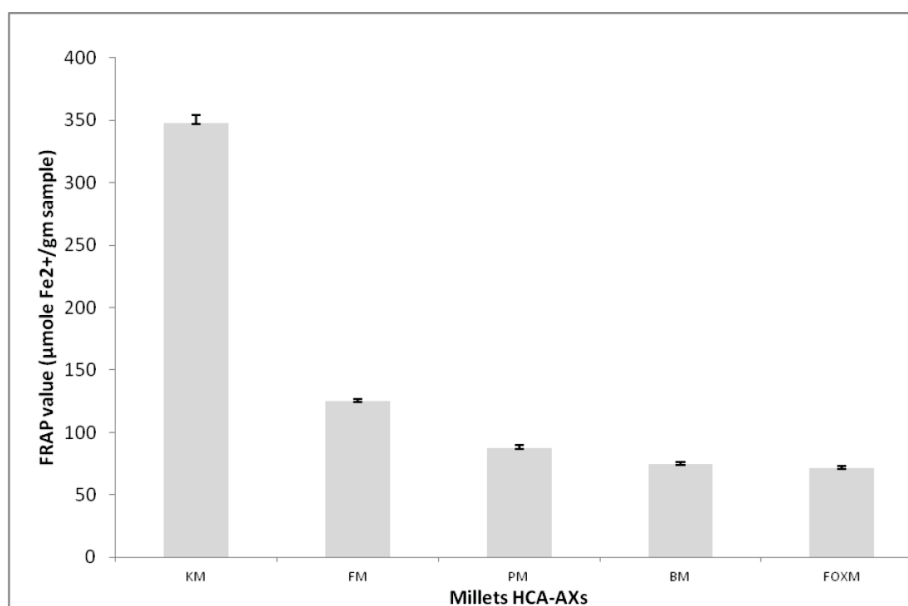


Figure 10: *In vitro* FRAP assay of millet HCA-AXs

### Future Perspectives

1. Understanding the relationship between the variability in the fine structures of the HCA-AXs and their antioxidant potential.
2. Develop functional foods and nutraceuticals with free radical scavenging and immuno-enhancing additives against various lifestyle diseases.

## 4.5 Application of dietary fibers as edible fruit coating materials

### Principal Investigator

Koushik Mazumder

### Research Fellow

Usman Ali

### Introduction

Edible films are defined as a thin layer of material which can be consumed and provides a barrier to moisture, oxygen, aroma and solute movement for the food. Water soluble and biodegradable polysaccharides provide thickening effect and can be used as alternative to the synthetic coating materials to extend the shelf life of fruits and maintaining the sensory quality and safety of fruits and other food products. Currently, only few carbohydrate based coating materials are available mainly from cellulose and chitosan, but due to their poor moisture barrier property and hydrophilic nature these coating materials pose limitations for their application as coating material. Therefore, novel strategies to structurally modify carbohydrates (polysaccharides) by derivatization to improve their physical properties such as viscosity, moisture barrier property and biological properties such as antimicrobial and antioxidant property will be adopted. Further, clinical and safety studies of these chemically modified coating materials will be carried out, so it can be used as safe, effective and health promotive coating materials for fresh fruits.

### Objectives

1. Extraction of polysaccharides from agricultural by-product/crop residues.
2. Chemical modification of polysaccharides to their corresponding derivatives using various chemical reactions.
3. Determination of physical properties of the modified carbohydrates such as moisture barrier property, film forming ability.
4. Determination of health promotive biological activity of the coating materials such as anti microbial and antioxidant property using various *in vitro* and *in vivo* model.

### Research Progress

In this study, arabinoxylan was extracted from the wheat straw by alkali extraction method and  $\beta$ -glucan was extracted from the oat by citric acid extraction method. In order to introduce moisture barrier property of the coating material,  $\beta$ -glucan was esterified to prepare different fatty acid conjugated derivatives (lauroyl, stearoyl, palmitoyl and oleoyl). FTIR analysis of various fatty acid conjugated  $\beta$ -glucan was done which showed the presence of IR stretching frequency of CO group at  $1741\text{ cm}^{-1}$  and confirmed the conjugation of fatty acid with polysaccharide.

Further, the edible films were made by preparing homogeneous mixture of esterified  $\beta$ -glucan and arabinoxylan. Water vapour rate transmission (WVRT) analysis of the mixture of palmitoyl acid conjugated  $\beta$ -Glucan (30%) and wheat arabinoxylan (45%) showed high moisture barrier property with WVRT value of  $110.8\text{ g/m}^2/\text{d}$  at  $24^\circ\text{C} / 60\% \text{ RH}$  compared to control (WVRT value of  $657.4\text{ g/m}^2/\text{d}$  for native  $\beta$ -Glucan: arabinoxylan mixture). Detailed studies on WVRT, gas transmission and mechanical properties of coating materials are in progress.

### Salient Achievements

1. Extraction protocols for arabinoxylan and  $\beta$ -glucan were standardized. Extracted  $\beta$ -glucan was conjugated with various fatty

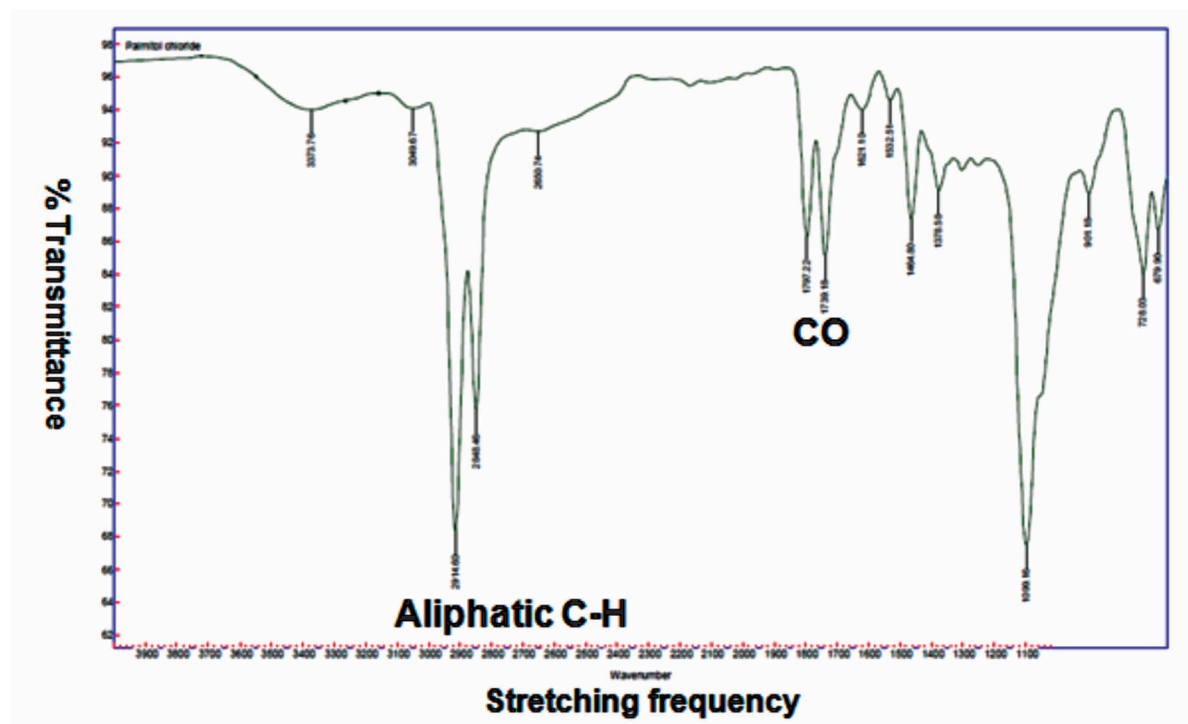


Figure 11: IR spectrum of palmitic acid conjugated  $\beta$ -glucan

acids to prepare hydrophobic derivatives.

- WVRT analysis of coating materials suggested better moisture barrier property of the mixture of palmitoyl acid conjugated  $\beta$ -Glucan (30%) and wheat arabinoxylan (45%). Detailed studies on the various physico-chemical and mechanical property of the coating materials are in progress.

#### Future Perspectives

Development and commercialization of safe, effective and health promotive coating materials for fresh fruits using biodegradable carbohydrates.

#### *4.6 Development of liposomes encapsulated novel compound to ameliorate expression of iron deficiency by suppressing expression and action of Hepcidin*

#### Principal investigator

Nitin Singhal

#### Research fellow

Stanzin Angmo

#### Introduction

Iron homeostasis is maintained through meticulous regulation of circulating hepcidin levels. Hepcidin levels that are inappropriately low or high result in iron overload or iron deficiency, respectively. The liver peptide hepcidin controls iron flux to plasma from enterocytes and macrophages through degradation of the cellular iron exporter ferroportin. The hepcidin-ferroportin axis is essential to maintaining iron homeostasis.

The present study was aimed for development of encapsulated GDP (liposomes) to study iron homeostasis through *in vitro* and *in vivo* analysis. Encapsulated GDP directly interfered with hepcidin binding to its receptor, ferroportin, by blocking ferroportin essential for hepcidin binding. Consequently, encapsulated GDP prevented hepcidin-induced ferroportin ubiquitination, endocytosis, and degradation *in vitro* and allowed continuous cellular iron export despite the presence of hepcidin. GDP is a unique antagonist of hepcidin *in vitro* that could serve as a template for the development of drug candidates that inhibit the hepcidin-ferroportin interaction. GDP seems to be interesting compound to combat anaemia but there was shortcomings of less bioavailability and toxicity with GDP, so we encapsulated the GDP in form of liposomes to treat all iron related disorder and iron deficiency. Further we aimed to study the encapsulated GDP effect of liposomes *in-vivo* for iron homeostasis.

### Objectives

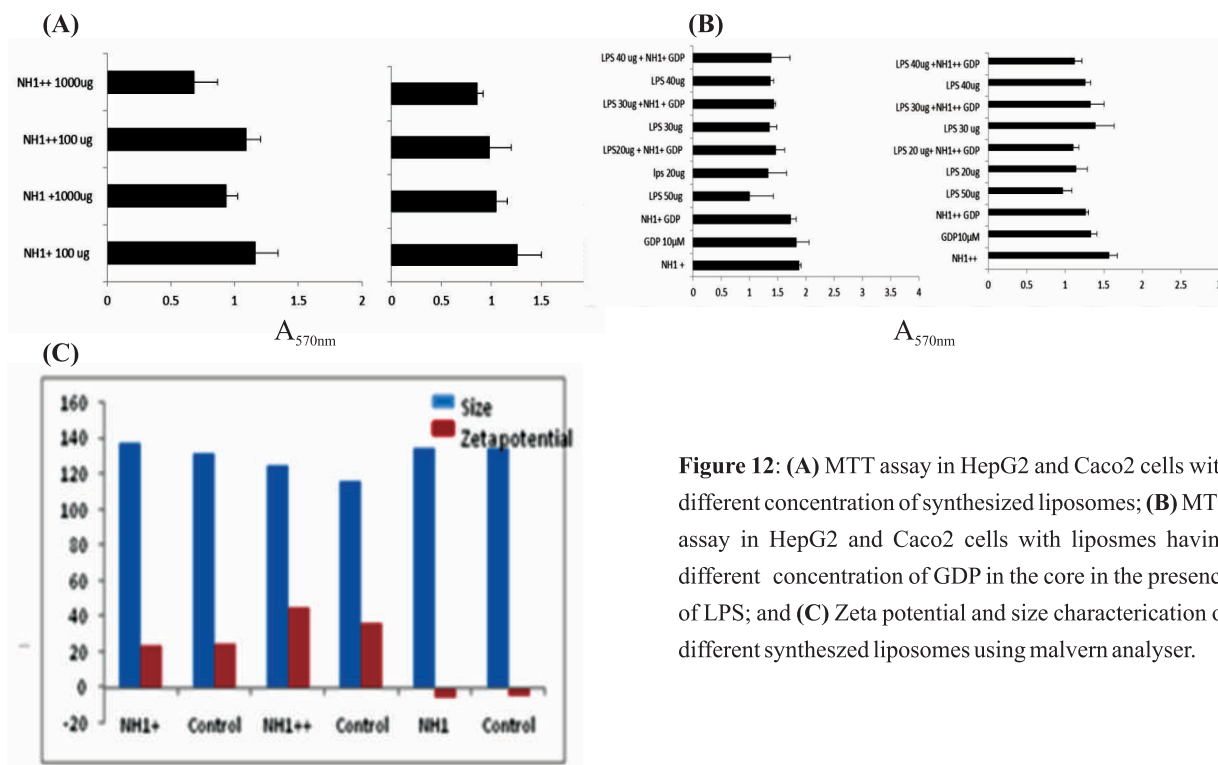
1. Development of Nanoparticle (liposomes) encapsulated GDP to increase iron bioavailability and further toxicity analysis of GDP with or without encapsulation of liposomes
2. Biological function of selected compound to increase iron uptake and development of encapsulated GDP to combat iron deficiency.
3. Investigation of encapsulated GDP effects in iron bioavailability on Caco-2 and HepG2 cell lines.
4. Chronic effects of encapsulated-GDP treatment on iron homeostasis in mice.

### Research Progress

1. We synthesize various types of liposome (Table 1), based on their charge by varying the amount of phospholipids to encapsulate GDP. Characterization studies of synthesized liposomes have been carried out using Malvern zetasizer and summarized in Figure 12.
2. MTT assay to assess the viability of two cell lines that is HepG2 and Caco2 have been done with synthesized liposome having no GDP in the core Figure 1a. Secondly, MTT assay has been done further to evaluate the effect of liposome's for same cell lines after incorporating the GDP in the presence of various concentrations of lipopolysaccharides (LPS) Figure 12B.

Table 1: Composition of various synthesized liposomes

S. No.	NAME	LIPOSOME COMPOSTION	CHARGE
1	NH1+	DOPE 60,DOTAP 30,lacPE10 (GDP10μM)	+
2	Control	DOPE 60,DOTAP 30,lacPE10	+
3	NH1++	DOPE 50,DOTAP 40,lacPE10(GDP10 μM)	++
4	Control	DOPE 50,DOTAP 40,lacPE10	++
5	NH1	DOPC45,DOPE45, Cholestrol10 (GDP10μM)	Neutral
6	Control	DOPC45,DOPE45, Cholesterol 10	Neutral



**Figure 12:** (A) MTT assay in HepG2 and Caco2 cells with different concentration of synthesized liposomes; (B) MTT assay in HepG2 and Caco2 cells with liposomes having different concentration of GDP in the core in the presence of LPS; and (C) Zeta potential and size characterization of different synthesized liposomes using malvern analyser.

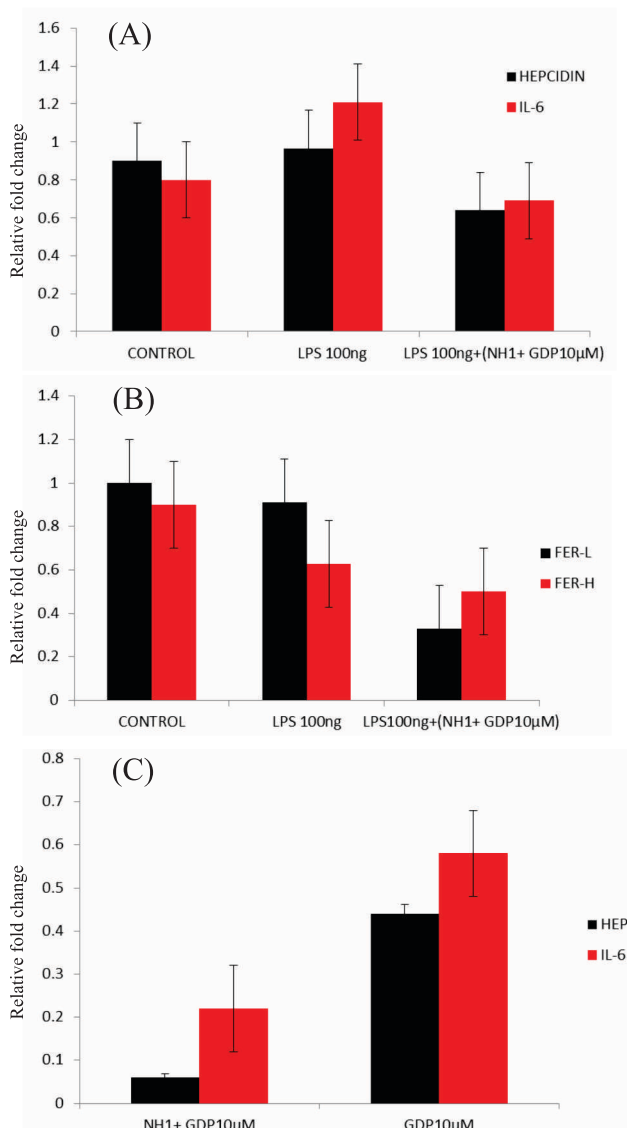
Hepcidin and IL-6 gene expression has been dramatically induced during in HepG2 cells with LPS treatment in response of acute inflammatory process. However, after treatment with liposome GDP shows less transcript gene expression level of hepcidin, proving that GDP is a robust antagonist. Ferritin expression analysis with LPS showed high transcript levels of hepcidin which lead to proteosomal degradation of iron transporting channel (ferroportin). This suggested that iron transported across the basolateral membrane of intestinal brush border gets accumulated and was not released or absorbed in plasma. Further in liposomes encapsulated GDP there is a decrease in iron accumulation in which the GDP binding to hepcidin inhibit the degradation of ferroportin and the iron was transported across the membrane. Currently, we are analysing the role of liposome having GDP to slow down the effect of inflammation to enhance bioavailability of iron to combat iron related disorders (Figure 13).

