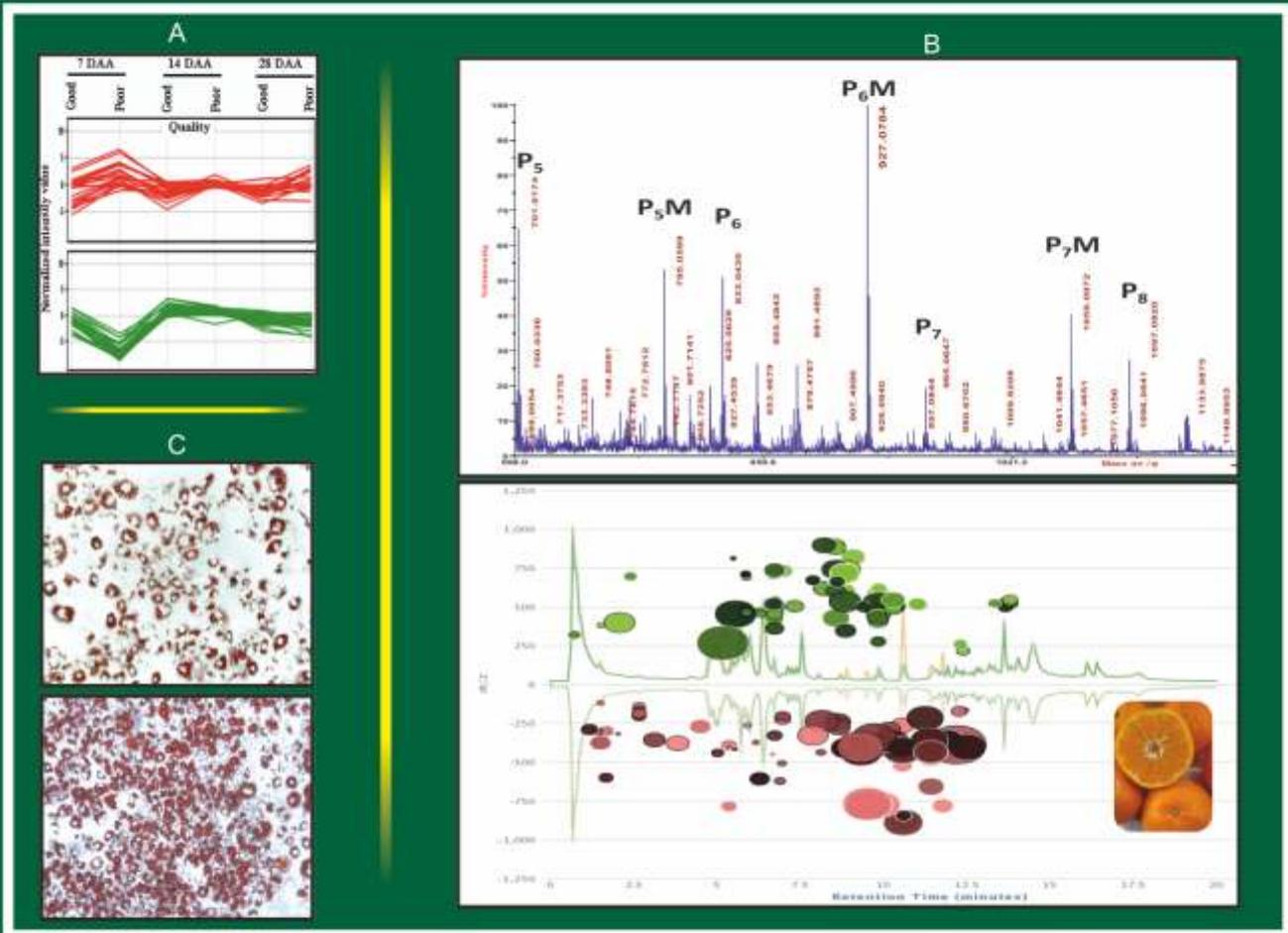


वार्षिक प्रतिवेदन ANNUAL REPORT 2012 - 2013



राष्ट्रीय कृषि-खाद्य जैव प्रौद्योगिकी संस्थान
National Agri-Food Biotechnology Institute

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

Published by:
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National Agri-Food Biotechnology Institute (NABI)
C-127, Industrial Area, Phase 8, Ajitgarh
(Mohali), Punjab, India - 160071

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Acknowledgment:
Scientists & Administration
for suggestions and for
providing information

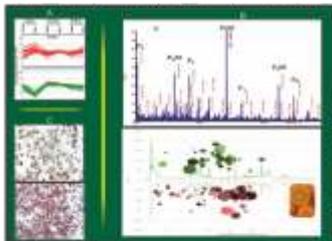


Image on Cover Page:

The cover page portrays: Amalgamation of research on (A) Agri - Biotechnology, (B) Food Biotechnology & (C) Nutrition Biotechnology. Detailed captions of respective image described on page -
A: 13, B (Upper) : 43, B (Lower) : 29 & C: 40.

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2012-2013



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FROM THE DESK OF THE EXECUTIVE DIRECTOR

The third year of NABI's growth has been a great learning experience. The team of twelve faculty members carried the Institute on the multi dimensional trajectory of growth, embodying diverse expectations of the stakeholders - DBT, State S & T Department, Governing Body, SAC & PACs, public, neighborhood institutes, and personal ambitions of the scientists and research fellows. The complex decision making processes and sub optimally engaging work environments compounded their efforts further. One issue that faced NABI unexpectedly was the mistakes in the boundary of the 50-acre piece of land allotted to the DBT by the State authorities. Decision related to allotting land with corrected boundary delayed the construction of NABI campus by several months, till Greater Mohali Area Development Authority gave the desired approvals in February 2013. The delay impacted budgetary requirements for construction of the campus. Meanwhile, the concept architectural plan had to keep waiting. A revised Expenditure Finance Committee approval had to recast the campus building plans in alignment with the allocated funds and dynamics of national economy. As NABI entered its fourth year, we were holding our breaths, awaiting the meeting of EFC scheduled on April 30th, 2013. The Institute continued to generate curiosity and excitement in the minds of its stakeholders. A number of them engaged with NABI in putting up a variety of technical queries. Some were very interesting and indicative of public expectations from NABI. An enthusiast from Mumbai inquired if NABI could guide how to make stretchable variants of Mozzarella cheese processed for low fat Indian pizza. An animal welfare organization from Bangalore inquired if NABI was researching on technologies for tissue-engineered meat or for mocking the nutrition and texture of meat through vegetarian route.

Emerging entrepreneurs made inquiries about technologies related to low cost, safe and preferably edible post harvest treatments to enhance shelf life of fruits and vegetables; nutraceuticals; non-fattening, low-glycaemic

sweeteners; high value molecules from agri surplus and agri waste; high quality food grade proteins from deoiled seed cakes; services related to food quality analyses; national preparedness to release GM food



crops etc. Institutes in the neighborhood - especially IISER, NIPER, PGIMER, IIT(Ropar) & PU very generously continued to extend their technical facilities to NABI. Emphatic discussions were held for working with the DBT to establish a functionally convergent cluster of institutes to take the Knowledge City region to a higher pedestal in biological sciences. The establishment of shared state-of-art facilities was discussed, for chemical and structural analysis of molecules, molecular interactions, experimental animal models and advanced microscopy. These would lead to taking up multidisciplinary approaches to advanced biomaterials for food, health and nutrition; clinical studies; tissue and genome engineering; non transgenic GM crops and high value molecules from agriculture.

Dr. R.S. Sangwan took charge of the Bioprocessing Unit as Chief Executive Officer in May, 2012. This expanded the opportunities for NABI to work in synergy for the development of cellular and enzymatic technologies for smarter bioprocesses. A cluster EFC proposal was submitted to DBT, with a vision to facilitate growth of the region in more daring areas of agriculture, health, entrepreneurship development, bio incubators and human resource advancement on the strength of transformational innovations through inter institutional synergy, aimed at bio based economy. The advise from the Scientific Advisory Committee and the Programme Advisory Committees, discussions with the institutes in neighborhood, and inquiries from public continued to enrich the vision and future plans of NABI and prepare it to take up bigger responsibilities and connect more visibly to

societal priorities. Some of the important research projects and initiatives taken at NABI are described in this report. Impact of the institute increased. It became more visible in form of increasing number of inquiries made by aspiring research fellows, competitively sponsored research projects awarded to NABI, research publications and attention from international and national organizations. By end of the year, NABI had 19 research fellows working with the 12 faculty members. Increasing attraction for the institute suggested that young researchers, ambitious of doing PhD at NABI were poised to increase by two fold next year, and we may soon fall short of the laboratory space. Commensurate with the increasing level of activity, the faculty was awarded five research projects (from one last year) through competitive extramural grants. Global networking increased. A new initiative, sponsored by BIRAC was taken on nutritional improvement of banana, jointly with four other national laboratories and Queensland University of Technology, Australia. Synchrotron based studies on mineral localization in wheat grain gave interesting results in joint efforts with the Slovenian and French facilities. The genomic studies on wheat continued with the Canadian group, and expanded in dimensions at NABI, covering parameters related to processing quality, starch granule structure, grain development and phytic acid pathway for iron bioavailability. A lot of transcriptomic sequencing was taken up, which will capture attention next year through publications.

By end of the year, Dr. Hari Om Yadav joined NABI as Ramalingaswamy Fellow, bringing to us his rich experience at NIH, on nutrigenomics and bioactive molecules. This will further strengthen the area of nutrition biology research at NABI, currently engaged in identifying dietary molecules that modulate metabolic responses leading to adipogenesis, and iron nutrition. Another important gain to NABI this year was Dr. Santosh Upadhyay who joined us as INSPIRE Faculty with experience in molecular mechanisms of action of insecticidal proteins. Santosh accepted to strengthen research on RNA- guided genome editing - an approach that may overtake future applications of plant molecular biology, and the development

of non-transgenic GM crops. Last year's finding at NABI, of a mastrevirus that causes dwarfing disease in wheat unraveled more surprises. This became the first discovery of a mastrevirus with associated alpha- and betasatellites, and possibly the first such virus that infects both monocots and dicots. These finding may open exceptional opportunities at NABI for developing novel suppression and expression vectors with broad host range, for functional genomic research.

Other areas that continued progress at NABI were: resolving the diversity in soluble carbohydrates and bioavailable iron in major and minor cereal grains, genes involved in seed development in litchi and custard apple, root stock-scion signaling, post harvest changes in tropical fruits and nanomaterials for bioavailability, coatings and diagnostics. Details of the research findings in various projects are given in the report. NABI faculty published six research papers this year (from one last year), based on the research projects initiated at this young institute. Based on their affiliations at other institutes, the total number of research publications by NABI faculty was close to 50 - speaking volumes about their creative potential.

The Governing Body of NABI, along with the Scientific Advisory Committee and the Programme Advisory Committees guided us in the selection and strengthening of research programmes. The invaluable support from NABI Society and DBT made it possible for a handful of faculty at NABI to place before you a rich bag of research findings, described in this Annual Report.

I would like to place on record my thanks for their visionary guidance and encouragement. NABI has embarked upon the journey on the strength of its faculty, research fellows, advisory committees and well wishers. Each of you adds immense value to our performance, creativity and growth.



(Dr Rakesh Tuli)

Executive Director

National Agri-Food Biotechnology Institute

VISION AND MISSION STATEMENT

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 Functional genomics for enhancing mineral nutrition and processing quality in wheat

1.1.1 Iron distribution and tissue - specific transcriptomics in grains of contrasting wheat genotypes

Principal Investigator:

Rakesh Tuli

Co-Investigator:

Sudhir P. Singh

Research Fellow:

Raja Jeet

Introduction:

Iron deficiency is the most prevalent micronutrient insufficiency, affecting more than 1.6 billion people worldwide, particularly women and children. In wheat grain, iron is located in the outer layer, called bran and is lost substantially during milling and processing. The wheat flour is almost devoid of iron. The prospect of developing wheat grain with iron-enriched endosperm is of great interest. We have been trying to understand the bottlenecks preventing iron translocation from the outer bran layer into the endosperm in wheat. Barriers to mineral transport within grain can be explored by examining the turnover rate for metals between different grain tissues in contrasting genotypes. Dynamics for the distribution pattern of iron (Fe), phosphorus (P) and sulphur (S) was investigated in grain tissues of wheat genotypes with contrasting grain iron concentration by using μ -X ray Fluorescence (μ -XRF) and μ -Proton Induced X-ray Emission (μ -PIXE). Proportion of divalent and trivalent forms and local chemical environment of Fe metal was determined in wheat grains by employing X-ray absorption near-edge spectroscopy (XANES).

Plant genome encodes families of metal transporters which control metal distribution in plants by different expression pattern and cellular localization. It is essential to examine differences in temporal and spatial expression of mineral metabolism related pathways in the tissues of developing grains of contrasting wheat genotypes. The expression or suppression of candidate gene/s

may lead to accumulation of bioavailable iron in the endosperm of wheat grains.

Research Progress:

1. Iron distribution and its chemical forms in seed tissues of wheat genotypes with contrasting grain iron concentration. Most of the Fe was localized in aleurone, scutellum and embryo, whereas, endosperm exhibited very low signals for Fe in the four contrasting genotypes (Figure 1).

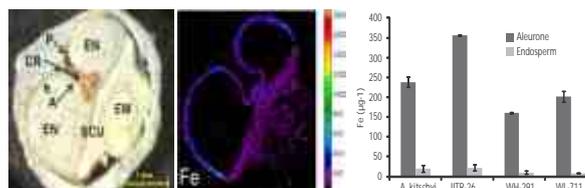


Figure 1: Imaging of iron (Fe) in the transverse section of mature wheat grain. The bar diagram showing iron concentration aleurone vs endosperm in four genotypes. EN - endosperm, A - aleurone, SCU - scutellum, EM - embryo, CR - crease, P - peripheral tissues (Pericarp and seed coat).

2. Iron distribution pattern in specific cell types of crease region which transports minerals from maternal to filial grain tissues (vascular strand, nucellar projection, aleurone transfer cell) showed contrasting differences between the high and low iron genotypes. (Figure 2). Fe and P were localized in different tissues of crease region in wild and wheat land race, whereas, co-localized in aleurone transfer cells in wheat cultivars. In addition, high concentration of S was noticed in the nucellar projection in the low iron wheat cultivars, whereas, it was consistently present in all the tissues of mineral transport route of high iron genotypes (Figure 2).
3. Bulk XANES on intact grains resolved the energy position of the Fe K-edge in the intact grain samples close to the edge position of Fe³⁺ compounds (Figure 3A). The linear combination fitting analysis (LCF) determined iron speciation as being, 77-86 (± 3) % ferric and the remainder as ferrous in mature seeds of the examined genotypes

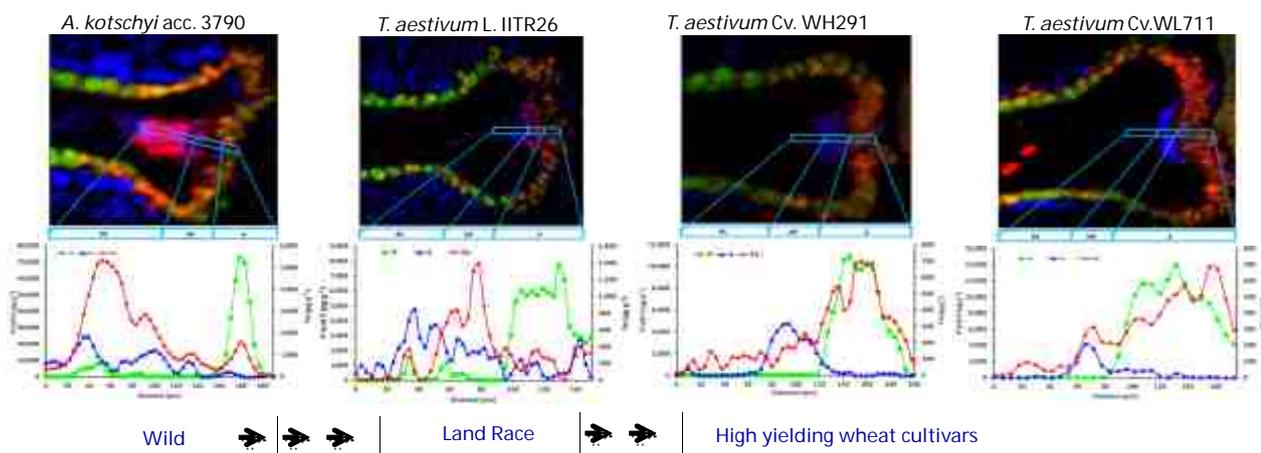
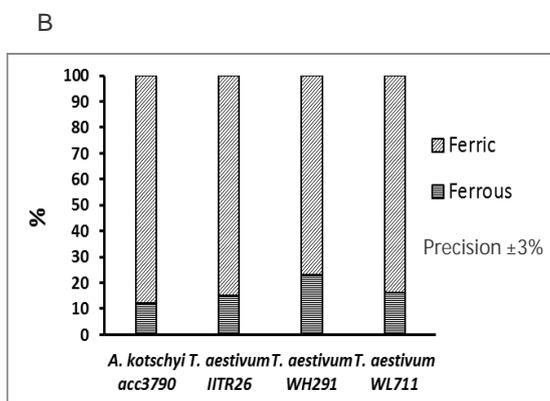
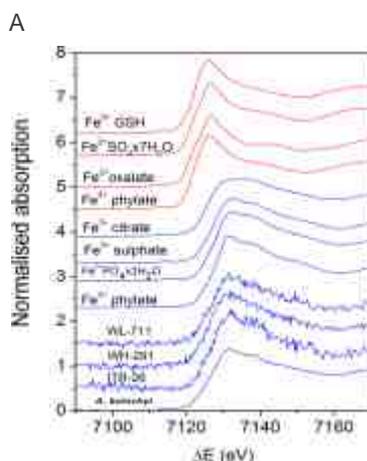


Figure 2: Iron (red), phosphorous (green) and sulphur (blue) co-localization map and μ -PIXE profile in crease tissues (vascular tissue, nucellar projection, aleurone transfer cells) of grains of four genotypes.

(Figure 3B). It also revealed the abundance of oxygen (O), phosphorus (P) or sulphur (S) in the local chemical environment of Fe metal in grains, as Fe-O-P-R and Fe-O-S-R coordination (Figure 3C). The results suggest the storage of ferrous iron, possibly as Fe (II)-phytate. Most of the iron was in Fe (III) form in association with O and P. About 19 % of trivalent iron was in coordination of O and S. Thus P and S are major constituents of iron binding compounds in grains and phytic acid as a predominant form of Fe, O, P compounds in grains.

4. Tissue specific differential transcriptomes of aleurone and endosperm of contrasting wheat genotypes have been sequenced. The transcriptomes analysis of the tissues and specific cell types of developing grain is in progress.
5. Aleurone and endosperm specific expression cassettes are being utilized to restrict the sequestration of iron in aleurone and to enhance the efflux of iron from aleurone to endosperm (Figure 4).



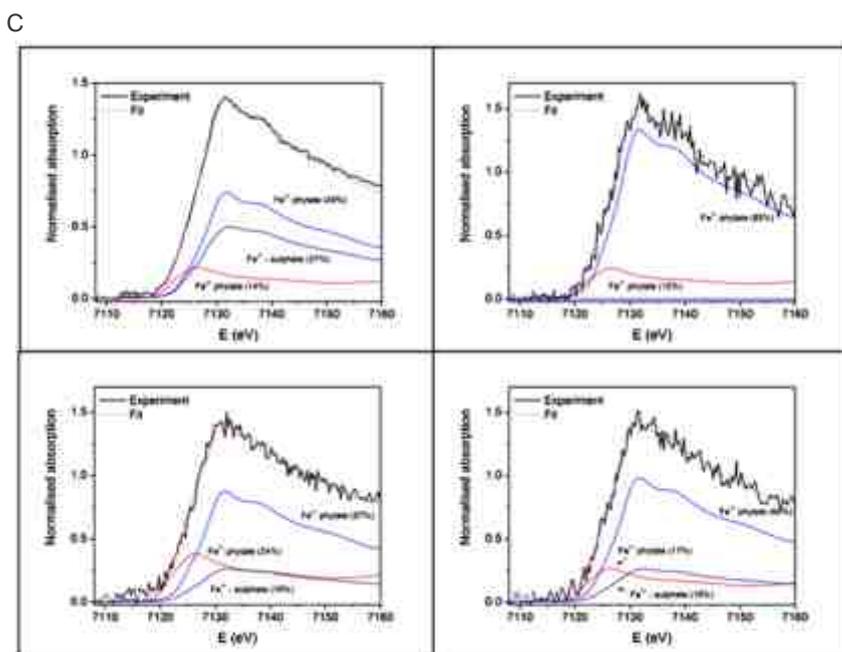


Figure 3: Spectral analysis and proportion of different forms of iron in wheat grains. (A) Fe K- edge XANES spectra measured on the intact grains of *A. kotschy* acc. 3790. (B) Proportion of Fe³⁺ and Fe²⁺ iron in the grains. (C) Linear combination fitting analysis solid black lines, experiment; dashed magenta line, best-fit linear combination of XANES profiles of Fe (II) phytate, Fe (III) phytate, and Fe (III) sulphate.

