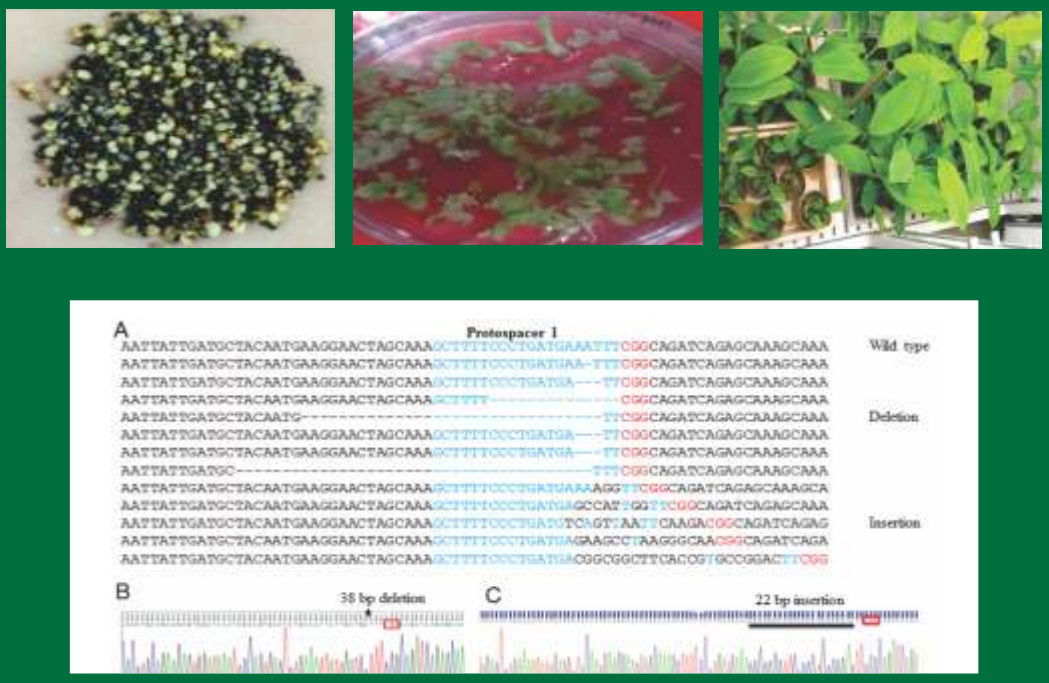
वार्षिक प्रतिवेदन
ANNUAL REPORT
2013 - 2014



राष्ट्रीय कृषि-खाद्य जैव प्रोद्योगिकी संस्थान

National Agri-Food Biotechnology Institute

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

Published by:
Executive Director
National Agri-Food Biotechnology Institute (NABI)
C-127, Industrial Area, Phase 8, S.A.S. Nagar,
Mohali, Punjab, India - 160071

Publication Committee:
Dr. Ajay K. Pandey
Sh. Shrikant Mantri
Dr. Mahendra Bishnoi
Sh. Arun Kumar

Acknowledgment:
Scientists & Administration
for suggestions and
providing information

Figure on Cover:
Upper Panel : The cover page portrays different steps of banana transformation (details of the study is given on page 30).
Lower Panel : Utilization of CRISPR-Cas technique for genome editing. (details of the study is given on page 46).

वार्षिक प्रतिवेदन
ANNUAL REPORT
2013-2014

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

(An Autonomous Institute of Department of Biotechnology, Government of India)
C-127, Industrial Area, Phase 8, Ajitgarh (Mohali), Punjab, India - 160071
EPABX: +91-172-4990145, Fax: 0172-4604888
Website : www.nabi.res.in

CONTENTS

FROM THE DESK OF THE EXECUTIVE DIRECTOR

National Agri-Food Biotechnology Institute (NABI) completes four years of its establishment with the basic objectives of promoting and coordinating research of high calibre in basic and applied aspects at the interface of Agriculture, Food and Nutrition. This year was very significant for NABI, in terms of expanding our research, publications and initiating successful collaboration with the neighbouring institutes.

One of major research work in the area of agri-biotechnology focuses on addressing the possible role of genes related to processing quality and their interaction (quality x development) in wheat. Genome-wide transcriptome study was used to decipher the candidate genes associated with such traits. Furthermore, polymorphism in the genes related to the grain hardness and softness was explored and an updated database was created. New chromosome specific translocation lines of wild species (*Agropyron elongatum*) with potential for better bread making quality have been generated. Marker assisted breeding to transfer good processing quality gene/s to high yielding cultivar is in progress. In an approach to understand and design strategy for enhancing micronutrient-bioavailability, localization of iron in contrasting wheat cultivars was reported. Similarly, genes responsible for accumulation of anti-nutrients like phytic acid in wheat were also isolated. Basic biology of seed development is another major area of research. Dominant negative and root-scion approaches have been expanded now in developing or controlling the seedlessness nature of target fruit crops. Utilization of CRISPR-Cas system for targeted mutagenesis has been demonstrated in wheat suspension cells. A newly identified wheat virus, reported last year, was modified and advancement was made to apply for targeted gene silencing thus enabling the development of high-throughput functional screening.

A multi-institutional program to develop nutritionally enriched (high pro-vitamin-A) transgenic varieties of Indian banana is on its way. Post harvest science is another important area where the institute is focussing towards development of some processing technologies to prevent fruit crop losses. Quality, safety and postharvest stability of fresh fruits are areas of research in the food biotechnology. The discovery and validation of biomarker(s) linked to artificial fruit ripening in mango and banana fruits are under progression. High-throughput analysis of metabolites responsible for flavour, nutrition and processing quality of 'Kinnow' mandarins has revealed the impact of various pre- and post-harvest factors on fruit metabolome and its consequences on suitability of fruit for fresh and processing industries.

Food and Nutrition areas focus on preventing both kinds of malnutrition (under and over nutrition). Micronutrient under nutrition, especially of iron, leads to anaemia in pregnant and lactating women, children and other populations. Multiple approaches such as enhancing iron bioavailability using nano-biotechnology and identifying natural compounds that could maintain iron homeostasis are in progress at the institute to address micronutrient deficiency. Iron alginate encapsulated ferric saccharate microemulsions were synthesized, characterized and currently evaluation studies are being taken-up. Simultaneously, in the area of over-nutrition and associated complications, dietary components from Indian millet varieties, spices, and prebiotics have been studied for their beneficial effects using both *in vitro* and *in vivo* model systems. The beneficial effects of finger millet whole grain and bran have been investigated in an animal model of obesity, prominently via anti-inflammatory activity, anti-oxidant activity and gut microbial modulation. Nutrigenomic and molecular mechanisms of Transient Receptor Potential (TRP) channel mediated dietary

modulation using capsaicin (red chillies), cinnamaldehyde (cinnamon), allicin (garlic and onion) have been reported. Further, structural characterization of arabinoxylans from millets and their biological activity with parameters relating to the variability in the fine structures of phenolic acids bound arabinoxylans from Indian millet varieties and their consequence on anti-oxidant and anti-obesity/adipogenesis activity is underway.

To enhance our genomics analyses capabilities in the area of animal and crop biotechnology, the institute has undertaken a task for development of advanced algorithms, databases, tools and pipeline for data mining and comparative analysis of food crop genomes. Small RNA based regulation has been targeted during seed development. The 10.1 teraflop supercomputing facility has accelerated the transcriptome and genome based analysis. User friendly web-interface has been developed to facilitate the use of this supercomputing facility by biologists.

Human resource development is one of our priorities. The institute currently has 19 Ph.D. students and also 20 junior research fellows and project assistants. Besides, NABI is also involved in providing research training to young students in the diverse areas of Agriculture, Food and Nutrition Biology. Recently, NABI in collaboration with PGIMER, Chandigarh has been recommended for extramural funding for initiation of integrated Masters-Ph.D. program in Nutritional Biology. The institute is currently hosting 12 extramural funded grants from different national and international agencies.

In short span of 4 years, NABI has been able to make significant progress in terms of initiating high quality research programs, securing extramural grants from multiple funding agencies, pursuing collaborative programs with national and international partners and developing well trained human resource. The support from all concerned is highly appreciative and we look forward to move ahead.

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

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 Gene discovery for improvement of processing and nutrition quality in wheat

Principal Investigator

Joy K Roy

Research Fellows

Anuradha Singh
Monica Sharma
Pankaj Kumar

Introduction

Wheat flour is processed into a wide range of end-use food products, whose complex quality mainly depends on biochemical composition of grains such as storage protein, starch, phenolics, lipid, etc. The wheat grains contain about 70% starch which also requires to be improved into nutritive starch, for example, high amylose-starch or resistant starch for healthy wheat diets. The present varieties require improvement both in nutrition and processing quality to meet the increasing demand of healthy wheat diets for consumers and better processing quality for baking and processing industries. Genetical and molecular knowledge of genes and their regulators underlying nutrition and processing related quality traits and their interaction are important for improvement of processing and nutrition quality in wheat. In this project, candidate genes and then a subset of the candidate genes are targetted to identify causal genes and their coding and 5' non-coding regulatory single nucleotide polymorphisms (SNPs) and then their functional validation would be done to identify causative genes and their SNPs for wheat improvement and/or enhancement through molecular breeding.

Objectives

1. Identification of candidate genes using 55K transcripts present on Affymetrix® wheat microarrays.
2. Identification of additional candidate genes through transcriptome and small RNA sequencings.
3. Phenotyping of processing and nutrition related traits on a subset of wheat germplasm collection.
4. Identification of causal genes and their SNPs implementing association study approaches.

Research Progress

1. A set of 110 candidate gene probesets was identified among 55K wheat transcripts through two-way ANOVA analysis in four Indian wheat varieties (C306, Lok1, Sonalika, and WH291) (Singh et al. 2014). Several key genes related to processing and nutrition quality traits were also identified in this study.
2. It is also important to understand the spatial distribution of expression of the candidate genes for designing tissue and growth specific functional validation experiments. The spatial distribution of expression of the candidate genes was studied in 10 development stages such as germination, seedling growth, tillering, stem elongation, booting, inflorescence emergence, anthesis, milk development, dough development, and ripening and 22 wheat tissues including endosperm, glume, caryopsis, embryo, leaf, root, coleoptile, mesocotyl, seedling, sheath, shoot, shoot apex, flag leaf, crown, inflorescence, spikelet, pistil, anther, glumes etc. through meta analysis of 1405 samples which were available in the Affymetrix®'s *Triticum aestivum* microarray database.
3. Gene expression analyses (qRT-PCR) of a subset of candidate genes are being done to validate their differential expression in diverse wheat genotypes (Table 1, Figure 1).

Table 1: Fold change values of expression of two candidate genes between good (C306) and poor (Sonalika) quality varieties estimated through qRT-PCR & wheat microarray (*DAA=Days after anthesis).