We are aiming to develop the Caco-2 model by introducing human liver cells (HepG2) to Caco-2 cells. The Caco-2 and HepG2 epithelia were separated by a liquid compartment, which allowed for epithelial interaction. We will quantify the amount of ferritin and intracellular iron content in the Caco-2 cells after liposomes treatment to assess the role of this model. The Caco-2/HepG2 model will provides an alternative approach to *in vitro* iron absorption studies in which the hepatic regulation of iron transport must be considered.

#### Salient Achievements

1. Development of encapsulated GDP in form of liposomes having better pharmacokinetics behaviour with less toxicity.
2. Biological function of encapsulated GDP interaction with hepcidin to increase iron uptake.





**Figure 13:** Gene expression analysis after adding LPS and treatment with liposomes encapsulated GDP in HepG2 cells (A) gene expression for hepcidin & IL6; (B) gene expression for FER-H & FER-L; & (C) gene expression comparison between GDP free and liposome encapsulated GDP.

#### Future Perspectives

1. Role of encapsulated GDP for prevention or treatment of iron related disease.
2. Developing a natural agonist novel compound and functional therapeutics to combat iron deficiency.
3. Encapsulated GDP in form of drug to treat iron deficiency.

### 4.7 Functionalized gold nanorods as potential biosensor to detect food borne pathogens

#### Principal Investigator

Nitin Singhal

#### Research Fellow

Parul Upadhyay

#### Introduction

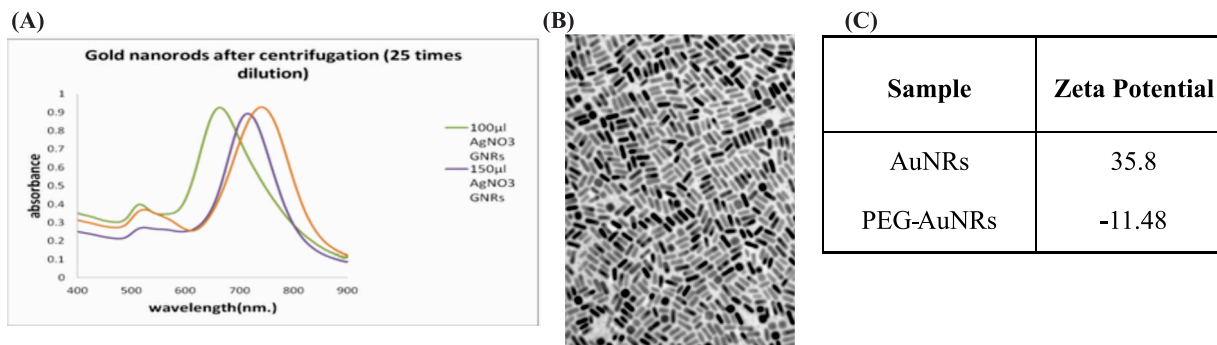
Nanotechnology has significantly enhanced sensing strategies for detecting bacteria through the use of nanoparticles, using unique physicochemical properties that are absent from their macroscale counterparts. Gold nanorods (AuNRs) are excellent candidates as biosensing materials because they are highly refractive index sensitive to trace amount of bio-molecules and exhibit LSPR properties. Among various bio-sensing technologies, both surface plasmon resonance (SPR) and localized surface plasmon resonance (LSPR) sensing technologies have

been widely explored and reported due to their attractive features such as high sensitivity, label-free detection, fast response and real-time monitoring. Carbohydrate based gold nano biosensor have advantages over the low reproducing signals and slightly less stable enzyme based detections as the stronger affinity between lectin-carbohydrate. In this study, we intend to design a reliable carbohydrate based functionalized AuNRs using aminophenyl boronic acid (APBA) and Bovine serum albumin (BSA) conjugate. This APBA-BSA conjugate behaves as lectin mimetics and therefore turned to be potential candidate for detection of pathogens containing glycans as cell wall content. It can be preferred well above the high cost antigen antibody based biosensors.

#### Objectives

1. Synthesis of AuNRs by seed mediated growth and characterization of the AuNRs according to different aspect ratios.





**Figure 14 :** (A) UV spectra showing different aspect ratios AuNRs with different volumes of silver nitrate; (B) TEM image of AuNRs (Aspect Ratio 2); and (C) DLS results showing zeta potential of AuNRs and PEG-AuNRs.

2. Fabrication and characterization of AuNRs with the poly (ethylene glycol) 2-mercaptoethyl ether acetic acid as linker.
3. Conjugation of BSA-BA with the PEG modified AuNRs and sensing of bacteria using the BSA-BA conjugated AuNRs by carbohydrate-boronic acid interactions.

### Research Progress

In our study, we have synthesized and characterized the different aspect ratio AuNRs by changing the concentration of silver nitrate. We confirmed the attachment of linker poly (ethylene glycol) 2-mercaptoethyl ether acetic acid to AuNRs by UV visible spectroscopy and zeta potential of conjugated gold nanorods. As the DLS results showed that the CTAB (Cetyl trimethylammonium bromide) capped AuNRs has positive zeta potential as 35.8 while the zeta potential turns negative i.e.-11.3 after attachment of PEG linker. The UV spectra of PEG –AuNRs showed shifts in wavelength in comparison to AuNRs peak (Figure 14).

We are currently synthesizing the BSA-aminophenylboronic acid conjugate and attaching the BSA-APBA conjugate with the help of PEG linker to AuNRs in order to build biosensor sensing bacteria by APBA-carbohydrate interaction. Alizarin Red S (ARS) dye is highly recognized to bind boronic acid with high association constant, ARS when bound to boronic acid a dramatic change in color was

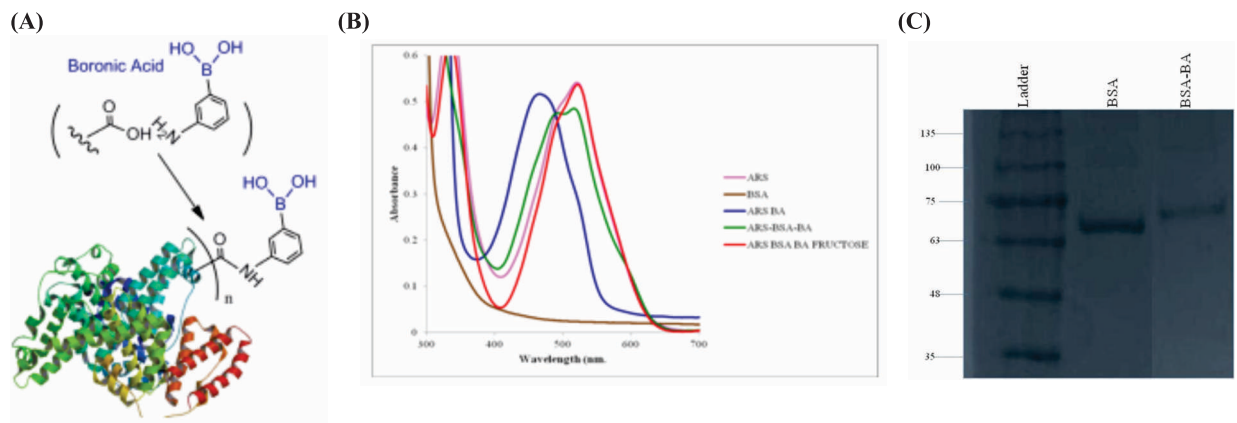
observed (Figure 15A). Thus, the ARS assay has been used extensively to quantify the boronic acid and also determine the sugar binding affinity to boronic acid ligands by ARS displacement assay. ARS when bound to BSA-BA and also shift in wavelength from around 520nm to 460 nm by UV absorption in PBS (pH 7.4) buffer and then by adding high concentration (1 M) of fructose, the fructose-boronic acid complex is formed to release ARS and the shift in wavelength back to approximately 520 nm. The BSA-BA was characterized by SDS-PAGE where the increase in molecular weight of the BA conjugated BSA was observed significantly (Figure 15C).

### Salient Achievements

1. The UV studies showed the synthesis of different aspect ratios AuNRs and the DLS results confirm the successful conjugation of the PEG linker to gold nanorods.
2. BSA-APBA conjugation with PEG-AuNRs as biosensor is currently in progress using carbamide covalent bond and will be characterized using FTIR, UV and SDS PAGE results.

### Future Perspectives

1. Using the ABPA- BSA conjugate as lectin mimetics in order to form a APBA-BSA AuNRs as sensing nose for bacterial detection.
2. Development of paper strip based cheap and quick carbohydrate gold nanobiosensor for detection of food borne pathogens.



**Figure 15:** (A) Pictorial representation of BSA-APBA conjugate; (B) UV spectra showing shifts in wavelength with ARS assay for BSA-APBA conjugate. (C) SDS Gel showing BSA 0.1mg/ml and the BSA-BA conjugate after purification on Sephadex G-50.



## **COMPUTATIONAL BIOLOGY APPROACHES FOR MARKER AND GENE DISCOVERY FOR NUTRITION AND PROCESSING TRAITS IN FOOD CROP GENOME**

## 5.1 Development of advanced algorithms, databases, tools and pipeline for data mining and comparative analysis of food crop genomes, transcriptome and small RNA based regulation.

### Principal Investigator

Shrikant Subhash Mantri

### Research Fellows

Anoop Kishor Singh Gurjar

Rajinder Gupta

### HPC Application Support Engineer

Abhijeet Singh Panwar

## I. Augmenting the pathway knowledge by adding newly identified biological entities

### Introduction

High-throughput next generation sequencing based genome and transcriptome research has led to discovery of new entities (gene/protein/non-coding RNA) from various organisms but it takes time till these get a place in a pathway(s) and network(s). Utilization of this information doesn't happen overnight; it takes time till we get acquainted with the new entity or it gets popular among the researchers. The time lag is too big to ignore in a quickly growing era of knowledge. Literature mining to find new entities is not a painless task. Orthology based extrapolation of gene functions can accelerate discovery of new networks and pathways.

### Objectives

1. Development of literature mining applications to identify new biological entities and its relationships.
2. Enriching pathway database for plants (viz. wheat, rice, maize, annona and litchi).
3. Development of framework for gene regulatory networks.

### Research progress

1. Text mining: Literature mining pipeline has been developed for searching new entities mentioned in published text viz. Pubmed. The task is accomplished using Regex(es) and comparing against Uniprot (Figure 1 ). New entities are identified that are not present in our Pathway repository.
2. Comparing web-repositories: A direct comparison against different gene / protein / non-coding RNA entity web-repositories and our local pathway repository to give a list of newly identified entities.

Furthermore the new entities so predicted from related species are checked for sequence similarity and if they qualify they are added to the database.

### Salient Achievements

1. New entities (gene/protein/non-coding RNA) reported in Wheat (*Triticum aestivum*) and Rice (*Oryza sativa*) are identified. Before comparing, duplicate sequences having 100% similarity were removed from Uniprot and GENE list and Regex(es) were made to differentiate between biological entity names and language words using in-house developed scripts.
2. Sequence similarity of newly identified proteins and IWGSC annotated genes is established.

### Future Perspectives

1. Mapping of in-house and publically available transcriptome on the enhanced pathways.
2. Development of Gene co-expression networks and Gene regulatory networks.

## II. Development of plant miRNA expression atlas database and web applications

### Introduction

Plant small RNA, 18-24 nt long, plays an important role in different developmental stages of plants and also during stress conditions (biotic

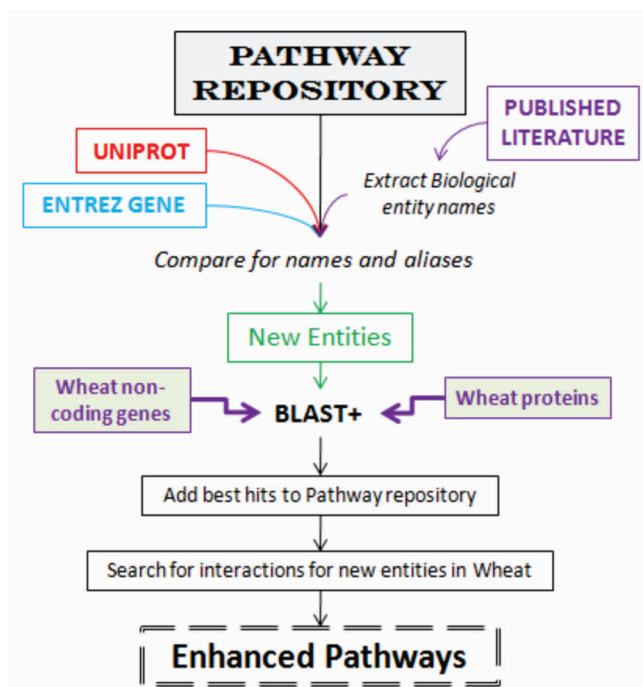


Figure 1: Analysis workflow

and abiotic). Conservation of miRNA sequences in different species implies that they play important role during the course of evolution. Identification of miRNAs in large number of diverse plant species is important to understand the evolution of miRNAs and miRNA-targeted gene regulations. Computational approaches are successful in identifying conserved miRNAs in many plants species. Understanding gene regulatory networks involving plant miRNAs are critical to design biotechnology based approaches for crop improvement.

### Objectives

1. Development of non-redundant database of mature miRNA sequences of plants.
2. Study of plant miRNA sequence and regulation conservation.
3. Differential expression analysis of plant miRNA to develop co-expression and regulatory networks.

### Research progress

1. The miRNA sequences of three majorly cultivated food crops namely wheat, rice and

maize were retrieved from miRBase release 20, PMRD and few from recent publications. Redundant miRNA sequences were removed and found 155 non redundant (NR) miRNA for wheat, 2309 NR miRNA for rice and 233 NR miRNA for maize. The NGS sequencing data of small RNA of wheat, rice and maize were collected from SRA database publicly available from NCBI website. These comprise of 21 small RNA datasets for 4 types of wheat tissues (leaf, spike, generic sample, whole plant), 53 small RNA datasets for 9 types of rice tissues (whole plant, root, shoot, leaf, panicle, anther, embryo, endosperm, seedling.) and maize 43 sRNA datasets for 11 types of tissues (whole plant, root, shoot, leaf, ear, anther, tassel, pollen, silk, 5Day old coleoptile, seedling), total 2.4 billion+ sequence reads.

2. Collected non redundant mature miRNA were used as query against respective wheat, rice and maize sRNA databases. Developed matrix for miRNA abundance following stringent criteria of 100 % identity, 0 mismatches and 100% query coverage.

Normalization was done by converting hit counts into transcript per million (TPM) counts. Heatmaps were developed after log2 transformation of TPM values (Figure 2).

3. The conserved miRNA sequences across multiple species were filtered on the basis of sequence homology. Micro RNA showing tissue preferential expression were screened by the TPM 80 fold greater than the mean TPM from other tissues along with Shannon entropy calculations using ROKU package.
4. PmiRExAt database was developed using open source Web 2.0 technologies, Java EE 6 standard, Bootstrap front end framework to support various screen sizes, Ajax to asynchronously call server, Highcharts API and MySQL at backend. PmiRExAt users can do desired data-mining in this rich processed resource. Apart from availability of intuitive web server interface, PmiRExAt also caters a SOAP web service which allows other programmers to remotely invoke the methods written for doing search operations on database.

#### Salient Achievements

1. A non redundant database of wheat 155 miRNA, rice 2309 miRNA and maize 233 miRNA developed from miRBase, PMRD, PNRD and few miRNA from recent publications and the expression abundance matrixes were synchronized with MySQL for querying.
2. On the basis of cumulative expression in all datasets, there were 31 miRNA in wheat, 57 miRNA in rice and 17 miRNA of maize which were showing very high expression.
3. We found 35 conserved miRNA sequences in wheat rice and maize. Conserved miRNA sequences belong to 24 miRNA families (miR156, miR159, miR160, miR164, miR166, miR167, miR168, miR169, miR171, miR172, miR2118, miR319, miR390, miR393, miR394, miR395, miR396, miR399, miR408, miR437, miR444, miR528, miR827, miRf10461).

4. Data-mining led to identification of 2 miRNA preferentially expressing in leaves and 2 in spikes of wheat; for rice 26 miRNA in root, 50 miRNA in leaf, 25 miRNA in anther and 38 miRNA in endosperm tissue; for maize 1 miRNA in root, 2 in shoot, 5 in leaf, 4 in anther, 3 in ear, 2 in pollen, 2 in tassel, 4 in silk and 10 in coleoptile were showing tissue preferential expression.
5. There were 62 miRNA in wheat, 27 in rice and 17 in maize that were showing constitutive miRNA expression.