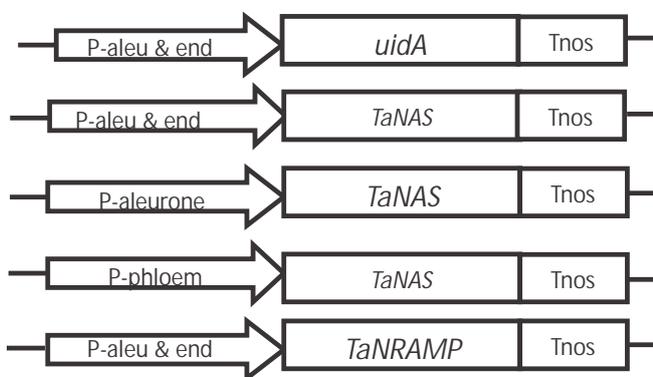
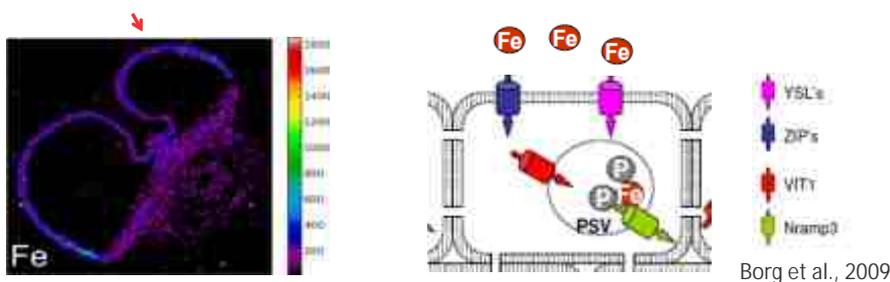


Figure 4: Tissue specific expression of candidate genes to restrict the sequestration of iron in aleurone and to enhance its efflux from aleurone to endosperm.

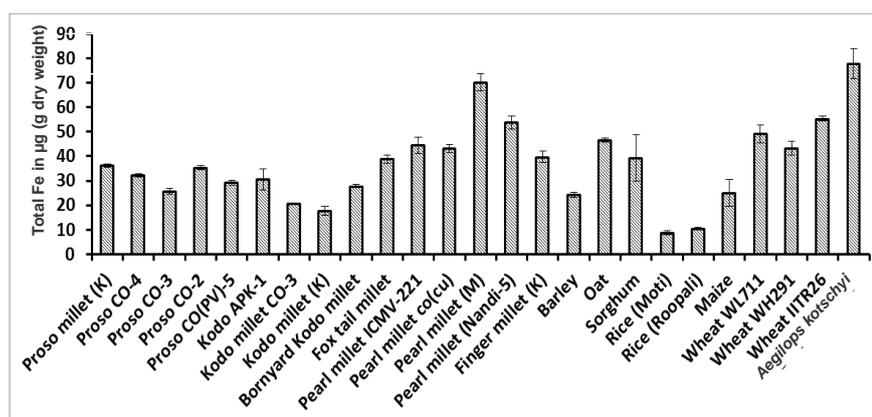


Figure 5: Iron concentration in different cereal grains.

6. A variety of cereal grains (wheat, rice, millets, maize etc.) are being analyzed for total iron concentration (Figure 5). The investigation of iron bioavailability in the cereal grains is in progress.

Salient Achievements:

1. Differences were noticed in tissue specific relative localization of Fe, P and S among the different genotypes, with contrasting grain iron content, suggesting possible effect of localization pattern on iron bioavailability.
2. A shift in iron distribution from maternal to filial tissues of grains was observed during the evolution of wheat from its wild relatives to the present-day cultivated varieties. The study suggests the value of detailed physical localization studies in varietal improvement programmes for food crops.
3. The proportion of divalent and trivalent forms of Fe in the wheat grains is, 77-86 (±3) % ferric and the remainder as ferrous. The study also revealed the abundance of oxygen, phosphorus, and sulphur in the local chemical environment of Fe in grains, as Fe-O-P-R and Fe-O-S-R coordination.

Future Perspectives:

1. Tissue specific transcriptomics in developing wheat grains.
2. Bioavailability and localization studies of iron in different cereals grains (wheat, rice, millets, maize etc.)

3. Development of transgenic wheat with iron enriched endosperm.

1.1.2 Gene discovery for improvement of processing and nutrition quality in wheat

Principal Investigator:

Joy K. Roy

Co-Investigator :

Shrikant Mantri

Research Fellows:

Anuradha Singh

Monica Sharma

Introduction:

Wheat is one of the most important staple food crops, and wheat flour is processed into a wide range of baked and processed foods. The present high yielding varieties require improvement in processing and nutrition related quality traits to meet the increasing demand of healthy wheat diets by consumers and better processing quality by baking and processing industries. Better understanding of genes and regulators of metabolic pathways and their interaction is required for the improvement of nutrition and processing quality.

Research Progress:

1. A set of 40 Indian wheat varieties including C306, LOK1, Sonalika, and WH291 are being evaluated for processing and nutrition related traits, specially starch, amylose and

amylopectin structures, grain and starch proteins, and phenolic acids.

- The gene expression data of about 25,000 unigenes (Affymatrix wheat arrays) were collected on a set of four Indian wheat varieties differing in processing quality during three seed development stages, namely, 7, 14, and 28 days after anthesis (DAA).
- Two-way ANOVA analysis of the gene expression data identified a set of 35,472 probesets after multiple testing corrections ($p < 0.05$). The variation in gene expression was partitioned into three sets of genes i.e.

differential expression between good and poor quality varieties. Several wheat seed storage protein genes such as glutenins and gliadins showed differential gene expression at the early stage of seed development.

- The expressions of the 236 differentially expressed genes were clustered to identify genes with similar expression profiles at three development stages (7, 14, and 28 DAA) in each of three groups of gene sets (quality, seed development, and interaction (Figure 7)
- The processing quality was affected by size

Table 1: Summary of two-way ANOVA of the expression data of the 60,130 probesets

Number of probesets whose expression varied at corrected <i>p</i> values			
Parameters for variation	<i>P</i> < 0.05	<i>P</i> < 0.01	<i>P</i> < 0.0010
Processing quality	3,126	772	189
Seed development	34,604	27,555	20,813
Quality x development	1,732	350	40
Expected by chance	156	7	0

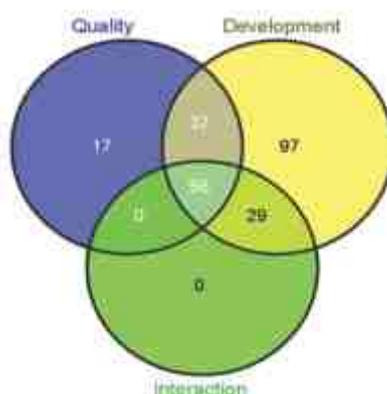


Figure 6: Partition of variation in expression of the 236 genes (at least 10-fold), between good and poor processing quality varieties into quality, seed development, and interaction (quality and seed development).

variation in expression due to quality, seed development, and interaction (quality seed development) (Table 1, Figure 6).

- A set of 236 potential genes (out of 35,472) was identified, showing at least 10-fold

and distribution of starch granules. Starch granules were visualized under light microscope and scanning electron microscope. The four varieties showed differences in size and distribution. The starch structures are currently being investigated in a set of 40 Indian wheat

varieties.

7. Amylose content was estimated in a set of 40 wheat genotypes which ranged from 22 to 30% (Figure 8). Further, structural and functional properties of starch are being estimated in these varieties.
8. Amylose content was estimated during seed development stages in four Indian wheat varieties differing for processing quality (Figure 9). The proteins within starch granules have been extracted from these varieties for proteomic analysis.
9. Free phenols and bound phenols were extracted from the seeds of the two diverse

wheat varieties, C306 and Sonalika. These showed differences in free phenolics than bound phenolics. The wheat variety C306 (86.9 to 104 GAE/100g) had more free phenolic content in comparison with Sonalika (63.9 to 68.8 GAE/100g). The two varieties showed LC-MS based differences in the types of phenolic compounds.

Salient Achievements:

1. A set of 236 potential genes were identified for processing quality using wheat microarrays. The subset of 40 wheat varieties will be utilized for preliminary association studies.

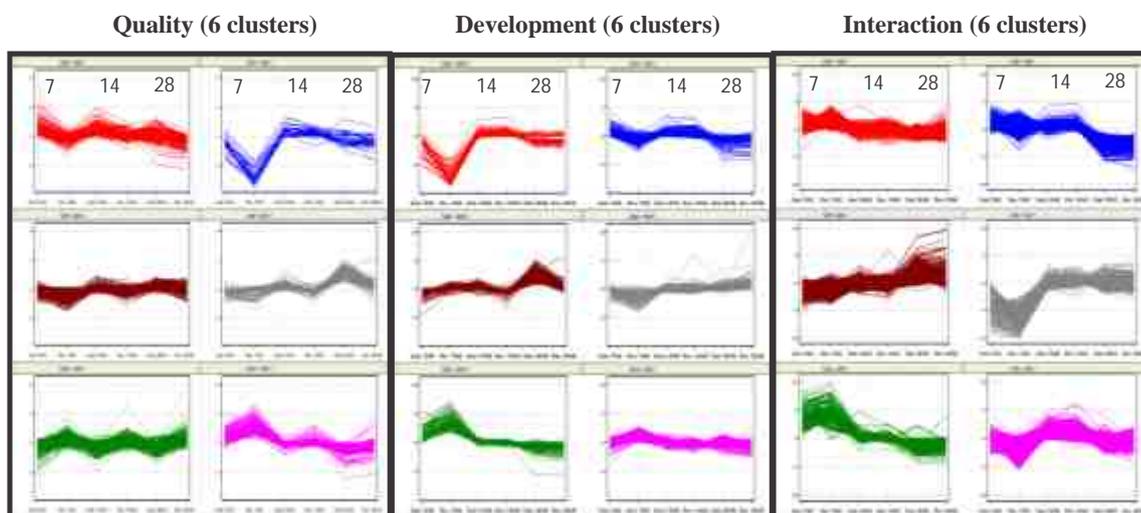


Figure 7: Clustering of the 236 differentially expressed genes into 6 clusters to identify genes with similar expression profiles in good and poor quality varieties at three development stages (7, 14, and 28 DAA) in three sets of genes (quality, development, and interaction).

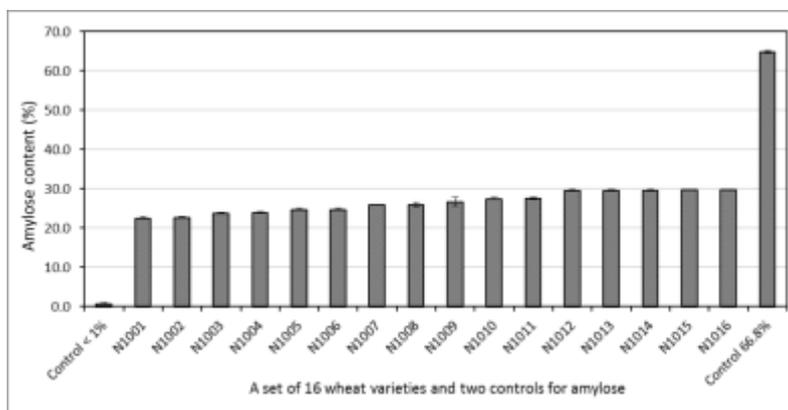


Figure 8: Variation in amylose content in a subset of 16 Indian wheat varieties.

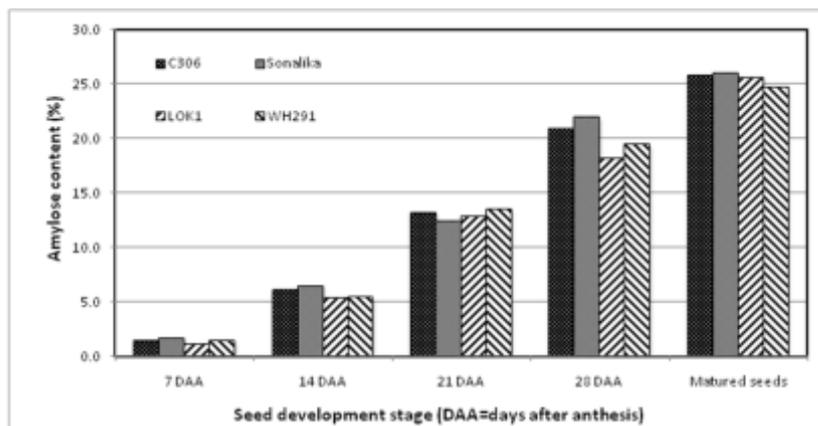


Figure 9: Variation in amylose content during seed development stages in four Indian wheat varieties differing for processing quality.

2. Transcriptome and small RNA sequencing have been done on 454 and Hiseq platform to identify potential pathways and regulators for processing quality.
3. An EMS induced M2 population has been developed for functional genomics studies.

Future Perspectives:

1. Starch and phenylpropanoid pathway genes are being studied for sequence variation.
2. Metabolic profiling will be performed on HPLC and NanoLC-QTRAP to identify metabolites and their variation in a subset of Indian wheat varieties.
3. Proteomic analysis of starch proteins are being done on NanoLC-Triple-TOF.
4. Mutation analysis will be conducted on an EMS induced M2 population to develop TILLING population

1.1.3 Molecular characterisation of wheat dwarf India virus and development of virus induced gene silencing (VIGS) vector and its applications in studying gene function in wheat

Principal Investigator:

Rakesh Tuli

Research Fellows:

Jitendra Kumar
Jitesh Kumar

Vishnu Shukla
Shashank Singh

Introduction:

The large and complex genome and recalcitrance to transformation limit the use of functional genomics approaches, such as mutagenesis, T-DNA knockout libraries, T-DNA activation tagging or transposon gene-tagging, in wheat. Despite the demonstrated usefulness, RNAi in wheat cannot yet be applied to large scale projects, as it requires the generation of transgenic lines. As an alternative, VIGS is of great importance in wheat as it can potentially speed up the characterization of candidate genes. A recombinant virus that infects plant tissue and spreads systemically can be very useful in the expression of small RNA and silencing of targeted endogenous genes. The target transcript is degraded by Post-Transcriptional Gene Silencing (PTGS). VIGS can validate the function of a specific gene within a single generation and obviates the need for screening large populations to identify mutations in specific genes. Being a transient method, it does not require the generation of stable transgenic plants. The project aims at developing a good VIGS vector for wheat.

Research Progress:

1. Utilization of WDIV-VIGS vector for studying function of genes involved in

mineral translocation and localization in wheat: Candidate genes have been shortlisted from literature, having positive and negative roles in mineral uptake and accumulation in grains. Silencing of at least five promising candidate genes will be done constitutively as well as specifically in developing seed. Methods are being developed to introduce virus infection in developing wheat grains, targeted to silencing of genes responsible for accumulation of minerals in bran.

2. **Functional annotation of upregulated and downregulated transcripts upon WDIV and satellite infections:** Unigenes upregulated and downregulated more than five folds are being analyzed for finding their putative functions in virus infection and symptom recovery.
3. **Investigation of the silencing suppressor activity of WDIV-ORFs and associated satellites for development of efficient VIGS and VOX (virus mediated overexpression) system:** Transient expression constructs are being developed for investigating the suppressor activity of viral open reading frames (ORFs) as-well-as satellite ORFs.

4. **Prevalence of WDIV in the country:** A survey has been conducted for investigating the incidence and prevalence of wheat disease caused by WDIV in different parts of the country (Figure 10). Based on the preliminary results WDIV has been detected in all parts of the country investigated during survey. Association of the satellites and estimation of yield loss are being analyzed.

Salient Achievements:

1. Detection and characterisation of first mastrevirus in India: The WDIV detected in this study is the first mastrevirus reported from India. This is also the first wheat virus from India for which nucleotide sequence has become available.
2. Detection of the first mastrevirus associated satellite: The study reported the first mastrevirus associated satellites (alpha- and betasatellite). The associated satellites helped in virus accumulation.
3. Development of the VIGS vector: This is the first indigenous VIGS vector for functional genomics of wheat. The WDIV-VIGS produced no/weaker symptoms on wheat; hence it will be useful for the functional genomics of wheat.

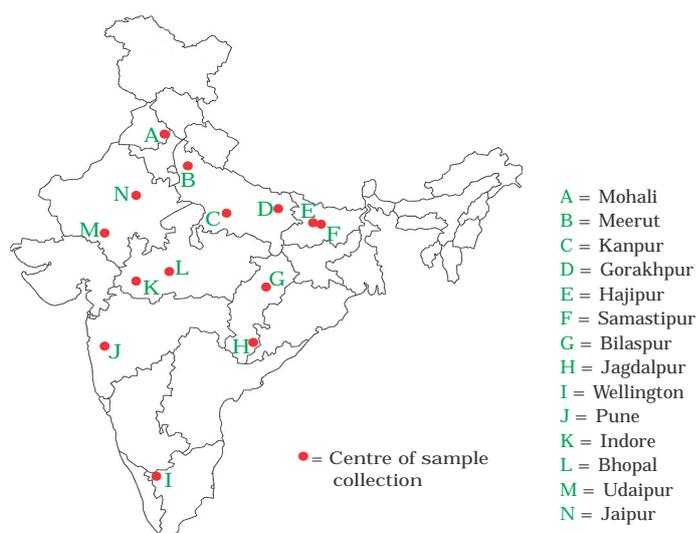


Figure 10: Different locations in India from where the suspected wheat plant samples were collected for the detection of the WDIV.

Future Perspectives:

1. Functional genomics of the genes involved in iron biosynthesis pathway: Candidate genes have been shortlisted from transcriptome sequencing as well as literature. They are supposed to have positive and negative roles in mineral uptake and accumulation in grains. Silencing of the candidate genes will be done in order to explore their function.
2. Development of kit for the detection of the WDIV at field scale: We are using DNA and protein based approaches for the development of detection kits.
3. Study of the mechanism of pathogenesis of the WDIV and satellites: Expression analysis is being done to know the plant regulators involved in the pathogenesis of the WDIV and satellites.
4. Development of approach for generating resistance against WDIV and other geminiviruses: Resistance against geminiviruses has been a challenge and at present approaches to achieve durable resistance are not available. Innovative approaches will be attempted for developing resistance against WDIV and other geminiviruses.

1.1.4 Efficient genetic transformation of wheat

Principal Investigator:

Siddharth Tiwari

Research Fellow:

Anshu Alok

Introduction:

Efficient regeneration and genetic transformation protocols are pre-requisites for crop improvement through genetic engineering. The aim of the study undertaken is the establishment of efficient genetic transformation protocol for wheat. The protocols for callus as well as direct multiple shoot mediated *in-vitro* regeneration and *Agrobacterium*-mediated genetic transformation of wheat have been optimized. Transgenic plants with reporter (GUS-Intron) gene expression have

been developed. PCR analysis of T₀ transgenic plants confirmed the transgene integration in the genome. Further molecular analysis of transgenic plants and improvement of the efficiency of transformation are in progress.

Research Progress:

1. **Establishment of genetic transformation:** Several factors were considered for the optimization of *Agrobacterium*-mediated genetic transformation. Mature embryo derived callus and direct multiple shoot induction based *in-vitro* regeneration were used for reporter (GUS-Intron) gene expression studies in transgenic wheat.
2. **Stable expression of reporter (GUS-intron) gene:** The stable expression of reporter gene was noticed as blue colour on the callus-derived and without callus mediated shoot buds, respectively (Figure 11 A & C). No expression of GUS was noticed in the control shoot bud(s) and elongated shoots (Figure 11 B & D).
3. **Detection of transgenic plants:** Total genomic DNA was isolated from *in-vitro* raised transgenic and non-transgenic plant leaves by using DNeasy Plant Maxi kit (Qiagen). Putative (T₀) transformants were screened for the presence of *gusA* and *hptII* by using gene specific primers. The PCR product was fractionated by electrophoresis on a 0.8% agarose gel, detected by ethidium bromide staining and photographed under ultraviolet light. The results showed amplification of the predicted 800 bp *gusA* as well as 1027 bp *hptII* fragments of genes in transgenic plants (Figure 12 A & B). The positive control (PCR with plasmid PCAMBIA1301) also gave similar size amplicons (lane 4). No amplification was observed with two negative controls, one was containing reaction mixture without template (lane no. 2) and other was untransformed (lane no. 3) plants (Figure 12).