Gene/seed development stage*	Differential gene expression between C306 and Sonalika (Fold Change)	
	qRT-PCR	wheat microarrays
Pre α/β-gliadin		
7 DAA	74.7	100.7
14 DAA	1.0	-1.0
28 DAA	1.4	1.2
γ gliadin		
7 DAA	47.6	38.8
14 DAA	6.4	-1.4
28 DAA	1.7	-2.0

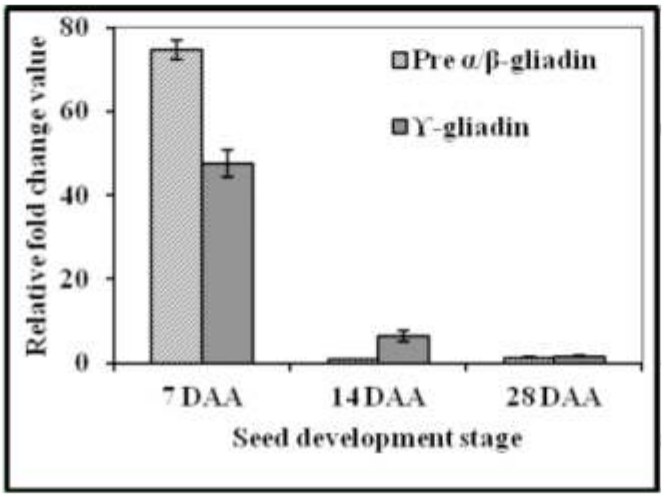


Figure 1: Validation of differential expression (fold change) of two candidate genes (pre-α/β-gliadin and γ-Gliadin) between good (C306) & poor (Sonalika) quality varieties at three seed development stages using qRT-PCR.

4. Of 63 probe sets of phenylpropanoid biosynthesis pathway genes on Affymetrix® wheat arrays, thirty-five showed at least two fold changes between good (C306) and poor (Sonalika) quality varieties at three seed developmental stages (7, 14, and 28 DAA). For example, the expression of naringenin chalcone synthase (Ta.9172.1.S1_at and Ta.9172.1.S1_x_at) was significantly lower in middle stages of seed development (14DAA) and higher in later stage of seed development (28DAA) in C306 with respect to Sonalika.

5. Comparative analysis of transcriptome and small RNA sequences at 3 seed development stages in two diverse wheat genotypes are being done to identify additional candidate genes. The nucleotide sequence of 110 candidate gene probesets were physically mapped to wheat chromosomes through blast using IWGSC's wheat genome sequence database.

6. Processing quality related to starch thermal properties such as onset, peak, conclusion temperature, and enthalpy were estimated on a set of 44 wheat diverse gentypes using Differential Scanning Calorimetery (DSC) (Figure 2). Starch quality affec

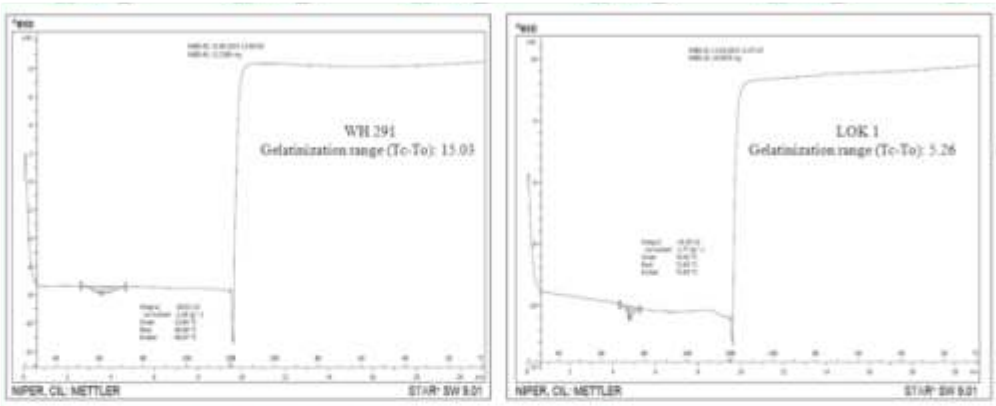


Figure 2: Thermograms of wheat starch samples showing low (WH 291) and high (LOK 1) onset temperature (To) of starch thermal property.

gelatinization, shelf life, softness, stickiness, and moistness maintainace of end-use products. The genotypes showed variation in the above parameters.

7. Processing quality related to dough properties such as medline peak time, peak value, and peak width indicating gluten strength were estimated on a set of 38 wheat genotypes using a mixograph. The variation in the above parameters identified wheat varieties with weak, medium, and strong dough.

8. Two diverse Indian wheat varieties i.e. C306 and Sonalika were used for phenolic compound identification and quantitative estimation. Many phenolic compounds showing presence/absence in free or bound forms between the two varieties were identified (Figure 3).

9. Other traits such as starch granule size, total grain protein content, total starch content, amylose content, seed length and width, grain weight, etc were calculated on a set of 55 wheat genotypes.

10. Many SNPs were identified between the two diverse wheat varieties, C306 and Sonalika for the coding regions of the 110 candidate genes by comparing their nucleotide sequences extracted from their transcriptome data generated on 454 and Illumina platforms.

Salient Achievements

1. A set of 110 candidate genes has been identified using Affymetrix® wheat microarrays. Physical mapping of the candidate genes was determined using IWGSC's wheat genome sequence data.

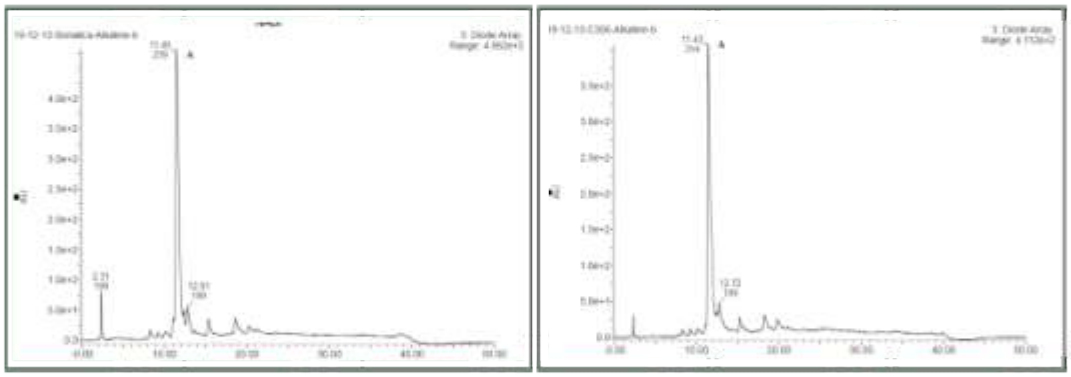


Figure 3: HPLC chromatograms (MS spectra) of alkaline hydrolyzed phenolics of Sonalika (Left) and C306 (right).

2. Processing quality related to starch thermal properties and dough properties were estimated on a sub set of wheat germplasm.

3. Other traits such as starch granule size, total grain protein content, total grain starch content, amylose content, seed length and width, grain weight, etc. were calculated on a set of 55 wheat genotypes.

4. An EMS treated M3 population of the Indian wheat variety, C306, has been developed for functional validation of the candidate genes.

Future Perspectives

1. Processing quality related to starch thermal properties and dough properties will be estimated in replications on a set of 50 wheat genotypes. Other traits such as starch granule size, total grain three replications on the above set.

2. Phenolic compounds profiling will be done to identify metabolites and their variation in the germplasm set.

3. Identification of single nucleotide polymorphisms (SNPs) in coding and 5' non-coding regions of candidate genes through sequencing on the germplasm set.

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

Principal Investigator

Ajay K. Pandey

Co-Investigator

Siddharth Tiwari

Research Fellows

Kaushal K Bhati
Sipla Aggarwal
Shivani Sharma
Mandeep Kaur

Introduction

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

Objectives

1. Identification and functional characterization of PA pathway genes from wheat.

2. Development of low phytate wheat crop by using RNAi approach.

Research Progress

1. Genes involved in the late phases of PA biosynthesis pathway are well-known in crops like maize, soybean, rice and barley but none have been reported from wheat.

2. Our *in silico* analysis identified six wheat genes that might be involved in the biosynthesis of inositol phosphates. Four of the genes were inositol tetraphosphate kinases (*TaITPK1*, *TaITPK2*, *TaITPK3*, and *TaITPK4*), and the other two genes encode for inositol triphosphate kinase (*TaIPK2*) and inositol penta*kis*phosphate kinase (*TaIPK1*).

3. Additionally, we identified a homolog of *Zmlpa-1*, an ABCC subclass multidrug resistance-associated transporter protein (*TaMRP3*) that is putatively involved in PA transport. Analyses of the mRNA expression levels of these seven genes showed that they are differentially expressed during seed development, and that some are preferentially expressed in aleurone tissue. These results suggest selective roles during PA biosynthesis, and that both lipid-independent and -dependent pathways are active in developing wheat grains (Figure 4).

4. *TaIPK1* and *TaMRP3* were able to complement the yeast *Scdipk1* and *Scdvcf1* mutants, respectively, providing evidence that the wheat genes have the expected biochemical functions (Figure 5). This is the first comprehensive study of the wheat genes involved in the late phase of PA biosynthesis (Figure 6). Knowledge generated from these studies could be utilized to develop strategies for generating low phyate wheat.