#### Future Perspectives

1. Detection of novel miRNA in wheat, rice and maize. Improving annotations of miRNA in existing databases.
2. Development of miRNA co-expression networks.

### *5.2 Molecular interaction studies and predictions for the function and designing of foods*

#### Principal Investigator

Shrikant Subash Mantri

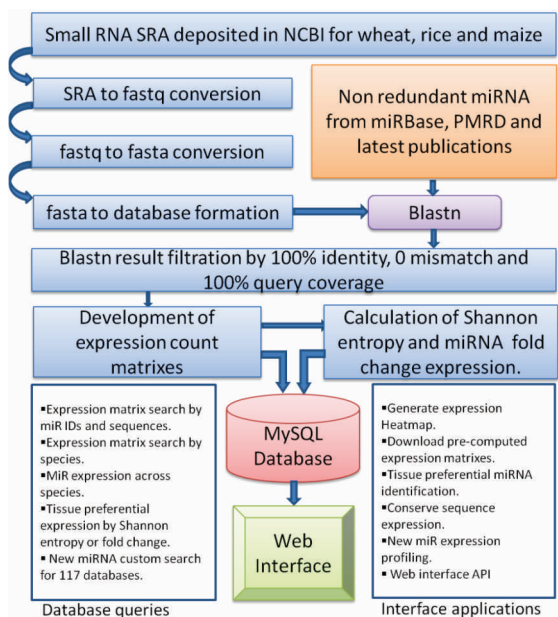
#### Co-Investigator

Shailesh Sharma

#### Introduction

Annotation is a process of embellishing raw DNA sequences with predictions of features such as genes and transcription factor binding sites. Annotations are necessary to identify the important gene functions and to enable comparative analysis. What is presently available are the web servers which allows public submission of genomes for high quality automated annotation. However, there have been fewer options available for the open source tools developed that allow users to assemble sequences and to run annotation on their own at their genomic labs. DIYOWAA is a "do it in your own way assembler and annotator" arose out of the





**Figure 2:** Stepwise data processing, expression abundance database formation and integration of database with web interface for searching and browsing pre-computed data.

desire of our group to be able to assemble and annotate genomes of interest on our own servers as soon as possible after generating raw sequence data.

### Objective

To develop a highthroughput assembly and annotation pipeline specially for *Triticum aestivum*.

### Research Progress

We are developing DIIYOWAA which is written in a high-level Python programming language and uses BLASTx/n program, CAP3: third generation DNA sequence assembly program, 22 different biological databases, of which some are in house developed and some are publicly available, 1 text search for biological pathways in a file having Uniprot ids and biological pathways and CNFpred1.66: A single-template protein threading package using context-specific information and Conditional Neural Fields. Installation and configuration of DIIYOWAA pipeline require some basic knowledge of Linux /Unix and Python and it can be executed at the command line. . Many genomes can be annotated

simultaneously by running in batch mode using the cluster.

DIIYOWAA is composed of steps that are executed in the specified order and each step is a Bioinformatic application which will analyze sequences and produce output. Complete algorithm is of DIIYOWAA is shown in Figure 3

### Salient Achievements

1. With DIIYOWAA we annotated 40, 2619 and 212 hypothetical gene models of *Triticum aestivum*, *Oryza sativa* and of *Arabidopsis thaliana* respectively.
2. Additionally DIIYOWAA is providing secondary and tertiary structure of first BLASTx hit protein sequences against Protein non reductant database.
3. Domain content in the protein sequence is included in the annotation information.

### Future Perspectives

1. DIIYOWAA will be launched for the research community through NABI website.
2. We will use DIYOWAA to study and annotate genomes of other food crops in high-throughput way.

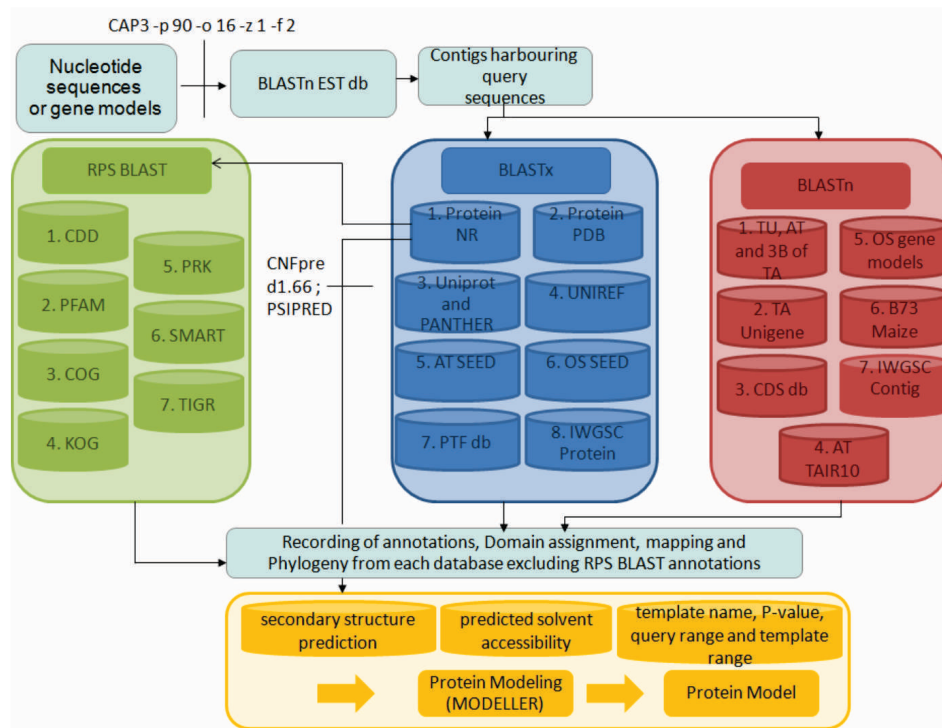


Figure 3: Pictorial representation of DIYOWAA algorithm



## EXISTING MOU FOR COLLABORATIONS & NETWORKING

1. NABI and Central University of Punjab, Bathinda signed a MOU on March 28th, 2013 for the promotion of quality research and high end research programmes between two institutes.
2. A MOU was signed with National Institute of Pharmaceutical Education and Research (Mohali), Indian Institute of Scientific Education and Research (Mohali), Post Graduate Institute of Medical and Education Research (Chandigarh), Panjab University (Chandigarh), Central Scientific Instruments Organization (Chandigarh), Indian Institute of Technology (Ropar) and Punjab Agriculture University (Ludhiana) on November 26th, 2012 to establish a Bioscience Cluster at Mohali.
3. NABI and Punjab Technical University, Jalandhar signed a MOU on October 19th, 2012 to promote academic and research interactions in the areas of science & technology to intensify the high priority programmes.
4. NABI and National Research Centre for Litchi (NRCL), Muzaffarpur, Bihar signed a MOU on September 16th, 2012 to share R&D facilities and carry out joint research projects.
5. NABI and Punjab Agricultural University, Ludhiana signed a MOU on August 14th, 2012 to jointly carry out research in the areas of agriculture and allied sciences.
6. NABI and NIPER signed a MOU on February 2<sup>nd</sup>, 2012 to undertake joint research work in the area of mutual interest besides imparting training to staff, students and technical personnel within the area of cooperation.
7. The following MOUs were signed with two Universities in neighborhood to catalyse networking, R & D collaborations, human resource development and award of degree to students who pursue Ph.D research at NABI.
  - (i) MOU with Punjab University, Chandigarh on May 27th, 2011.
  - (ii) MOU with Guru Jambheshwar University of Science & Technology, Hissar on March 29th, 2011.
8. The following three MOUs were signed with Canadian institutes, for co-operation in S&T on November 24th, 2010.
  - (i) MOU with National Research Council, Plant Biotechnology Institute, Saskatoon.
  - (ii) MOU with University of Saskatchewan, Saskatoon.
  - (iii) MOU with Genome Prairie, Saskatoon.

## EXTRAMURAL GRANTS AND FUNDINGS

S.No.	Project Investigator	Title of the Project	Funding Agency
1.	Dr. Sudhir P Singh	A novel strategy for developing scion plants of desired phenotype by using an RNAi delivering rootstock.	SERB, DST, Govt. of India
2.	Dr. Siddharth Tiwari	Transfer and evaluation of Indian banana with Pro - Vitamin A (PVA) constructs. This project is a part of the multi-institutional core project entitled development and transfer of technology from Queensland University of Technology (QUT), Australia to India for biofortification and disease resistance in banana.	Biotechnology Industry Research Assistance Council (BIRAC), Department of Biotechnology, Govt of India
3.	Dr. Ajay K Pandey	Metabolic engineering of phtytic acid pathway to enhance iron bioavailability in wheat.	Department of Biotechnology, Govt. of India
4.	Dr. Kanthi Kiran	Effects of finger millet and kodo millet arabinoxylan on adipogenesis and associated inflammatory markers- a nutrigenomic study	Department of Biotechnology, Govt. of India
5.	Dr. Kanthi Kiran	A nutrigenomic study to assess the role of polyphenols from <i>Eleusine coracana</i> (finger millet) and <i>Paspalum scrobiculatum</i> (kodo millet) on the regulation of adipogenesis.	SERB, DST, Govt. of India
6.	Dr. Mahendra Bishnoi	Studies of transient receptor potential (TRP) channel mediated modulation of adipogenesis and obesity by dietary molecules.	SERB, DST, Govt. of India
7	Dr. Mahendra Bishnoi - PI Dr. Kanthi Kiran - Co-PI	Nutrigenomic approach to understand the role of TRP channel activating food components in adipose tissue inflammation.	Department of Biotechnology, Govt. of India
8	Dr. Koushik Mazumder	Variability in the fine structures of feruloyl arabinoxylans from Indian millet varieties and their consequence on anti-oxidant activity.	SERB, DST, Govt. of India
9	Dr. Sukhvinder P Singh	Metabolomics approach to discovery and validation of biomarkers for artificial fruit ripening induced through prohibited and acceptable ripening elicitors.	SERB, DST, Govt. of India
10	Dr. Monika Garg	Identification of celiac disease epitopes in Indian wheat cultivars and their modulation by RNAi and breeding approaches.	Department of Biotechnology, Govt. of India
11	Dr. Monika Garg	Chromosome specific wide hybridization for improvement of bread making quality of wheat.	SERB, DST, Govt. of India
12	Dr. Siddharth Tiwari	Identification, cloning and functional characterization of myo-inositol oxygenase (MIOX) from wheat.	SERB, DST, Govt. of India
13	Dr. Hariom Yadav	Development of Novel components for treatment and type 2 diabetes.	SERB, DST, Govt. of India



## PROGRESS OF INFRASTRUCTURE AT MAIN CAMPUS



- First row from left : Residential Apartments and Students hostel.
- Second row from left : Construction status of lab building.
- Third row from left : Director's Bungalow and Green house building



## PARTICIPATION IN NATIONAL/INTERNATIONAL CONFERENCES/WORKSHOPS:

1. Sh. Dharendra Pratap Singh attended the International Brain Research Organization (IBRO) conference on "Advanced School on Neuroscience" held at Chinese University of Hong Kong, Hong Kong, on June 8 - 21, 2014.
2. Sh. Dharendra Pratap Singh presented a poster in "30th Hong Kong Society of Neuroscience Meeting in conjunction with the Annual Scientific Meeting of the Biophysical Society of Hong Kong, held at The University of Hong Kong on June 16, 2014.
3. Dr. Joy K. Roy was invited to deliver a lecture on "Genome wide selection and genotyping by sequencing for crop improvement" in DBT sponsored national symposium held on July 7th, 2014 at Eternal University, Baru Saheb, HP.
4. Dr. Ajay K. Pandey delivered a talk on "Identification, expression and functional analysis for late phase of phytic acid biosynthesis genes from Wheat" at the National Conference on Biotechnology for Sustainable Agriculture (NCBSA 2014) held at Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, MP during September 8-9, 2014.
5. Dr. Mahendra Bishnoi attended and chaired a poster session at International Brain Research Organization (IBRO) school organized at University Institute of Pharmaceutical Sciences (UIPS), Panjab University, Chandigarh during October 19<sup>th</sup> - 24<sup>th</sup>, 2014.
6. Sh. Ritesh Kumar attended 1st IBRO/APRC Chandigarh Neuroscience School held at University Institute of Pharmaceutical Sciences on November 2nd-8th, 2014.
7. Dr. Monika Garg visited Norwich, UK to attend "Total food 2014" conference and explore opportunity of research partnership with UK institutes working on agri-food sector, co-sponsored by DBT and British high commission during November 11<sup>th</sup> -13<sup>th</sup>, 2014.
8. Dr. Sudhir Pratap was invited to deliver a talk on "Distribution in maternal and filial grain tissues of wheat and related genotypes, and molecular studies to enhance mineral bioavailability at a national symposium organized at Sam Higginbottom Institute of Agriculture Technology and Sciences, Deemed University, Allahabad during December 2<sup>nd</sup>, 2014.
9. Sh. Dharendra Pratap Singh attended the Flowcytometry Workshop held at Department of Biochemistry, Punjab University, Chandigarh on December 5th-6th, 2014
10. Dr. Mahendra Bishnoi attended and delivered a lecture at pre-conference workshop at 47th Annual Conference of the Indian Pharmacological Society (IPSCON 2014), Guwahati, Assam during December 27<sup>th</sup> -30<sup>th</sup>, 2014.
11. Dr. Koushik Mazumder, Dr. Monika Garg, Dr. Kanthi Kiran & Sh. Shrikant Mantri participated in "29th Carbohydrate Conference" organized by Center of Innovative and Applied Bio-processing (CIAB), Mohali from December 29<sup>th</sup> -31<sup>th</sup>, 2014.
12. Sh. Rohit Kumar (DBT-SRF) attended and presented his poster titled, "Trimodal

- distribution of starch granules, their structure and properties in soft and hard wheat lines", in CARBO-XXIX organized by Center of Innovative and Applied Bio-processing (CIAB), Mohali from December 29<sup>th</sup> -31<sup>th</sup>, 2014
13. Dr. Mahendra Bishnoi attended and deliver an invited lecture entitled "Linking diet and obesity: Public health perspective" at 9th Chandigarh Science Congress (CHASCON) – 2015, held at Panjab University, Chandigarh during February 25th-27th, 2015.
  14. Sh. Shrikant Mantri attended the workshop on "High Performance Computing" and delivered a talk on "Peta-scale Bioinformatics: Understanding scientific computing challenge in plant science and developing capability to solve it" organized by Department of Physics, Panjab university during 16th-17th March, 2015.
  15. Dr. Kanthi Kiran was invited as a panel judge for Research scholars conventions 2K15: Challenges Ahead for Microbiologists and Bio-technologists, organized by the Department of Microbiology, Panjab University, on 25th March 2015 in Association with Microbiologists of India, Chandigarh Unit.
  16. Dr. Mahendra Bishnoi attended and delivered an invited lecture at International Conference on Molecular Pharmacology, Drug Discovery and Nano-pharmaceuticals (MPDDNP-2015) held at Chitkara College of Pharmacy, Chitkara University, Rajpura, Patiala, Punjab during March 27th – 28th, 2015).