Salient Achievements :

1. Callus mediated *in-vitro* regeneration in the

four high yielding (PBW621, PBW550, HD2697 and Lok1) cultivated varieties of wheat was optimized.

2. Direct multiple shoot induction without an intermediary callus in high yielding (PBW621, PBW550) cultivars of wheat was optimized.
3. Protocol for *Agrobacterium*-mediated genetic transformation for the transient and

stable expression of reporter (GUS-Intron) gene was optimized.

Future Perspectives :

1. Molecular and segregation studies of transgenic plants with reporter (GUS-Intron) gene expression are in progress.
2. Development of transgenic wheat with reduced phytic acid.

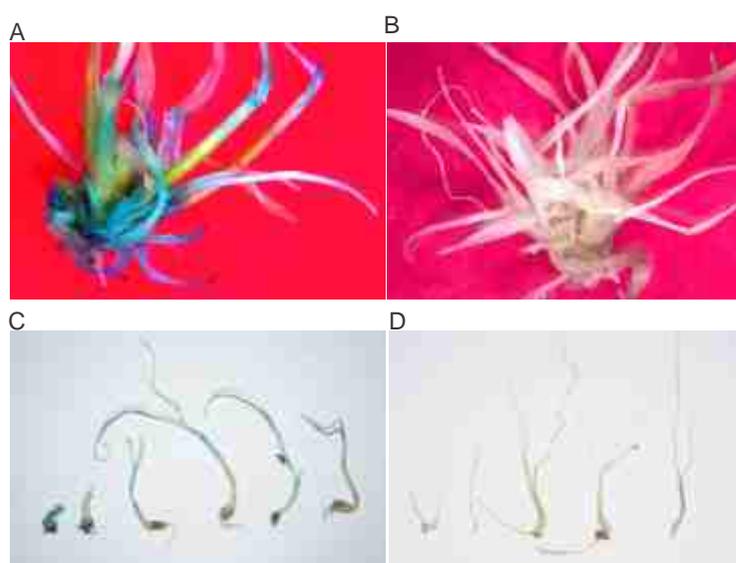


Figure 11: GUS histochemical assay. (A) Stable expression in callus-derived shoot buds (blue color). (B) Non-transgenic shoot buds. (C) Transformed germinated shoots (blue color). (D) Non-transformed shoots.

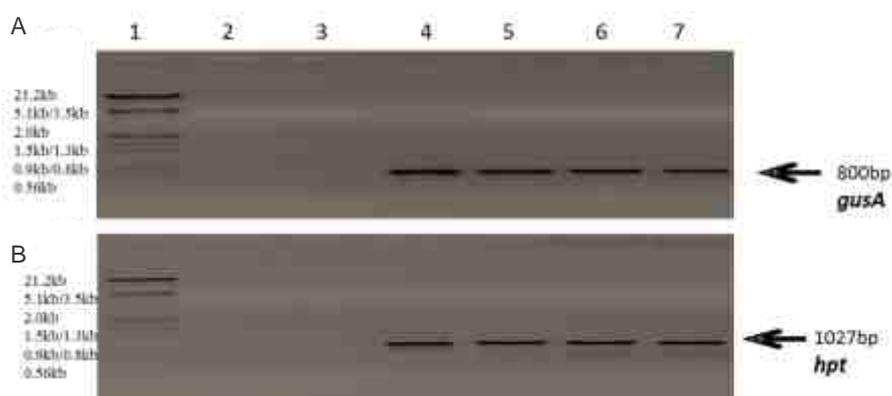


Figure 12: Analysis of Transgene Plants: PCR analysis of genomic DNA from T0 *gusA* (A) and *hptII* (B) positive transgenic plants. Lane 1: Lambda DNA *HindIII* & *EcoRI* digest. Lane 2: Without template (DNA). Lane 3: Untransformed plant. Lane 4: Positive control (pCAMBIA1301 vector). Lane 5-7: Transgenic plants showing 800 bp amplicon from *gusA* gene and 1027 bp amplicon of *hptII* gene.

1.1.5 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 Aggrawal

Shivani Sharma

Introduction:

Low phytic acid (*lpa*) has become a desirable trait for grain crops. Previous studies indicated that some of the early and late genes involved in phytic acid (IP₆; PA) pathway could be potential targets to achieve low phytic acid plants with enhanced micronutrient bioavailability. Such approach has been successful in barley, maize, soybeans and other legumes, but considerable progress is lagging in wheat. Understanding the genes involved in biosynthesis pathway and metabolically engineering them to control the accumulation of PA in plant seeds is of importance for molecular breeding of crop plants especially in wheat. In this research work an attempt will be made first to identify the genes/signaling components that contribute for the PA accumulation in wheat grains. The identified genes will then be evaluated functionally for their effect on phytic acid accumulation and subsequently will be targeted for stable suppression or mutations so as to reduce the total

phytic acid content in the grains, thus expecting an increase in iron bioavailability.

Research Progress:

1. To identify the late PA pathway genes homologs of wheat, Blastx analysis was performed using the query sequences from barley, maize and Arabidopsis. Cereal DB (<http://www.cerealsdb.uk.net/>) and EST sequences from NCBI were used to gather sequence information. The corresponding EST sequences were used to design primers for PCR amplification and further sequence analysis was performed.
2. We utilized barley (AM404177; *HvIpk*) and rice (AM410634; *OsIpk*) gene sequences to search wheat homologs. Our searches resulted in identification of four est sequences
viz. *TaItpk1* (UniGene ID: Ta.70767), *TaItpk2* (EST ID: CA618510.1), *TaItpk3* (UniGene ID: Ta.39455) and *TaItpk4* (UniGene ID: Ta.36061). Similarly, for *Ta-IPK* rice (*OsIpk1*; AK102842) and Arabidopsis (*AtIpk1*; ATG5G42810) were used to perform blast against wheat EST and cereal database. Single *TaIpk1* from wheat with a UniGene ID: Ta.41955 was identified. The predicted amino acid of *TaIpk1* showed identity with *OsIpk1*, *ZmIpk1* and *AtIpk1* of 83.4, 78.7 and 53.2 % respectively.
3. Rice (*OsIpk2*; AK072296) and Arabidopsis (*AtIpk2*; AY147935) were used to perform

Table 2: List of putative phytic acid biosynthesis pathway genes identified from C306

Name	Annotated Function	Unigene ID
<i>Ta-ITPK-1</i>	Inositol tetra-phosphate kinase	Ta.70767
<i>Ta-ITPK-2</i>	Inositol tetra-phosphate kinase	CA618510.1
<i>Ta-ITPK-3</i>	Inositol tetra-phosphate kinase	Ta.39455
<i>Ta-ITPK-4</i>	Inositol tetra-phosphate kinase	Ta.36061
<i>Ta-IPK-1</i>	Inositol pentakis-phosphate kinase	Ta.41955
<i>Ta-IPK2</i>	Inositol tris-phosphate kinase/inositol polyphosphate kinase	Ta.35113

blast against wheat EST and cereal database. Balstx analysis identified only one *TaIpk2* (UniGene ID: Ta.35113) gene having all the conserved domains as in other gene family. Domain analysis reveals the presence of typical inositol phosphate and ATP binding site (Table 2).

4. Phytic acid and free phosphate quantification was performed during the grain filing stage of C306. Our analysis suggests an increase in PA accumulation with the development of the grain. From 35

negatively correlated with the free phosphate.

Future Perspectives :

1. Targeted silencing will be done for the genes involved in late stage of PA synthesis. Generated transgenic lines will be evaluated for the bioavailability of iron.
2. Gene/s involved in the synthesis of IP_7/IP_8 will be studied and their possible role in the regulation of enhanced phosphate uptake in seeds will be studied.

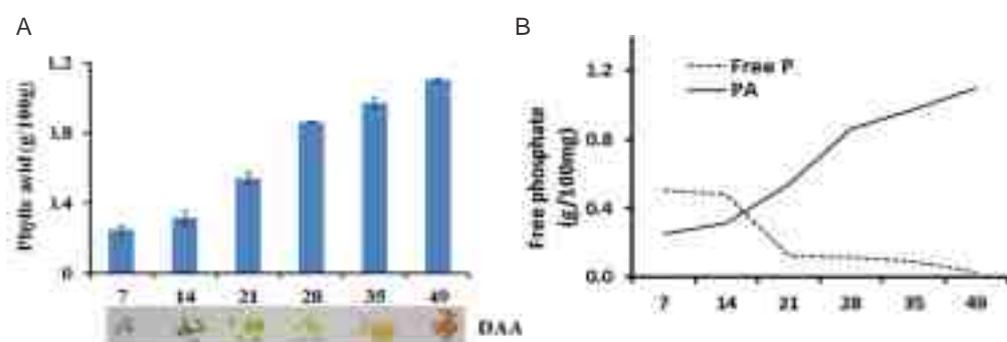


Figure 13: Accumulation of PA (A) and free phosphate (B) during the grain development in wheat cultivar C306. The error bars indicate standard errors.

to 49, the accumulation rate of PA was lower than during the early stages of grain development (Figure 13A). On the contrary free phosphate level drastically decreased from 14 DAA till the stage of seed maturation i.e. 49 DAA (Figure 13 B).

These studies coupled with detailed gene expression analysis during seed development and tissue level will help in prioritizing the target candidate genes for their possible functional role in wheat.

Salient Achievements :

1. Genes for the wheat phytic acid pathway were identified and further characterization during grain development is underway.
2. PA analysis suggested a linear increase in the IP_6 accumulation with the grain development and this accumulation is

1.2 Accelerated breeding for quality improvement

1.2.1 Marker development and its utilization for accelerated breeding

Principal Investigator:

Monika Garg

Research Fellow:

Rohit Kumar

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 upon their processing quality (milling and baking characteristics), marker development and utilization of validated markers.

Processing quality of wheat depends upon 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 and 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.

Research Progress:

1. Marker discovery

Three traits (bread, biscuit and chapatti making) and factors responsible for their good quality are under study. Involvement of granule bound starch synthase 1 (GBSS1) enzyme in the formation of good quality chapatti making was identified in this work. Briefly, transcriptomics studies at different developmental stages of good (C306 and LOK1) and poor chapatti making varieties (Sonalika and WH291) indicated that among the earlier reported genes affecting wheat processing quality, GBSS1 was several folds down-regulated in good chapatti varieties. GBSS1 is involved in amylose starch synthesis. Genomic variation of GBSS1 was studied in several wheat cultivars. Allelic variation of GBSS1 in Indian cultivars indicated that GBSS-A1 and GBSS-D1 genes were non-polymorphic and present in all the cultivars studied.

GBSS-B1 gene was polymorphic based on presence/absence of alleles. Preliminary study on different cultivars and lines with known chapatti making quality indicated that absence of GBSS-B1 gene was correlated with good chapatti making quality. Among starch pasting characteristics, breakdown in viscosity was found to be correlated with good chapatti making quality. For improvement of biscuit making quality major genes (Puroindoline and HMW glutenin genes) responsible for grain softness were characterised in selected lines. PCR multiplexing for selection of grain softness genes in the backcross lines were standardised.

2. Accelerated breeding

For improvement of bread making quality, we are utilizing wild species of wheat and their genetic stocks (addition lines, substitution lines and translocation lines) as donors. For improvement of biscuit making quality, we are utilizing soft wheat landraces IITR67 as donor of grain softness. For chapatti making old cultivars C306, LOK1 (well known for their good chapatti quality) are being utilized. Factors/genes from the donors are being transferred to agronomically superior background by accelerated breeding approach.

1. For improvement of chapatti making quality, good chapatti making old cultivars were crossed with high yielding recent cultivars (PBW343, PBW550 and PBW621). BC₂ seeds were backcrossed at DWR Regional Station, Dalang Maidan, Lahaul, Himachal Pradesh during off season. BC₃ plants were screened for absence of GBSS-1B in the main season at NABI, Mohali. Negative plants were selfed. BC₃F₂ seeds will be sown at Dalang Maidan, for generation advancement.
2. For improvement of biscuit making quality donor landraces were crossed with high yielding recent cultivars (PBW343, PBW550 and PBW621). Crossed seeds were backcrossed at Dalang Maidan in the

off season. BC₃ plants were selected based on presence of puroindoline gene *PinaD1a* and selfed. BC₃F₂ will be checked for grain hardness and selected seeds will be sown at Dalang Maidan for generation advancement.

3. For improvement of bread making quality wild species/genetic stocks of *Ag. elongatum*, *Ae. longissima*, *Ae. searsii* and *Ag. intermedium*, are being utilized. Our target is to transfer HMW-GS genes related to high grain strength from wild species to chromosome 1A of wheat (translocation lines), as later has some alleles that contribute negatively to bread

- i. During this study substitution line of *Ae. longissima* 1S¹(1A) was developed in the background of Chinese Spring and checked for quality traits including farinograph, hardness, gluten and bread loaf volume. Results indicated that highly significant increase in gluten index was accompanied by relatively smaller increase in bread loaf volume and loaf quality (Table 3). It might be due to the fact that Chinese Spring is a soft wheat and not suitable for bread making. Good bread requires hard wheat with strong gluten. It will be interesting to transfer this substitution and create translocation in hard wheat background and study its

Table 3: Grain, flour and product properties of DSL1S1(1A) in comparison to Chinese Spring

Line	Protein %	Hardness (kg)	Gluten			Bread	
			Wet	Dry	GI	Loaf Vol.	Score
CS	11.1 ^a	10.0 ^a	23.3 ^a	9.3 ^a	7.7 ^a	450.0 ^a	4.5 ^a
1S1(1A)	11.4 ^a	7.4 ^b	27.3 ^b	8.8 ^b	82.6 ^b	465.3 ^b	5.1 ^b

^a and ^b Indicates significantly different values at p<0.05

making quality. For this purpose marker assisted selection and transfer to agronomically superior cultivars is in progress. Genetic material received for this purpose (addition, substitution and translocation lines) was screened by sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) of storage proteins from endosperm half of seeds. Emryo halves of the seeds expressing selected wild species' HMW-GSs were selected for sowing in the NABI field. Crossing/backcrossing of selected material carried out. Several rounds of selection, sowing and crossing were carried out to generate genetic lines in order to understand the role of different genes in processing quality. Attempts were also made to transfer useful genes to high yielding wheat cultivars.

effect on quality.

- ii. Chromosome specific translocation line of *Ag. Elongatum* (i.e. long arm of chromosome 1E translocated into short arm of chromosome 1A i.e. 1EL(1AS)) was used as starting material. Accelerated breeding was performed and HMW glutenin protein of *Ag. elongatum* was utilized as marker. This line was backcrossed three times with soft wheat cultivar Norin 61 (N61) and further selfed. BC₃F₃ plants were morphologically similar to recipient cultivar N61 (Figure 14). Seeds of this line were studied for rheological and bread making quality. This line showed significantly improved gluten index and loaf score. But loaf volume was lower than the recipient cultivar due to lower protein content in this line. Moreover N61 is a soft wheat



Figure 14: Morphological similarity of spikes of BC₃F₃ translocation line 1EL(1AS) in comparison to background cultivar N61.

cultivar. We are now crossing this line with high yielding hard wheat cultivars, to exploit its positive effect on bread making quality at the commercial level.

1.2.2 Development of Puroindoline gene database of Indian cultivars

Puroindoline genes have been reported to be strongly associated with grain softness; a trait associated with processing quality of wheat. Two genes *Pina-D1* and *Pinb-D1* located on chromosome 5D have been found to be associated with grain softness. *Pina-D1* and *Pinb-D1* encode small (13 kDa) protein called puroindoline A and B (PINA and PINB) which exhibit the characteristics of alpha helical structure, ten cysteine residue, a tryptophan rich domain and basic nature (PI 10.5). The soft texture of the common wheat requires both wild type PINA and PINB. Diverse alleles with single nucleotide polymorphism (SNPs), small insertions/deletions and /or megabase deletions have been associated with hard texture of common wheat. There is no information available on sequences of puroindoline genes in the Indian germplasm. This study was planned to generate a database of puroindoline genes of around 500 Indian cultivar/lines that can be utilized by Indian breeders. This will also help to find out structure function relationship and involvement of different genes in grain hardness.

PCR amplification of *Pina-D1* gene from 551 Indian germplasm indicated amplification and thus presence *Pina-D1a* functional allele in 53 lines i.e. 9.6% of total lines. Sequencing of *Pina-D1a* allele from these cultivars revealed 2 new *Pina-D1* alleles (Table 4). Sequence variations in the new alleles of *PinaD1* were located in the signal peptide. One at position-15 caused no functional change (Pina new1), other at position -1 and -7 may or may not have resulted in functional change being only slightly lower in hardness (SKCS 57) than the lower limits of hard wheats (>60). *Pinb-D1* allele in all the new *Pina-D1* allele cultivars was non-function *Pinb-D1b* type.

Research Progress :

1. PCR amplification of *Pinb-D1* gene from 551 Indian germplasm indicated amplification in all the lines studied. Sequencing of *Pinb-D1* gene from different Indian cultivars indicated sequence variations. *PinbD1* gene was sequenced from 84 cultivars. Out of these, 63 cultivars had functional *Pinb-D1a* allele. This allele was present in two different combinations of *PinaD1* i.e. eleven with functional *PinaD1a* and fifty two (largest number) with non-functional *PinaD1b* allele.
2. Non-functional *Pinb-D1b* allele resulting from guanine to adenine nucleotide mutation leading to glycine to serine amino acid mutation at 46th position was observed in nine cultivars. *PinbD1e* allele that results from glycine to alanine nucleotide mutation leading to change of tryptophan amino acid to stop codon at 39th position was present in seven wheat cultivars with *PinaD1a* allele.
3. In addition, four new mutations (*Pin b-D1new1* to *Pinb-D1new4*) were identified as listed in table 2. Among the new alleles at *pinbD1* locus, one had stop codon at 49th position and resulted in functional change (*Pin b-D1new1*), other one is in the hydrophobic domain at 95th position and this also resulted in functional change (*Pin b-D1new2*). Two of the new alleles (SNPs) did not result in functional change, as one

nucleotide change did not result in amino acid change (*Pin b-D1new3*) and in other case the changed amino acid was at the 2nd position (*Pin b-D1new4*, Table 4).

- 75% of Indian cultivars have wild type *Pinb-D1a* allele. Mutant *Pinb-D1b* allele commonly found in Australian wheat was present in only 10% Indian cultivars. Six new alleles have been identified in Indian germplasm that may contribute more sources of variation.

Salient Achievements :

- Correlation of absence of GBSS-B1 with good chapatti making quality has been identified.

- Advanced breeding material for improvement of chapatti and biscuit making quality has been generated
- BC3F3 translocation line 1EL (1AS) with improved gluten strength and loaf score has been generated in the background cultivar N61.
- Six new alleles of puroindoline gene have been identified in Indian wheat cultivars.

Future Perspectives :

- Generation of breeding material with improved processing quality by utilizing of existing markers.
- Discovery, validation and interaction pattern study of new markers.

Table 4: New *Pin a-D1* and *Pin b-D1* genes identified from Indian cultivars.

S. No.	Cultivar	Allele	No. of entries	Mismatch position Nucleotide/ Amino acid	Base change/ AA change	Hardness in SKCS units
1.	NP715	<i>Pin b-D1new1</i>	2	232/ 49	G----T/Glu acid----Stop	76,81
2.	HS277	<i>Pin a-D1new1</i>	1	41/-15	C----T/Val----Ala	73
3.	K0710	<i>Pin b-D1new2</i>	1	371/95	T----C/Leu----Pro	95
4.	Sarbati Sonara	<i>Pin a-D1new2</i>	1	65/-7 86/-1	G----C/Ser----Th A----G/Asp--Gly	57
5.	DBW17	<i>Pin b-D1new3</i>	1	92/no change	T----C/No change	87
6.	WH1073	<i>Pin b-D1new4</i>	1	93/2	T----A/Valine---Alanine	78



IMPROVING FRUITS FOR POST HARVEST QUALITY AND NUTRITION

2.1 Genetic transformation of banana for quality improvement

Principal Investigator:

Siddharth Tiwari

Co- Investigator:

Rakesh Tuli

Research Fellow:

Anshu Alok

Introduction:

Banana contributes significantly towards the food security and is known as poor man's staple food in developing countries. Most of the cultivated varieties of banana are triploid in nature, hence sterile and provide natural barrier to cross pollination. Therefore, genetic improvement of banana through conventional methods is limited. Biotechnological tools such as transgenic approach hold high promise for the biofortification of this crop and are biologically safe to introduce desired traits. Metabolic pathway engineering to enrich germplasm with micronutrients is a promising approach to biofortification. We have received a project for the Development of Indian Banana with Pro-Vitamin A (PVA) Constructs with four year (November 2012 to October 2016) support from Biotechnology Industry Research Assistance Council (BIRAC), Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India (Sanction order number: BIRAC/Tech Transfer/08/12/QUT-BBF). This project is a part of the multi-institutional project

entitled "Development and Transfer of Technology from Queensland University of Technology (QUT), Australia to India for Biofortification and Disease Resistance in Banana". It is proposed to utilize the experience and achievements of QUT for the development, validation and transfer of specific traits in two Indian banana varieties cv. Grand Nain and Rasthali by partner labs.