Salient Achievements

1. PA analysis suggested a linear increase in the IP_6 accumulation with the grain development and this accumulation is negatively correlated with the free phosphate.
2. Genes for the wheat phytic acid pathway were identified and TaIPK1 and TaMRP3 were checked for functional activity in yeast mutants.
3. Among the genes studied, *TaIPK1* and

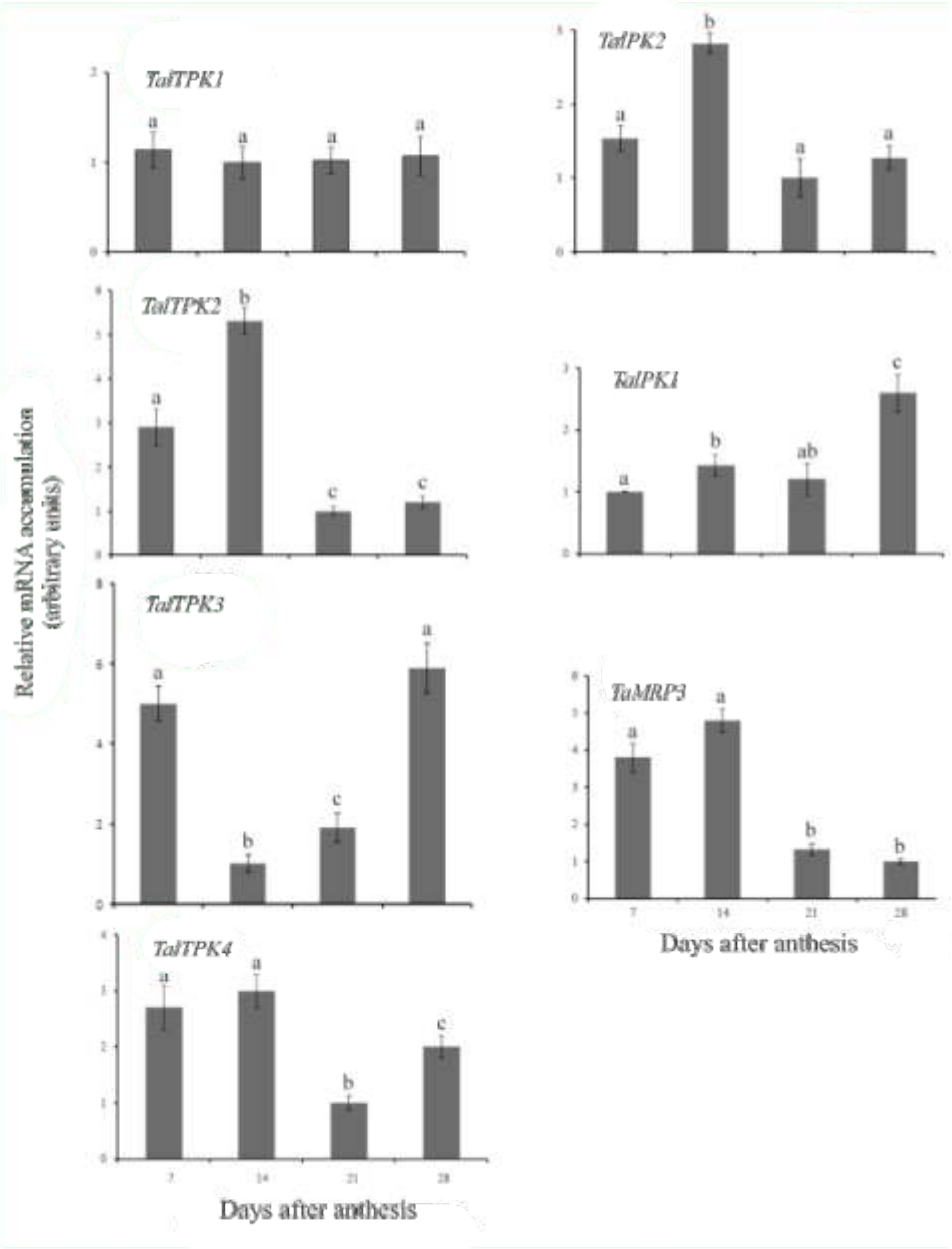


Figure 4: QRT-PCR analysis of genes involved in PA biosynthesis in wheat seeds.

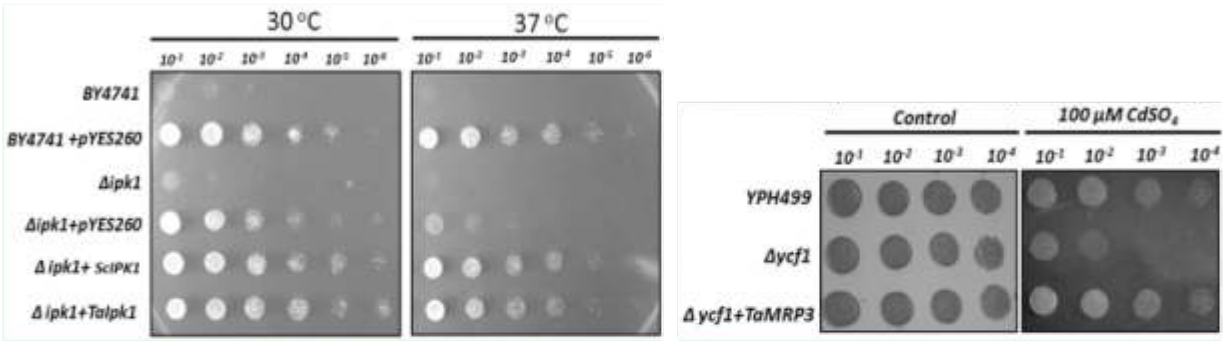


Figure 5: Complementation assays of yeast *Δipk1* and *Δycf1* mutants by *TaIPK1* and *TaMRP3*, respectively.

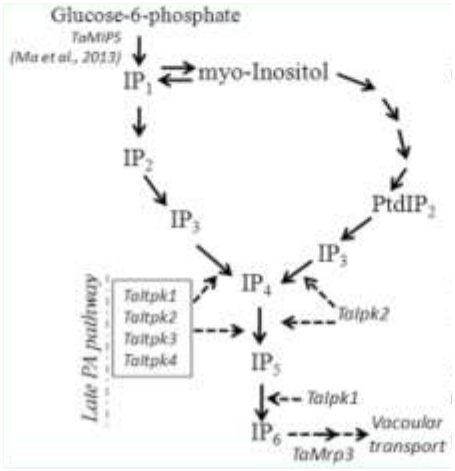


Figure 6: Schematic representation of genes contributing to the PA pathway in wheat. Inositol phosphates are synthesized via lipid-dependent (PtdIP) or lipid-independent (IP) pathways to form IP_6. Italicized wheat genes are indicated at the position adjacent to the probable steps they catalyze.

TaMRP3 could be the most suitable targets for genetic engineering, although role of other genes cannot be ignored.

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.

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

Principal Investigators

Rakesh Tuli
Sudhir P. Singh

Co-Investigator

Shrikant Mantri

Research Fellow

Raja Jeet

Introduction

Mineral deficiency is widespread in the world, especially in cereal diet-based countries. Most of the minerals are present in cereals in forms absorbed poorly from the diets. Wheat is one of the globally important cereal crops, with over 650 million tonnes harvested annually. This accounts for nearly 20% of the world's daily food supply. Though wheat is a significant source of minerals such as Fe, Zn, Mg, K, Ca, Mn, Cu, S and P, agronomically superior varieties often have lower mineral concentrations than their wild relatives. The high yielding varieties may be intrinsically less efficient in translocating minerals into the grains. A substantial portion of minerals in the whole grain is lost during processing of wheat flour because these are located primarily in the outer portion of the grain, called bran, which is often not consumed by people. The study around mineral transport, sequestration and localization in seed tissues is useful for designing varieties with higher mineral content in tissues that allow their efficient dietary uptake. Understanding molecular details of elemental localization can suggest new ways to design nutritionally improved crops with mineral elements enriched in endosperm rather than aleurone. Henee, it is desirable to study localization for all mineral elements in wheat cultivars and the related genotypes. It is essential to examine difference in temporal and spatial expression of genes within the tissues of developing grains of contrasting wheat genotypes.

Objectives

1. To investigate spatial distribution of minerals across the maternal and filial grain tissues of wheat and related genotypes.
2. To examine tissue specific transcriptome in developing grains of wheat.
3. To designe a strategy for mobilization of minerals from outer bran to endosperm.

Research Progress

1. Micro-PIXE elemental analysis was done on transverse sections of grains to generate quantitative distribution maps of macro (Mg, P, S, K and Ca) and micro (Fe, Zn, Mn and Cu) nutrients in two wheat cultivars (*Triticum aestivum* Cv. WH291 and Cv. WL711), a landrace (*T. aestivum* L. IITR26) and a wild relative of wheat (*Aegilops kotschyi* acc. 3790). Figure 7 shows elemental distribution maps of the representative samples of the four genotypes.
2. The two-way clustering of Pearson's correlation coefficients between P and cationic minerals revealed distinct patterns of tissue specific mineral co-localization among the genotypes. The highest level of co-localization was seen for P and K in all four genotypes, followed by P-Mg and P-Fe (Figure 8).
3. Cluster analysis (CA) was performed using concentrations of all the measured elements (Mg, P, S, K, Ca, Mn, Fe, Cu and Zn) in major grain tissues (aleurone, endosperm, scutellum and embryo region), based on Bray and Curtis distance/similarity measure and nearest neighbour clustering method. The four genotypes were grouped into three clusters, with WH291 and WL711 being the most similar (distance 0.112). The landrace-IITR26 formed a separate cluster (distance 0.122), and the most distant cluster is formed by *A. kotschyi* (distance 0.134) (Figure 9).
4. Transcriptome of aleurone and endosperm is being examined in high (IITR26) and low (WL711) mineral wheat genotypes. Several metal accumulation, transport and homeostasis related genes were found up-regulated in aleurone as compared to endosperm in both the genotypes. Some of the genes were expressed at higher level in IITR26 (Table 2).

Salient Achievements

1. The physical evidence of distribution pattern and qualitative localization of several nutritionally important minerals was