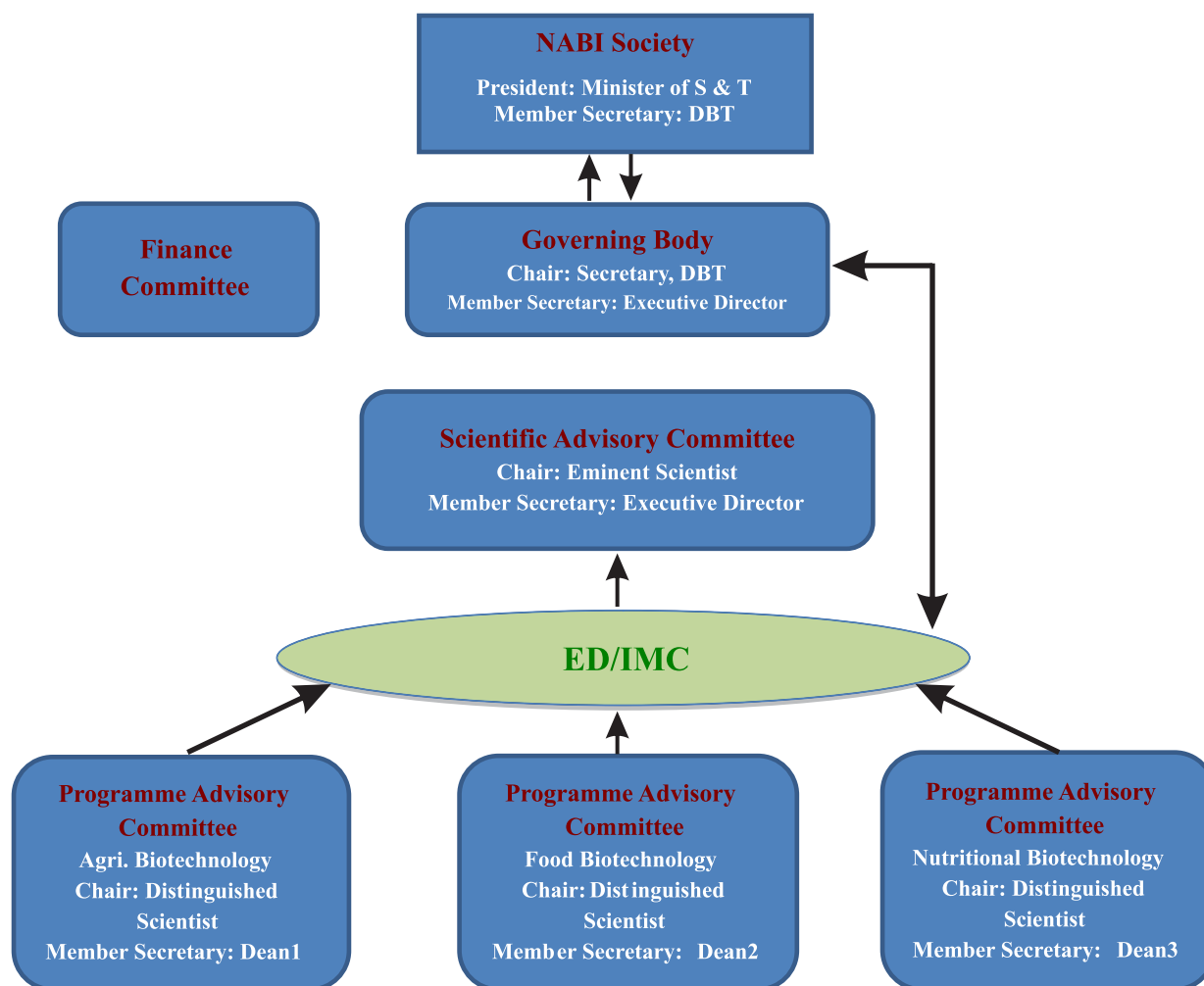
#### International visitors to NABI

1. The representative from European delegation comprising of Dr. Tim Foster, Professor in Food Structure, University of Nottingham; Dr. Martin Howarth, Director, National Center of Excellence for Food Engineering; Prof. Savvas Tassou, Director, Institute of Energy Futures; Dr. Alan Rowe, Food and Drink Industry Specialist, Rowett Institute of Nutrition and Health; Mr. Tim Ingmire, R&D Director, Food Innovation Pepsico; Mr. Neil Wilson, Knowledge Transfer Manager, Knowledge Transfer Network and Ms. Swati Saxena, Senior Science & Innovation Asvisor, British High Commission visited NABI on December 09th, 2014 for discussion on Food Processing Technologies.
2. An European delegation comprising of Dr. PR Shewry, Dr. M Hawkesford, Dr. S Griffiths and Dr. AM Allen visited NABI during on March 16<sup>th</sup>, 2015 to explore the opportunities of future collaborations.





## GOVERNANCE



## MANAGEMENT OF THE INSTITUTE

### A. Members of NABI Society

**Dr. Harsh Vardhan**

Hon'ble Minister of Science & Technology,  
Ministry of Science & Technology,  
Govt. of India  
New Delhi  
(**President**)  
(9<sup>th</sup> November '14 to till date)

**Sh. Jitendra Singh**

Hon'ble Minister of Science & Technology,  
Ministry of Science & Technology,  
Govt. of India  
New Delhi  
(**President**)  
(26<sup>th</sup> May '14 to 9 November '14)

**Sh. Sudini Jaipal Reddy**

Hon'ble Minister of Science & Technology,  
Ministry of Science & Technology,  
Govt. of India  
New Delhi  
(**President**)  
(28<sup>th</sup> Oct '12 to 26 May '14)

**Dr. K. VijayRaghavan**

Secretary,  
Department of Biotechnology,  
Ministry of Science & Technology,  
New Delhi  
(**Chairman**)

**Sh. J.B Mohapatra**

Financial Advisor,  
Department of Biotechnology,  
Ministry of Science & Technology,  
New Delhi

**Dr. N. Sathyamurthy**

Director,  
Indian Institute of Science & Education Research,  
Mohali – 160065

**Dr. G. Venkateshwara Rao**

Former Director,  
Central Food Technological  
Research Institute (CFTRI ),  
Mysore – 570026  
(From 1<sup>st</sup> July '14)

**Dr. H.S. Gupta**

Director,  
Indian Agricultural Research Institute (IARI),  
Pusa Campus,  
New Delhi -110012  
(From 1<sup>st</sup> July '14)

**Dr. R.S. Paroda**

(Former Director General – ICAR)  
Trust for Advancement of Agricultural Sciences,  
New Delhi - 110012

**Dr. R.S.Sangwan**

Chief Executive Officer,  
Center of Innovative & Applied Bioprocessing  
C- 127, Ind Area, Phase-VIII,  
Mohali – 160071  
(From 1<sup>st</sup> July '14)

**Dr. Umesh Kapil**

Professor,  
All India Institute of Medical Science (AIIMS),  
Ansari Nagar East, Gautam Nagar,  
New Delhi-110029  
(From 1<sup>st</sup> July '14)

**Dr. Harsh Vardhan Batra**

Director,  
Defense Food Research Laboratory,  
Buddha Marg, Bannur Rd, Siddarth Nagar,  
Chamundi Vihar Layout, Mysore  
Karnataka-570011  
(From 1<sup>st</sup> July '14)

**Dr. S. Nagarajan**

Former Chairperson,  
Protection of Plant Varieties and Farmers'  
Rights Authority,  
New Delhi  
(Upto 30<sup>th</sup> June '14)

**Dr. V. Prakash**

Former Director, CFTRI  
Distinguished Scientist,  
Council of Scientific and Industrial Research,  
Mysore  
(Upto 30<sup>th</sup> June '14)



**Dr. Rajesh Kapur**

Advisor,  
Department of Biotechnology,  
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New Delhi

**Dr. Vikas Rishi**

Scientist-E,  
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**Dr. Joy K. Roy**

Scientist- D,  
National Agri-Food Biotechnology Institute,  
C-127, Ind Area, Phase-VIII,  
Mohali- 160071

**Dr. S.P Singh**

Scientist-C,  
National Agri Food Biotechnology Institute (NABI),  
C- 127, Ind Area, Phase-VIII,  
Mohali - 160071

**Dr. B. Sesikera**

Former Director,  
National Institute of Nutrition,  
Hyderabad  
(Upto 30<sup>th</sup> June '14)

**Prof. Akhilesh Kumar Tyagi**

Executive Director,  
National Agri-Food Biotechnology Institute,  
C-127, Ind Area, Phase-VIII  
Mohali- 160071  
(*Member Secretary*)

## B. Governing Body

### **Dr. K. Vijay Raghavan**

Secretary,  
Department of Biotechnology,  
Ministry of Science & Technology,  
New Delhi  
(*Chairman*)

### **Sh. J.B Mohapatra**

Financial Advisor,  
Department of Biotechnology,  
Ministry of Science & Technology,  
New Delhi

### **Dr. Manju Sharma**

(Former Secretary, DBT)  
President & Executive Director,  
Indian Institute of Advanced Research,  
Gujarat  
(*Upto 30<sup>th</sup> June '14*)

### **Dr. C.R. Bhatia**

Former Secretary,  
Department of Biotechnology,  
New Delhi  
(*Upto 30<sup>th</sup> June '14*)

### **Dr. Ashok D. B. Vaidya**

Research Director,  
Kasturba Health Society Medical &  
Research Centre,  
Mumbai  
(*Upto 30<sup>th</sup> June '14*)

### **Dr. B. Siva Kumar**

Former Director  
National Institute of Nutrition,  
Secunderabad  
(*Upto 30<sup>th</sup> June '14*)

### **Ms. Seema Jain**

Secretary,  
Deptt. Science & Technology and Environment,  
6th Floor, Room No. 616,  
Mini Secretariat, Sector -9, Chandigarh.  
(*From 1<sup>st</sup> July '14*)

### **Dr. G. Venkateshwara Rao**

Former Director,  
Central Food Technological Research Institute  
(CFTRI),  
Mysore – 570026  
(*From 1<sup>st</sup> July '14*)

### **Dr. H.S. Gupta**

Director,  
Indian Agricultural Research Institute (IARI),  
Pusa Campus,  
New Delhi -110012  
(*From 1<sup>st</sup> July '14*)

### **Dr. R.S. Paroda**

(Former Director General – ICAR)  
Trust for Advancement of Agricultural Sciences,  
New Delhi - 110012

### **Dr. R.S. Sangwan**

Chief Executive Officer,  
Center of Innovative & Applied Bioprocessing  
C- 127, Ind Area, Phase-VIII,  
Mohali – 160071  
(*From 1<sup>st</sup> July '14*)

### **Dr. Umesh Kapil**

Professor,  
All India Institute of Medical Science (AIIMS),  
Ansari Nagar East, Gautam Nagar,  
New Delhi-110029  
(*From 1<sup>st</sup> July '14*)

**Dr. S. Nagarajan**

Former Chairperson,  
Protection of Plant Varieties and Farmers'  
Rights Authority,  
New Delhi

*(Upto 30<sup>th</sup> June '14)*

**Dr. N.K. Ganguly**

(Formerly Director General- ICMR),  
Distinguished Professor of Biotechnology,  
Translational Health Science &  
Technology Institute,  
New Delhi

*(Upto 30<sup>th</sup> June '14)*

**Dr. J.S. Pai**

(Former Director- UICT)  
Executive Director,  
Protein Foods & Nutrition Development  
Association of India,  
Mumbai

*(Upto 30<sup>th</sup> June '14)*

**Dr. V. Prakash**

Former Director, CFTRI  
Distinguished Scientist,  
Council of Scientific and Industrial Research,  
Mysore

*(Upto 30<sup>th</sup> June '14)*

**Dr. B. Sesikeran**

Former Director,  
National Institute of Nutrition,  
Hyderabad

**Dr. N. Sathyamurthy**

Director,  
Indian Institute of Science &  
Education Research,  
Mohali -160065

**Dr. Harsh Vardhan Batra**

Director,  
Defense Food Research Laboratory,  
Buddha Marg, Bannur Rd, Siddarth Nagar,  
Chamundi Vihar Layout, Mysore

Karnataka-570011

*(From 1<sup>st</sup> July '14)*

**Dr. Rajesh Kapur**

Advisor,  
Department of Biotechnology,  
Ministry of Science & Technology,  
New Delhi

**Dr. Vikas Rishi**

Scientist-E,  
National Agri Food Biotechnology Institute (NABI),  
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**Dr. Joy K. Roy**

Scientist- D,  
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**Dr. S.P Singh**

Scientist-C,  
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Mohali - 160071

**Prof. Akhilesh Kumar Tyagi**

Executive Director,  
National Agri-Food Biotechnology Institute,  
C-127, Ind Area, Phase-VIII  
Mohali- 160071

*(Member Secretary)*

## C. Finance Committee

**Dr. K. VijayRaghavan**

Secretary,  
Department of Biotechnology,  
Ministry of Science & Technology,  
New Delhi  
(Chairman)

**Sh. J.B Mohapatra**

Financial Advisor,  
Department of Biotechnology,  
Ministry of Science & Technology,  
New Delhi

**Prof. Akhilesh Kumar Tyagi**

Executive Director,  
National Agri-Food Biotechnology Institute,  
Mohali

**Dr. Rajesh Kapur**

Advisor,  
Department of Biotechnology,  
Ministry of Science & Technology,  
New Delhi

**Dr. R.S Sangwan**

Chief Executive Officer  
Centre for Innovative and Applied Bioprocessing  
Mohali

**Dr. Vikas Rishi**

Scientist- E,  
National Agri-Food Biotechnology Institute,  
Mohali

**Dr. Joy K. Roy**

Scientist- D,  
National Agri-Food Biotechnology Institute,  
Mohali

**Sh. Shrikant Subhash Mantri**

Scientist- C,  
National Agri-Food Biotechnology Institute,  
Mohali

**Sh. Suneet Verma**

Finance Officer,  
National Agri-Food Biotechnology Institute,  
Mohali  
(Non-Member Secretary)

## D. Scientific Advisory Committee (SAC)

**Dr. R.S. Paroda**

(Former Director General – ICAR)  
Trust for Advancement of Agricultural Sciences,  
New Delhi  
(Chairman)

**D. C.R. Bhatia**

Former secretary,  
Department of Biotechnology,  
New Delhi

**Dr. Deepak Pental**

Former Vice Chancellor,  
University of Delhi,  
New Delhi

**Dr. B. Siva Kumar**

Former Director,  
National Institute of Nutrition,  
Secunderabad

**Dr. V. Prakash**

Former Director, CFTRI  
Distinguished Scientist,  
Council of Scientific and Industrial Research,  
Mysore

**Dr. Imran Siddiqi**

Scientist,  
Centre for Cellular & Molecular Biology,  
Hyderabad

**Dr. Akshay Kumar Pradhan**

Professor,  
Department of Genetics,  
University of Delhi,  
New Delhi

**Dr. Anura V. Kurpad**

Dean,  
St. John's Medical College,  
Bangaluru

**Dr. H.P.S. Sachdev**

Senior Consultant (Paediatrics),  
Sitaram Bhartia Institute of Science &  
Research,  
New Delhi

**Dr. G. Venkateshwara Rao**

Former Director,  
Central Food Technological Research  
Institute,  
Mysore

**Dr. Arun Sharma**

Outstanding Scientist (Food  
Technology),  
Bhabha Atomic Research Centre,  
Mumbai

**Dr. Rajesh Kapur**

Advisor,  
Department of Biotechnology,  
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New Delhi

**Prof. Akhilesh Kumar Tyagi**

Executive Director,  
National Agri-Food Biotechnology  
Institute,  
Mohali



## E. Programme Advisory Committee (PAC) : Agri-Biotechnology

**Dr. C.R. Bhatia**

Former secretary,  
Department of Biotechnology,  
New Delhi  
(Chairman)

**Dr. Kailash Chander Bansal**

Director,  
National Bureau of Plant Genetics Resources,  
New Delhi

**Dr. G.K. Garg**

Former Director, ITR  
Krishidhan Research Foundation Pvt. Ltd,  
Aurangabad Road,  
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**Dr. Sunil K. Mukherjee**

Scientist,  
International Centre for Genetic Engineering &  
Biotechnology,  
New Delhi

**Dr. Kiran K. Sharma**

Principal Scientist (Cell Biology).  
International Crops Research Institute for the  
Semi-Arid Tropics,  
Hyderabad

**Dr. T. Mohapatra**

Director,  
Central Rice Research Institute,  
Cuttack

**Dr. Ramesh Sonti**

Deputy Director,  
Centre for Cellular & Molecular Biology,  
Hyderabad

**Dr. Ashok K. Singh**

Sr. Scientist & Programme Leader (Rice),  
Division of Genetics,  
Indian Agricultural Research Institute,  
New Delhi

**Dr. Rajesh Kapur**

Advisor,  
Department of Biotechnology,  
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New Delhi

**Prof. Akhilesh Kumar Tyagi**

Executive Director,  
National Agri-Food Biotechnology Institute,  
Mohali

## F. Programme Advisory Committee (PAC): Food and Nutrition Biotechnology

**Dr. V. Prakash**

Former Director, CFTRI  
Distinguished Scientist,  
Centre Food Technological Research Institute,  
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Former Director,  
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*(Chairman– Nutrition Biotechnology)*

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National Dairy Research Institute,  
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Deputy Director,  
National Institute of Nutrition,  
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**Dr. S.K. Roy**

Emeritus Professor & Consultant FAO  
Indian Agricultural Research Institute,  
New Delhi

**Dr. H.P.S. Sachdev**

Senior Consultant (Paediatrics),  
Sitaram Bhartia Institute of Science & Research,  
New Delhi

**Dr. H.N. Mishra**

Professor,  
Agriculture & Food Engineering Department,  
Indian Institute of Technology,  
Kharagpur

**Dr. Bhupendar Khatkar**

Chairman,  
Department of Food Technology,  
Guru Janbheshwar University of Science &  
Technology,  
Hisar

**Dr. M.C. Varadraj**

Chief Scientist,  
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**Dr. Rajesh Kapur**

Advisor,  
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Ministry of Science & Technology,  
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**Prof. Akhilesh Kumar Tyagi**

Executive Director,  
National Agri-Food Biotechnology Institute,  
Mohali

## G. Building Committee

**Dr. V.S. Chauhan**

Director,  
International Centre for Genetic Engineering and  
Biotechnology,  
New Delhi  
(Chairman)

**Prof. Akhilesh Kumar Tyagi**

Executive Director,  
National Agri-Food Biotechnology Institute,  
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**Dr. R.S. Sangwan**

Chief Executive Officer,  
Center of Innovative & Applied Bioprocessing  
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Mohali - 160071

**Dr. R.S. Khandpur**

Director General,  
Pushpa Gujral Science City  
Chandigarh

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**Er. N.K. Verma**

Chief Engineer,  
Council of Scientific and Industrial Research,  
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Financial Advisor,  
Department of Biotechnology,  
Ministry of Science & Technology,  
New Delhi

**Sh. Sreeshan Raghavan**

Joint Secretary,  
Department of Biotechnology,  
New Delhi

**Dr. Jagdeep Singh**

Additional Director,  
Department of Higher Education,  
Chandigarh

**Dr. K.K. Kaul**

Former Chief Town Planner,  
Greater Mohali Area Development Authority,  
Chandigarh

**Dr. A. Vamsi Krishna**

Scientist - C,  
Department of Biotechnology,  
New Delhi

**Sh. Hardip Singh**

Administrative Officer,  
National Agri-Food Biotechnology Institute,  
Mohali  
(Member Secretary)



## RESEARCH PUBLICATIONS

### 2015

1. Singh S.P., Singh S.P., Pandey T, Singh R.R., and Sawant S.V. (2015). A novel male sterility-fertility restoration system in plants for hybrid seed production. *Scientific Reports* 5, 11274
2. Upadhyay S.K., Sharma S, Singh H, Dixit S, Kumar J, Verma P.C., and Chandrashekar K. (2015). Whitefly Genome Expression Reveals HostSymbiont Interaction in Amino Acid Biosynthesis. *Chandrashekar PLOS ONE* | DOI:10.1371/journal.pone.0126751
3. Kumari A., Kumar J., Kumar A., Chaudhari A, Singh S.P. (2015). Grafting triggers differential responses between rootstock and scion. *PLoS ONE* 10(4): e0124438
4. Gupta Y., Pathak A.K, Singh K., Mantri S.S., Singh S.P., and Tuli R. (2015). De novo assembly and characterization of transcriptomes of early-stage fruit from two genotypes of *Annona squamosa* L. with contrast in seed number. *BMC Genomics* 16, 86
5. Kumar J., Kumar J., Singh S., Shukla V., Singh S.P, and Tuli R. (2015). Prevalence of Wheat dwarf India virus in wheat in India. *Current Science* 108, 260-265
6. Singh S.P., Srivastava R., and Kumar J. (2015). Male sterility systems in wheat and opportunities for hybrid wheat development. *Acta Physiologiae Plantarum* 37, 1713
7. Kumar R., Arora S., Singh K., and Garg M. (2015). Puroindoline allelic diversity in Indian wheat germplasm and identification of new allelic variants. *Breeding science*. (Accepted).