NABI had initiated work on the collection of banana germplasm and development of protocol for *in-vitro* regeneration from immature male flowers of Grand Naine and Rasthali. Embryogenic Cell Suspension (ECS) culture will be used for the *Agrobacterium-tumefaciens* mediated genetic transformation of gene constructs involved in carotenoid biosynthesis pathway.

ResearchProgress:

1. **Germplasm collection and plantation at NABI research field**
 - i. Suckers of around fifteen established banana cultivars have been collected from different places and grown at NABI research field for establishing germplasm (Figure 1A).
 - ii. Several tissue culture raised plants of Grand Nain and Rasthali cultivars have been generated and grown at NABI research field for collection of immature male flower buds for explant source (Figures 1 A & B).



Figure 1: Banana germplasm at NABI research field (A). Tissue culture raised Grand Naine and Rasthali plants (B).

2. Establishment of Embryogenic Cell Suspension (ECS) culture and regeneration of embryos

- i. Immature male flower buds of two cultivars (Grand Naine and Rasthali) were collected from TNAU, Coimbatore and NRCB, Trichy for the preparation of explants and

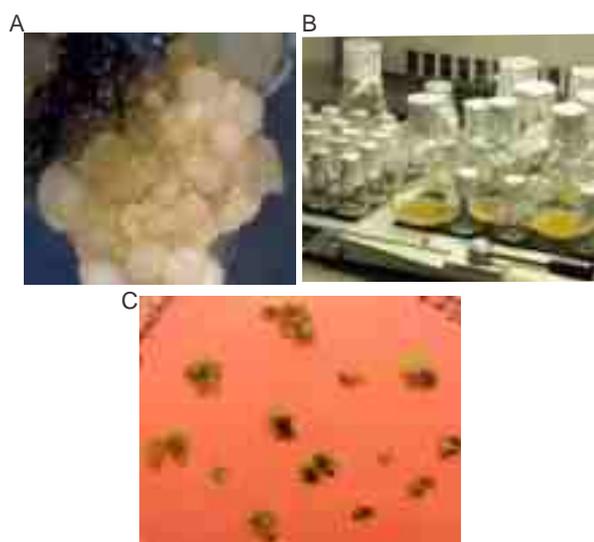


Figure 2: Stages of Embryogenic Cell Suspension (ECS) culture development for somatic embryogenesis. Embryogenic callus (A). Cell suspension culture maintained in a shaker (B). Regeneration of embryos (C).

initiation of embryogenic callus (Figure 2A).

- ii. Embryogenic callus used for ECS development and regeneration of embryos (Figures 2 B & C).

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. Banana cultivars have been collected from different places and grown at NABI research field for establishing germplasm.
3. Efficient ECS of Grand Nain and Rasthali were developed.

Future Perspectives:

Development of pro-vitamin A (β-carotene) rich biofortified Indian bananas, bioavailability study, nutritional analysis and agronomical field trials of transgenics.

2.2 Quality enhancement and postharvest stability of tropical fruits

Principal Investigator:

Sukhvinder P. Singh

Research Fellow:

Manpreet Kaur

Introduction:

High perishability of fresh fruits accounts for qualitative and quantitative losses during postharvest supply chain. Their profoundly diverse physical, physiological and biochemical attributes often present unique challenges in developing postharvest strategies. Our understanding of the basic biology of these perishable commodities has advanced considerably over recent years. The recent development of 'omics' (viz. genomics, transcriptomics, proteomics, and metabolomics) has greatly accelerated our understanding of the fundamental biology of fresh produce and highlighted opportunities for developing more efficient and robust strategies for improvement. The next-generation sequencing technologies have revealed the genomes and transcriptomes of several fruit and vegetable crops. However, there has been limited translation of this knowledge into developing new products with modified postharvest traits. The application of metabolomics is assisting our understanding of the fate of thousands of metabolites in relation to postharvest quality, ripening and development of physiological disorders. The high throughput 'omics' techniques and their integration in system-wide approaches are complementing traditional postharvest research.

The prime objective of this research program is to understand biological basis of fruit quality and generate basic knowledge to develop pre- and

postharvest strategies for extending the storage stability and maintaining produce quality. The research efforts are directed to expand the horizon of measurement, definition and meaning of 'quality' of fresh fruits. The genetic, physiological and biochemical diversity in the fresh produce require the commodity-specific approach to develop postharvest solutions.

Research Progress:

Citrus

'Kinnow' mandarin is a commercially important fruit crop for North India, especially for the Punjab state. It contributes more than 60% of the total fruit production in the state; while Abohar and Hoshiarpur being the epicentres of Kinnow production. However, fruit quality from these two regions is known to contrast in attributes such as fruit size, peel thickness and acid content. Fruit quality is dependent on several factors including geographical location. Recently, metabolomics applications have shown that food commodities grown in different geographical locations can be grouped to establish their provenance. It was hypothesised that 'Kinnow' mandarins grown in different locations may differ in their metabolome which ultimately leads to differences in various quality traits.

1. To study the influence of geographical location on fruit quality, Kinnow mandarins were collected from commercial orchards in different locations viz., Abohar (Fazilka), Lambi (Muktsar), Tahliwala Jattan

(Fazilka), Chhauni Kalan (Hoshiarpur), Bhunga (Hoshiarpur), Amritsar and Una. The untargeted global metabolomics approach was followed by employing UPLC-QTOF (ABSciex Triple TOF 5600) platform to cluster the fruits from different locations based on their metabolome. The data were generated, extracted for peak alignment, normalized using internal standard and subjected to multivariate analysis using Analyst, Peakview and Markerview software packages.

2. About 8000 features for each data set were detected in the mass range of 100-1000 m/z. In data reduction strategy, the t-test was conducted from the first to last samples and the features (~2000) showing differences at $p < 0.01$ were selected for further multivariate analysis. The principal component analysis (PCA; unsupervised), PC-discriminate analysis (PCDA; supervised) and PC variable group (PCVG) analysis were performed using Markerview software. Figure 3 shows that fruits from Hoshiarpur and Amritsar region grouped together closely while Abohar was quite distinct from all other locations. Lambi and Tahliwala Jattan also tended to lie in the same PC. The PCA score and loading lots clearly indicate that metabolomics applications can be employed to establish provenance of food commodities.

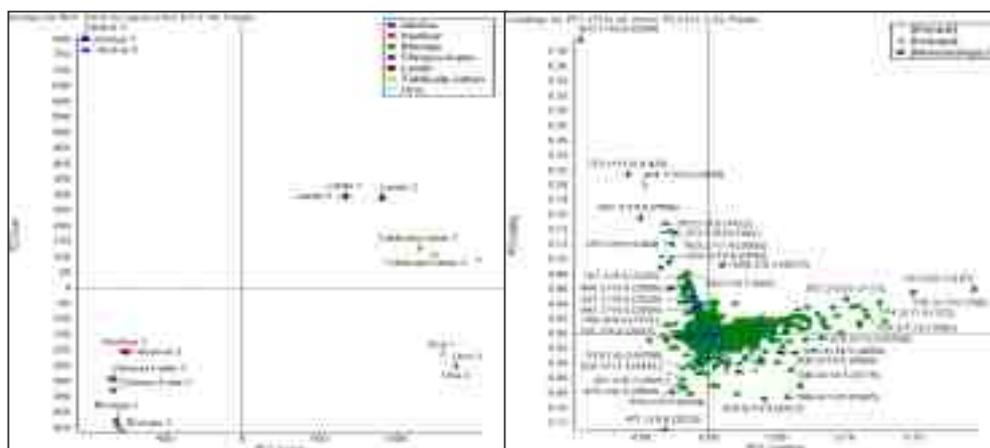


Figure 3: PCA score (left) and loadings (right) plots for global metabolomics data sets for Kinnow mandarins from different locations.

- Global metabolomics data can be effectively visualized using a recently developed tool, called 'Cloud plot' (Patti et al., 2013). The uniqueness of metabolome of the fruit grown in a particular location can be explained with a set of metabolites which are up-and/or down-regulated. The metabolome data from Abohar and Chhauni Kalan were subjected to XCMS analysis for deriving the cloud-plot to understand the pattern of fold change of various metabolites and their identification was performed by matching their MS/MS spectra with either authentic standards or databases such as Metlin, Massbank, ChemSpider etc. (Figure 4).
- The cloud plot was constructed for 177 metabolites having significant differences at $p < 0.0001$ level with 1.5 to 10.0 fold change in their expression level for two locations. Metabolites responsible for uniqueness for each location/region have been identified. Additionally, the quantitative analysis of major metabolites such as limonoids and phenolics has been conducted for conclusive evidence in favour of geographical location effect on fruit quality.

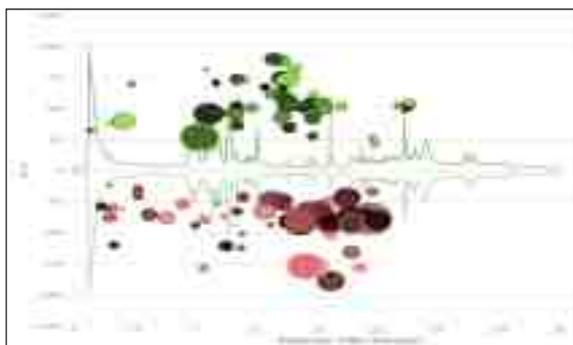


Figure 4: Cloud plot of Kinnow metabolome data set obtained from Abohar and Chhauni Kalan locations, 177 features with p -value < 0.0001 and fold change 1.5 includes visualization of the p value, the directional fold change, the retention time, and the mass-to-charge ratio of features. Also shown are the total ion chromatograms for each sample and the time dependent composition of the mobile phase. Features whose intensity is increased are shown on the top plot in green, whereas features whose intensity is decreased are shown on the bottom plot in red.

Mango

Postharvest phytosanitary barriers imposed by several countries such as the United States of America (USA), Japan, Australia and New Zealand have impacted the fresh mango fruit industry. In recent years, heat-based phytosanitary treatments have received regulatory approvals from Japan, Australia and New Zealand which led to the establishment of these facilities in the major mango producing and exporting zones of the country. However, there are certain quality concerns associated with these treatments which can affect consumer acceptance of the exported fruit in the international markets. Our objective was to assess the impact of heat treatment protocols on quality, fruit physiology and postharvest behaviour of mango fruit. In 2012 season, experiments on vapour heat treatment (VHT) of commercial mango cultivars ('Amrapalli', 'Dashehri', 'Langra' and 'Chausa') of North India were conducted in a VHT facility at Saharanpur, U.P.

- The post-VHT physiological responses of mango cultivars were studied in terms of respiration and ethylene production rates. The respiration and ethylene production behaviour of all mango cultivars except 'Langra' did not differ significantly among VHT and control fruit. Climacteric ethylene production rate was slightly higher in 'Langra' mango (Figure 5).
- 'Dashehri' and 'Langra' cultivars reached eating ripe stage in 7 days compared to 9 and

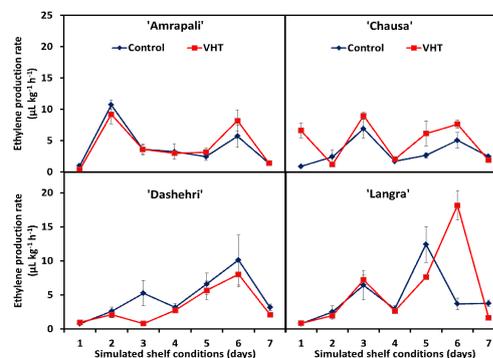


Figure 5: Ethylene production rates of VHT and untreated mango fruits of different cultivars during fruit ripening

- 12 days in 'Chausa' and 'Amrapali', respectively.
3. The fruit quality in terms of soluble solids concentration was almost similar in both treated and control fruits.
 4. To gain better understanding into metabolic changes in response to heat treatments, the fruit were subjected to target metabolite profiling for small molecules such as sugars, organic acids and water soluble vitamins. The untargeted metabolomics approach using LC-MS has to be completed so as to derive a set of metabolites up- and down-regulated in response to heat treatments in different cultivars.

Salient Achievements:

1. The application of metabolomics tool in postharvest research program has provided insights into the whole metabolome of 'Kinnow' fruit collected from different geographical locations.

2. The high throughput analytical platforms have been used to gain better understanding of the factors affecting postharvest behaviour of fresh fruits such as mango, 'Kinnow' and litchi.

Future Perspectives:

1. The research efforts will be directed to discover and validate biomarkers for artificially ripened fruit using prohibited ripening agents. To improve the postharvest stability of fresh fruits, the application of surface coatings developed from indigenously available biopolymers will be explored for 'Kinnow', mango, litchi and guava fruits.
2. The horizon of metabolomics applications will be extended to postharvest fruit quality, screening germplasm for discovering novel phytochemical sources, prediction of physiological disorders and food safety aspects.



BASIC BIOLOGY FOR CROP IMPROVEMENT

3.1 Biology of seed development in custard apple and litchi

Principal Investigator:

Rakesh Tuli

Co-Investigator:

Sudhir P. Singh

Research Fellows:

Yogesh Gupta

Ashish Kumar

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. Sugar (or Custard) apple, a popular fruit throughout the tropics, belongs to genus *Annona*. The *Annona* fruit develops from the cluster of

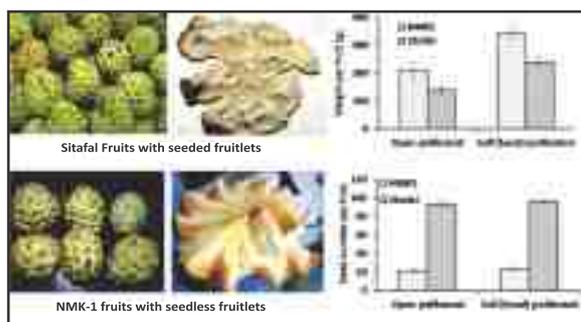


Figure 1: Fruit, weight per fruit and seed per fruit in contrasting genotypes of *Annona squamosa*; Sitafal and NMK-1.

fertilized carpels, thus the aggregate fruit contains several fruitlets. Out of the multiple fruitlets, a few develop naturally, without seeds. *Annona squamosa* produces fruits with greater number of seeded fruitlets, sixty to eighty seeds per fruit. A contrasting genotype, named NMK-1 has fewer numbers of seeded fruitlets. This many fruitlets are seedless (Figure 1).

The variety was selected from the seedling population of *Annona squamosa* by a farmer, Shri N. M. Kaspate of Madhuban nursery, Solapur, Maharashtra. The aim of the project is to understand the molecular basis of the development of seeded and seedless fruitlets in the same fruit of *Annona squamosa*. The major approach is to do tissue specific differential transcriptomics in developing

fruitlets of *Annona* species for identifying genes involved in seed development.

Litchi is another crop where seedlessness is a desirable trait. Some litchi accessions, popularly known as 'Seedless' or 'Bedana', have seeds of very small size with well-developed fleshy pulp, in comparison to the common litchi varieties. The project aims to identify genes related to small size of seeds by doing tissue specific differential transcriptomics of contrasting accessions of litchi.

Research Progress:

1. A genotype, NMK-1, was identified with more number of seedless fruitlets at Madhuban Nursery, Solapur, Maharashtra (Figure 1). Self pollination was performed in the contrasting genotypes NMK-1 and Sitafal at farmer's field (Solapur, Maharashtra). The developing fruits were harvested. Differential transcriptome analysis at 0 and 8 DAP revealed several genotype specific genes in both the stages (Figure 2).

The expression pattern of some auxin related genes was examined. Distinct pattern of expression was observed in case of auxin-repressed protein (ARP). The gene plays important role in arresting the growth of tissues. Higher expression level of ARP in NMK-1 was in agreement with higher number of small sized ovules.

2. Litchi contrasting genotypes, showing differences in seed development and size were screened for candidate genes to develop molecular approaches to seedlessness. Polymorphism was noticed in a candidate gene responsible for small seed phenotype in Litchi. Further study on molecular details is in progress.
3. Developing ovules of large and small seeded litchi genotypes have been collected at different stages of development (Figure 3). Transcriptome sequencing has been done and the analysis is in progress.
4. Amplified fragment length polymorphism

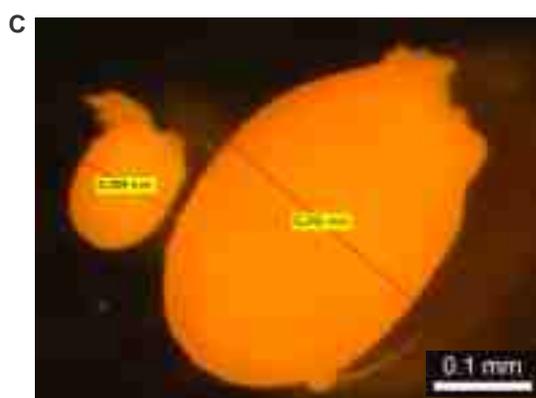
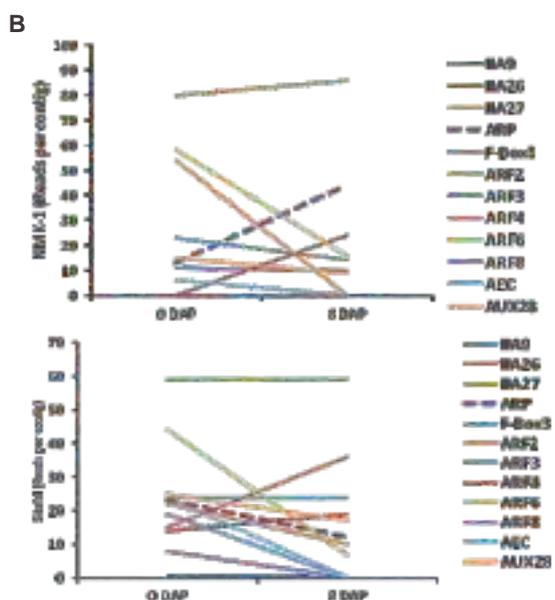
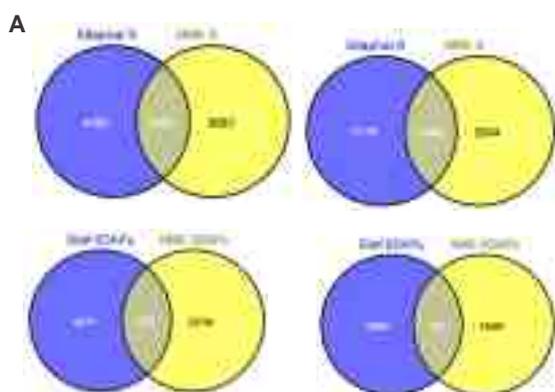


Figure 2: (A) Gene expression statistics in developing fruits of contrasting genotypes at 0, 8 DAP. (B) Expression pattern of auxin related genes. Higher level of expression of auxin-repressed protein (ARP) gene in NMK-1, in contrast to Sitafal. (C) Small and large ovules within a fruit of same stage in *Annona squamosa*.

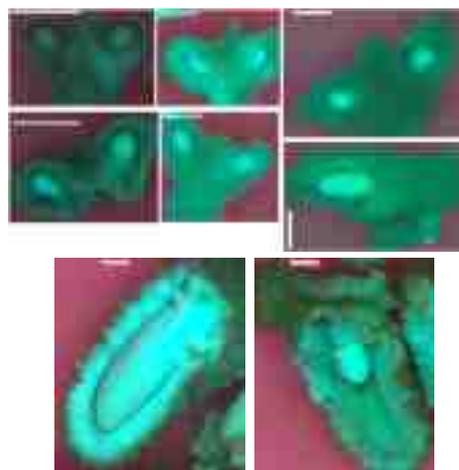


Figure 3: Developing ovules of small and large seeded litchi fruits at early developmental stages.