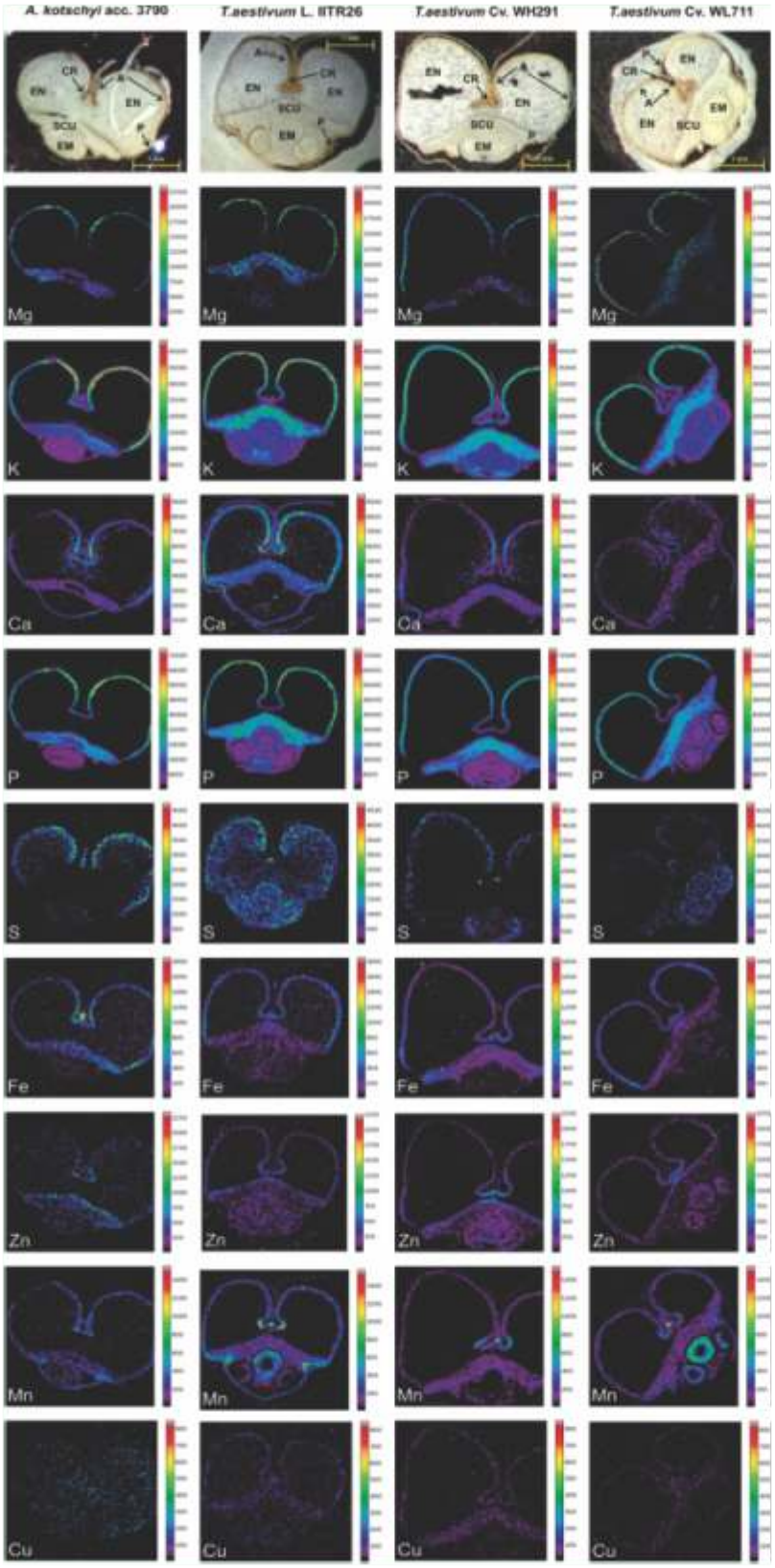


Figure 7: Micro-PIXE quantitative element distribution maps of a representative grain cross-sections of *A. kotschyi*, IITR26, WH291 and WL711. Bars next to the images represent concentration of an element in $\mu g\ g^{-1}$ dry mass. A- aleurone, CR- crease, EM- embryo region, EN- endosperm, P- peripheral tissues (pericarp and seed coat), SCU- scutellum.

investigated in filial and maternal tissues of grains of *T. aestivum* and *A. kotschyi* genotypes.

2. The cellular signatures of minerals have the potential to be useful biomarkers related to the biological and agronomic differences among diverse crop varieties.

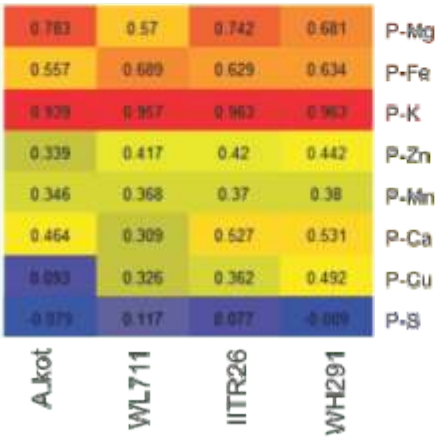


Figure 8: Heat maps constructed by two-way clustering of Pearson's correlation coefficients (depicting the level of co-localization between P and other minerals), using μ-PIXE data, in major grain tissues (aleurone, scutellum, endosperm and embryo region).

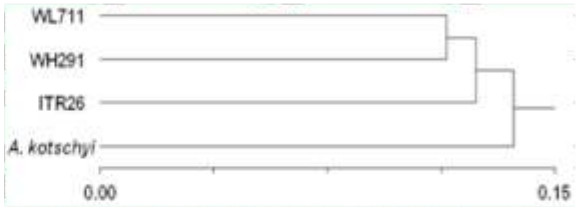


Figure 9: Cluster analysis based on Bray and Curtis distance/similarity measure and nearest neighbour clustering method. Micro-PIXE concentrations of Mg, P, S, K, Ca, Fe, Zn, Mn and Cu in the major grain tissues were used as the attributes for clustering.

3. Differential expression of several transcripts related to metal transport, accumulation and homeostasis were obtained. Further analysis is in progress.

Future Perspectives

1. Identification of candidate pathways and genes for mineral accumulation in wheat grains at higher level.
2. Validation of the function of the gene through viral vector.
3. To develop a strategy for mobilization of minerals from outer bran to endosperm.

Table 2: Differential expression of metal homeostasis genes identified in aleurone and endosperm, at 14 days after anthesis, of wheat genotypes with high (IITR26) and low (IITR26) grain mineral concentration.

Genes / Product Id	Reads Per Kilobase per Million reads (RPKM)			
	Aleurone_IITR26	Aleurone_WL711	Endosperm_IITR26	Endosperm_WL711
Metallothionein_Ta	3379.163	3149.639	549.124	428.391
Metallothionein_Hv1	590.766	0.000	0.000	0.000
Metallothionein_Xh	11.135	0.000	0.000	0.000
Metallothionein like protein3_Hv	99.684	179.329	1.200	5.422
Metallothionein_Hv2	3.979	35.587	0.000	0.000
Metal ion binding protein_Ta	4.176	0.000	0.000	0.000
Ferritin	46.8833	28.9053	6.3828	8.4630
metal tolerance protein 1_Bd	4.116	0.000	0.000	0.000
Metal tolerance protein5_Bd	6.903	7.386	0.000	0.000
Metal tolerance protein5_Ta	4.653	3.099	0.000	0.650
Metal tolerance protein7_Bd	70.989	26.044	4.992	6.218
Iron-sulfur protein_Zm	18.654	20.856	1.173	1.969
Iron-sulfur assembly protein IscA-like 1_Hv	12.990	16.044	1.976	2.948

Iron-sulfur assembly protein IscA-like 2_Bd	44.295	7.629	0.000	3.531
Iron-sulfur protein NUBPL_Bd	4.308	5.034	0.655	1.611
Iron-sulfur cluster assembly protein ISCU_Bd	140.714	85.100	8.802	11.616
Zinc transporter_(ZIP4)_Ta	16.107	9.513	0.000	0.902
Zinc transporter (ZIP1)_Ta	8.360	5.974	0.774	2.561
Zinc transporter_(ZIP2)_Bd	10.231	0.000	0.000	0.000
Zinc transporter protein ZIP7_Hv	2.847	0.000	0.000	0.000
Cadmium/zinc-transporting ATPase3_Bd	9.270	4.875	1.051	0.000
Cation diffusion facilitator transporter_Ta	11.005	14.061	0.848	1.176
Vacuolar iron transporter2_Bd	4.909	3.138	0.000	0.843
Vacuolar iron transporter5_Bd	3.420	1.685	0.000	0.000
Nicotianamine sythase3_Ta	181.070	109.525	0.000	0.000
Nicotianamine synthase 5_Hv	6.2468	9.1888	1.1033	0.2303
Nicotianamine aminotransferase_Hv	51.521	37.294	0.598	0.728
Nicotianamine transporter YSL2_Bd	23.902	24.075	4.614	8.915
Nramp domain-containing protein_Ta	60.893	54.771	9.119	7.782
Metal transporter Nramp1_Bd	1.130	0.384	0.000	0.163
Metal transporter Nramp2_Bd	4.738	6.785	1.554	0.707
Metal transporter Nramp3_Bd	50.320	51.670	2.728	4.062
Metal transporter Nramp4_Bd	0.922	0.112	0.106	0.000
Integral membrane protein NRAMP	17.365	12.678	0.000	1.410
Magnesium transporter MRS2	23.855	1.192	0.000	4.144
Membrane magnesium transporter 1_Bd	44.517	50.662	0.000	0.000
Magnesium transporter NIPA2_TA	6.048	7.551	3.675	2.583
Magnesium transporter NIPA2_Bd	1.571	2.465	0.000	0.000
Magnesium transporter NIPA2_Bd	1.375	1.043	0.000	0.000
Boron transporter4_Bd	8.429	2.541	6.685	7.449
Boron transporter2_Bd	1.653	0.000	0.000	0.000
Potassium transporter23_Bd	7.208	4.382	0.000	0.262
High-affinity potassium transporter_Ta	4.005	9.807	0.167	0.765
Potassium transporter 17_Bd	6.751	9.821	3.397	0.643
Potassium transporter 13_Bd	2.558	2.466	1.307	1.172
Potassium transporter_Pa	1.796	0.516	0.000	0.000
High-affinity potassium uptake transporter_Ta	0.671	0.168	0.000	0.100
Potassium channel_Hv	6.493	0.954	0.152	0.000
Potassium channel KAT3_Bd	2.046	0.467	0.000	0.000
Potassium channel KOR2_Bd	3.090	2.742	0.361	0.261
Potassium channel KOR2 like_Bd	3.090	2.742	0.361	0.261
Copper-transporting ATPase PAA1_Bd	2.757	2.616	0.235	0.000
Cation-chloride cotransporter1_Bd	10.319	13.934	4.106	2.705

1.1.4 Efficient genetic transformation of wheat

Principal Investigator
Siddharth Tiwari

Research Fellows
Anshu Alok
Harsimran Kaur

Introduction

The protocols for callus as well as direct multiple shoot mediated *in-vitro* regeneration and *Agrobacterium*-mediated genetic transformation with reporter (GUS-Intron) gene of wheat have been optimized. Eventually optimized protocol will be used for generation of stable transgenic plants with desirable traits.

Objectives

Establishement of genetic transformation protocol of wheat.

Research Progress

1. **Stable expression of reporter (GUS-intron) gene:** The stable expression of reporter gene was noticed as blue colour on the flag leaves of transgenic plants (Figure 10 A). The results further confirmed by PCR analysis showed amplification of the predicted 800 bp *gusA* as well as 1027 bp *hptII* fragments of genes in transgenic plants (Figure 10 B).

Salient Achivements

Agrobacterium-mediated genetic transformation for the stable expression of reporter (GUS-Intron) gene was optimized.

Future Perspectives

Optimized transformation protocol will be used as a tool for validation and generation of transgenic wheat with the desirable traits.