### 2014

1. Baboota R.K., Murtaza N., Jagtap S., Singh D.P., Karmase A., Kaur J., Bhutani K.K.,

Boparai R.K., Premkumar L.S., Kondepudi K.K., et al. (2014). Capsaicin-induced transcriptional changes in hypothalamus and alterations in gut microbial count in high fat diet fed mice. *The Journal of nutritional biochemistry* 25, 893-902.

2. Baboota R.K., Singh D.P., Sarma S.M., Kaur J., Sandhir R., Boparai R.K., Kondepudi K.K., and Bishnoi M. (2014). Capsaicin induces "brite" phenotype in differentiating 3T3-L1 preadipocytes. *PLOS ONE* 9, e103093.
3. Bhati K.K., Aggarwal S., Sharma S., Mantri S., Singh S.P., Bhalla S., Kaur J., Tiwari S., Roy J.K., Tuli R., and Pandey A.K. (2014). Differential expression of structural genes for the late phase of phytic acid biosynthesis in developing seeds of wheat (*Triticum aestivum* L.). *Plant science : an international journal of experimental plant biology* 224, 74-85.
4. Kumar J., Gunapati S., Kumar J., Kumari A., Kumar A., Tuli R., and Singh S.P. (2014). Virus-induced gene silencing using a modified betasatellite: a potential candidate for functional genomics of crops. *Archives of virology* 159, 2109-2113.
5. Kumar J., Kumar J., Singh S.P., and Tuli R. (2014). Association of satellites with a mastrevirus in natural infection: complexity of Wheat dwarf India virus disease. *Journal of virology* 88, 7093-7104.
6. Kumar J., Kumar J., Singh S.P., and Tuli R. (2014). betaC1 is a pathogenicity determinant: not only for begomoviruses but also for a mastrevirus. *Archives of virology* 159, 3071-3076.
7. Murtaza N., Baboota R.K., Jagtap S., Singh D.P., Khare P., Sarma S.M., Podili K., Alagesan S., Chandra T.S., and Bhutani K.K., et al. (2014). Finger millet bran supplementation alleviates obesity-induced oxidative stress, inflammation and gut microbial derangements in high-fat

- diet-fed mice. The British journal of nutrition 112, 1447-1458.
8. Sharma P., and Mantri S.S. (2014). WImpiBLAST: web interface for mpiBLAST to help biologists perform large-scale annotation using high performance computing. PLOS ONE 9, e101144.
9. Sharma S., and Upadhyay S.K. (2014). Functional Characterization of Expressed Sequence Tags of Bread Wheat (*Triticum aestivum*) and Analysis of CRISPR Binding Sites for Targeted Genome Editing American Journal of Bioinformatics Research p-ISSN: 2167-6992 e-ISSN: 2167-6976 2014; 4(1): 11-22 doi:10.5923/j.bioinformatics.20140401.03
10. Singh A., Mantri S., Sharma M., Chaudhury A., Tuli R., and Roy J. (2014). Genome-wide transcriptome study in wheat identified candidate genes related to processing quality, majority of them showing interaction (quality x development) and having temporal and spatial distributions. BMC genomics 15, 29.
11. Singh S.P., Jeet R., Kumar J., Shukla V., Srivastava R., Mantri S.S., and Tuli R. (2014). Comparative transcriptional profiling of two wheat genotypes, with contrasting levels of minerals in grains, shows expression differences during grain filling. PLOS ONE 9, e111718.
12. Singh S.P., and Saini M.K. (2014). Postharvest vapour heat treatment as a phytosanitary measure influences the aroma volatiles profile of mango fruit. Food chemistry 164, 387-395.
13. Upadhyay S.K., and Sharma S. (2014). SSFinder: high throughput CRISPR-Cas target sites prediction tool. BioMed research international 2014, 742482.
14. Singh S.P., Vogel-Mikus K., Vavpetic P., Jeromel L., Pelicon P., Kumar J., and Tuli R. (2014). Spatial X-ray fluorescence micro-imaging of minerals in grain tissues of wheat and related genotypes. Planta 240, 277-289.
15. Thakur N., Upadhyay S.K., Verma P.C., Chandrashekar K., Tuli R., and Singh P.K. (2014). Enhanced whitefly resistance in transgenic tobacco plants expressing double stranded RNA of v-ATPase A gene. PLOS ONE 9, e87235.
16. Pathak A., Singh S.P., and Tuli R. (2014). AFLP fingerprinting to identify genetic relatedness among lychee cultivars, and markers associated with small seeded cultivars. Journal of The American Society for Horticultural Science 139(6), 657-668
17. Srivastava R., Rai K.M., Srivastava M., Kumar V., Pandey B., Singh S.P., Bag S.K., Singh B.D., Tuli R., and Sawant S.V. (2014). Distinct role of core promoter architecture in regulation of light mediated responses in plant genes. Molecular Plant 7(4), 626-641
18. Garg M., Kumar R., Singh R.P., and Tsujimoto H. (2014). Development of an *Aegilops longissima* substitution line with improved bread-making quality. Journal of cereal science. 60: 389-396.
19. Garg M., Yanaka M., Tanaka H., and Tsujimoto H. (2014). Introgression of useful genes from *Thinopyrum intermedium* to wheat for improvement of bread-making quality. Plant breeding. 133: 327-334.
20. Singh D.P., Kondepudi K.K., Bishnoi M., Chopra K. (2014). Altered monoamine metabolism in high fat diet induced neuropsychiatric changes in rats. J Obes Weight Loss Ther. 4:4.





# HUMAN RESOURCE



## I. Research Faculty

S. No	Name	Designation	Date of Joining
<b>Regular Faculty</b>			
1	Prof. Akhilesh K. Tyagi	Executive Director	01-10-2013
2	Dr. Vikas Rishi	Scientist E	01-03-2012
3	Dr. Joy K. Roy	Scientist D	09-08-2010
4	Dr. Ajay K. Pandey	Scientist D	14-11-2011
5	Dr. Siddharth Tiwari	Scientist C	28-07-2010
6	Sh. Shrikant Subhash Mantri	Scientist C	18-08-2010
7	Dr. (Ms.) Monika Garg	Scientist C	30-11-2010
8	Dr. Sukhvinder P. Singh	Scientist C	06-12-2010
9	Dr. Kanthi Kiran	Scientist C	02-09-2011
10	Dr. Mahendra Bishnoi	Scientist C	16-12-2011
11	Dr. Koushik Mazumder	Scientist C	01-02-2012
12	Dr. Nitin K. Singhal	Scientist C	02-03-2012
<b>Contractual Faculty</b>			
13	Dr. Shailesh Sharma	Project Scientist	02-01-2012
14	Dr. Sudhir P. Singh	Project Scientist	16-01-2012
15	Dr. Hariom Yadav	Ramalingaswami Fellow	14-12-2012
16	Dr. Santosh Kumar Upadhyay	INSPIRE Fellow	01-03-2013
17	Dr. Ashutosh Pandey	Project Scientist	04-12-2013

## II. Technical and Engineering Support

S. No	Name	Designation	Date of Joining
1	Ms. Aakriti Gupta	Senior Technical Assistant	22-02-2011
2	Sh. Jagdeep Singh	Senior Technical Assistant	01-03-2011
3	Sh. Sukhjinder Singh	Technical Assistant (Computers)	23-02-2012
4	Sh. Jaspreet Singh	Assistant Engineer	19-03-2012
5	Sh. Sushant Vatsa	Assistant Engineer	02-04-2012
6	Dr. Mainpal Singh	Senior Technical Assistant	24-12-2012
7	Sh. Atul Kesarwani	Senior Technical Assistant	21-01-2013
8	Sh. Kamalendra	Senior Technical Assistant	18-03-2013
9	Sh. Pankaj Pandey	Senior Technical Assistant	29-04-2013

## III. Administration

S. No	Name	Designation	Date of Joining
1	Sh. S. Krishnan	Store & Purchase Officer	10-03-2010
2	Sh. Suneet Verma	Finance Officer	15-09-2011
3	Sh. Virendra K. Banerjee	Former Administrative Officer	21-02-2013
4	Sh. Hardip Singh	Administrative Officer	01-10-2014
5	Sh. Sabir Ali	Management Assistant (Admin.)	21-01-2011
6	Ms. Hema Rawat	Management Assistance (Accounts)	01-04-2011
7	Sh. Vishal Kumar	Management Assistant (Accounts)	08-09-2011
8	Sh. Ashish Arora	Management Assistant (Admin.)	15-06-2012
9	Sh. Arun Kumar	Management Assistant (Public Relation)	21-06-2012
10	Ms. Anukiran Sabharwal	Library Assistant	19-12-2012

## IV. Human Resource Development

### (i) Research Scholars:

S. No	Name	Area of Research	Awarding University/Institute
<b>Student awarded Ph.D degree:</b>			
1	Sh. Jitendra Kumar	Development of virus induced gene silencing vector and its application in studying gene function in wheat ( <i>Triticum aestivum</i> )	Barkatullah University, Bhopal, MP  (Degree Awarded)
<b>Students enrolled for Ph.D degrees:</b>			
1	Sh. Yogesh Gupta	Gene discovery for seedlessness in <i>Annona</i> species	Panjab University, Chandigarh, Punjab
2	Ms. Anuradha Singh	Expression analysis of starch biosynthesis pathway genes and their effects on starch quality.	Guru Jambheshwar University of Science & Technology, Hisar, Haryana
3	Sh. Rohit Kumar	Allelic variation in puroindolines in Indian wheat cultivars, their association with hardness and starch granule properties.	Panjab University, Chandigarh, Punjab
4	Sh. Anshu Alok	Cloning and functional characterization of myo -inositol oxygenase (MIOX) from wheat ( <i>Triticum aestivum</i> )	Barkatullah University, Bhopal, MP
5	Ms. Anita Kumari	Modulation of scion through graft transmissible signals from rootstock, using <i>Arabidopsis thaliana</i> as a model system	Guru Jambheshwar University of Science & Technology, Hisar, Haryana
6	Ms. Monica Sharma	Genomic characterization & biochemical analysis of genes involved in phenylpropanoid pathway & their effect on nutritional & processing qualities of wheat.	Panjab University, Chandigarh, Punjab
7	Sh. Ritesh Kumar Baboota	Studies on modulation of adipogenesis, obesity and related complications by capsaicin	UIET Punjab University, Chandigarh

S.No.	Name	Designation	Date of Joining
1	Sh. Jitesh Kumar	Senior Research Fellow	09-09-2011
2	Ms. Manpreet Kaur Saini	Senior Research Fellow	09-09-2011
3	Sh. Kaushal Kumar Bhati	Senior Research Fellow	14-11-2011
4	Sh. Raja Jeet	Senior Research Fellow	12-03-2012
5	Sh. Ashish Kumar Pathak	Senior Research Fellow	08-08-2012
6	Ms. Sipla Aggarwal	Senior Research Fellow	16-08-2012
7	Sh. Prateek Jain	Senior Research Fellow	31-08-2012
8	Ms. Stanzin Angmo	Senior Research Fellow	11-02-2013
9	Ms. Shivani Sharma	Senior Research Fellow	12-02-2013
10	Sh. Shashank Singh	Junior Research Fellow	22-02-2013
11	Sh. Vishnu Shukla	Junior Research Fellow	25-02-2013
12	Ms. Mandeep Kaur	Junior Research Fellow	18-03-2013
13	Ms. Shivani	Junior Research Fellow	11-05-2013
14	Ms. Shelley Sardul Singh	Junior Research Fellow	16-07-2013
15	Ms. Parul Upadhayay	Junior Research Fellow	01-08-2013
16	Sh. Anoop Kishore Singh Gurjar	Junior Research Fellow	05-08-2013
17	Sh. Aman Kumar	Junior Research Fellow	05-08-2013
18	Sh. Koushik Shah	Junior Research Fellow	05-09-2013
19	Sh. Dharendra Pratap	Junior Research Fellow	11-09-2013
20	Sh. Pragyanshu Khare	Junior Research Fellow	23-09-2013
21	Sh. Siddhartha M. Sharma	Junior Research Fellow	25-09-2013
22	Ms. Harsimran Kaur	Junior Research Fellow	26-09-2013
23	Ms. Vandana	Junior Research Fellow	14-10-2013
24	Ms. Navneet Kaur	Junior Research Fellow	30-08-2013
25	Ms. Navneet Kaur	Junior Research Fellow	28-01-2014
26	Sh. Nand Kishore Sharma	Junior Research Fellow	29-01-2014
27	Sh. Pankaj Kumar	Junior Research Fellow	25-02-2014
28	Sh. Usman Ali	Junior Research Fellow	13-03-2014
29	Ms. Flowerika	Junior Research Fellow	04-04-2014
30	Ms. Diksha Sharma	Junior Research Fellow	03-09-2014
31	Sh. Rajinder Gupta	Junior Research Fellow	15-09-2014
32	Sh. Venkatesh Chunduri	Junior Research Fellow	25-09-2014
33	Ms. Saloni Sharma	Junior Research Fellow	30-09-2014
34	Ms. Preeti	Junior Research Fellow	10-02-2015
35	Ms. Ankita Mishra	Junior Research Fellow	13-02-2015



## (ii) Project Assistants:

S.No.	Name	Designation	Date of Joining
1.	Sh. Vikrant Sharma	Project Assistant	01-04-2013
2.	Sh. Prateek Kumar	Project Assistant	16-09-2013
3.	Ms. Meenakshi Chawla	Project Assistant	28-01-2014
4.	Sh. Anil Kumar	Project Assistant	06-09-2014
5.	Ms. Jaspreet Kaur	Project Assistant	10.02.2015

## (iii) Trainees:

S. No	Name	Designation	Date of Joining
1	Ms. Maninder Kaur	Trainee	01-07-2014
2	Sh. Venkatesh Chunduri	Trainee	01-04-2014
3	Ms. Sujata Thakur	Trainee	01-07-2014
4	Ms. Bindu Punia	Trainee	01-07-2014
5	Ms. Anjali	Trainee	04-07-2014
6	Ms. Shivani Sharma	Trainee	04-07-2014
7	Ms. Pallavi	Trainee	04-07-2014
8	Ms. Shalini Sharma	Trainee	02-01-2015
9	Ms. Jyoti Guleria	Trainee	02-01-2015
10	Sh. Zeetendra Singh	Trainee	03-01-2015
11	Ms. Banita Kumari	Trainee	15-1-2015



## PHOTO GALLERY OF IMPORTANT EVENTS



## Celebration of Independence Day: August 15<sup>th</sup>, 2014



Dr. R.S. Sangwan, CEO, CIAB hoisted the National flag at NABI Interim Facility and addressed the staff.



Independence Day celebrations at the NABI Interim Facility.



## Celebration of Hindi Pakhwada – September 1<sup>st</sup> - 15<sup>th</sup>, 2014



Participants at “Hindi Pakhwada ” which was organized in the institute during September 1<sup>st</sup> -15<sup>th</sup>, 2014.