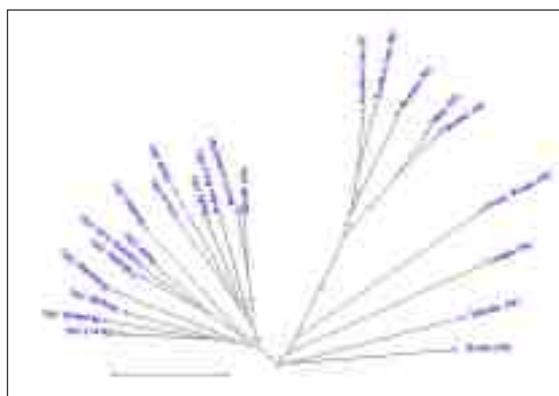


Figure 4: Growing root neighbor-joining tree of litchi cultivars based on jaccards coefficient using 306 polymorphic AFLP markers. Bootstraping values are given for the clusters. M: NRCL, Muzarffarpur; H: FRS, Hoshiarpur; P: Yadvendra Garden, Pinjore. Software pack: DAR win 5.0.158

analysis established genetic relatedness among the popular commercial Litchi cultivars of India collected from National Research Centre for Litchi (ICAR), Mujjafarpur, Bihar, Fruit Research Station (PAU), Hoshiarpur, Punjab and Yadvendra Garden (Horticulture Department), Pinjaur, Haryana (Figure 4).

Salient Achievements:

1. Contrasting genotypes have been identified in *Annona squamosa* and *Litchi chinensis* for the trait seed number and seed size in fruit, respectively.
2. A gene, related to seed development, has

been identified showing SNPs in coding region among the contrasting litchi genotypes.

3. AFLP phylogeny analysis revealed unique fingerprints between bold seeded and small seeded Litchi cultivars.

Future Perspectives:

1. Transcriptomics of developing ovules in the contrasting genotypes of *Annona squamosa* and *Litchi chinensis*.
2. Validation of the SNPs for their role in small seed trait in *Litchi chinensis*.

3.1.1 Development of approaches for the modulation of seedlessness through rootstock signaling

Principal Investigator:

Rakesh Tuli

Co-Investigator:

Sudhir P. Singh

Research Fellow:

Anita Kumari

Introduction:

Fruit crops are mostly propagated through asexual reproduction by grafting. In grafting, one plant is selected for its superior root related traits and is called rootstock. The other plant is selected for its stems, leaves, flowers or fruits and is called the scion. Scion–rootstock interaction affects the physiology and thus phenotype of the scion plant. The research project anticipates establishment of a technology which can lead to the development of a new approach to use transgenic rootstocks for delivering specific signals, like siRNAs in non-transgenic scions for the modification of economically important traits. In the present case, the principle will possibly lead to prevention of seed development and enhancing the productivity and biomass. The rootstock would transmit siRNAs, targeted for silencing of specific genes involved in the development of embryo and endosperm.

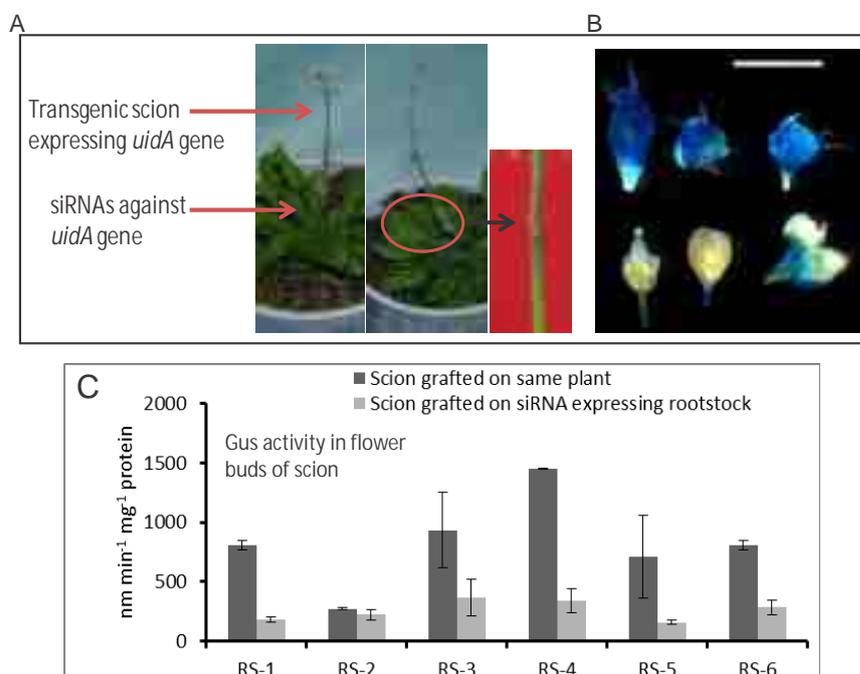


Figure 5: Grafting of reporter gene (*uidA*) expressing scion onto the rootstock which expresses siRNA against the reporter gene (A). Histochemical GUS assay in flower buds of *uidA* expressing scion grafted onto the same plant (up) and in the flower buds of *uidA* expressing scion grafted onto the siRNA expressing rootstock (down), scale 500µM (B). Bar diagram showing Gus activity in the flower buds of *uidA* expressing scion grafted onto the same plant (dark) and in the flower buds of *uidA* expressing scion grafted onto the siRNA expressing rootstock (light) (C).

Research in Progress:

1. Transgenic *Arabidopsis* lines expressing reporter gene (GUS) constitutively were developed. Transgenic *Arabidopsis* lines expressing (constitutive and phloem specific) double stranded RNA-hairpin homologue of the reporter gene were raised. The GUS expressing lines were grafted onto the rootstock which expresses siRNAs against GUS gene. Silencing was observed in flower buds of the grafted scion (Figure 5).
2. Transgenic lines expressing siRNA against a gene for seed development have been developed. Further analysis is in progress.

Salient Achievements:

Silencing was achieved in the flower buds of GUS expressing scion grafted onto the rootstock which delivers siRNAs against the reporter gene GUS gene.

Future Perspectives:

1. Silencing of ovule specific gene by siRNA delivered by rootstock.
2. Development of a Virus induced gene silencing vector (VIGS) for gene silencing in ovules after agro-inoculation of VIGS in stem or leaf.





DIET AND HEALTH

4.1 Effect of dietary constituents on adipogenesis

4.1.1 Role of non-starch dietary fibers from millets in regulating adipogenesis: A nutrigenomic study

Principal Investigator:

Kanthi K. Kiran

Co-Investigator:

Mahendra Bishnoi

Introduction:

Obesity is a worldwide epidemic and has a significant negative impact on health, mortality and related costs. Diet and genetic factors including gene mutations and also certain gut microbes contributes to the development of obesity. Untreated, it may lead to chronic diseases, such as type II diabetes, hypertension, cardiovascular disease and cancer. Anti-obesity medications pose serious side effects on prolonged usage. Functional food ingredients such as dietary polyphenols, proteins and fibers are important constituents of plants and have tremendous health benefits. Non-starch hemicellulosic dietary fibers (NSDFs) have been reported to have potential health benefits in alleviating disease symptoms such as diabetes, atherosclerosis and colon cancer. In the present study, the role of NSDFs from cereals in

regulating adipogenesis and their metabolic fate in animal models is being studied.

Research Progress:

1. Using chemical and enzymatic treatments, NSDFs were extracted from cereals and their efficacy in inhibiting adipogenesis was evaluated *in vitro* using 3T3-L1 preadipocytes. NSDF was added at the time of differentiation in DMEM till mature adipocytes were formed.
2. Lipid accumulation was quantified using oil red-o staining. Different degrees of adipogenesis inhibition upon treatment with NSDF extracted from different cereals were observed (Figure 1A). Viability of the cells was not altered upon treatment with NSDF at different concentrations (Figure 1B).

Salient Achievement:

SDFs with different structures exhibited different levels of inhibition of lipid accumulation.

Future Perspectives:

1. Intervention of adipogenesis with NSDF and quantification of key adipose tissue associated genes.
2. *In vivo* studies on antiobesity effects of NSDF supplemented along with high fat diet in mice.

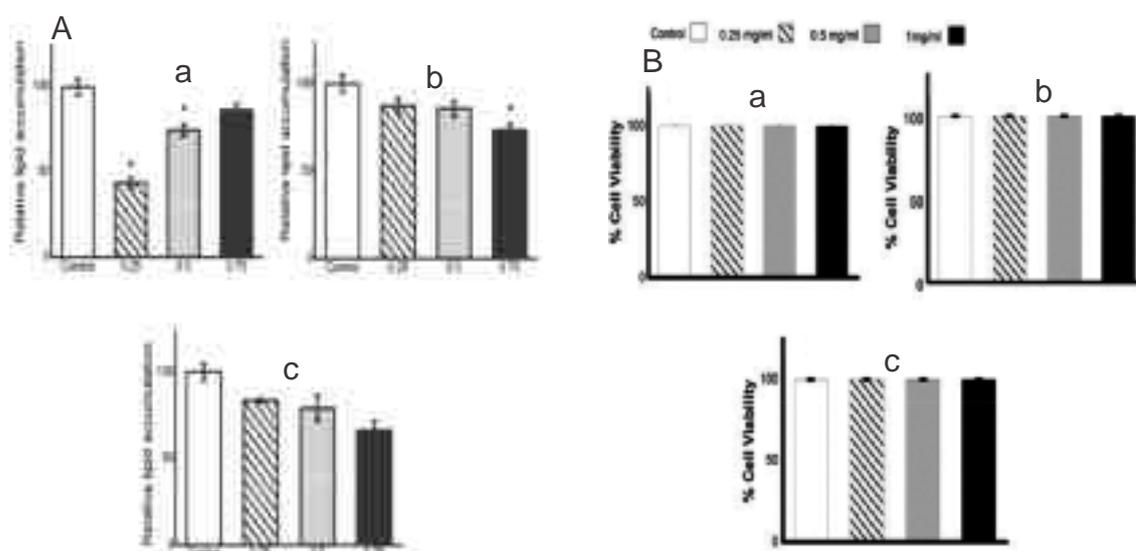


Figure 1: Effect of NSDF from (a) 'Cereal 1' (b) 'Cereal 2' and (c) 'Cereal 3' on lipid accumulation (A) by adipocytes and preadipocytes viability (B).

4.1.2 Transient Receptor Potential (TRP) channel mediated modulation of adipogenesis & obesity by dietary molecules

Principal Investigator:

Mahendra Bishnoi

Co-Investigator:

Kanthi K. Kiran

Research Fellow:

Ritesh K Baboota

Introduction:

Current anti-obesity medications are pharmacological agents which can reduce or control weight. These drugs affect one of the fundamental processes of the weight regulation in human body i.e. altering appetite, metabolism or consumption of calories. There is only one anti-obesity medication, Orlistat, approved by FDA. It acts through inhibition of pancreatic lipase enzyme. Rimonabant, acting through blockade of the endocannabinoid receptor and Sibutramine, acting through brain by inhibiting neurotransmitter metabolism were previously approved drugs which have been withdrawn from the market in several countries and regions including India ("Banned Medicines" (Press release), Ministry of Health and Family Welfare, February 10th, 2011). The potential side effects (increased cardiovascular concerns, stroke, suicidality and depression) of these drugs are much more than their beneficial effects. Over the years it has been seen that the best and most effective options available for overweight and obese individuals are dieting and physical exercise. It is important to have dietary regulations to prevent life style problems rather than to search for the treatment. Literature suggests that Transient Receptor Potential Vanilloid (TRPV1), Ankyrin (TRPA1), Metastatin (TRPM8) channels are possible candidates to regulate energy metabolism and thermogenesis, which can lead to

calorie consumption and prevention of obesity. Common dietary spices like chilli pepper, black pepper, clove, garlic, cinnamon and their constituents (capsaicin, piperine, eugenol, allicin, cinnamaldehyde, menthol) can activate the TRP channels. Using the TRP channel receptor system we intend to come up with dietary constituents that may modulate the molecular mechanism associated with the process of adipogenesis, adipose tissue related hormonal secretion and release of pro-inflammatory mediators and lipolysis.

Research Progress:

1. Based on our qRT-PCR data, we established that multiple TRP channel genes are expressed in mouse 3T3-L1 preadipocytes (high to moderate expression: TRPP2 (PKD2), TRPC1, TRPV2, TRPM2, TRPV4, TRPV1, TRPV3, TRPV6, TRPC4; low expression: TRPA1, TRPC6, and TRPM8) and adipocytes (high to moderate expression: TRPP2 (PKD2), TRPV2, TRPC1, TRPV4, TRPM2; low expression: TRPA1, TRPV3, TRPV6, TRPC4, TRPV1, TRPC6, and TRPM8).
2. Further, these channels are also expressed in murine white adipose tissue (WAT) (high to moderate expression: TRPP2 (PKD2), TRPV2, TRPC6, TRPC1, TRPV4, TRPM2, TRPV3, TRPC4; low expression: TRPV1, TRPV6, TRPM8 and TRPA1) and brown adipose tissue (BAT) (high to moderate expression: TRPP2 (PKD2), TRPV2, TRPC6, TRPC1, TRPV4, TRPM2, TRPV3, TRPC4, TRPV6; low expression: TRPV1, TRPM8, and TRPA1). Critical analysis of TRPV1, TRPA1 and TRPM8 gene has shown decreased expression of these genes with adipocyte differentiation, suggesting the role of these channels or permeable calcium through these channels in differentiation process (Figure 2).

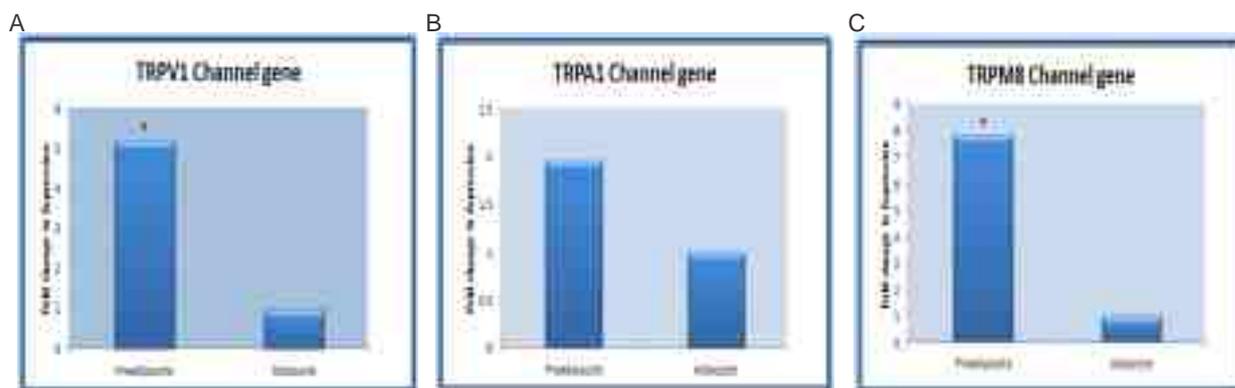


Figure 2: Change in expression of TRPV1(A), TRPA1(B) and TRPM8(C) genes in 3T3-L1 preadipocytes and differentiated adipocytes (Significant with p value ≤ 0.05).

- Experiments with multiple dietary constituents TRP channels activating capsaicin (TRPV1 activator), cinnamaldehyde (TRPA1 activator) and menthol (TRPM8 activator) on 3T3-L1 murine preadipocytes *in-vitro* showed different degrees of adipogenesis inhibition (Figures 3, 4 and 5) without significantly affecting cell viability.
- Nutrigenomic studies with these dietary constituents using *in-vitro* and *in-vivo* animal models are in progress. Based on our initial *in-vitro* studies, capsaicin (1 μM) caused the upregulation of anti-adipogenic genes whereas capsaicin (100 μM) significantly caused the down regulation of these genes (Figure 6).

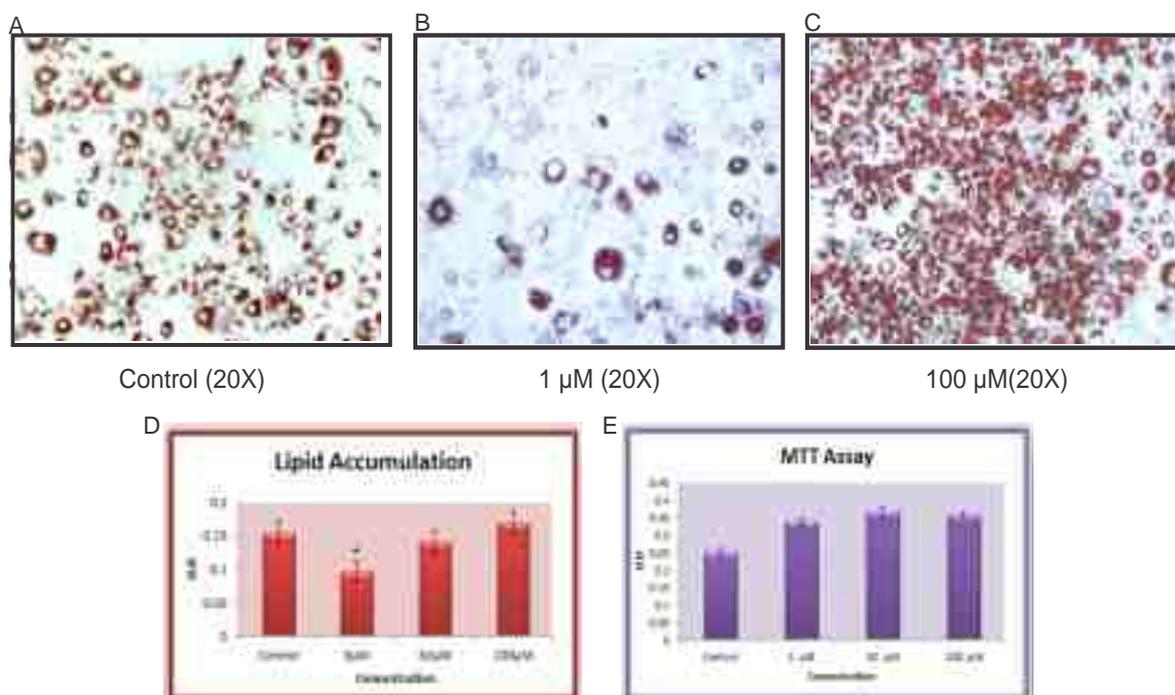


Figure 3: Effect of capsaicin on lipid accumulation (A, B, C and D) and cell viability (E) during the differentiation of 3T3-L1 murine preadipocytes to adipocytes (significant with p value ≤ 0.05).

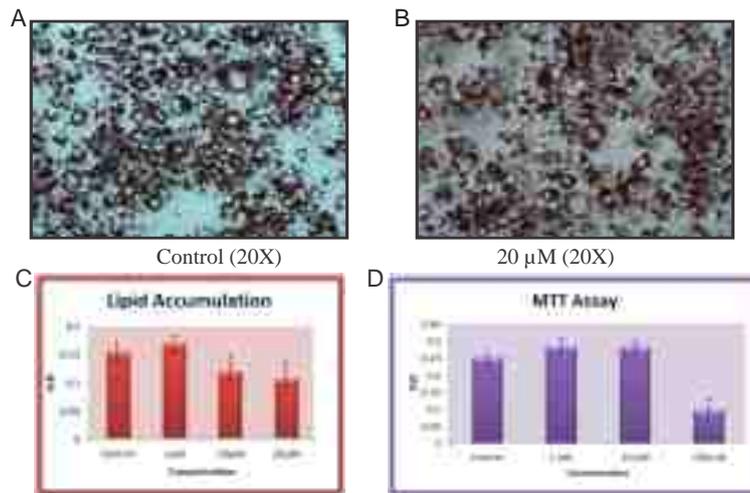


Figure 4: Effect of cinnamaldehyde on lipid accumulation (A, B and C) and cell viability (D) during the differentiation of 3T3-L1 murine preadipocytes to adipocytes (Significant with p value ≤ 0.05).

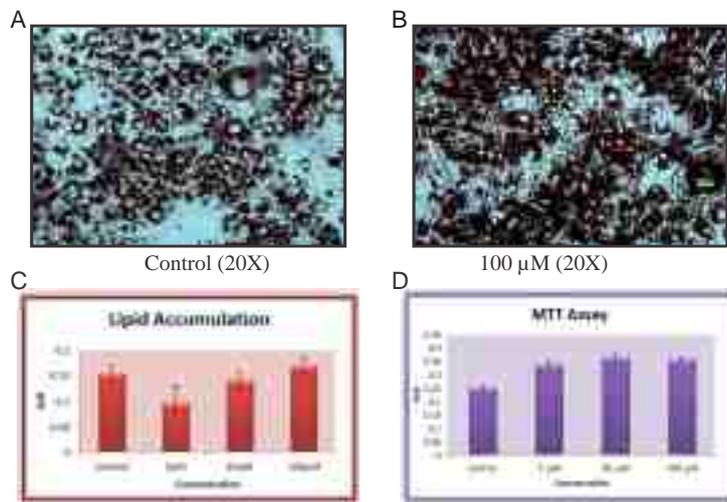


Figure 5: Effect of menthol on lipid accumulation (A, B and C) and cell viability (D) during the differentiation of 3T3-L1 murine preadipocytes in adipocytes (Significant with p value ≤ 0.05).