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

Principal Investigator:

Rakesh Tuli

Research Fellows
Jitendra Kumar
Jitesh Kumar
Vishnu Shukla
Shashank Singh

Introduction

Plant viral vectors are valuable tools for suppression (VIGS) and heterologous gene expression (virus mediated overexpression; VOX). Viral vectors are especially useful for plants such as wheat (*Triticum aestivum*) that has large and complex genome and are recalcitrant to transformation, which limit the use of functional genomics tools such as mutagenesis, T-DNA knockout libraries, T-DNA activation tagging or

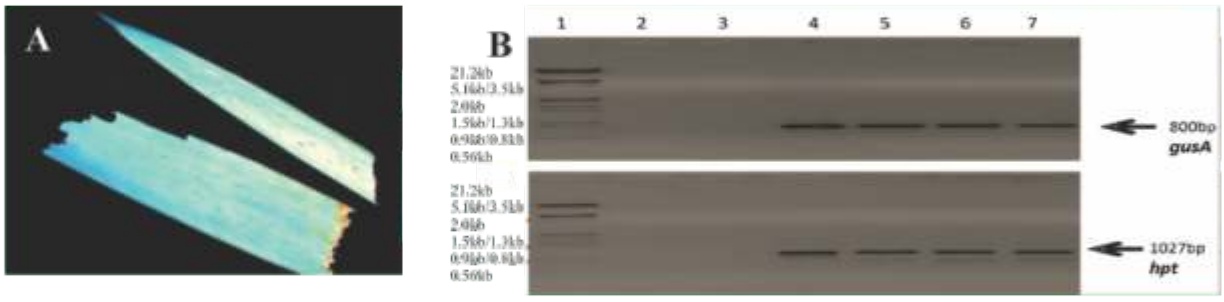


Figure 10: Stable expression and PCR analysis of GUS in transgenic plants. **(A)** Histochemical assay showed GUS expression in flag leaves of transgenic plants. **(B)** PCR analysis of transgenic plants showed transgenes integration in the genome. Lane 1: Lambda DNA *HindIII & EcoRI* digest, Lane 2: 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.

transposon gene-tagging and even the RNAi. VIGS is of great importance in wheat as it can potentially speed up the characterization of candidate 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 viral vector for suppression (VIGS) and expression (VOX) vector for wheat.

Objectives

1. Investigating incidence and prevalence of the WDIV disease complex in India.
2. Development and validation of VIGS vector and its further utilization for functional assessment of iron biosynthesis related genes.

Research Progress

1. **Detection and characterization of WDIV associated satellites from wheat:** The wheat samples found positive for WDIV were investigated for the presence of subgenomic components or satellites. Two types of satellites (alpha and beta) were detected, whereas no begomovirus was detected from WDIV positive wheat samples (Figure 11).
2. **Assessment of effect of alpha and beta satellites on the symptom induction by virus:** The phenotype was observed for assessing the effects of the satellites on the virus symptom induction process. The plants were observed showing more dwarfism in the presence of satellite in comparison to the virus alone (Figure 12).

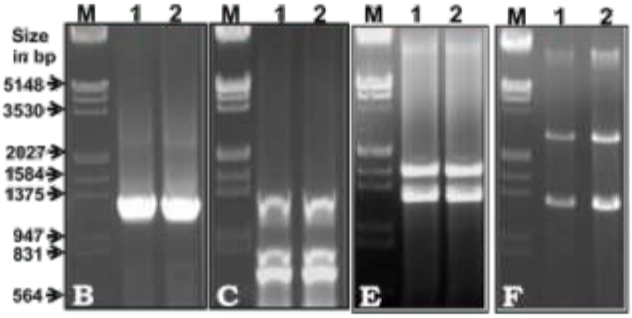


Figure 11: Amplification for beta satellite and alpha satellite.

3. **Assessment of effect of alpha and beta satellites on WDIV and WDIV-specific small RNA accumulation:** Southern

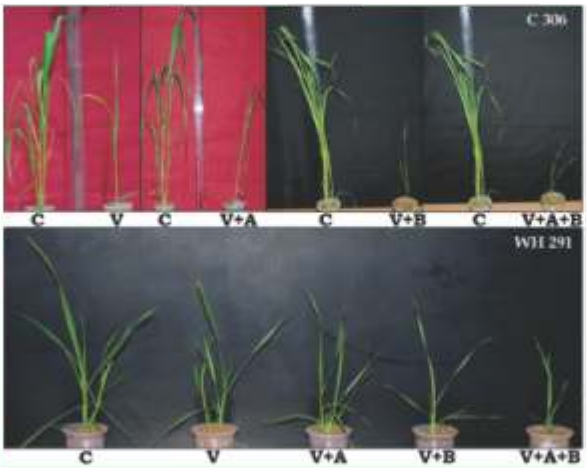


Figure 12: Agroinoculated wheat plants at 42 day post inoculation. C= mock inoculated, V= virus infectious clone and V+A+B= virus+alpha+beta satellite infectious clone inoculated wheat plants.

hybridization to assess the effect of the satellites on the virus accumulation inside the wheat plant. The accumulation of virus was found high in the presence of the satellites (Figure 13). Production of virus-specific small RNA is plant defence against viruses. Virus and satellites encoded ORFs exhibit silencing suppressor activity to suppress plant silencing machinery. Northern hybridization was performed to access the accumulation of WDIV-specific small RNA, which showed that accumulation of WDIV-specific small RNA was lower in plants inoculated with WDIV and satellites (Figure 13).

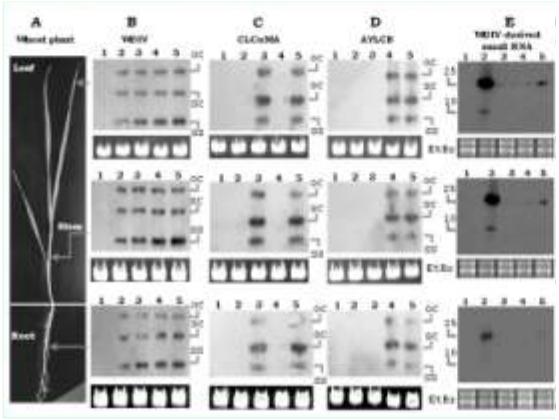


Figure 13: Southern hybridization based quantification of WDIV (B), alphasatellite (C) and betasatellite (D) in agroinoculated wheat plant (A). Coat protein gene was used as probe to investigate virus accumulation from different parts of wheat plants (root, stem and leaf). Accumulation of WDIV-specific small RNA (E) in different parts of wheat plant (A).

4. **Assessing the effect of associated satellites on wheat plant gene expression using microarray analysis:** Expression analysis using microarray revealed that many unigenes were up regulated and down regulated more than 5-fold by its expression value. Presence of betasatellite supported the gene regulation pattern of virus, whereas alphasatellite regulated different sets of unigenes.

5. **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. Wheat plants at different pathogenicity scales of 0-9 were found at different locations (Table 3). Broad occurrence of WDIV has been concluded on the basis of survey. Association of satellites was found at all the locations.

6. **Correlation of pathogenicity scale with productivity:** The number of tillers was less at disease scale 8-9. The length of ear was also reduced at scale 8-9 (Table 4). Significant variations were observed in TKW at different disease scales. TKW at scale 0 was 44 ± 5 g and gradually decreased to 35 ± 4 g at higher disease scale (Table 4). Grain formation was nil in the spikes at scale 8-9 (Table 4).

Table 3: Geographical co-ordinates of sample collection sites, percentage of infected wheat plants and disease severity in field.

S. No.	Centre of sample collection	Geographical co-ordinates	No. of plants in 10 Sq. Ft.	No. of infected plants in 10 Sq. Ft.*	Incidence (%)	No. of sample collected	No. of sample found positive**	Positive sample (%)	Prevalence (%)	Pathogenicity scale found
			Average of 5 locations							
1.	Mohali	30° 47' North; 76° 41' East	261	35	13.4	317	263	82.9	10.9	1 to 8
2.	Meerut	28° 58' North; 77° 42' East	240	26	10.8	67	59	88	9.5	2 to 6
3.	Kanpur	26° 45' North; 80° 31' East	246	28	11.3	30	23	76.6	8.6	2 to 6

S. No.	Centre of sample collection	Geographical co-ordinates	No. of plants in 10 Sq. Ft.	No. of infected plants in 10 Sq. Ft.*	Incidence (%)	No. of sample collected	No. of sample found positive**	Positive sample (%)	Prevalence (%)	Pathogenicity scale found
			Average of 5 locations							
4.	Gorakhpur	29° 45' North; 75° 66' East	275	23	8.3	24	18	75	6.2	2 to 5
5.	Samastipur	25° 80' North; 85° 67' East	253	36	14.2	40	36	90	12.7	2 to 8
6.	Hajipur	25° 68' North; 85° 22' East	270	29	10.7	20	17	85	9.1	1 to 3
7.	Bilaspur	22° 4' North; 82° 9' East	244	20	8.2	45	33	85.1	6.9	2 to 6
8.	Jagdalpur	20° 37' North; 81° 35' East	236	23	9.7	40	28	70	6.7	1 to 5
9.	Wellington	11° 22' North; 76° 47' East	233	45	19.3	78	71	91	17.5	1 to 9
10.	Pune	18° 6' North; 74° 18' East	237	26	10.9	75	70	93.3	10.1	1 to 7
11.	Indore	22° 43' North; 75° 49' East	242	38	15.7	60	54	90	14.1	2 to 9
12	Bhopal	23° 12' North; 77° 27' East	247	22	8.9	20	15	75	6.6	1 to 4
13.	Udaipur	24° 34' North; 73° 38' East	251	28	11.1	77	55	71.4	7.9	1 to 9
14.	Jaipur	26° 5' North; 75° 47' East	245	36	14.6	70	50	71.4	10.4	1 to 8
15.	Average across India		248.5	29.6	11.9	68.7	56.5	81.7	9.8	1 to 9

* Infected samples suspected on the basis of visible symptoms.

** Samples found positive on the basis of amplification of 2.8 kb PCR fragment using WDIV specific primers.

Table 4: Correlation of pathogenicity scale with productivity traits.

S. No.	Pathogenicity scale	No. of plants collected	No. of virus positive plant	% positive plants	Prevalence at scale (%)	Average of 10 independent observations				
						No. of tillers /plant	No. of ears/ plant	Length of ear	Total grain weight/ plant	Thousand grain weight
1.	8 to 9	15	14	93.3	1.4	5 - 6	5-6	5 - 8 cm	No grain	No grain
2.	5 to 7	136	127	93.3	13.5	8 - 15	8 - 15	7 - 10 cm	8 - 15g	35g± 4g
3.	3 to 4	360	333	92.5	35.5	6 - 13	6 - 13	7 - 10 cm	11 - 26g	41g± 4g
4.	1 to 2	452	315	69.7	33.6	5 - 15	5 - 15	8 - 12 cm	12 - 37g	41g± 4g
5.	0	42	2	4.7	NA	5 - 16	5 - 16	8 - 14 cm	13 - 39g	44g± 5g

7. **Development of VIGS vector by modifying the viral genome** : Several modifications have been done in viral genome by removing a small stretch of nucleotide and inserting Multiple Cloning Sites (MCS) at the same positions.

stages of wheat is being optimized with different VIGS constructs in wheat (Figure 15). The vector constructs exhibiting good silencing activity in different developmental stages will be utilised for silencing of gene of interest.