Dr. R.S. Sangwan, CEO, CIAB Distributing the prizes to winners.

## Swachh Bharat Mission – October 2<sup>nd</sup>, 2014



NABI & CIAB staff taking pledge during Swachh Bharat Mission



All staff members took initiative and participated voluntarily in clean India drive



## Republic Day Celebrations at NABI: January 26<sup>th</sup>, 2015



Dr. R.S. Sangwan, CEO, CIAB hoisted the National flag at Interim Facility



NABI staff, celebrating the Republic day with their family members

## Fifth Foundation Day: February 18<sup>th</sup>, 2015



**First row from left:** Dr. Vikas Rishi, Sct-D, NABI; Dr. K.C. Bansal, Director, NBPGR; Prof. Akhilesh K. Tyagi, Executive Director, NABI and Dr. R.S. Sangwan, CEO, CIAB  
Dr. K.C. Bansal, was the Chief Guest on the occasion & lighting the lamp

**Second row from left:** Prof. Akhilesh K. Tyagi, lighting the lamp and addressing the gathering

**Third row from left:** Dr. R.S. Sangwan presenting shawl to Dr. K.C. Bansal  
Dr. Vikas Rishi, giving the vote of thanks







# FINANCIALS

## FORM OF FINANCIAL STATEMENTS (NON PROFIT ORGANIZATION) NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE C-127 INDUSTRIAL AREA PHASE-8 S.A.S. NAGAR, MOHALI


### BALANCE SHEET AS ON 31<sup>st</sup> MARCH 2015

CORPUS/ CAPITAL FUND AND LIABILITIES	Schedule	Current Year	Previous Year
Corpus/Capital Fund	1	77,80,36,197	52,18,04,081
Reserves and Surplus	2	-	-
Earmarked / Endowment Funds	3	1,36,66,356	1,56,86,198
Secured Loans and Borrowings	4	-	-
Unsecured Loans and Borrowings	5	-	-
Deferred Credit Liabilities	6	-	-
Current Liabilities and Provisions	7	69,23,985	78,12,307
<b>TOTAL</b>		<b>79,86,26,538</b>	<b>54,53,02,587</b>
<b>ASSETS</b>			
Fixed Assets	8	22,67,73,187	26,70,98,974
Capital Work in Progress	8	41,53,54,248	6,46,01,217
Investments- from Earmarked/Endowment funds	9	1,08,19,176	1,01,29,167
Investments - Others	10	-	-
Current Assets, Loans & Advances etc.	11	14,56,79,926	20,34,73,228
<b>TOTAL</b>		<b>79,86,26,538</b>	<b>54,53,02,587</b>
Significant Accounting Policies	24		
Contingent liabilities and notes on accounts	25		

As per our separate report of even date attached

For National Agri-Food Biotechnology Institute

For U.K. Mehta & Associates  
Chartered Accountants

  
Finance Officer  
Suneet Verma / सुनीत वर्मा  
Finance Officer / वित्त अधिकारी  
National Agri-Food Biotechnology Institute  
Govt. of India / भारत सरकार  
Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग  
Mohali, Punjab / मोहाली, पंजाब - 160071  
Dated: 05-6-15  
Place: Mohali

  
Executive Director  
प्रो. अखिलेश कुमार त्यागी  
Prof. Akhilesh Kumar Tyagi  
कार्यकारी निदेशक/Executive Director  
राष्ट्रीय कृषि स्वयं सेव प्रौद्योगिकी संस्थान  
National Agri-Food Biotechnology Institute  
जैव प्रौद्योगिकी विभाग, भारत सरकार  
मोहाली - 160071 पंजाब, भारत  
Department of Biotechnology, Govt. of India  
Mohali-160071 Punjab, INDIA

  
(U.K. Mehta), FCA  
Chartered Accountants  


**FORM OF FINANCIAL STATEMENTS (NON-PROFIT ORGANISATIONS)**  
**NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE**  
**C-127, INDUSTRIAL AREA, PHASE-8, S.A.S. NAGAR, MOHALI**  
**INCOME AND EXPENDITURE ACCOUNT**  
**FOR THE YEAR ENDED 31<sup>st</sup> MARCH 2015**

(Amount in Rs.)

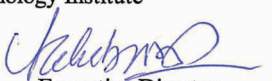
INCOME	Schedule	Current Year	Previous Year
Income from Sales/Services	12	-	-
Grants in aid /subsidies	13	9,90,00,000	5,10,00,000
Fees/subscriptions	14	-	-
Income from Investments (Income on investment from earmarked/endowment funds transferred to funds)	15	-	-
Income from Royalty, Publication etc.	16	-	-
Interest Earned	17	1,78,47,784	79,60,094
Other Income	18	21,13,416	53,66,152
Increase/decrease in stock of finished goods & work- in-progress	19	-	-
<b>TOTAL(A)</b>		<b>11,89,61,200</b>	<b>6,43,26,246</b>
<b>EXPENDITURE</b>			
Establishment Expenses	20	2,10,85,227	2,26,03,635
Other Administrative Expenses	21	3,61,65,297	3,73,77,666
Research & Development Expenditure (Incl. Grants, Subsidies etc)	22	2,32,16,983	1,90,24,350
Interest	23	-	-
Depreciation (net total at the year end-corresponding to schedule 8)		4,22,61,578	5,20,72,764
<b>TOTAL(B)</b>		<b>12,27,29,085</b>	<b>13,10,78,415</b>
Balance being surplus/ (deficit) carried to Capital Fund(A-B)		-37,67,885	-6,67,52,169
Significant Accounting Policies	24		
Contingent liabilities and notes on accounts	25		

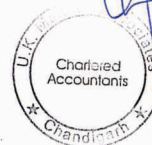
As per our separate report of even date attached

For National Agri-Food Biotechnology Institute

For U.K. Mehta & Associates  
Chartered Accountants

  
**Finance Officer**  
 Suneet Verma / सुनीत वर्मा  
 Finance Officer / वित्त अधिकारी  
 National Agri-Food Biotechnology Institute  
 Govt. of India / भारत सरकार  
 Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग  
 Mohali, Place: Mohali - 160071

  
**Executive Director**  
 प्रो. अखिलेश कुमार त्यागी  
 Prof. Akhilesh Kumar Tyagi  
 कार्यकारी निदेशक/Executive Director  
 राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान  
 National Agri-Food Biotechnology Institute  
 जैव प्रौद्योगिकी विभाग, भारत सरकार  
 मोहाली - 160071 पंजाब, भारत  
 Department of Biotechnology, Govt. of India  
 Mohali-160071 Punjab, INDIA



(U.K. Mehta), FCA



## Form of Financial Statements for the Central Autonomous Bodies (Non- Profit Organizations and similar Institutions)

**NATIONAL AGRI FOOD BIOTECHNOLOGY INSTITUTE**  
 C-127 INDUSTRIAL AREA PHASE -8, S.A.S. NAGAR, MOHALI
**RECEIPTS AND PAYMENTS ACCOUNT FOR THE PERIOD/YEAR ENDED ON 31.03.2015**


(Amounts in Rs.)

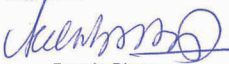
RECEIPT	Current Year	Previous Year	PAYMENT	Current Year	Previous Year
<b>Plan Grants</b>			<b>Expenditure</b>		
(A) Opening Balance			(A) Establishment Expenses		
a) Cash in Hand			1. Manpower Salaries and Fellowships	2,44,27,291	2,46,71,253
b) Bank Balances			2. Staff Welfare Exp./Seminars	17,932	64,593
i) In current accounts			(B) Other Administrative Expenses		
ii) In deposit Accounts	7,18,36,045	12,29,96,757	1. Carriage & Carriage inward	800	750
iii) In Savings Accounts	22,72,358	68,93,306	2. Allowances & Bonus (Honorarium)	5,32,964	6,26,404
			3. Electricity & Diesel Charges	68,95,461	67,01,334
			4. Rent	1,49,05,894	1,51,85,616
(B) Grant-in-Aid			5. Vehicles Running & maintenance	32,786	1,48,023
(a) Grant from DBT	35,90,00,000	20,10,00,000	6. Postage, Telephone & Comm charges	5,55,439	6,89,154
			7. Printing & stationery	4,79,995	5,45,896
(C) Interest Incomes			8. Travelling & conveyance expenses	15,57,012	18,40,443
(a) Interest Income	1,81,17,447	95,34,338	9. Outsourcing	75,64,130	69,55,057
			10. Professional Charges	70,793	32,416
(D) Other Incomes			11. Advertisement	2,17,500	13,31,386
(a) Application Fees		1,95,696	12. Building Renovation		
(b) Misc. Income	22,623	2,35,663	13. Repair & Maintenance	13,91,434	19,81,816
(c) Farm Income		2,60,906	14. Office and Admn Expenses	3,77,504	2,87,143
(d) Tender Fees	66,000	14,100	15. Bank Charges	-	1,617
(e) Guest house income	68,450	23,660	16. Guest House Expenditure	37,916	48,010
(f) RTI Fee	30	490	17. Insurance	8,662	10,740
(g) Car Charges		15,513	18. Library Books	4,45,182	5,486
(h) Licence Fee		2,46,526			
(i) Project Income	3,71,865	6,74,008	(C) Research & Deveopment Expenditure.		
(E) Other Projects Receipt	1,75,71,762	1,32,63,400	1. Chemicals & Consumables	1,70,97,795	1,60,28,656
			2. Computer Software & Accessories	12,50,801	2,58,525
(F) Other Receipt			3. Research Work Expenses	46,050	50,050
(a) Security Deposit		82,954	4. Field Expenses	73,439	2,24,626
			(D) Non-Recurring Expenditures		
			1. Development of Main Campus	32,39,12,085	4,67,02,318
			2. Scientific Equip & Research Acce	8,25,522	54,56,177
			2.1. Equipment WIP		84,029
			3. Computers & Books	6,43,784	1,38,350
			4. Furniture & Fixture	19,701	90,262
			5. Office Equipment	28,085	1,45,622
			(E) Other Payments		
			(a) External Project Expenses	1,94,50,158	1,46,40,299
			(b) Expenses Payable		4,31,843
			(c) TDS Receivable	10,822	6,609
			(d) Earnest Money Deposit	3,84,556	84,433
			(e) Refund of Security Deposits	3,11,209	
			(F) Loan & Advances		
			(a) Advance to RITES Ltd.		13,54,89,163
			(b) Advance to NIPER		1,25,562
			(c) DBT (Brain Storming)		2,21,904
			(d) Advance to Employees	1,76,779	23,349
			(e) Advance to NCCS, Pune	1,500	
			(f) M/s Gurukirpa Refrigeration	400	
			(G) Closing Balance		
			a) Cash in Hand		
			b) Bank Balances		
			i) In Current Accounts		
			ii) In Deposit Accounts	4,13,50,830	7,18,36,045
			iii) In Savings Accounts	42,24,369	22,72,358
<b>Grand Total</b>	<b>46,93,26,580</b>	<b>35,54,37,317</b>	<b>Grand Total</b>	<b>46,93,26,580</b>	<b>35,54,37,317</b>

In terms of separate report of even date attached

 Dated: 05.6.15  
 Place: Mohali

For National Agri-Food Biotechnology Institute

  
 Finance Officer  
 Suneet Verma / सुनीत वर्मा  
 Finance Officer / वित्त अधिकारी  
 National Agri-Food Biotechnology Institute  
 Govt. of India / भारत सरकार  
 Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग  
 Mohali, Punjab / मोहाली, पंजाब-160071

  
 Executive Director  
 प्रो. अखिलेश कुमार त्यागी  
 Prof. Akhilesh Kumar Tyagi  
 कार्यकारी निदेशक/Executive Director  
 राष्ट्रीय कृषि एवं जैव प्रौद्योगिकी संस्थान  
 National Agri-Food Biotechnology Institute  
 जैव प्रौद्योगिकी विभाग, भारत सरकार  
 मोहाली-160071 पंजाब, भारत  
 Department of Biotechnology, Govt. of India  
 Mohali-160071 Punjab, INDIA

For U.K. Mehta & Associates  
Chartered Accountants

  
 U.K. Mehta, FCA  
 Chartered Accountants  
 Chandigarh



**FORM OF FINANCIAL STATEMENTS (NON-PROFIT ORGANISATIONS)**  
**NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE**  
**C-127, INDUSTRIAL AREA, PHASE-8, S.A.S. NAGAR, MOHALI**

**SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31.03.2015**

**SCHEDULE-1**  
**CORPUS/CAPITAL FUND**

(Amount In Rs.)

Particulars	Current Year	Previous Year
Balance as at the beginning of the year	52,18,04,081	43,85,56,250
Add : Contributions towards corpus/capital fund	26,00,00,000	15,00,00,000
Less/(Deduct) : balance of net expenses transferred from the income & expenditure a/c	-37,67,885	-6,67,52,169
<b>BALANCE AS AT THE YEAR -END</b>	<b>77,80,36,197</b>	<b>52,18,04,081</b>


**SCHEDULE-2**  
**RESERVES AND SURPLUS**

(Amount in Rs.)

Particulars	Current Year	Previous Year
1.Capital Reserves:	-	-
2.Revaluation Reserve	-	-
3.Special Reserve	-	-
4.General Reserve	-	-
<b>TOTAL</b>	<b>-</b>	<b>-</b>

for National Agri-Food Biotechnology Institute  
Dated: 05.6.15  
Place: Mohali

For U.K. Mehta & Associates  
Chartered Accountants

  
**Finance Officer**  
Suneet Verma / सुनीत वर्मा  
Finance Officer / वित्त अधिकारी  
National Agri-Food Biotechnology Institute  
Govt. of India / भारत सरकार  
ptl. of Biotechnology/ जैवप्रौद्योगिकी विभाग  
Mohali, Punjab / मोहाली, पंजाब-160071

  
**Executive Director**  
प्रो. अखिलेश कुमार त्यागी  
Prof. Akhilesh Kumar Tyagi  
कार्यकारी निदेशक/Executive Director  
राष्ट्रीय कृषि स्वायत्त जैव प्रौद्योगिकी संस्थान  
National Agri-Food Biotechnology Institute  
जैव प्रौद्योगिकी विभाग, भारत सरकार  
मोहाली-160071 पंजाब, भारत  
Department of Biotechnology, Govt. of India  
Mohali-160071 Punjab, INDIA

  
**U.K. Mehta, FCA**  
Chartered Accountants  


SCHEDULE 03-EARMARKED/ENDOWMENT FUNDS				Additions				Utilisation/Expenditure					NET BALANCE AT THE YEAR END
Sr. No.	Project Name	a) Opening balance of the Fund	b) Additions during the Year	c) Accrued Interest / Invested	TOTAL (a+b+c)	i) Capital Expenditure	Fellowships	Chemical & Consumable	Contingency Exp/Travel etc	Overhead Exp/ refunded	TOTAL	TOTAL EXP	
1	A Novel strategy for developing scion plants of desired phenotype (e.g. seedless, early flowering) by using an RNAi delivering rootstock	1,39,705	4,00,000	11,063	5,50,768			4,11,660		65,560	4,77,220	4,77,220	73,548
2	Queensland University of Technology, Australia to India for Bio-fortification and Disease Resistance in Banana	1,22,07,819	42,08,000	9,47,219	1,73,63,038	1,67,135	11,29,892	6,67,507	9,81,670		27,79,069	29,46,204	1,44,16,834
3	Metabolic Engineering of Phytic Acid Pathway for Improving Iron Bioavailability in Wheat	5,37,388	4,04,900	10,604	9,52,892		4,51,751	4,17,021	33,984		9,02,756	9,02,756	50,136
4	Effect of Finger Millet and Kodo Millet	2,11,807	8,76,800	18,241	11,06,848		2,42,169	6,34,268	20,583		8,97,020	8,97,020	2,09,828
5	A Nutritional study to access the role of polyphenols constituents	44,227	5,00,000	7,659	5,51,886		3,96,875				3,96,875	3,96,875	1,55,011
6	Studies on transient receptor potential (TRP) channel mediated modulation	1,63,028	4,00,000	4,619	5,67,647		1,72,800		3,06,542	51,264	5,30,606	5,30,606	37,041
7	Development of Novel compounds for treatment of obesity and type 2 diabetes	2,75,273			2,75,273			95,303		1,79,970	2,75,273	2,75,273	-
8	Transient Receptor Potential	4,44,347	8,54,700	19,097	13,18,144		2,49,600	9,03,804	32,459		11,85,863	11,85,863	1,32,281
9	Variability in the fine structure of feruloyl arabinosylans from Indian Millet varieties and their consequence on anti-oxidant activity	4,06,891	1,50,000	9,092	5,65,983		2,49,600	1,59,978		20,454	4,30,032	4,30,032	1,35,951
10	Metabolomics approach to discovery and validation of biomarkers for artificial fruit ripening induced through prohibited and acceptable ripening elicitors	2,32,382		2,814	2,35,196		87,129			1,48,067	2,35,196	2,35,196	-
11	Identification of celiac disease epitopes in Indian wheat cultivars and their modulation by RNAi and breeding approach	10,28,043		22,440	10,50,483	2,99,121	2,80,349	3,88,731	34,719		7,03,799	10,02,920	47,563
12	Chromosome specific wide hybridization for improvement of bread making quality of wheat	3,76,720	3,00,000	10,916	6,87,636			5,52,196		50,000	6,02,196	6,02,196	85,440
13	Identification, cloning and Functional characterization of MIOX from Wheat		9,00,000	15,391	9,15,391	1,81,800		4,41,530		94,170	5,35,700	7,17,500	1,97,891
14	Developing glycoconjugates capped multifunctional gold nanorod based nanobiosensor for detection of multiple food borne bacteria		2,25,500	641	2,26,141					2,696	2,696	2,696	2,23,445
15	Ramalinga Swami fellowship (DBT)	-1,52,199	3,36,110		1,73,911		1,16,774	57,137			1,73,911	1,73,911	-
16	DST Inspire Fellowship	3,63,275	16,91,680	49,415	21,04,370		4,76,443	68,125		15,59,802	21,04,370	21,04,370	-
17	IC BOSE National Research Fellowship	8,51,116		36,939	8,88,055					8,88,055	8,88,055	8,88,055	-
18	Department of Biotechnology (DBT) JRF/SRF fellowships	-12,73,593	34,62,544		21,88,951		38,36,302				38,36,302	38,36,302	-16,47,351
19	Indian Council of Medical Research (ICMR) JRF/SRF Fellowships	9,809	10,89,972		10,99,781		13,66,123				13,66,123	13,66,123	-2,66,342
20	UGC Fellowship	-1,79,840	4,71,710		2,91,870		2,87,670				2,87,670	2,87,670	4,200
	Council of Scientific & Industrial Research (CSIR)		3,08,012		3,08,012		4,97,132				4,97,132	4,97,132	-1,89,120
21	JRF/SRF Fellowships	1,56,86,198	1,65,69,928	11,66,150	3,34,22,276	6,48,056	97,53,480	48,84,389	14,09,957	30,60,038	1,91,07,864	1,97,55,920	1,36,66,356
Total													