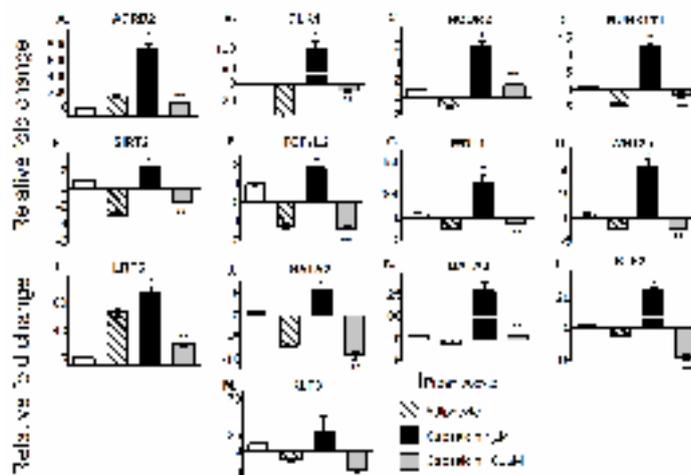


Figure 6: Effect of different doses of capsaicin on anti-adipogenic gene expression during 3T3L1 preadipocyte differentiation into adipocytes (Significant with p value ≤ 0.05).

4.1.3 Characterization of arabinoxylans in cereal grains

Principal Investigator:

Koushik Mazumder

Introduction:

Cereals, the staple food for millions of people across the world, are the chief source of soluble dietary fibers. Several epidemiological studies have clearly demonstrated that increased consumption of whole grain cereals and soluble dietary fibers has been associated with a reduced risk of many life style disorders and chronic diseases such as type-II diabetes and obesity. In cereal grains, AXs are the major non-starchy polysaccharides which constitute cell wall residue in cereal grains. They make up an important physicochemical and physiological property, which is beneficial to the health of consumers and for this reason, their use as food ingredient has increased rapidly. Although AXs share the same basic chemical structure, but the pattern and degree of substitution of arabinose along the xylan backbone vary with cereal sources. Therefore, the AXs exhibits a great deal of structural heterogeneity and variability with respect to molecular mass, xylose to arabinose ratio and branching pattern, distribution of arabinose residue and substitution with glucuronic acid/4-O-methyl glucuronic acid. There is no documented report about the comparative study of arabinoxylan poly and oligo-saccharides from Indian millet varieties, such as finger millet, kodo millet, branyard millet, foxtail millet and proso millet regulating biological activities. Hence in the present study the variability in the structures of the AXs, isolated from various Indian millet varieties and their role in regulating the biological activities will be evaluated using *in vitro* and *in vivo* model. This will allow us to understand the structure-function relationship of the AXs in Indian millet varieties with respect to biological activities.

Research Progress:

1. In our studies, we have standardized the extraction protocol for the isolation of AXs from bran of five Indian millet varieties (finger millet, kodo millet, branyard millet,

foxtail millet, proso millet). The compositional analysis of the polysaccharides have been carried out (GC, GC-MS analysis) as monomeric alditol acetate derivative with inositol as internal standard.

2. The compositional analysis showed the presence of arabinoxylan as major constituent (~70-80%) together with starch

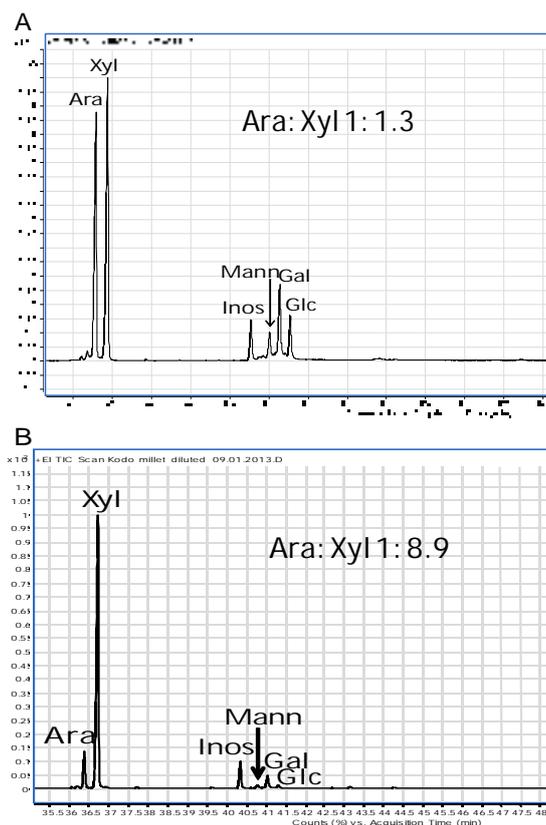


Figure 7: GC chromatogram of the monomeric sugar residues of extracted arabinoxylan fraction from Finger millet (A) and kodo millet (B) bran (Ara: Arabinose, Xyl: Xylose, Inos: Inositol, Gal: Galactose, Glc: Glucose, Mann: Mannose).

and galactomannan as minor component in the five millet varieties. The GC and GC-MS analysis of the extracted AXs showed the presence of highly substituted arabinoxylan polysaccharides in the finger millet (arabinose: xylose ratio 1:1.3), where as the low arabinose: xylose ratio (1:8.9) in the kodo millet indicated the presence of mostly un-substituted xylan backbone with fewer arabinose substitutions at the side chains

(Figure 7).

- AXs extracted from various millet varieties were treated with endo-xylanase to generate oligosaccharides (AXOS) with various degree of polymerization (DP). In finger millet highly substituted AXs produced AXOS with degree of polymerization ranging from 5 to 15, containing arabinose,

- The variability in the composition, degree of substitution and degree of polymerization of the AXs and AXOS from different millet varieties may pose different biological properties.

Salient Achievements:

- Arabinoxylan polysaccharides (AXs) and

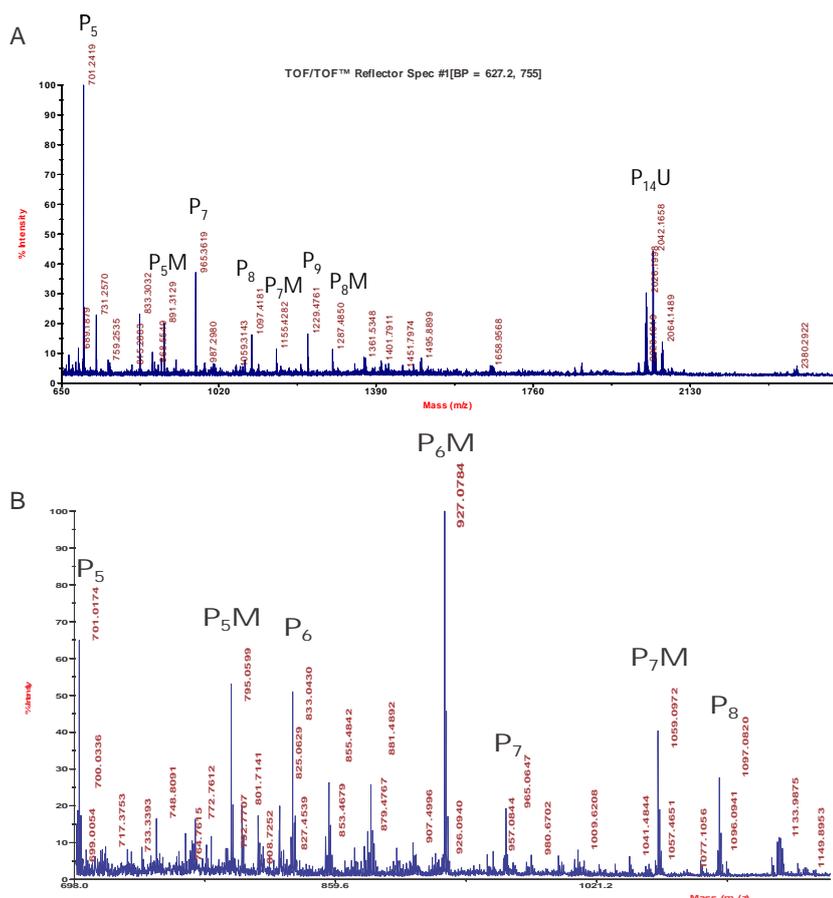


Figure 8: MALDI-TOF-MS spectrum of the AXOS isolated from Finger millet bran (A). MALDI-TOF-MS spectrum of the AXOS isolated from Kodo millet bran P: pentose (arabinose and xylose), M: 4-O-methyl glucuronic acid, U: uronic acid residues (B).

xylose, glucuronic acid and 4-O-methyl glucuronic acid as major sugar residues (Figure 8A). Whereas kodo millet AXs having lower degree of substitution generated AXOS with lower degree of polymerization ranging from 5 to 8, containing arabinose, xylose and only 4-O-methyl glucuronic acid as major constituent sugar residues (Figure 8B).

oligosaccharides from the bran of Indian millet varieties were isolated, purified and characterized by various chemical and modern analytical methods (GC-MS, HPLC, MALDI-TOF-MS, ESI-MS/MS and NMR).

- Comparative structural characterization of arabinoxylans poly and oligo-saccharides from Indian cereals and millet (wheat, finger

millet, kodo millet, branyard millet, foxtail millet and proso millet) showed significant differences in the molecular weight, xylose to arabinose ratio, branching pattern, distribution of arabinose residues and substitution with glucuronic acid/4-O-methyl glucuronic acids. AXs with different chemical structures isolated from different sources may impart different biological functions.

Future Perspectives:

1. Establish the role of structural features of arabinoxylyan poly and oligosaccharides in regulating different biological functions.
2. Development of nutraceutical health foods based on dietary fibers enriched with AXs.

4.1.4 Characterization of poly and oligo-saccharides from Chlorophytum borivilianum and their application as effective coating material for fresh fruits

Principal Investigator:

Koushik Mazumder

Introduction:

Natural polymers can be an alternative source of packaging due to their permeability and biodegradability. Edible coatings and films have emerged an alternative for synthetic plastic for food application. These natural biodegradable polymers can prevent the product's deterioration and extend their shelf life, while maintaining the sensory and safety of several food products. The structure of polysaccharides can be easily modified in order to improve their physical, physicochemical and mechanical properties.

Research Progress:

1. The poly and oligo-saccharides were isolated from the root of Chlorophytum borivilianum using 80°C hot water extraction (~70% yield). Qualitative compositional analysis of extracted material was performed using GC (Figure 9A) and GC-MS which showed the presence of fructose and glucose as major constituent sugar residues. The MALDI-TOF-MS

analysis of the extracted material showed the presence of oligosaccharides (mainly fructo-oligosaccharides, Figure 9B) with degree of polymerization (DP) from 4 to 13.

2. Furthermore, the hot water extracted poly and oligosaccharides were derivatized to prepare carboxymethylated derivatives to reduce the viscosity of the material. The standardization of the derivatization reaction protocol is under progress.

Salient Achievements:

1. Structural characterization of the extracted material from Chlorophytum borivilianum showed presence of fructo (~50%) and gluco (~10%) poly and oligo-saccharides as major constituent sugar residues .
2. Various derivatisations such as carboxymethylation of the extracted carbohydrate material were carried out to improve the physical (solubility, viscosity) and physicochemical properties.

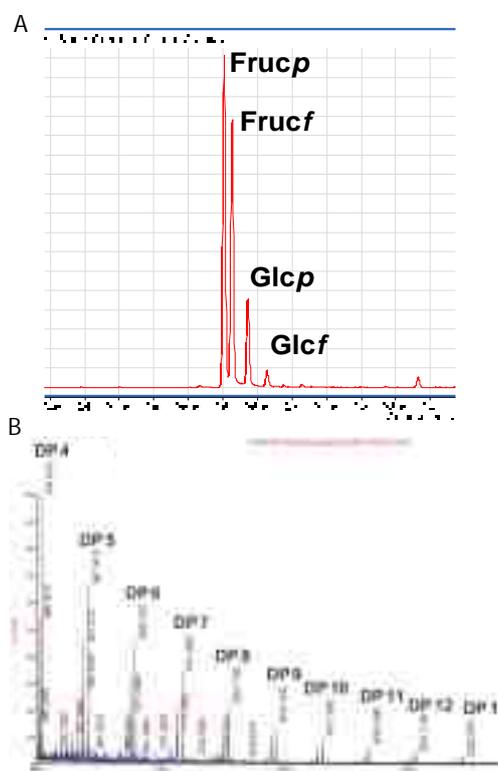


Figure 9: GC (A) and MALDI-TOF-MS (B) spectrum of the hot water extract of *Chlorophytum borivilianum* (Fruc : Fructose, Glc : Glucose).

Future Perspective:

Development of effective coating materials for fresh fruits using edible and biodegradable carbohydrates.

4.1.5 Establishment of dietary constituents to regulate iron absorption/ homeostasis and their use against iron deficiency

Principal Investigator:

Hariom Yadav

Research Fellow:

Stanzin Angmo

Introduction:

India is one of the countries with very high prevalence of anaemia in the world. Almost 58 per cent of pregnant women in India are anaemic and it is estimated that anaemia is the underlying cause for 20–40 per cent of maternal deaths in India. India contributes to about 80 per cent of the maternal deaths due to anaemia in South Asia. Nutritional anaemia is a major public health problem in India and is primarily due to iron deficiency. The increased prevalence of iron deficiency is because of unavailability of adequate strategies to combat iron deficiency in Indian population and lack of complete knowledge about the pathophysiology of iron homeostasis in Indians. Majority of Indian population depends on vegetarian diet. Although, iron is present in vegetarian diet (non-heme) but it is less bioavailable, therefore increasing bioavailable iron content in Indian foods will be one of the important future research aspects at NABI. We will pursue these goals with the close collaboration of various scientists working on plant molecular biology and genetics to improve the varieties of crops i.e. wheat to improve the iron bioavailability.

Absorption of iron in human body is tightly regulated by various feedback mechanism(s). Hepcidin is a central regulator of iron absorption in mammals, and inhibits iron absorption as well as release from iron storing cells (i.e. intestinal epithelial cells, macrophages, hepatocytes) when iron is needed to RBC formation. Hepcidin binds

with Ferroportin (an iron transporter to release intracellular iron) and leads to degradation of Hepcidin-Ferroportin complex. Therefore, blocking interaction of Hepcidin and Ferroportin may be an important target to develop strategies to combat iron deficiency. My future goals in this direction is to establish novel compounds that can inhibit hepcidin expression or block the action and finally incorporate these compounds in Indian foods to increase iron absorption.

Research Progress:

1. Our meta-analysis study indicated that circulating Hepcidin levels are dramatically higher in Indian population than other countries. This suggests that along with consuming less bioavailable iron, there seems to be blockage of iron absorption in Indian population through increased hepcidin levels. Therefore, we have started screening of natural compounds present in food that can inhibit hepcidin expression and/or can block the action of hepcidin.
2. We have predicted certain compounds that show potential action on hepcidin target (Figure 10). Hence our group is specifically targeting three strategies to increase iron absorption; 1) increase, 2) decrease the hepcidin expression and 3) inhibit hepcidin action using natural compounds.

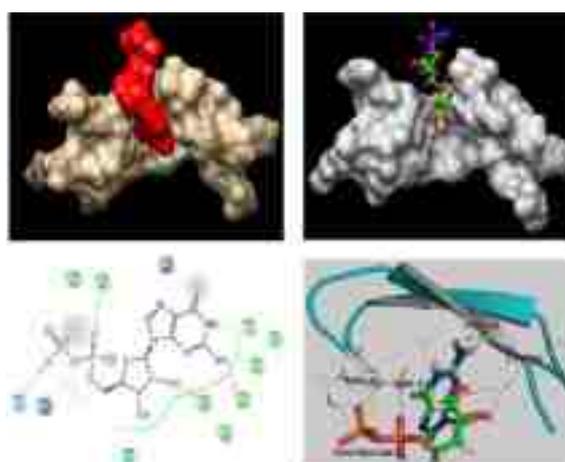


Figure 10: Interaction of selected compounds with hepcidin.

Salient Achievement:

We hope to develop novel natural compounds that may inhibit the expression and action of Hepcidin.

Future Perspectives:

1. Develop new dietary formulations that can enhance iron absorption and homeostasis to combat iron deficiency.
2. Develop novel compounds to target hepcidin-ferroportin complex and increase iron bioavailability.
3. Establish beneficial effects of selected natural compounds and dietary formulations in human population using clinical trial protocols.



**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

Coordinator:

Rakesh Tuli

Principal Investigators:

Shrikant Subhash Mantri

Joy K Roy

Project Scientist:

Shailesh Sharma

Research Fellow:

Anuradha Singh

Introduction:

Genome level understanding of functioning and dynamics is challenging with respect to a) huge amount of information b) its visualisation c) its interpretation etc. Next Generation Sequencing has made it possible to generate genome and transcriptome data at an unprecedented pace. To make biological sense of giga/terra scale data, new algorithms for accelerated analysis are required. Analysis rate can be increased by designing faster algorithms and by using parallel computing clusters. Food crop genomes are huge in terms of genome size. Wheat genome is around 17 Giga-bases, almost more than five times that of human genome. We at NABI computational biology lab have developed HPC cluster for accelerating analysis of transcriptome and genome data.

Research Progress:

2012 was an exciting year for Computational Biology Lab at NABI. We got our Super Gadget: 10 Tera FLOPs (10^{12}) High Performance Computing Cluster. Thanks to DBT for funding and CDAC Pune, HPC Team for their help in setting up this Supercomputing facility. wiki: Serendipity means a "happy accident" or "pleasant surprise"; specifically, the accident of finding something good or useful while not specifically searching for it. We hope NABI's new HPC cluster usage will accelerate important discoveries, some through genuine quest and some through chance

findings.

Hence, we have named this cluster as 'Serendipity Cluster' (Figure 1). We are using this 'Serendipity Cluster' for accelerating discovery through annotation of transcriptomes to identify new genes and their roles. Biology works through macromolecules interacting with each other to perform important functions. By studying transcriptome we try to see the digital transcriptional snapshot of full movie of these dynamically interacting macromolecules. To understand this snapshot, we use many open source software tools and algorithms, thanks to the giants of computational biology. Using these available software tools, we try to see and unravel the complex picture. There were instances where we landed in no man's land where no algorithm/ tools were available to resolve some snapshots. We grabbed such opportunities to develop our own source code for analysis and data mining.

Young scholars got opportunity to use 'Serendipity Cluster' and got hands on experience to solve some small unsolved problems. We hope to develop an effective team of young skilled minds who don't fear to write own programs, develop own algorithms, who can dive into ocean of data to find meaningful patterns and new knowledge. We look forward to nurture appropriate individual talent and challenge them to be intensely creative in answering complex challenging questions. So far, NABI has mainly invested resources to generate transcriptome, proteome and metabolome data. Very soon we will venture into plant genomics and epigenomics research. At Computational Biology Lab we are constantly working towards integrating information from all these high throughput experiments and trying to infer meaningful knowledge to answer complex questions.

Salient Achievements:

1. **Commissioning of HPC data centre and HPC cluster "Serendipity Cluster":** Brief Specifications: 32 Nodes 448 core CPU, 1.8 TB RAM and ~100 TB storage (Computational capacity 10.9 TF). It ranks 33rd in Top Supercomputer in India List (<http://topsupercomputers-india>).

iisc.ernet.in/jsp/june2013/index.html)

2. **Wheat deleterious non synonymous nsSNP Database development:** Using public domain more than 1 Million ESTs, 3290 putative high quality nsSNP were discovered of which 939 nsSNP were predicted to be functionally deleterious.
3. **Novel gene discovery in wheat seed aleurone and endosperm specific transcriptomes:** In house we analysed published Gillies et al. data (Gene expression in the developing aleurone and starchy endosperm of wheat. Plant Biotechnol J. 2012 August;10(6):668-79) and were able to identify novel contigs which are unreported. These findings will improve the understanding of the gene expression changes during the development of aleurone and starchy endosperm in wheat seed.
4. **In-house transcriptome data analysis and database development:** Early 2013 we started getting 454 and Illumina transcriptome for NABI samples. Comprehensive analysis and database development is in progress.
5. **Towards understanding the function of hypothetical and unannotated transcripts:** In-house scripts for annotating the hypothetical and unannotated transcripts have been developed. Rigours testing and optimisation is in progress.

Future Perspectives:

1. Visualization of differential expressed

transcriptome data for wheat is challenging using existing tools. We are developing new knowledgebase to visualise pathways. These efforts will help in long term objective of identifying genes involved in important traits.

2. Molecular Biology scholars can use HPC cluster more effectively through web based user interface for computationally intensive tasks. We are developing a secure web user interface which can be used by non IT expert for using NABI HPC cluster for annotation of genes/sequences.