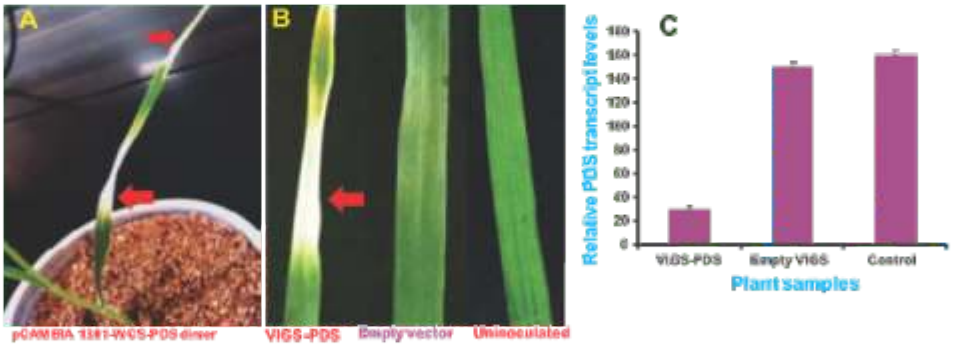


Figure 14: Panel A: partial view of the VIGS-PDS constructs inoculated wheat plant. Panel B: comparison between leaves of VIGS-PDS, empty VIGS-vector inoculated and uninoculated control wheat plants. Panel C: real-time PCR based amplification of relative PDS transcripts.

8. **Validation of VIGS vector by silencing of visual marker gene**: Phytoene desaturase gene (PDS) from wheat was amplified, cloned and sequenced. It was inserted into the VIGS vector. Agroinoculation of leaf tissues with VIGS-PDS construct resulted in efficient silencing (Figure 14).

9. **Optimization of silencing in different developmental stage of wheat:** Silencing of PDS gene in different developmental stages of wheat is being optimized with different VIGS constructs in wheat (Figure 15). The vector constructs exhibiting good silencing activity in different developmental stages will be utilised for silencing of gene of interest.

10. **Utilization of WDIV-VIGS vector for studying function of iron nutrition related genes in wheat:** Candidate genes have been short-listed from literature and also from the transcriptomics data. Silencing of two candidate genes is under process in developing seed. Methods are being developed to introduce infection in developing wheat grains.

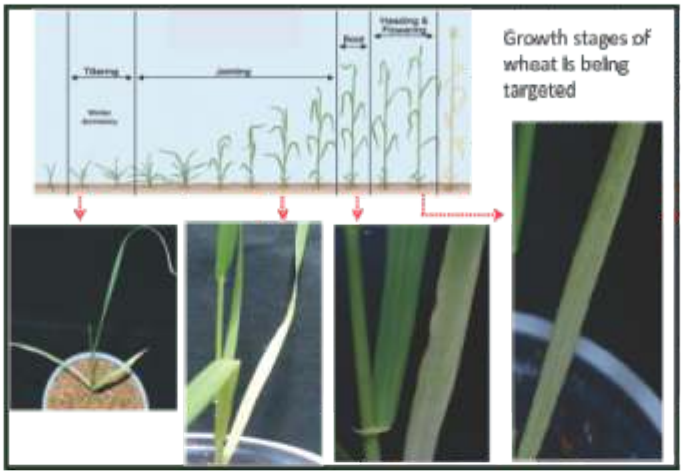


Figure 15: Silencing of PDS gene in different developmental stages of wheat.

Salient Achievements

1. We have detected a novel mastrevirus with novel genome organisation and association of subgenomic components (satellites). We have detected the association of satellites with a mastrevirus for the first time. Role of the associated satellites in viral disease enhancement was documented.
2. WDIV genome was modified in order to achieve a good silencing in wheat.

Future Perspectives

1. Functional annotation of upregulated and downregulated transcript upon WDIV and satellite infections.
2. Optimization of silencing in different developmental stage of wheat and utilization of WDIV-VIGS vector for functional genomics in wheat.

1.2 Accelerated breeding for quality improvement of processing and nutritional quality in wheat

Principal Investigator

Monika Garg

Research Fellows

Rohit Kumar
Aman 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 on processing quality (milling and baking characteristics), marker development and utilization of validated markers.

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

Objectives

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

Research Progress:

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

   (I) For improvement of chapatti making quality, good chapatti making old cultivars were crossed with high yielding recent cultivars (PBW343, PBW550 and PBW621). F_1 seeds of BC_1 and other crosses (Table 5) were sown at NABI and around 600 morphologically selected plants were screened first by linked marker WMC313 and subsequently for absence of GBSS-1B. Negative plants were screened for morphologically superior plants. Selected lines/plants will be subjected to background screening and generation advancement.

   ii For improvement of Biscuit making

Table 5: Screening and selection of F_3 plants/lines of BC_2 and other crosses for good chapatti making quality.

Cross	No. of Homozygous lines	No. of Homozygous Plants
C3333	2	11
5C555	-	6
5C5HH	-	1
6C666	1	15
C6666	-	2
3L333	-	2
L33HH	-	3
6L666	-	1
6L6HH	2	-

Where 3-PBW343, 5-PBW550, 6-PBW621, H-HD2967, C-C306, L-LOK1

quality donor landraces were crossed with high yielding recent cultivars (PBW343, PBW550, PBW621 and HD2967). F_3 seeds of different crosses (Table 6) were sown at NABI and around 1150 morphologically selected plants were screened based on presence of puroindoline gene *PinaD1a*. Positive plants were screened for morphologically superior plants. Selected lines/plants will be subjected to background screening and generation advancement.

Table 6: Screening and selection of F_3 plants/lines of different crosses for good biscuit making quality

Cross	No. of Homozygous lines	No. of Homozygous Plants
I3333	1	34
3I333	-	4
I5555	-	3
I55HH	1	13
5I555	-	14
I6666	-	13
I66HH	1	10
6I6HH	-	1

Where 3-PBW343, 5-PBW550, 6-PBW621, H-HD2967, I-IITR67

(iii) For improvement of bread making quality genetic stocks of wild species *Ag. elongatum, Ae. longissima, Ae. searsii and Ag. intermedium,* are being crossed with high yielding recent cultivars (PBW550, PBW621 and HD2967). 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 making quality. Genetic material (addition, substitution and translocation lines) was screened by sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) of storage proteins from endosperm half of seeds. Embryo halves of the seeds expressing selected wild species' HMW-GSs were sown in the NABI field. Crossing/backcrossing of selected material is in progress.

Chromosome specific translocation

line of *Ag. elongatum* i.e. long arm of chromosome 1E translocated to short arm of chromosome 1A i.e. 1EL (1AS) in the background of Japanese cultivar Norin 61 was generated during this study. This line showed improved dough strength gluten index, and bread making quality. Microsatellite based background screening of BC_3F_4 (Line 1) and BC_4F_3 (Line 2) translocation line 1EL (1AS) is in progress (Figure 16 and 17).

Total 287 SSR markers were used for background screening and identification of translocations in BC_3F_4 (Line 1) and BC_4F_3

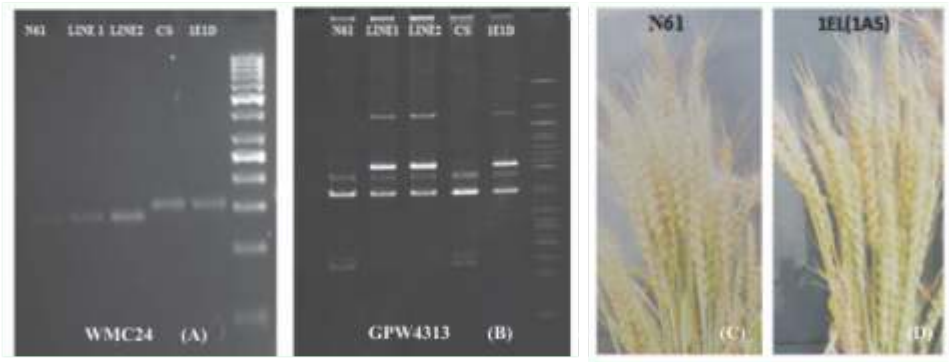


Figure 16: Characterization of translocation line and microsatellite based background screening of BC_3F_4 (Line 1) and BC_4F_3 (Line 2) 1EL(1AS) translocation lines showing pattern of **(A)** background cultivar N61. **(B)** *Th. elongatum*. Translocation line. **(C)** CS-Chinese spring (Donor cultivar) and substitution line 1E(1D) were used as checks. **(D)** was morphologically similar to background cultivar N61.

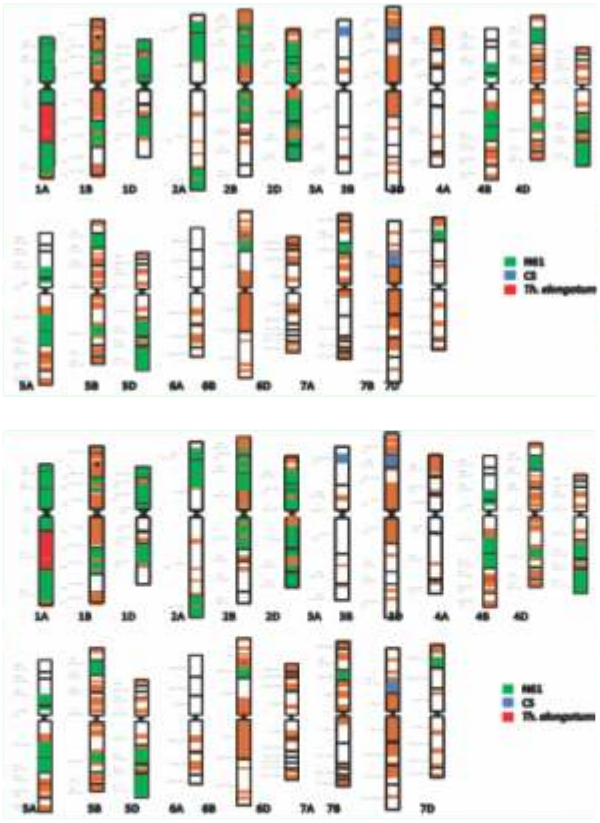


Figure 17: Schematic microsatellite marker based map of 1EL(1AS) translocation line. Donor cultivar was CS and recipient cultivar was N61.

(Line 2) plants of translocation line 1EL (1AS). Out of which 64 markers were found polymorphic. Most of the polymorphic markers showed N61 pattern (Table 1, Fig. 1) indicating good background recovery after three backcrosses. Four markers on long arm of chromosome 1A showed patterns suggesting presence of *Th.elongatum* translocation. These were BARC83, WMC469, BARC17 and WMC312. Marker WMC469 was present near HMW-G locus. SSR maker data indicated presence of interstitial translocation, rather than whole arm translocation, former being better than later due to reduction in linkage drag.