For National Agri-Food Biotechnology Institute

For U.K. Mehta & Associates  
Chartered Accountants

प्रो. अखिलेश कुमार त्यागी  
Prof. Akhilesh Kumar Tyagi  
अध्यक्ष/प्रमुख/Executive Director  
राष्ट्रीय कृषि खाद्य और जैवोपार्श्व संस्थान  
National Agri-Food Biotechnology Institute  
जैव प्रौद्योगिकी विभाग, भारत सरकार  
मोहली - 140071 पंजाब, भारत  
Department of Biotechnology, Govt. of India

सुखदेव शर्मा  
Sukhdev Sharma  
वित्त अधिकारी/वि. अधिकारी  
National Agri-Food Biotechnology Institute  
Govt. of India / भारत सरकार  
Dept. of Biotechnology / जैवप्रौद्योगिकी विभाग

Date: 05.6.15  
Place: Mohali



**SCHEDULE-4**  
**SECURED LOANS & BORROWINGS**

(Amount in Rs.)		
Particulars	Current Year	Previous Year
1. Central Government		-
2. State Government(specify)		-
3. Financial Institutions		
4. Banks:		
5. Other Institutions & agencies		-
6. Debentures & bonds		-
7. Others(specify)		-
<b>TOTAL</b>		-

**SCHEDULE-5**  
**UNSECURED LOANS & BORROWINGS**

(Amount in Rs.)		
Particulars	Current Year	Previous Year
1. Central Government		-
2. State Government(specify)		-
3. Financial Institutions		
4. Banks:		
5. Other Institutions & agencies		-
6. Debentures & bonds		-
7. Others(specify)		-
<b>TOTAL</b>		-

**SCHEDULE-6**  
**DEFERRED CREDIT LIABILITIES**

(Amount in Rs.)		
Particulars	Current Year	Previous Year
1. Acceptances secured by hypothecation of capital equipment		-
2. Others		-
<b>TOTAL</b>		-

for National Agri-Food Biotechnology Institute For U.K. Mehta & Associates

Dated: 03.6.15

Place: Mohali

Chartered Accountants

  
Finance Officer

Suneet Verma / सुनीत वर्मा  
Finance Officer / वित्त अधिकारी  
National Agri-Food Biotechnology Institute  
Govt. of India / भारत सरकार  
Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग  
Mohali, Punjab / मोहाली, पंजाब-160071

  
Executive Director

प्रो. अखिलेश कुमार त्यागी  
Prof. Akhilesh Kumar Tyagi  
कार्यकारी निदेशक/Executive Director  
राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान  
National Agri-Food Biotechnology Institute  
जैव प्रौद्योगिकी विभाग, भारत सरकार  
मोहाली-160071 पंजाब, भारत  
Department of Biotechnology, Govt. of India  
Mohali-160071 Punjab, INDIA

  
U.K. Mehta, FCA



**SCHEDULE-7**  
**CURRENT LIABILITIES & PROVISIONS**

(Amount in Rs.)

Particulars	Current Year	Previous Year
<b>A)CURRENT LIABILITIES</b>		
1. Sundry Creditors		
a) For goods/Equipment	18,29,560	18,29,560
b) For Securities	1,14,923	4,26,132
c) Earnest Money Deposit	6,23,011	10,07,567
2. Advances received from External Projects		
3. Interest accrued but not due on:		
a) Secured Loans/Borrowings		-
b) Unsecured Loans/Borrowings		-
4. Statutory Liabilities		
a) Overdue		-
5. Other Current Liabilities		
a) Manpower (Salary) Payable	19,34,581	14,99,859
b) Other Expenses Payable	12,10,075	11,82,127
c) TDS Payable	4,58,235	11,73,415
d) Fellowship Payable	7,53,600	6,93,647
<b>TOTAL(A)</b>	<b>69,23,985</b>	<b>78,12,307</b>
<b>B) PROVISIONS</b>		
1. Gratuity		
2. Superannuation/Pension		-
3. Leave Encashment		-
<b>TOTAL(B)</b>		-
<b>TOTAL(A+B)</b>	<b>69,23,985</b>	<b>78,12,307</b>


for National Agri-Food Biotechnology Institute

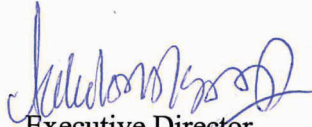
For U.K. Mehta &amp; Associates

Dated: 05.6.15

Chartered Accountants

Place: Mohali

  
**Finance Officer**  
 Suheet Verma / सुनीत वर्मा  
 Finance Officer / वित्त अधिकारी  
 National Agri-Food Biotechnology Institute  
 Govt. of India / भारत सरकार  
 Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग  
 Mohali, Punjab / मोहाली, पंजाब-160071

  
**Executive Director**  
 प्रो. अखिलेश कुमार त्यागी  
 Prof. Akhilesh Kumar Tyagi  
 कार्यकारी निदेशक/Executive Director  
 राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान  
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 मोहाली-160071 पंजाब, भारत  
 Department of Biotechnology, Govt. of India  
 Mohali-160071 Punjab, INDIA

  
**U.K. Mehta, FCA**  
  
 Chartered Accountants  
 Chandigarh



NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE  
C-127 INDUSTRIAL AREA PHASE-8 S.A.S. NAGAR, MOHALI  
SCHEDULE-8

Sl.No.	Description	Depreciat on Rate	Cost/Valuation as at beginning of the year	Additions during the year	UPTO 30.09.14	After 30.09.14	Deduction during the year	Cost/Valuation at the year end	As at the beginning of the year	Depreciation during the year	Total at the year end	As at the Current Year End	NET BLOCK As at the Previous Year End
Sl.No.			1st April 2014	UPTO 30.09.14	30.09.14	2014-15	2014-15	31st March 2015	1st April 2014	2014-15	31st March 2015	31st March 2015	31st March 2014
A	FIXED ASSETS												
I	LAND												
	a) Free Hold	0.00%	-	-	-	-	-	-	-	-	-	-	-
	b) Lease Hold	0.00%	-	-	-	-	-	-	-	-	-	-	-
II	BUILDINGS												
	a) On Freehold Land	10.00%	83,57,674	-	-	-	-	83,57,674	15,87,958	6,76,972	22,64,930	60,92,744	67,69,716
	b) On Leasehold Land	10.00%	-	-	-	-	-	-	-	-	-	-	-
	c) Ownership Premises	10.00%	-	-	-	-	-	-	-	-	-	-	-
	d) Other Superstructures	10.00%	-	-	-	-	-	-	-	-	-	-	-
III	PLANT, MACHINERY & EQUIPMENT	15.00%	35,77,34,407	1,20,848	6,63,476	-	-	35,85,18,731	10,79,94,267	3,75,28,909	14,55,23,176	21,29,95,555	24,97,40,140
IV	VEHICLES	15.00%	6,62,497	-	-	-	-	6,62,497	2,89,090	56,011	3,45,101	3,17,397	3,73,408
V	FURNITURE & FIXTURES	10.00%	35,57,193	7,681	12,020	-	-	35,76,894	9,61,135	2,60,975	12,22,110	23,54,784	25,96,058
VI	COMPUTER/PERIPHERALS	60.00%	2,03,28,981	13,625	6,30,159	-	-	2,09,72,765	1,56,95,346	29,77,404	1,86,72,750	23,00,015	46,33,635
VII	LIBRARY BOOKS	100.00%	17,291	-	4,59,897	-	-	4,77,188	17,291	4,59,897	4,77,188	-	-
VIII	OFFICE EQUIPMENT	10.00%	38,68,026	28,085	-	-	-	38,96,111	8,82,008	3,01,410	11,83,418	27,12,692	29,86,017
	TOTAL OF CURRENT YEAR (A)		39,45,26,069	1,70,239	17,65,552	-	-	39,64,61,860	12,74,27,096	4,22,61,578	16,96,88,674	22,67,73,187	26,70,98,974
XI	PREVIOUS YEAR												
	a) Expenditure on Assets/Fixed Assets		-	-	-	-	-	-	-	-	-	-	-
	b) Expenditure on Plan Activities		-	-	-	-	-	-	-	-	-	-	-
	TOTAL OF PREVIOUS YEAR		-	-	-	-	-	-	-	-	-	-	-
XII	CAPITAL WORK-IN-PROGRESS												
	a) Main Campus At Sec 81		6,45,15,344	18,59,54,178	16,47,98,853	-	-	41,52,68,375	-	-	-	41,52,68,375	6,45,15,344
	d) Equipment		85,873	2,710	-	2,710	-	85,873	-	-	-	85,873	85,873
	TOTAL OF CURRENT YEAR (CWIP) (B)		6,46,01,217	18,59,56,888	16,47,98,853	2,710	-	41,53,54,248	-	-	-	41,53,54,248	6,46,01,217
	TOTAL (A+B)		45,91,27,286	18,61,27,127	16,65,64,405	2,710	-	81,18,16,108	12,74,27,096	4,22,61,578	16,96,88,674	64,21,27,435	33,17,00,191

Date: 05.06.15  
Place: Mohali

*Suresh Kumar*  
Finance Officer  
National Agri-Food Biotechnology Institute  
C-127, Industrial Area Phase-8, S.A.S. Nagar, Mohali

For National Agri-Food Biotechnology Institute

*Abhinav Kumar*  
Executive Director  
National Agri-Food Biotechnology Institute  
C-127, Industrial Area Phase-8, S.A.S. Nagar, Mohali

For U.K. Mehta & Associates  
Chartered Accountants

*U.K. Mehta*  
Chartered Accountants  
U.K. Mehta & Associates  
FCA



**SCHEDULE-9**  
**INVESTMENTS FROM EARMARKED/ENDOWMENT FUNDS**

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. In Government Securities	-	-
2. Other approved securities	-	-
3. Shares	-	-
4. Debentures & Bonds	-	-
5. Subsidiaries & Joint Ventures	-	-
6. Others(to be specified)	1,08,19,176	1,01,29,167
<b>TOTAL</b>	<b>1,08,19,176</b>	<b>1,01,29,167</b>

**SCHEDULE-10**  
**OTHER INVESTMENTS**

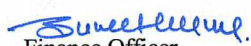
(Amount in Rs.)

Particulars	Current Year	Previous Year
1. In Government Securities	-	-
2. Other approved securities	-	-
3. Shares	-	-
4. Debentures & Bonds	-	-
5. Subsidiaries & Joint Ventures	-	-
6. Others(to be specified)	-	-
<b>TOTAL</b>	<b>-</b>	<b>-</b>

for National Agri-Food Biotechnology Institute

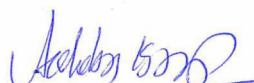
Dated: 05.6.15

Place: Mohali



Finance Officer

Suneet Verma / सुनीत वर्मा  
Finance Officer / वित्त अधिकारी  
National Agri-Food Biotechnology Institute  
Govt. of India / भारत सरकार  
Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग  
Mohali, Punjab / मोहाली, पंजाब-160071



Executive Director

प्रो. अखिलेश कुमार त्यागी  
Prof. Akhilesh Kumar Tyagi  
कार्यकारी निदेशक/Executive Director  
राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान  
National Agri-Food Biotechnology Institute  
जैव प्रौद्योगिकी विभाग, भारत सरकार  
मोहाली-160071 पंजाब, भारत  
Department of Biotechnology Govt. of India

For U.K. Mehta & Associates  
Chartered Accountants


U.K. Mehta, FCA



**SCHEDULE-11**  
**CURRENT ASSETS, LOANS & ADVANCES**

(Amount in Rs.)

Particulars	Current Year	Previous Year
<b>A) CURRENT ASSETS</b>		
<b>1. Inventories</b>		
a) Stores & Spares		-
b) Loose Tools		-
c) Stock-in-trade		-
<b>2. Sundry Debtors</b>		
<b>3. Cash balances in hand</b>		
<b>4. Bank balances:</b>		
a) With Scheduled Banks:		
-On Current accounts		
-On Fixed Deposit accounts	3,05,31,654	6,21,62,845
-On Savings accounts		
(i) State Bank of India A/c	42,24,369	22,72,358
<b>TOTAL(A)</b>	<b>3,47,56,023</b>	<b>6,44,35,203</b>
<b>B) LOANS, ADVANCES AND OTHER ASSETS</b>		
<b>1. Loans</b>		
<b>2. Advances and other amounts recoverable</b>		
a) On Capital Account		-
b) Advances		-
(i) Deposite with M/s RITES Ltd	10,79,57,152	13,54,89,163
(ii) Advance to CFTRI	375	375
(iii) NCCS Pune	1,500	
c) Recoupable form Govt. Agencies		
(i) Director NIPER	615	1,25,562
(ii) DBT (Brain Storming Project)	2,21,904	2,21,904
(iii) Advance to CDAC Pune	-	9,97,285
d) Advance to Employees	2,51,775	74,996
e) Others(specify)		
(i) Security for Rent	50,000	50,000
(ii) IDA Deposit with IMTECH		
(iii) TDS Receivable	2,36,272	2,25,450
(v) PSEB Elelct Security for Main Campus	11,12,090	11,12,090
(vi) Electricity Security of Interim facility	7,41,200	7,41,200
(vii) M/s Gurukripa Refrigeration	400	
<b>3. Income accrued:</b>		
a) on investments from earmarked/endowment funds		
b) Interest On Saving and Fixed Deposits	1,86,304	
c) on loans & advances		
d) others(Accrued Interest from GAPs)	1,64,316	
<b>4. Claims Receivable</b>		
<b>TOTAL(B)</b>	<b>11,09,23,903</b>	<b>13,90,38,025</b>
<b>TOTAL(A+B)</b>	<b>14,56,79,926</b>	<b>20,34,73,228</b>

for National Agri-Food Biotechnology Institute

Dated: 05.6.15

Place: Mohali

*Suneet Verma*  
Finance Officer

Suneet Verma / सुनीत वर्मा

Finance Officer / वित्त अधिकारी  
National Agri-Food Biotechnology Institute

Govt. of India / भारत सरकार  
Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग  
Mohali, Punjab / मोहाली, पंजाब-160071

*Prof. Arvind Kumar Tyagi*  
Executive Director

Prof. Arvind Kumar Tyagi  
कार्यकारी निदेशक/Executive Director  
राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान  
National Agri-Food Biotechnology Institute

जैव प्रौद्योगिकी विभाग, भारत सरकार  
मोहाली - 160071 पंजाब, भारत  
Page 10 of 18  
Department of Biotechnology, Govt. of India  
Mohali-160071 Punjab, INDIA

For U.K. Mehta & Associates

Chartered Accountants

*U.K. Mehta*  
U.K. Mehta, FCA



**SCHEDULE-12**  
**INCOME FROM SALES/SERVICES**

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Income from sales		
2. Income from services	-	-
<b>TOTAL</b>	-	-

**SCHEDULE-13**  
**GRANTS/SUBSIDIES**

(Amount in Rs.)