Figure 1: Serendipity cluster facility.





EMERGING AREAS

1. A designed dominant negative protein that inhibits the DNA binding activities of a number of B-ZIP transcription factors involved in seed formation and development in *Arabidopsis*

B-ZIP transcription factors are a family of transcription factors (TF's) found only in eukaryotes. They are involved in a plethora of gene regulation activities including growth, development and differentiation. The B-ZIP protein motif is a long bipartite α -helix. The C-terminal half is an amphipathic α -helix that dimerizes to form a parallel dimeric coiled-coil termed as leucine zipper. The N-terminal half is a basic region that binds sequence specific DNA (Figure 1).

There are approximately 81 B-ZIP transcription factors in *Arabidopsis*. Ten B-ZIPs are recognized as involved in seed formation, maturation and development in *Arabidopsis*. These B-ZIPs are

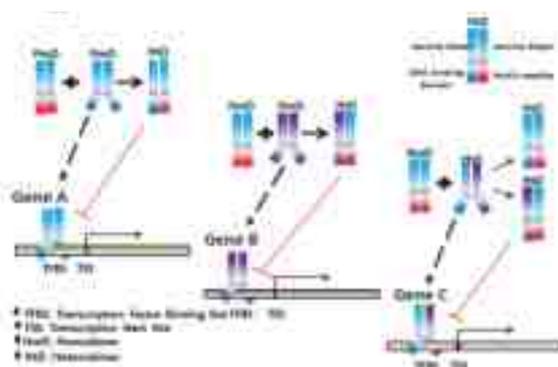


Figure 1: A dominant negative approach to study gene regulation.

AtbZip1, AtbZip10, AtbZip15, AtbZip25, AtbZip39, AtbZip53, AtbZip62, AtbZip64, AtbZip67, and AtbZip72. Traditional gene-knock down/out studies have resulted in feeble or no phenotype suggesting redundancy in these B-ZIP functions- a phenomena common in biological systems. Instead of targeting one gene at a time we have taken an approach of aiming all 10 TFs using a dominant negative approach. A dominant negative (DN) protein called A-ZIP in which the basic DNA binding region of a B-ZIP transcription factor (TF) is replaced by a rationally designed acidic extension has been used successfully in mammalian systems to study the role of B-ZIP in

growth, development and differentiation. These DN heterodimerize with the wild type proteins and inhibit their function by not allowing them to bind to promoter of a gene. DN is biologically active reagent because a heterodimer is more stable than a B-ZIP homodimer bound to DNA. Currently our group is in the process of cloning these 10 B-ZIPs from *Arabidopsis*. We are designing a DN protein against these B-ZIPs using the rules and fundamentals we have learned from our previous studies with the mammalian systems.

2. Developing carbohydrate and lipid derivative based nano molecular vehicles for bioavailability of micronutrients

Micronutrient deficiency such as iron deficiency may arise from inadequate intake. Impaired absorption leads to anaemia, reduce cognitive development and lower work capacity. Although leading iron deficiency therapy with oral ferrous salts is widespread, the bioavailability of iron from such salts is poor. Anti-nutrient components, such as phytate in whole grain and bran, oxalic acid in spinach, phosphates in practically all foods, and tannins in black tea and coffee may decrease the bioavailability of iron.

FeSO₄ is water soluble compound, but is easily oxidized to low soluble Fe³⁺ form after contact with oxygen. This may often cause unacceptable change in the color and taste of foods. Moreover, Fe²⁺ frequently causes gastrointestinal side effects. It is highly desirable to find a better alternative iron source that can overcome the gastrointestinal side effects of iron. Iron oxide/hydroxide nanoparticles with neutral and hydrophilic carbohydrate nanosize shell are used as alternative to ferrous salts. In these formulations, gastrointestinal side effects are rare because hundreds of Fe³⁺ atoms are safely packed in nanoscaled cores surrounded by the solubilizing shell. There are some evidences that particles in the range of nanometre show different properties compared to larger particles. This and the fact that very large surface area of nanoparticles allows iron to be much more bioavailable, suggest the potential of designing and producing particles of nano dimension. Nanoparticle based delivery systems can boost iron bioavailability and play a

vital role in food applications in future for treating micronutrient deficiencies.

3. RNA guided genome editing in plants

Bacteria and archaea have evolved several mechanisms to protect themselves against phage infections. Among different mechanisms, they have also developed an immune system. They acquire resistance against plasmids or phages by integrating short pieces of invader's DNA (i.e. proto-spacers) into bacteria's own genome locus called "clustered regularly interspaced short palindromic repeats" (CRISPRs). In the type II system of *Streptococcus pyogenes* CRISPRs are transcribed and processed into several short CRISPR derived RNAs (crRNAs) which are assembled with another transcribed trans-activating-RNA (tracrRNA) and the CRISPR associated protein 9 (Cas9). The tracrRNA/crRNA/Cas9 complex can target and cleave invading double stranded DNA, mostly resulting in DNA destruction. For correct functioning proto-spacer adjacent motifs (PAMs) are necessary which are short sequence motifs present in the invader's DNA adjacent to the integrated sequence. It has been demonstrated that in-vitro target DNA cleavage can be achieved using only Cas9 protein and a chimera of the tracrRNA and the crRNA (called guide RNA). Recently it has been showed that this system can be used beyond bacteria for targeted DNA cleavage and genome modifications in mammalian cells, Zebrafish and yeast. We are adapting the Cas9/guideRNA system for genome engineering approaches in plants for improved genetic traits. In future this system might also evolve to develop non-transgenic mode of genome editing.

4. Establishment of probiotic therapy against obesity and diabetes

Obesity and diabetes is associated with excess caloric intake and reduced energy expenditure

resulting in a negative energy balance. The complex metabolic pathogenesis has rendered the current treatment modalities inadequate to effectively combat these diseases. Diabetes incidence has reached epidemic proportions and childhood diabetes and obesity is on an alarming increase. Therefore, it is important to develop safe, easily deliverable and economically viable treatment alternatives for these diseases. Our previous studies provide data supporting the candidacy of probiotics as a therapeutic modality against obesity and diabetes. Probiotics are live bacteria that colonize the gastrointestinal tract and impart health beneficial effects. However, their widespread prescription as medical therapies is limited primarily due to paucity in our understanding of their mechanism of action. Our recent studies demonstrated that administration of a probiotic VSL#3 can prevent and treat obesity and diabetes in several mouse models. VSL#3 suppressed body weight gain and insulin resistance via modulation of the gut flora composition. With this current research we tend to focus on isolation and characterization of new food/ human origin probiotic strains with anti-obese/anti-diabetic potential and explore the target genes of probiotics action(s) and their correlation with gut-flora modulation. Identity of such target genes is unknown and we will attempt to uncover the general and tissue-specific probiotics targets that regulate glucose homeostasis and their correlation with probiotics mediated modulation of gut flora. Pre-clinical and clinical efficacy of probiotics in weight loss will be studied. This will give a unique opportunity to use probiotics in weight management regimens and help to fight against obesity and diabetes.

SYNERGY THROUGH COLLABORATIONS & NETWORKING

1. NABI and National Research Centre for Litchi (NRCL), Muzaffarpur, Bihar signed a MOU on September 15th, 2012 to share R&D facilities and carry out joint research projects.
2. 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.
3. 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.
4. 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.



EXTRAMURAL GRANTS AND FUNDINGS

S.No.	Project Investigator	Title of the Project	Funding Agency
1.	Dr. Ajay K Pandey	Metabolic engineering of phytic acid pathway for improving iron bioavailability in wheat.	DBT
2.	Dr. Santosh Kumar Upadhyay	Identification, isolation and characterization of novel insecticidal proteins from lower plant diversity. .	DST
3.	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.	BIRAC DBT
4.	Dr. Hariom Yadav	Probiotic mediated gut flora modulation can protect obesity and diabetes.	DBT
5.	Dr. Hariom Yadav	Development of novel compounds for treatment of obesity and type 2 diabetes.	DST
6.	Dr. Mahendra Bishnoi	Studies of transient receptor potential (TRP) channel mediated modulation of adipogenesis & obesity by dietary molecules.	DST
7.	Dr. Kanthi K Kondepudi	Nutrigenomic study to assess the role of polyphenols from Indian millet varieties on the regulation of adipogenesis.	DST





PROGRESS OF INFRASTRUCTURE AT MAIN CAMPUS



Proposed Master Plan of the main campus, Sector-81, Mohali.



Model of NABI-BPU upcoming campus in sector -81, Mohali.

Participation in National/International Conference/Workshops:

1. Dr. Rakesh Tuli attended a seminar on “Secondary Agriculture Opportunities in India” held on April 9th, 2012 at FICCI, New Delhi.
2. Dr. Joy K. Roy was invited to deliver a lecture on “Immunogenic Moieties in Wheat and Genes Confer Antigenticity to Wheat” at DBT sponsored brain storming session held on May 28th, 2012 at Vellore, Tamil Nadu.
3. Dr. Monika Garg was invited to deliver a lecture on “Potential of Biotechnological Approaches for Reduction of Gluten in Wheat” at DBT sponsored brain storming session held on May 28th, 2012 at Vellore, Tamil Nadu.
4. Sh. Shrikant Mantri attended a advanced faculty training workshop on “Think Parallel – Parallel Programming for Engineers and Scientists” held during June 12th-22nd, 2012 at CDAC, Bangalore.
5. Dr. S.P. Singh visited the Centre of Excellence for Postharvest Biotechnology and Centre for Future Crop Research at University of Nottingham, Malaysia Campus, Kuala Lumpur, Malaysia on June 28th, 2012.
6. Dr. Monika Garg visited Beijing, China to attend "11th International Gluten workshop" during August 12th-15th, 2012 for oral presentation "Towards Understanding Genetic Basis of Chapatti Making Quality" and Poster Presentation "Transfer of HMW Glutenin Genes From *Th. elongatum* for Improvement of Bread Making Quality". This visit was jointly supported by DBT-CTEP, CICS and conference organizers (CAAS, CAS, CIMMYT).
7. Dr. Monika Garg was invited to deliver a lecture on “Emerging Agri-Food Biotechnology Sectors” at Shaheed Udham Singh College of Engineering and Technology, Tangori, Mohali on September 19th, 2012.
8. Sh. Shrikant Mantri attended the 19th DeLCON steering and negotiation meeting held on November 2nd, 2012 at ICGEB, New Delhi.
9. Dr. Ajay K. Pandey attended an international conference on “Recent Trends in Lathyrus Sativus Research” held at National Institute of Nutrition, ICMR, Hyderabad during November 8th-9th, 2012.
10. Dr. Siddharth Tiwari attended the complete capacity enhancement training at Queensland University of Technology (QUT), Brisbane, Australia on regeneration methodology (Stewardship Stage 1) held during December 10th -14th, 2012. This training was performed under the project on “Development and Transfer of Technology from Queensland University of Technology (QUT), Australia to India for Biofortification and Disease Resistance in Banana”, externally funded by Biotechnology Industry Research Assistance Council (BIRAC), Government of India.
11. Dr. Rakesh Tuli, was invited to participate in a DAE-BRNS life sciences symposium 2012 on “Trends in Plant, Agriculture and Food Sciences” held during December 17th -19th, 2012 at BARC, Mumbai. He delivered a lecture entitled “The Problem of Mineral Nutrition in Cereals and Molecular Approaches to Enhance Bio-availability”.
12. Dr. Rakesh Tuli, was invited to deliver a lecture on “Biotechnology for Designer Plants and Foods” held on February 3rd, 2013 at KB Patil College, Mumbai.
13. Sh. Shrikant Mantri attended a workshop on “National Mission on Supercomputing” held on

February 8th, 2013 at CDAC, Pune.

14. Dr. Rakesh Tuli attended a conference on “Innovation for Equitable Growth” held at NIPER, Mohali during February 21st - 22nd, 2013.
15. Dr. Joy K. Roy attended a session on “Biosciences and Biotechnology” organized by CHASCON -07 on March 2nd, 2013. He delivered a lecture on “Development of Nucleic-acid Based Markers Through Next-gen Sequencing”.
16. Dr. Vikas Rishi was invited to participate in the symposium sponsored by UGC-SAP on “Recent Trends in Cancer Research” held on March 18th, 2013 at Panjab University, Chandigarh. He delivered a lecture on “B-ZIP Transcription Factors as Molecular Targets for Cancer Prevention.

International visitors to NABI

1. Ms. Lessely Torrence, Dr. Ankush Prashar and Dr. Mark Taylor from James Hutton Institute visited NABI on May 11th, 2012 and discussed about plant pathology, genetic & abiotic stress.
2. Dr. Chris Barker, Genome Prairie, Saskatoon, Canada visited NABI on October 18th, 2012 and discussed about flax genomics.
3. Dr. Munish Puri, Associate Professor, Deakin University, Victoria, Australia visited NABI on December 4th, 2012 and discussed about bioactive molecules research in plants.
4. Mr. Bui Quoc Khanh, Embassy of the Socialist Republic of Vietnam, visited NABI for research collaboration in agriculture with India on December 12th, 2012.
5. Mr. Ian Dean, CEO, Groman Consulting, presented his topic about change leadership in R& D Institutes at NABI on January 18th, 2013.
6. Professor Louis S. Premkumar, Department of Pharmacology, Southern Illinois University, School of Medicine, Springfield, IL delivered a lecture on “Role of TRP channels in different disease pathologies” at NABI on February 7th, 2013.
7. Dr. Abhinav Verma, Steinbuch Center for Computing, Karlsruhe Institute of Technology, Karlsruhe, Germany visited NABI on March 7th 2013 and talked about “Molecular Simulations of protein structure, folding, and dynamics”.





NEWLY JOINED FACULTY

(BRIEF PROFILES)

Name: Dr. Hariom Yadav

Date of Joining: 14-12-2012

Designation: Ramalingaswami Fellow

Area of Research Interest: Investigation of the biological and molecular effects of novel nutraceuticals against chronic human diseases i.e. iron deficiency and metabolic diseases. Ultimate goal of my research is to develop functional foods that can ameliorate these human diseases.



Past Appointments:

1. **Research Fellow (4/2012-12/2012):** Diabetes, Endocrinology and Obesity Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA.
2. **Post Doctoral Visiting Fellow (2007-2012):** Diabetes, Endocrinology and Obesity Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA.

Major Publications:

1. **Yadav H, Jain S, Prasad GBKS and Yadav M (2007).** Preventive effect of diabegon; a polyherbal preparation during progression of diabetes in high fructose fed rats. **Journal of Pharmacological Sciences.** 105: 12-21.
2. **Yadav H, Jain S and Sinha PR (2007).** Anti-diabetic effect of probiotic dahi containing *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactococcus lactis* bacteria in high fructose diet fed rats. **Nutrition.** 72: 62-68
3. **Yadav H, Jain S and Sinha PR (2007).** Production of free fatty acids and conjugated linoleic acid in probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* during fermentation and storage. **International Dairy Journal.** 60: 1006-1010.
4. **Yadav H, Jain S and Sinha PR (2008).** Oral administration of dahi containing probiotic *Lactobacillus acidophilus* and *Lactobacillus casei* ameliorated the Streptozotocin-induced oxidative stress and dyslipidemia in rats. **Journal of Dairy Research.** 75: 189-195.
5. Lin HM, Lee JH, **Yadav H**, Kamaraju AK, Gavrilova O, Liu E, Vieira A, Kim SJ, Collins H, Matschinsky H, Harlan DM, Roberts AB and Rane SG (2009). TGF- β /Smad3 signaling regulates insulin gene transcription, pancreatic islet β -cell function and insulin action. **Journal of Biological Chemistry.** 284: 12246-57.
6. Kim YC, Kim SY, Mellado-Gil JM, **Yadav H**, Neidermyer W, Kamaraju AK and Rane SG (2011). RB regulates pancreas development by stabilizing Pdx1. **EMBO Journal.** 30: 1563-76.
7. **Yadav H**, Quijano C, Kamaraju AK, Gavrilova O, Malek R, Chen W, Zerfas P, Zhigang D, Wright E, Lonning S, Krause M, Skarulis M, Sumner A, Finkle T and Rane SG (2011). Protection from obesity and diabetes by blockade of TGF- β /Smad3 signaling. **Cell Metabolism.** 14: 67-79.

Name: Dr. Santosh Kumar Upadhyay

Date of Joining: 01-03-2013

Designation: DST-INSPIRE Faculty

Area of Research Interest: RNA guided genome editing in plants. Engineering and characterization of new insecticidal proteins and mode of action by protein-protein interaction and transcriptome dynamics.



Awards and Fellowship:

1. INSA Young Scientist Award 2013.
2. NASI Platinum Jubilee Young Scientist Award 2012.
3. DST-INSPIRE Faculty Fellowship 2012.
4. Alltech Young Scientist Award 2011 (IIIrd place in South Asia).

Past Appointment:

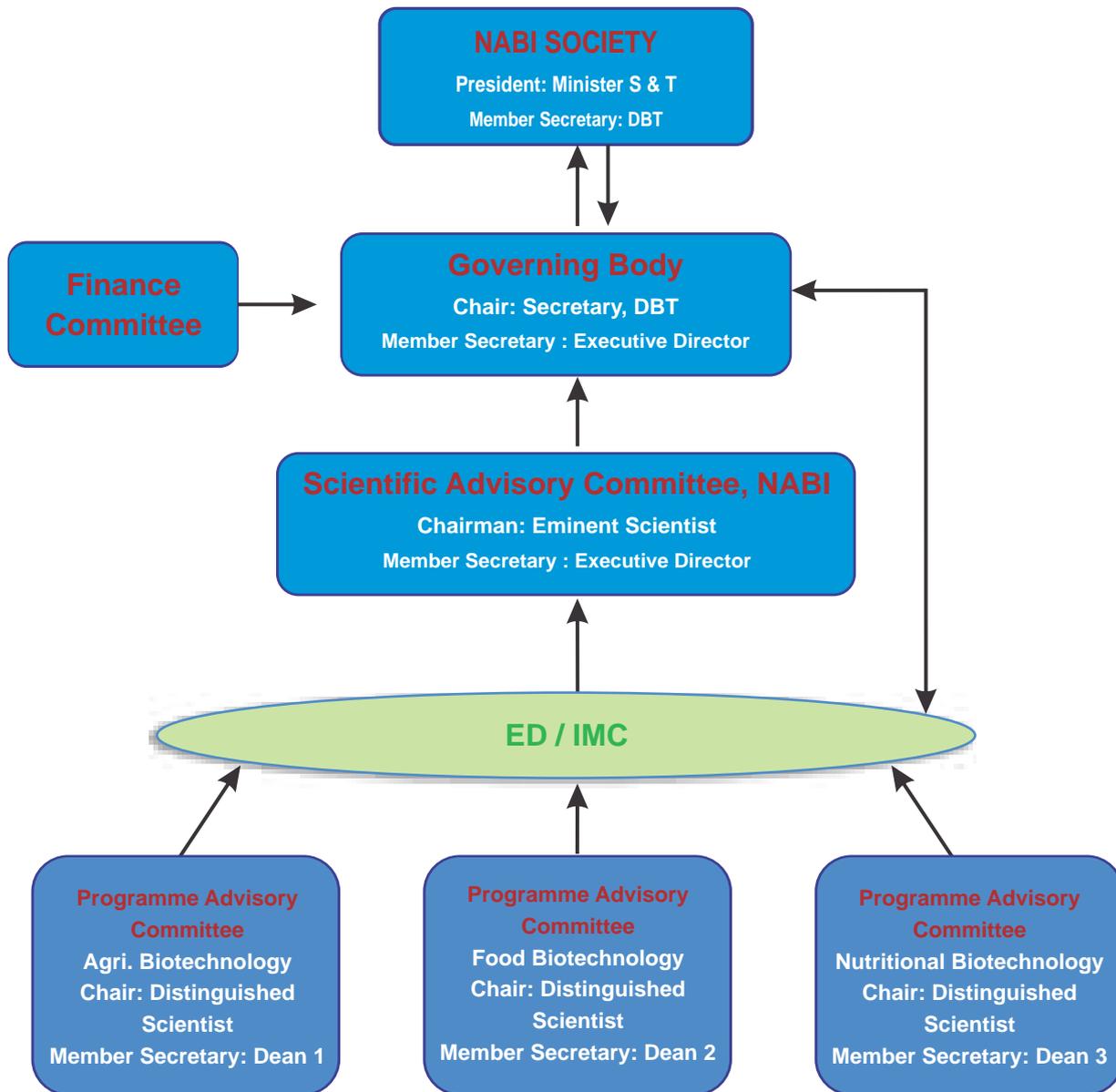
CSIR-Senior Research Fellow (2010-2013): CSIR-National Botanical Research Institute, Lucknow, India.