2. **Development of database for grain softness related gene (puroindoline) of Indian wheat cultivars:** Puroindolines (PINs) are important proteins that determine wheat grain texture and its end product quality. Puroindolines genes (*Pina* and *Pinb*) encoding these proteins are located on hardness locus (*Ha* locus) on the short arm of chromosome 5D of wheat. We investigated the genetic diversity of puroindoline genes in Indian wheat cultivars with the aim to understand the effect of different allelic variants on grain texture. Phenotypically large number of Indian wheat cultivars (91.5%) exhibited hard grain texture. These hard cultivars were better classified by Indian system of classification. Genotypically hard textured cultivars exhibited ten different allelic patterns (Table 7), with pattern of non-functional *PinaD1b* and functional *PinbD1a* being the most common (64.6%) and hardest. Soft textured Indian cultivars had functional *PinaD1a* and *PinbD1a* alleles. Among the known alleles, uncommon *PinaD1e* allele was observed in 7.3% cultivars. Six new *Pin* alleles were identified and named during the diversity study. Two of these six belonged to *Pina*, that are being named here as *Pina-D1w* and *Pina-D1x*. Rest four belonged to *Pinb* that are being named here as *Pinb-D1ad*, *Pinb-D1ae*, *Pinb-D1af* and *Pinb-D1ag*. Among the new alleles, *Pinb-D1af* resulted in functional change of the gene.

Table 7: Allelic variation of Puroindoline genes in Indian wheat cultivars

S. No.	Pina allele	Pinb allele	No. of cultivars/ percentage	Nucleotide mismatch position/	Amino Acid (AA) mismatch position/AA base change	Hardness (in SKCS Units)	Functional Change
1	*Pina-D1a*	*Pinb-D1a*	7/8.5%	Wild type	Wild type	21 - 36	No
2	***Pina-D1b***	*Pinb-D1a*	53/64.6%	Null allele	Null allele	70 -101	Yes
3	*Pina-D1a*	***Pinb-D1b***	8/ 9.7%	223/ G to A	46/ Gly to Ser	58 -84	Yes
4	*Pina-D1a*	***Pinb-D1e***	6/ 7.3%	204/ G to A	39/ Trp to stop	59- 82	Yes
5	*Pina-D1a*	***Pinb-D1r***	1/1.2%	Insertion 127/G	Frame shift and stop codon at 48	76	Yes
6	***Pina -D1w***	***Pinb-D1b***	1/ 1.2%	41/ C to T (Pina)	(-) 15/ Ala to val (Pina)	76	No
7	***Pina -D1x***	***Pinb-D1b***	1/ 1.2%	65/ G to C 86/ A to G	(-) 7/ Ser to Th 1/ Asp to Gly	57	?
8	*Pina-D1a*	***Pinb -D1ad***	1/ 1.2%	92/ T to C	2/ V to A	78	No
9	***Pina-D1b***	***Pinb -D1ae***	1/ 1.2%	93/ T to A	No change	87	No
10	***Pina-D1b***	***Pinb -D1af***	2/ 2.4%	232/ G to T	49/ Glu acid to stop	76, 81	Yes
11	***Pina-D1b***	***Pinb -D1ag***	1/ 1.2%	371/ T to C9	5/ Leu to Pro	95	No

3. **Study of supramolecular structure and physiochemical properties of starch granules in soft and hard wheat cultivars:** Starch constitutes the major carbohydrate in the endosperm of wheat grains and a major source of feed, fiber, biofuels, and biopolymers. During grain development, starch is deposited in the endosperm as discrete semi crystalline aggregates known as starch granules. It is composed of two glucose polymers, called amylose and amylopectin. The branching of the glucan chains of amylopectin occurs with regular periodicity and its length and pattern are critical for the proper formation of the starch granule and its properties. During wheat grain development starch is deposited in three types of granules, A- type granules (diameter > 9.9 µm), B-type granules (diameter < 9.9 µm) and C-type granules (diameter < 5 µm). The multimodal size distribution of starch in wheat is of great interest because the physiochemical properties of each type of starch granule vary and contribute to the food and industrial end-uses of starch. Starch was purified from soft (IITR67) and hard (C306) wheat lines. Purity of starch was checked by light microscope. Estimation of the amylose content of the total starch indicated that IITR 67 contains 26% and C306 contains 24% amylose. Total starch from hard and soft wheat lines was separated into A, B and C type granules. SEM images of different starch granules indicated that regularity in round shape of the granules decreased in the order A <B <C (Figure 18). Variations were found in Amylose/amylopectin content of within different type of starch granules and between hard and soft wheat. Further analysis is in progress.

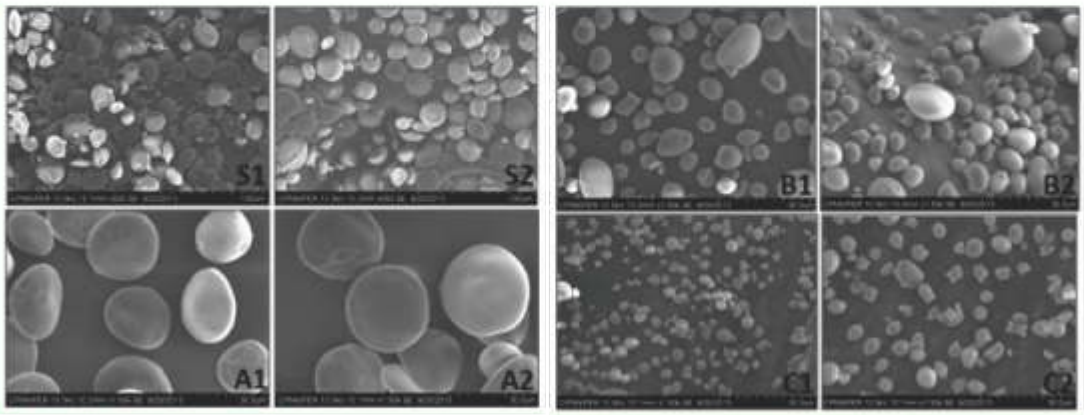


Figure 18: SEM images of Total starch, A-granules, B-granules and C-granules of soft wheat IITR67 (S1, A1, B1, C1) and hard wheat C306 (S2, A2, B2, C2).

Salient Achievements

1. Advanced breeding material for improvement of chapatti and biscuit and bread making quality has been generated.
2. Allelic variation of Puroindoline genes in Indian wheat cultivars has been studied.
3. Variation in structure and properties were observed in A, B and C type of starch granules.

Future Perspectives

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

IMPROVING FRUITS FOR POST HARVEST QUALITY AND NUTRITION

2.1 Genetic transformation of banana for quality improvement

Principal Investigator

Siddharth Tiwari

Project Scientist

Ashutosh Pandey

Project Fellows

Shivani
Navneet Kaur

Project Assistants

Vikrant Sharma
Prateek Kumar

Introduction

We have received funding for this project is a part of the multi-institutional international core project entitled "Development and Transfer of Technology from Queensland University of Technology (QUT), Australia to India for Biofortification and Disease Resistance in Banana" (Project sponsored by Biotechnology Industry Research Assistance Council (BIRAC), Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India). 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 Naine and Rasthali by Indian partner labs. Selected varieties of banana are triploid in nature, hence sterile and provide natural barrier to cross pollination. Transgenic approach hold high promise for the biofortification of this crop would be biologically safe and useful tool to introduce desirable traits. At the first stage, QUT has provided the best four pro-vitamin A (PVA) Generation 1 gene constructs, containing Asupina banana derived *phytoene synthase* (*APsy2a*) gene under the control of Exp1, Ubi, ACO and BT4 promoters for the genetic transformation of Indian banana varieties. Protocols for embryogenic cell suspension (ECS) culture of selected cultivars were optimized at NABI. Genetic transformation of ECS with PVA gene constructs received from QUT has been started for the generation of β-carotene rich transgenic banana.

Objective

1. To develop pro-vitamin A (PVA; β-carotene) rich biofortified and agronomically improved transgenic varieties of Indian bananas cv. Grand Naine and Rasthali.

Figure 1: Establishment of Banana germplasm and tissue culture raised plants at NABI Research Field. **(A)** Banana germplasm. **(B)** Banana Plants with fruits. **(C)** Tissue culture raised Grand Naine and Rasthali Plants.

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&B).

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 1C).

2. **Establishment of Embryogenic Cell**

Suspension (ECS) culture for regeneration of somatic embryos and genetic transformation

(i) Immature male flower buds of two cultivars (Grand Naine and Rasthali) were collected from NRCB, Trichy for the preparation of explants and initiation of embryogenic callus on semi-solid medium (Figures 2A & B).

(ii) Embryogenic callus used for ESC development & multiplication in liquid medium (Figures 2C, D, E & F).

(iii) ECS derived globular embryos developed and regenerated on semi-solid medium (Figures 2G & H).

(iv) Regenerated embryos germinated on germination medium (Figure 2I).

(v) Transient expression of reporter GFP observed in the transformed ECS (Figure 2J).

3. **Genetic transformation of ECS with PVA gene constructs (Generation one) received from QUT:**

Genetic transformation of ECS of Rasthali and Grand Naine with four PVA gene constructs performed at different time intervals for generation of several independent transgenic lines. Transgenic embryogenic cells survived and looked healthy on kanamycin selection (regeneration) medium (Figure 3A). Untransformed cells turned brown and did not survive on the selection medium (Figure 3B).

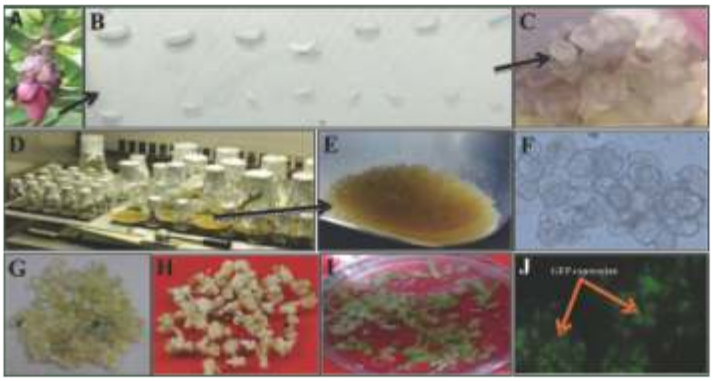


Figure 2: Stages of Embryogenic Cell Suspension (ECS) culture development for germination of somatic embryos and genetic transformation. **(A)** Immature male flower bud. **(B)** Immature male flower hands of rank 1 to 15 adjacent to the floral apex, used as explants. **(C)** Embryogenic callus induction. **(D & E)** ECS cultures in a shaker. **(F)** Microscopic observation of dense cytoplasmic embryogenic cells with starch granules. **(G & H)** Globular embryos developed from ECS on regeneration medium. **(I)** Embryos germinated on germination medium **J)** Microscopic observation of transient expression of GFP in the transformed ECS of Rasthali.