Particulars	Current Year	Previous Year
(Irrevocable Grants & subsidies received)		
1. Central Government	9,90,00,000	5,10,00,000
2. State Government	-	-
3. Government Agencies	-	-
4. Institutional /welfare bodies	-	-
5. International Organisations	-	-
6. Others (to be specified)	-	-
<b>TOTAL</b>	<b>9,90,00,000</b>	<b>5,10,00,000</b>

**SCHEDULE-14**  
**FEES/SUBSCRIPTIONS**

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Entrance Fees	-	-
2. Annual Fees / subscriptions	-	-
3. Seminar/program fees	-	-
4. Consultancy fees	-	-
5. Others	-	-
<b>TOTAL</b>	-	-

**SCHEDULE-15**  
**INCOME FROM INVESTMENTS**

(Amount in Rs.)

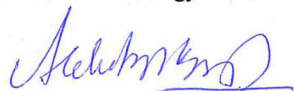
Particulars	Current Year	Previous Year
1. Interest	-	-
a) On Govt. securities		-
b) Other Bonds/Debentures		-
2. Dividends:	-	-
a) On shares		-
b) On Mutual Fund securities		-
3. Rents	-	-
4. Others (specify)	-	-
<b>TOTAL</b>	-	-

for National Agri-Food Biotechnology Institute

Dated: 05.6.15

Place: Mohali

  
Finance Officer  
Suneet Verma / सुनीत वर्मा  
Finance Officer / वित्त अधिकारी  
National Agri-Food Biotechnology Institute  
Govt. of India / भारत सरकार  
Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग  
Mohali, Punjab / मोहाली, पंजाब-160071

  
Executive Director  
प्रो. अखिलेश कुमार त्यागी  
Prof. Akhilesh Kumar Tyagi  
कार्यकारी निदेशक/Executive Director  
राष्ट्रीय कृषि स्वाथ जैव प्रौद्योगिकी संस्थान  
National Agri-Food Biotechnology Institute  
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मोहाली - 160071 पंजाब, भारत  
Department of Biotechnology, Govt. of India  
Mohali-160071 Page 11 of 18

For U.K. Mehta & Associates  
Chartered Accountants

  
U.K. Mehta, FCA  




**SCHEDULE-16**  
**INCOME FROM ROYALTY/PUBLICATIONS. ETC.**

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Income from Royalty	-	-
2. Income from Publications	-	-
3. Others(specify)	-	-
<b>TOTAL</b>	<b>-</b>	<b>-</b>

**SCHEDULE-17**  
**INTEREST EARNED**

(Amount in Rs.)

Particulars	Current Year	Previous Year
<b>1)On Term Deposits</b>		
a)With Scheduled Banks:		
i) Actual Received	47,35,303	70,36,078
ii) Accrued as on 31.03.2015	1,86,304	4,55,967
b)With Non-Scheduled Banks:		
<b>2)On Savings Accounts:</b>		
a)With Scheduled Banks:	1,91,825	3,53,106
b)With Non-Scheduled Banks:		
<b>3)On Loans</b>		
a)Employees/staff		-
b) Interest on Mobilisation Advance/Escrow Acc		-
<b>4)Interest on Debtors &amp; other Receivables</b>	1,27,34,352	1,14,943
<b>TOTAL</b>	<b>1,78,47,784</b>	<b>79,60,094</b>

**SCHEDULE-18**  
**OTHER INCOME**

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Profit on sale/disposal of assets		
a) Owned Assets		
b) Assets acquired out of grants,or received free of		
2. Export Incentives realized		
3. Fee for Miscellaneous Services (Overhead External Projects)	3,71,865	6,74,008
4. Miscellaneous Income		
a) Application Fees		1,95,696
b) Tender Fees	66,000	14,100
c) Misc Income	22,623	2,35,663
d) Guest House (Income)	68,450	23,660
e) Farm Income (Sale of Surplus Crop.)		2,60,906
f) RTI Fee	30	490
g) LD Charges	93,258	36,99,590
h) Car Charges		15,513
i) Licence Fee		2,46,526
j) Recovery-Land Lord Interim Facility	14,91,190	
<b>TOTAL</b>	<b>21,13,416</b>	<b>53,66,152</b>

for National Agri-Food Biotechnology Institute

For U.K. Mehta & Associates  
Chartered Accountants

Dated: 05.6.15  
Place: Mohali

*Suneet Verma*  
Finance Officer  
Suneet Verma / सुनीत वर्मा  
Finance Officer / वित्त अधिकारी  
National Agri-Food Biotechnology Institute  
Govt. of India / भारत सरकार  
Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग  
Mohali, Punjab / मोहाली, पंजाब-160071

*Akhilesh Kumar Tyagi*  
Executive Director  
Prof. Akhilesh Kumar Tyagi  
कार्यकारी निदेशक/Executive Director  
राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान  
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जैव प्रौद्योगिकी विभाग, भारत सरकार  
मोहाली-160071 पंजाब, भारत  
Department of Biotechnology, Govt. of India  
Punjab, INDIA

*U.K. Mehta*  
Chartered Accountants  
U.K. Mehta, FCA

**SCHEDULE-19****INCREASE/(DECREASE) IN STOCK OF FINISHED GOODS & WORK IN PROGRESS**

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Closing Stock	-	-
a) Finished Goods	-	-
b) Work-in-progress	-	-
2) Less: Opening stock	-	-
a) Finished Goods	-	-
b) Work-in-progress	-	-
<b>NET INCREASE/(DECREASE)(1-2)</b>	<b>-</b>	<b>-</b>

**SCHEDULE-20****ESTABLISHMENT EXPENSES**

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Manpower	2,10,67,295	2,25,39,042
2. Allowances & Bonus (Honorarium)		-
3. Contribution to provident fund		-
4. Staff welfare expenses/seminar	17,932	64,593
5. Contribution to other fund(specify)		-
6. Expenses on Employees Retirement & terminal benefits		-
7. Others(specify) (Outsourcing)		
<b>TOTAL</b>	<b>2,10,85,227</b>	<b>2,26,03,635</b>

for National Agri-Food Biotechnology Institute

Dated: 05.6.15

Place: Mohali



Finance Officer

Suneet Verma / सुनीत वर्मा

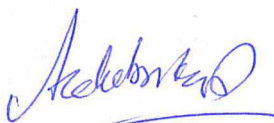
Finance Officer / वित्त अधिकारी

National Agri-Food Biotechnology Institute

Govt. of India / भारत सरकार

Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग

Mohali, Punjab / मोहाली, पंजाब-160071



Executive Director

प्रो. अखिलेश कुमार त्यागी  
Prof. Akhilesh Kumar Tyagi

कार्यकारी निदेशक/Executive Director

राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान

National Agri-Food Biotechnology Institute

जैव प्रौद्योगिकी विभाग, भारत सरकार

मोहाली-160071 पंजाब, भारत

Department of Biotechnology, Govt. of India

Mohali-160071 Punjab, INDIA

For U.K. Mehta &amp; Associates

Chartered Accountants


  
U.K. Mehta, FCA



**SCHEDULE-21**  
**OTHER ADMINISTRATIVE EXPENSES**

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Cartage & Carriage inward	800	750
2. Allowances & Bonus (Honorarium)	5,32,964	6,26,404
3. Electricity, power and Water charges	68,54,407	67,63,367
4. Rent of Interim Facility and Guest House	1,64,08,790	1,53,89,772
5. Vehicles Running & maintenance	41,448	1,58,763
6. Postage, Telephone & communication charges	5,55,974	6,95,499
7. Printing & stationery	4,79,995	5,45,896
8. Travelling & conveyance expenses	15,92,076	18,45,208
9. Outsourcing Manpower Exp	75,98,898	72,93,094
10. Legal & Professional charges	70,793	29,326
11. Advt. & publicity	2,16,810	13,32,076
12. Building Renovation		
13. Repair & Maintenance Building	13,95,197	23,54,251
14. Office & Admn Expenses	3,78,466	2,93,022
15. Others(specify)		
a) Bank charges	-	1,617
b) Guest House Expenditure	38,679	48,621
<b>TOTAL</b>	<b>3,61,65,297</b>	<b>3,73,77,666</b>

**SCHEDULE-22**

**RESEARCH & DEVELOPMENT EXPENDITURE (INCL. GRANTS, SUBSIDIES ETC.)**

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Chemical & Consumables	1,72,98,449	1,63,07,654
2. Fellowship	35,29,977	21,81,907
3. Computer Software & Accessories	22,73,580	2,59,533
4. Research Work Expenses	41,538	50,630
5. Field Expenses (Ploughing, RM & Other Job work)	73,439	2,24,626
<b>TOTAL</b>	<b>2,32,16,983</b>	<b>1,90,24,350</b>

**SCHEDULE-23**

**INTEREST**

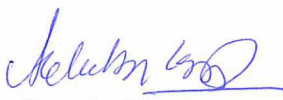
(Amount in Rs.)

Particulars	Current Year	Previous Year
1. On Fixed loans		-
2. On Other Loans		-
3. Others (Specify)		-
<b>TOTAL</b>		-

for National Agri-Food Biotechnology Institute  
Dated: 05.6.15  
Place: Mohali

For U.K. Mehta & Associates  
Chartered Accountants

  
**Finance Officer**  
Suneet Verma / सुनीत वर्मा  
Finance Officer / वित्त अधिकारी  
National Agri-Food Biotechnology Institute  
Govt. of India / भारत सरकार  
Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग  
Mohali, Punjab / मोहाली, पंजाब - 160071

  
**Executive Director**  
Prof. Akhilesh Kumar Tyagi  
कार्यकारी निदेशक / Executive Director  
राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान  
National Agri-Food Biotechnology Institute  
जैव प्रौद्योगिकी विभाग, भारत सरकार  
मोहाली - 160071 पंजाब, भारत  
Department of Biotechnology, Govt. of India  
Mohali-160071 Punjab, INDIA

  
**U.K. Mehta, FCA**  
  
Chartered Accountants  
Chandigarh

## FORM OF FINANCIAL STATEMENTS

# NATIONAL AGRI FOOD BIOTECHNOLOGY INSTITUTE

C-127 INDUSTRIAL AREA PHASE-8 S.A.S. NAGAR, MOHALI

### SCHEDULE 24

#### SIGNIFICANT ACCOUNTING POLICIES

##### A) ACCOUNTING CONVENTION

The Financial Statements are prepared on the basis of historical cost convention, unless otherwise stated and on the accrual method of accounting as per the Common Format of Accounting for all Central Autonomous Bodies.

##### B) INVENTORY VALUATION

Expenditure on purchase of chemicals, consumables, glassware, publications, stationery and other stores are accounted for as revenue expenditure, immediately on purchase of these items.

##### C) INVESTMENTS

There are no investments other than fixed deposits in the bank. No brokerage or other expenses have been incurred in making such investments.

##### D) FIXED ASSETS

Fixed assets are valued at cost of acquisition inclusive of inward freight, duties and taxes and incidental and direct expenses related to acquisition.

##### E) DEPRECIATION

Depreciation on fixed assets has been charged as per the rate prescribed in the Income Tax Act-1961 on written down value method.

##### F) MISCELLANEOUS EXPENDITURE

There is no deferred revenue expenditure during 2014-15

##### G) ACCOUNTING FOR SALES

Being an Institution there is no sales/services during the year under consideration.

##### H) GOVERNMENT GRANTS/ SUBSIDIES

As the Institute is funded by the Department of Biotechnology (DBT) , Ministry of Science and Technology, (Govt. of India) and the grants are treated as irrevocable, the same has been accounted for on receipt basis. During the FY 2014-15 , recurring grants amounting to Rs. 9,90,00,000/- has been received for the purpose as shown in schedule-13. Non-recurring Grants amounting to Rs. 26,00,00,000/- received from DBT have been shown as addition to Corpus/ Capital Fund (schedule-I).




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**I) Expenses payable and paid up to 30<sup>th</sup> April, 2015** pertaining to FY 2014-15 have been shown under expenses payable. Any expenditure which has not been claimed or for which bill has not been received pertaining to any expenditure relevant to the FY 2014-15, the same will be accounted for in the year of claim.

#### **J) RETIREMENT BENEFITS**

The institute is covered under New Pension Scheme of Government of India and is registered with the agency approved by Ministry of Finance. Institute is regularly depositing the monthly pension contribution (both employee and employer share) with appropriate authority.

#### **K) FOREIGN CURRENCY TRANSACTIONS**

Foreign Currency Transactions are accounted for at the rate of exchange prevailing on the dates of such transactions. Assets and Consumables acquired against foreign currency are recorded at the amount actually paid on their import.

For National Agri-food Biotechnology Institute

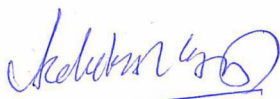
U. K. Mehta & Associates  
Chartered Accountants

  
Finance Officer

Dated: 05.6.15

Place: Mohali

Suneet Verma / सुनीत वर्मा  
Finance Officer / वित्त अधिकारी  
National Agri-Food Biotechnology Institute  
Govt. of India / भारत सरकार  
Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग  
Mohali, Punjab / मोहाली, पंजाब-160071



Executive Director

प्रो. अखिलेश कुमार त्यागी  
Prof. Akhilesh Kumar Tyagi  
कार्यकारी निदेशक/Executive Director  
राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान  
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मोहाली - 160071 पंजाब, भारत  
Department of Biotechnology, Govt. of India  
Mohali-160071 Punjab, INDIA



(U.K. Mehta), FCA



**FORM OF FINANCIAL STATEMENTS**  
**NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE**  
**C-127 INDUSTRIAL AREA PHASE-8 S.A.S. NAGAR, MOHALI**

**SCHEDULE 25**  
**NOTES ON ACCOUNTS**

The financial statement of accounts is prepared in three parts (i) The Balance Sheet. (ii) Income & Expenditure Accounts and (iii) Receipt & Payment Accounts,

**1. Receipt and Payment Accounts**

The Receipt & Payment Account carries the figures of actual receipts & actual payments of the Institute during the financial year 2014-15. It is virtually a copy of cash book / Institute's accounts. The total receipt as shown in receipt & payment account comes to Rs.39,52,18,177/- which include Rs. 35,90,00,000/- as grants from DBT and rest from other receipts.

**2. The Income and Expenditure Account**

The Income and Expenditure accounts are prepared on accrual basis. The total income is Rs. 11,89,61,200/- out of which includes Recurring Grant from DBT and rest is from Interest & Other Resources. Total Interest Income of Rs. 1,78,47,784/- includes an amount of Rs 1,27,34,352/- on account of Interest on Escrow Account as per the Utilisation Certificate issued by M/s RITES Ltd.

Total expenditure (before depreciation) comes to Rs. 8,04,67,507/- and depreciation of Rs. 4,22,61,578/- has been charged in the current FY 2014-15. A sum of Rs. 37,67,885/- being excess of expenditure over income has been transferred to Corpus/ Capital Fund (Schedule-1).

**3. Fixed Assets**

Fixed assets are stated at cost of acquisition less accumulated depreciation thereon.

**4. Depreciation**

Depreciation for the year 2014-15 has been provided and debited to the Income & Expenditure Account.

**5. Current Assets, Loans and Advances**

In the opinion of the management the current assets, loans & advances of the institute have a realizable value in the ordinary course at least to the extent shown in the accounts and the provisions of liabilities are adequate.



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## 6. Land

The Government of Punjab has provided approx. 35 acres of land at Mohali to the Institute, free of cost, on ownership basis.

## 7. Interim Facility

The institute has acquired on rental basis a portion of the building at C-127, Industrial Area, Phase VIII, SAS Nagar, Mohali on monthly rental of Rs. 13,17,343/- which has now been increased to Rs. 14,16,143/- w.e.f. February, 2015.

## 8. Exemption u/s 35(i)(ii) of The Income Tax Act,1961

The institute has been granted exemption u/s 35(i)(ii) of the Income Tax Act,1961 in the Category of 'Scientific Research Association vide notification no 21/2013 dated 20<sup>th</sup> March,2013.

9. There are no losses from casualties such as flood and fire.

10. Previous year figures have been re-grouped and rearranged where ever considered necessary to make them comparable with those of current year.

11. Government Grants have been recognized on the basis of sanctions issued by the Govt. of India.

For National Agri-food Biotechnology Institute

U. K. Mehta & Associates  
Chartered Accountants

  
Finance Officer

Dated: 05.6.15

Place: Mohali

Suneet Verma / सुनीत वर्मा

Finance Officer / वित्त अधिकारी

National Agri-Food Biotechnology Institute

Govt. of India / भारत सरकार

Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग  
Mohali, Punjab / मोहाली, पंजाब-160071

  
Executive Director

प्रो. अखिलेश कुमार त्यागी

Prof. Akhilesh Kumar Tyagi

कार्यकारी निदेशक/Executive Director

राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान

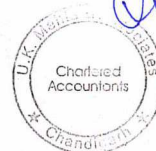
National Agri-Food Biotechnology Institute

जैव प्रौद्योगिकी विभाग, भारत सरकार

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C-127, Phase VIII, Industrial Area, S.A.S. Nagar,  
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**C-127, Industrial Area, Phase 8, Ajitgarh (Mohali), Punjab, India - 160071**

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