Major Publications:

1. **Upadhyay SK**, Chandrashekar K, Thakur N, Verma PC, Singh PK and Tuli R (2011). RNA interference (RNAi) for the control of whitefly (*Bemisia tabaci*). **Journal of Biosciences**. 36: 153–161.
2. **Upadhyay SK** and Singh PK (2011). Role of alkaline phosphatase in insecticidal action of Cry1Ac against *Helicoverpa armigera* larvae. **Biotechnol letters**. 33: 2027-2036.
3. **Upadhyay SK**, Sharad S, Rai P, Singh R, Chandrashekar K, Verma PC, Singh PK and Tuli R (2010). SUMO fusion facilitates expression and purification of garlic lectin but modifies some of its properties. **Journal of Biotechnology**. 146: 1-8.
4. **Upadhyay SK**, Mishra M, Singh H, Ranjan A, Chandrashekar K, Verma PC, Singh PK and Tuli R (2010). Interaction of *Allium sativum* leaf agglutinin (ASAL) with midgut BBMV proteins and its stability in *Helicoverpa armigera*. **Proteomics**. 10: 4431–4440.
5. **Upadhyay SK** and Singh PK (2011). Role of alkaline phosphatase in insecticidal action of Cry 1Ac against *Helicoverpa armigera* larvae. **Biotechnol let**. 33:2027-2036



GOVERNANCE





MANAGEMENT OF THE INSTITUTE

A. Members of NABI Society

Sh. Jaipal Sudini Reddy

Hon'ble Minister of Science and Technology and Earth Sciences,
Ministry of Science & Technology and Earth Sciences,
Government of India, New Delhi
(President)
(October 29th, 2012 to till date)

Sh. Vayalar Ravi

Hon'ble Minister of Science and Technology and Earth Sciences,
Ministry of Science & Technology and Earth Sciences,
Government of India, New Delhi
(President)
(August 14th, 2012 to October 28th, 2012)

Sh. Vilasrao Deshmukh

Hon'ble Minister of Science and Technology and Earth Sciences,
Ministry of Science & Technology and Earth Sciences,
Government of India, New Delhi
(President)
(July 12th, 2011 to August 14th, 2012)

Dr. K. VijayRaghavan

Secretary,
Department of Biotechnology,
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B. Governing Body

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Sh. R. L. Sharma

Associate Director,
National Agri-Food Biotechnology Institute,
Mohali
(Non-Member Secretary)



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Bioprocessing Unit,
Mohali

Dr. R.S. Khandpur

Director General,
Pushpa Gujral Science City,
Chandigarh

Dr. Rajesh Kapur

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

Er. N.K. Verma

Chief Engineer,
Council of Scientific and Industrial Research,
New Delhi

Dr. K. K. Kaul

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

Late Sh. N.S. Bhatti

Former Chief Engineer,
Punjab Administration,
Chandigarh

Ms. Anuradha Mitra

Financial Advisor,
Council of Scientific and Industrial Research,
New Delhi

Sh. Sreeshan Raghavan

Joint Secretary,
Department of Biotechnology,
New Delhi

Dr. Jagdeep Singh

Additional Director,
Department of Higher Education,
Chandigarh

Sh. R.L. Sharma

Associate Director,
National Agri-Food Biotechnology Institute,
Mohali

Dr. A. Vamsi Krishna

Scientist - C,
Department of Biotechnology,
New Delhi

Sh. Virendra K. Banerjee

Administrative Officer,
National Agri-Food Biotechnology Institute,
Mohali





RESEARCH PUBLICATIONS

I. Publications based on research initiated at NABI:

1. Baboota RK, **Bishnoi M**, Ambalam P, **Kondepudi KK**, Sarma SM, Boparai RK and Podili K (2013). Functional food ingredients for the management of obesity and associated co-morbidities– a review. **Journal of Functional Foods**. dx.doi.org/10.1016/j.jff.2013.04.014 (in press).
2. **Bishnoi M**, **Kondepudi KK**, Baboota RK, Dubey R and Boparai RK (2013). Role of Transient Receptor Potential (TRP) channels in adipocyte biology. **Expert Review of Endocrinology and Metabolism** (in press).
3. **Bishnoi M**, **Kondepudi KK**, Gupta A, Karmase A and Boparai RK (2013). Expression of multiple Transient Receptor Potential (TRP) channel genes in murine 3T3-L1 cell lines and adipose tissue. **Pharmacological Reports** (in press).
4. **Kumar J**, Gunapati S, **Singh SP**, Kumar A, Lalit A, Sharma NC, Puranik R and **Tuli R** (2013). A new betasatellite associated with cotton leaf curl Burewala virus infecting tomato in India: influence on symptoms and viral accumulation. **Archives of Virology**. DOI: 10.1007/s00705-013-1613-y.
5. **Kumar J**, **Singh SP**, Kumar A, Khan JA and **Tuli R** (2013). Detection and characterization of a new betasatellite: variation in disease symptom of Tomato leaf curl Pakistan virus-India due to associated betasatellite. **Archives of Virology**. 158: 257-261.
6. **Singh SP**, Vogel-Mikus K, Arcon I, Vavpetic P, Jeromel L, Pelicon P, **Kumar J** and **Tuli R** (2013). Pattern of iron distribution in maternal and filial tissues in wheat grains with contrasting levels of iron. **Journal of Experimental Botany**. DOI: 10.1093/jxb/ert160.
7. Raman M, Ambalam P, **Kondepudi KK**, Pithva S, Kothari C, Patel AT, Purama RK, Dave JM and Vyas BR (2013). Potential of probiotics, prebiotics and synbiotics for management of colorectal cancer. **Gut Microbes**. 4(3): 181-192.

II. Publications based on work done by faculty or initiated at their earlier institute:

2013

1. Das A, Saha T, Ahmad F, Roy KB and **Rishi V** (2013). Dodecamer d-AGATCTAGATCT and a homologous hairpin form triplex in the presence of peptide REWER. **PLoS ONE**. 8-5.
2. Dixit S, Yadav S, **Upadhyay SK**, Verma PC and Chandrashekar K (2013). A method to produce insect resistance in plant by altering amino acid content in sap. **International Journal of Biotechnology**. Res 3: 13-20.
3. Singh B, Singh K, Rao GR, Chikara J, Kumar D, Mishra DK, Saikia SP, Pathre UV, Raghuvanshi N, Rahi TS and **Tuli R** (2013). Agro-technology of *Jatropha curcas* for diverse environmental conditions in India. **Biomass & Bioenergy**. 48: 191-202.
4. **Singh SP** and Singh Z (2013). Dynamics of enzymatic and non-enzymatic antioxidants in Japanese plums during storage at safe and lethal temperatures. **LWT-Food Science and Technology**. 50: 562-568.
5. **Singh SP** and Singh Z (2013). Controlled and modified atmospheres influence chilling injury, fruit quality and antioxidative system of Japanese plums (*Prunus salicina* Lindell). **International Journal of Food Science and Technology**. 48(2): 363-374.
6. **Singh SP** and Singh Z (2013). Postharvest cold storage-induced oxidative stress in Japanese plums in relation to harvest maturity. **Australian Journal of Crop Science**. 7(3): 391-400.

2012

7. Gupta P, Idris A, **Mantri S**, Asif MF, Yadav HK, **Roy JK**, **Tuli R**, Mohanty CS, Sawant SV, (2012) Discovery and use of single nucleotide polymorphic (SNP) markers in *Jatropha curcas* L. *Molecular Breeding*. 30: 1325 -1335
8. Kumar J, Gunapati S, **Singh SP**, Lalit A, Sharma NC and **Tuli R** (2012). First report of 'Candidatus *Phytoplasma asteresis*' (16SrI group) associated with little leaf disease of brinjal in India. **New Disease Reports**. (Earlier BSPP Plant Pathology) 26- 21.
9. **Singh SP** and Singh Z (2012). Role of membrane lipid peroxidation, enzymatic and non-enzymatic antioxidative systems in the development of chilling injury in Japanese plums. **Journal of the American Society for Horticultural Science**. 137(6): 473-481.
10. **Singh SP** and Singh Z (2012). Postharvest oxidative behaviour of 1-methylcyclopropene treated Japanese plums (*Prunus salicina* Lindell) during storage under controlled and modified atmospheres. **Postharvest Biology and Technology**. 74: 26-35.
11. **Upadhyay SK**, Singh S, Chandrashekar K, Singh PK and **Tuli R** (2012). Compatibility of garlic (*Allium sativum* L.) leaf agglutinin and Cry1Ac -endotoxin for gene pyramiding. **Applied Microbiology and Biotechnology**. 93: 2365–2375.

Patent filed for research work initiated at NABI

1. **Tuli R, Kumar J and Kumar J** (2013). A recombinant mastrevirus as a viral vector system. (639/DEL/2013) submitted.





HUMAN RESOURCE

I. Research Faculty

S. No	Name	Designation	Date of Joining
Regular Faculty			
1	Dr. Rakesh Tuli	Executive Director	08-02-2010
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 K. 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
Contractual Faculty			
13	Dr. Shailesh Sharma	Project Scientist	02-01-2012
14	Dr. Nishima	Project Scientist	04-01-2012
15	Dr. Sudhir P. Singh	Project Scientist	16-01-2012
16	Ms. Vandana Mishra	Project Scientist	23-01-2012
17	Dr. Hariom Yadav	Ramalingaswami Fellow	14-12-2012
18	Dr. Santosh Kumar Upadhyay	INSPIRE Fellow	01-03-2013

II. Technical and Engineering Support

S. No	Name	Designation	Date of Joining
1	Sh. E. Subramanian	Computer Operator	27-02-2010
2	Ms. Aakriti Gupta	Senior Tech Assistant	22-02-2011
3	Sh. Jagdeep Singh	Senior Tech Assistant	01-03-2011
4	Sh. Sukhjinder Singh	Computer Operator	23-02-2012
5	Sh. Jaspreet Singh	Assistant Engineer	19-03-2012
6	Sh. Sushant Vatsa	Assistant Engineer	02-04-2012
7	Dr. Mainpal Singh	Senior Tech Assistant	24-12-2012
8	Sh. Atul Kesarwani	Senior Tech Assistant	21-01-2013
9	Sh. Kamalendra	Senior Tech Assistant	18-03-2013

III. Administration

S.No	Name	Designation	Date of Joining
1	Sh. Rattan Lal Sharma	Associate Director (Accounts and Finance)	25-5-2011
2	Sh. S. Krishnan	Store & Purchase Officer	10-03-2010
3	Sh. Vikram Singh	Administrative Officer	01-04-2011
4	Sh. Suneet Verma	Finance Officer	15-09-2011
5	Sh. Sabir Ali	Executive Assistant (Administration)	21-01-2011
6	Ms. Hema Rawat	Executive 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
<i>Students enrolled for Ph.D degrees:</i>			
1	Sh. Jitendra Kumar	Development of virus induced gene silencing vector and its application in studying gene function in wheat (<i>Triticum aestivum l.</i>)	Barkatullah University, Bhopal, MP
2	Sh. Yogesh Gupta	Gene discovery for seedlessness in <i>Annona</i> species.	Panjab University, Chandigarh, Punjab
3	Ms. Anuradha Singh	Expression analysis of starch biosynthesis pathway genes and their effects on starch quality.	Guru Jambheshwar University of Science & Technology, Hisar, Haryana
4	Sh. Rohit Kumar	Allelic variation in puroindolines in Indian wheat cultivars, their association with hardness and starch granule properties.	Panjab University, Chandigarh, Punjab

S.No	Name	Designation	Date of Joining
1	Sh. Jitesh Kumar	Junior Research Fellow	09-09-2011
2	Ms. Manpreet Kaur Saini	Junior Research Fellow	09-09-2011
3	Sh. Anshu Alok	Junior Research Fellow	09-09-2011
4	Sh. Kaushal Kumar Bhati	Junior Research Fellow	14-11-2011
5	Ms. Monica Sharma	Junior Research Fellow	01-03-2012

S.No	Name	Designation	Date of Joining
6	Sh. Raja Jeet	Junior Research Fellow	12-03-2012
7	Sh. Ashish Kumar Pathak	Junior Research Fellow	08-08-2012
8	Ms. Sipla Aggarwal	Junior Research Fellow	16-08-2012
9	Sh. Prateek Jain	Junior Research Fellow	31-08-2012
10	Sh. Ritesh Kumar Baboota	Junior Research Fellow	21-09-2012
11	Ms. Stanzin Angmo	Junior Research Fellow	11-02-2013
12	Ms. Shivani Sharma	Junior Research Fellow	12-02-2013
13	Sh. Shashank Singh	Junior Research Fellow	22-02-2013
14	Sh. Vishnu Shukla	Junior Research Fellow	25-02-2013
15	Ms. Mandeep Kaur	Junior Research Fellow	18-03-2013

ii. Trainees:

S.No	Name	Designation	Date of Joining
1	Ms. Vandana Bijalwan	Trainee	01-08-2012
2	Sh. Anil Kumar Saini	Trainee	01-01-2013
3	Ms. Harsimran Kaur	Trainee	01-01-2013
4	Ms. Meenakshi Katyal	Trainee	01-01-2013
5	Ms. Manpreet Kaur	Trainee	01-01-2013
6	Ms. Nida Murtaza	Trainee	01-01-2013
7	Ms. Renuka Maurya	Trainee	01-01-2013
8	Ms. Shumalya	Trainee	01-01-2013
9	Sh. Shiv Prasad	Trainee	01-01-2013
10	Ms. Sushma Vishwakarma	Trainee	02-01-2013
11	Sh. Paramvir Mann	Trainee	02-01-2013
12	Sh. Rahul Thakur	Trainee	03-01-2013
13	Ms. Tripathi Rungta	Trainee	03-01-2013
14	Ms. Preetika	Trainee	06-01-2013
15	Sh. Arun Malik	Trainee	07-01-2013
16	Sh. Harmanmeet Brar	Trainee	07-01-2013
17	Ms. Priyanka Chopra	Trainee	07-01-2013
18	Sh. Harnish	Trainee	08-01-2013
19	Ms. Jyoti Bhargava	Trainee	09-01-2013
20	Ms. Harleen Kaur	Trainee	15-01-2013
21	Ms. Navjit Kaur	Trainee	23-01-2013
22	Sh. Phanikanth Jogam	Trainee	25-01-2013
23	Ms. Ankita Mishra	Trainee	01-02-2013
24	Sh. Umang Gupta	Trainee	06-02-2013



PHOTO GALLERY OF IMPORTANT EVENTS

Celebration of Independence Day: August 15th, 2012



Dr Rakesh Tuli, Executive Director, NABI and Dr. R S Sangwan, CEO, BPU hoisted the National flag at NABI Interim Facility and addressed the staff.



Independence Day celebrations at the NABI Interim Facility.

Bioscience Cluster Meeting – August 21st, 2012



Bioscience Cluster meeting is being chaired by Dr. M.K Bhan, Secretary, DBT (Centre).

From Left: Dr. Dinakar M. Salunke, Director, RCB; Dr. Rakesh Tuli, Executive Director, NABI; Dr. M.K Bhan, Secretary, DBT; Dr. Karan Avatar Singh, Principal Secretary, Department of Industries and Commerce, Government of Punjab; Dr. Rajesh Kapur, Advisor, DBT.



Discussion among the heads of the institute from PU, IISER, PGIMER, ISB, Biotech Park, PSCST, NABI, BPU, NIPER, IMTech, PBTI, IIT (Ropar) and PAU.

Third PAC Food Biotechnology Meeting: September 15th, 2012



From Left: Dr. S. Nagarajan, Former Chairman, PPV&FR; Dr. V. Prakash, Former Director, CFTRI (Chairman PAC, Food Biotechnology) and Dr. Rakesh Tuli, Executive Director, NABI.



Faculties presenting their research work to the experts.

Collaboration with other Institutes



MOU was signed between Dr. Vishal Nath (left), Director, NRCL and Dr. Rakesh Tuli, Executive Director, NABI to undertake joint research work in the area of mutual interest.



MOU was signed between Dr. Jai Rup Singh, VC, CUPB and Dr. Rakesh Tuli, Executive Director, NABI to promote quality research and high end research programmes between two institutes.

Visitors to the Institute



Dr. Chris Barker, Genome Prairie, Saskatoon, Canada having discussions with NABI faculty on October 19th, 2012.



Dr. Sudhir P. Singh demonstrating LCM related research experiments to the delegates from Scotland on May 11th, 2012.



Mr. Bui Quoc Khanh, Embassy of the Socialist Republic of Vietnam visited NABI for collaboration in Agriculture with India on December 12th, 2012.



Mr. Ian Dean, CEO, Groman Consulting talking about the “Change Leadership in R&D Institutes” on January 18th, 2013.

Republic Day Celebrations: January 26th, 2013



Dr. Rakesh Tuli, Executive Director, NABI and Dr. R.S Sangwan, CEO, BPU hoisted the National Flag at Interim Facility.



NABI staff, celebrating the Republic Day with their family members at NABI campus.

Third PAC Agricultural Biotechnology Meeting: February 18th, 2013



Scientific discussion during the PAC (Agri Biotechnology) meeting. The session was chaired by Dr. C.R Bhatia, Former Secretary, DBT.

Third Foundation Day: February 18th, 2013



First row from left: Dr. C.R Bhatia, Former Secretary, DBT unveiled the NABI-BPU future campus model.

Dr. Rakesh Tuli, Executive Director, NABI explaining about the model to invited guests on the occasion.

Second row from left: Dr. V. Prakash, Former Director, CFTRI delivering Foundation Day lecture on “Biotechnology in Agri-Food Chain”.

Dr. Manju Sharma, Former Secretary, DBT was the Chief Guest on the occasion and delivered a lecture on “Biotechnological Tools for Nutritious Foods”.

Third row from left: Dr. C.R Bhatia, Former Secretary, DBT presided over the function.

Dr. Vikas Rishi, Scientist, NABI giving vote of thanks.

Meetings of PAC & SAC: March 11th, 2013



From Left: Dr. B. Sivakumar, Chairman - PAC, Nutrition Biotechnology and Dr. R.S Paroda, Chairman - SAC



Experts in Agri, Food and Nutrition Biotechnology having discussions with faculty during PAC and SAC meetings.



FINANCIALS

Annual Accounts for the year 2012-13:

- The financial resource of the institute is the core grant provided by the Department of Biotechnology, Govt. of India under non-recurring and recurring components.
- The institute received the core grant of Rs.2074.944 Lacs in the year 2012-13.
- Annual accounts for the year 2012-13 are prepared by the institute on the basis of accrual system of accounting using standard format of accounts prescribed by the Government of India for Central Autonomous Bodies.
- M/s Raj Gupta & co., (Chartered Accountant) Chandigarh, the Statutory Auditor of the institute have audited the accounts.

Financial Position

Figures in Rupees

S.NO.	Particulars	As on 31-03-2012	As on 31-03-2013
A.	CAPITAL FUND AND LIABILITIES		
1.	Capital Fund	36,08,87,705	43,85,56,250
2.	Earmarked/Endowment Funds	-----	1,70,63,097
3.	Current Liability and Provisions	69,72,351	1,00,80,443
	Total	36,78,60,056	46,56,99,790
B.	ASSETS		
1	Fixed Assets	18,07,26,363	31,35,37,217
2.	Capital Work-in-Progress	3,69,11,630	1,70,14,470
3.	Investment from Earmarked/Endowment Funds	-----	1,02,65,524
4.	Current Assets ,Loans & Advances etc.	15,02,22,063	12,48,82,579
	Total	36,78,60,056	46,56,99,790
C.	RECEIPT OF THE INSTITUTE		
1.	Grants from DBT	23,58,00,000	20,74,94,400
2.	Interest Earned	87,79,070	93,55,364
3.	Other Income	4,00,838	76,95,349
	Total	24,49,79,908	22,45,45,113
D.	UTILIZATION		
1.	Non-Recurring Expenses		
	a) Main Campus	----	83,57,674
	b) Equipment	7,42,44,835	15,71,97,112
	c) Vehicle	----	----
	d) Furniture & Fixtures	7,45,163	8,76,355
	e) Computer and other Fixed Assets	55,02,652	1,51,10,971
	f) Capital Work-In Progress	3,69,11,630	1,70,14,470
2.	Recurring Expenses	9,11,31,221	9,81,45,310
E	Payables	69,72,351	1,00,80,443
F	Advances / Receivables	1,65,25,554	32,27,829
G	Margin Money for LCs	8,27,47,849	13,74,802
H	Closing Bank Balance (Excluding Margin Money)	5,09,48,660	12,02,79,948

NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE
C-127, Phase VIII, Industrial Area, S.A.S. Nagar,
Mohali-160071, Punjab, INDIA



High Performance Computing Cluster facility at NABI

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