Salient Achievements

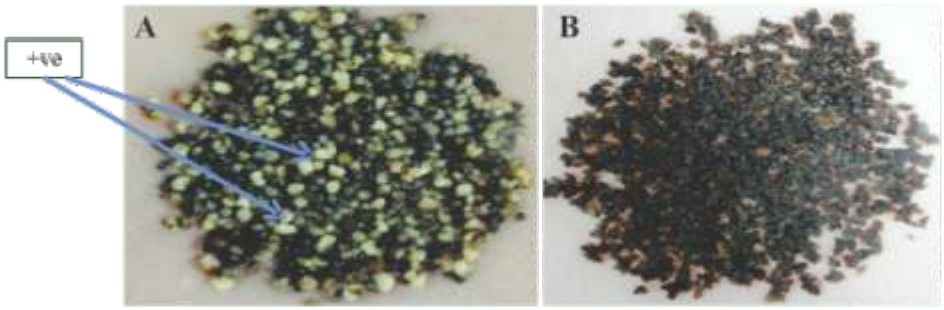


Figure 3: Visual observation of embryogenic cells on kanamycin selection (regeneration) medium after 3 months. **(A)** Transformed white and healthy globular embryogenic cells (Arrows indicated) with brown colored untransformed cells. **(B)** Untransformed (-ve control) embryogenic cells.

1. Tissue culture raised several plants of Grand Naine and Rasthali were generated and grown at NABI research field for the collection of explant for ECS development.
2. Protocol for ECS culture of Grand Naine and Rasthali cultivars were optimized.
3. Rasthali and Grand Naine ECS are maintain and multiplying for genetic transformation experiments.
4. Genetic transformation protocol was optimized by using reporter gene.Genetic transformation of ECS with gene constructs (Generation one) received from QUT has been started for the generation of β-carotene rich transgenic banana.

Future Perspectives

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

2.2 Quality enhancement and postharvest stability of tropical fruits

2.2.1 Metabolomics approach to discovery and validation of biomarkers for artificial fruit ripening induced through prohibited and acceptable ripening elicitors

Principal Investigator
Sukhvinder Pal Singh

Project Assistant
Veena Bains

Introduction

Artificial fruit ripening is a commercial practice in banana and mango fruits aimed at enhancing quality and regulating demand-supply equilibrium in the fresh fruits market. Exposure to low concentration of ethylene sourced from either a gas cylinder or catalytic generator is a recommended fruit ripening practice and globally accepted from regulatory perspective. Ethephon (2-chloroethylphosphonic acid), an ethylene releasing compound in liquid form, is also being used for fruit ripening, leaving harmful residues of ethephon and its degradation product, hydroxy ethylphosphonic acid (HEPA). This compound is not registered for postharvest ripening application, but the fruit ripened with ethephon can be identified by analytically determining the residues of ethephon and HEPA. In addition to ethylene gas and ethephon, commercial ripening procedures employed in India also use acetylene gas liberated from calcium carbide powder by the addition of water or by contact with moisture in the air. As an ethylene-action analogue, acetylene induces ethylene-like responses. The carcinogenicity and many other health hazards have been attributed to carbide toxicity. Considering the health risks associated with the use of calcium carbide for workers and consumers, its use has been banned for artificial fruit ripening in India. However, fruits artificially ripened with either calcium carbide (acetylene gas) or ethylene gas are quite similar in their appearance and taste quality, and are difficult to distinguish due to no residues. Currently, no diagnostic test/procedure is available to distinguish the fruit ripened with calcium carbide. The project is aimed at employing metabolomics approach to discovery and validation of biomarkers linked to artificial fruit ripening elicitors such as calcium carbide and ethylene. It is hypothesised that a comparison of metabolomes of the fruits subjected to artificial ripening (calcium carbide/acetylene versus ethylene) may elucidate biomarker(s) for diagnosis.

Objectives

1. Understanding the postharvest physiology and quality of banana and mango fruits in response to artificial ripening elicitors.
2. Discovery and validation of diagnostic biomarker(s) capable of diagnosing/ distinguishing fruits subjected to calcium carbide induced ripening agent under simulated postharvest conditions.
3. Application of metabolomics infocmatics for development of diagnostic tool and

guidelines for adoption of biomarkers in practice.

Research Progress:

The fundamental requirement for biomarker discovery is to offset the biological variation to a larger extent. The experiments were conducted to simulate the artificial ripening practices in the laboratory by employing different types of ripening elicitors. The concentrations of ethylene and its analogues (acetylene and propylene), ethephon and calcium carbide were optimised in order to achieve uniform and synchronous fruit ripening. The synchronous ripening in banana and mango fruits was achieved at 21-22°C, 90-95% RH with the different concentrations of ethylene (100 ppm), acetylene (2000 ppm), propylene (1000 ppm), calcium carbide (2.5 g/kg fruit) and ethephon (500 ppm). The differences in biological activities of ethylene and its analogues to induce fruit ripening responses were evident from their required concentrations for ripening. Physiological responses of banana fruit to different ripening elicitors revealed that acetylene and calcium carbide enhanced ethylene biosynthesis and its peak during the climacteric

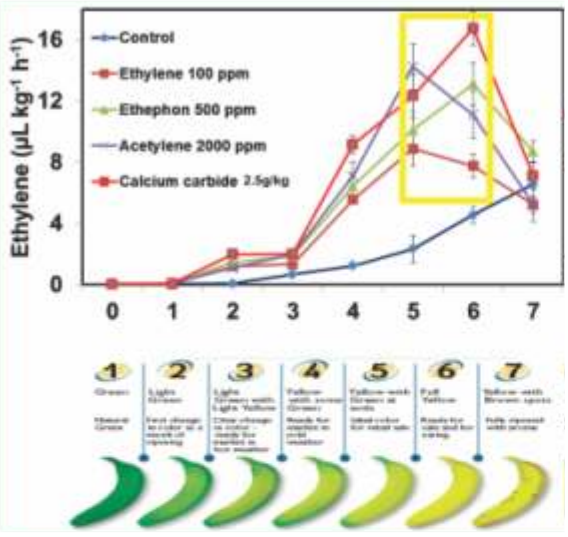


Figure 4: Ethylene biosynthesis rates of banana fruit subjected to artificial fruit ripening involving different ripening agents

phase of fruit ripening (Figure 4). The synchronous ripening pattern among all treatments except control was also observed in fruit ripening parameters such as skin colour, firmness, soluble solids, and acidity. Banana and mango fruit pulp sampled at the ripe stage was stored at -80°C until further analysis.

As per hypothesis of the project, the fruit ripened with ethylene and calcium carbide might differ in their fruit metabolome that comprises of several thousand small molecules present in low to high abundance with different degrees of polarity and volatility (volatiles, semi-volatiles and non-volatiles). Preliminary investigations were conducted to screen the contrasting banana and mango fruit samples from ethylene- and calcium carbide-induced ripening for their volatiles profiles. The volatiles from fruit pulp were extracted using headspace-solid-phase microextraction (HS-SPME) technique and analysed using a gas chromatograph-mass spectrometer (GC-MS). The chromatogram walk and overlay approaches showed that the intensities of volatiles differed between ethylene- and carbide-induced fruit ripening, but the presence/absence of a marker compound was not detected. This investigation does not completely rule out the possibility of a volatile marker associated with carbide-induced ripening as there are more sophisticated and sensitive analytical techniques other than GC-MS such as proton transfer reaction mass spectrometry (PTR-MS) which is capable of detecting volatile organic compounds at very low level.

The presence/absence of non-volatile biomarker(s) in the fruit pulp would be more reliable and realistic procedure compared to the volatile biomarker. Therefore, liquid chromatography-time-of-flight mass spectrometry (LC-TOF-MS) based global untargeted metabolomics approach was followed as the first step to biomarker discovery. Sample preparation, UPLC, and QTOF MS conditions were optimised for better coverage of the range of metabolites with increased scope. The extracted samples were injected into an UHPLC-QTOF mass spectrometer operated in an independent data acquisition mode enabling generation of TOF-MS and MS/MS fragmentation data. Mass

spectral output analysis involved peak alignment, normalization against an internal standard using Analyst™ and PeakView™ software packages. About 7,000 and 5,800 features for each data set of banana and mango, respectively, were detected in the mass range of 100-1000 m/z. MarkerView™ software package was employed to process and visualise the data in different perspectives based on the principles of biomarker discovery generally followed in clinical chemistry. Following data pre-processing and data reduction based on different levels of confidence ($P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$), multivariate statistical modelling was performed.

In banana, the principal component analysis (PCA) showed that the first two principal components (PCs) could explain 82.3% of the total variance of banana fruit metabolome. The segregation of fruits ripened with ethylene and calcium carbide in different quadrants could be visualized in the score plot which indicates the possibility of distinguishing the fruit based on whole metabolome (Figure 5). The closer grouping of carbide- and acetylene-ripened fruit further strengthens the results towards the discovery stage.

In mango, the PCA showed that the first two PCs contributed to 73.4% of the total variance of mango fruit metabolome. The fruit ripened with calcium carbide and acetylene though showed distinct grouping, but those ripened with ethephon also tended to cluster closer to carbide group (Figure 6).

Subsequent to the PCA, a further data-reduction strategy to shortlist potential biomarkers was

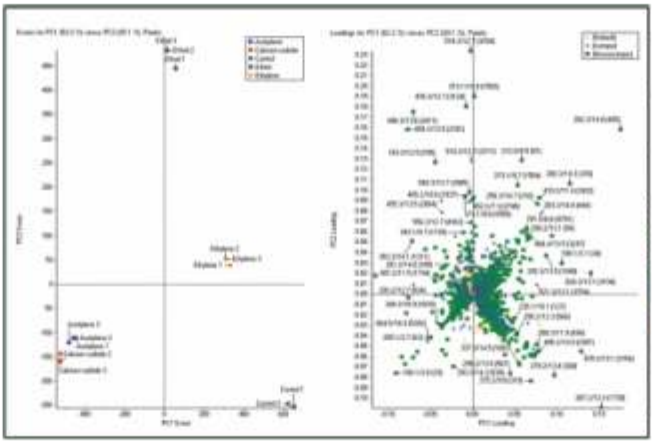


Figure 5: Principal component analysis (PCA) score (left) and loadings plots (right) revealing segregation of banana fruits ripenened with different ripening elicitors.

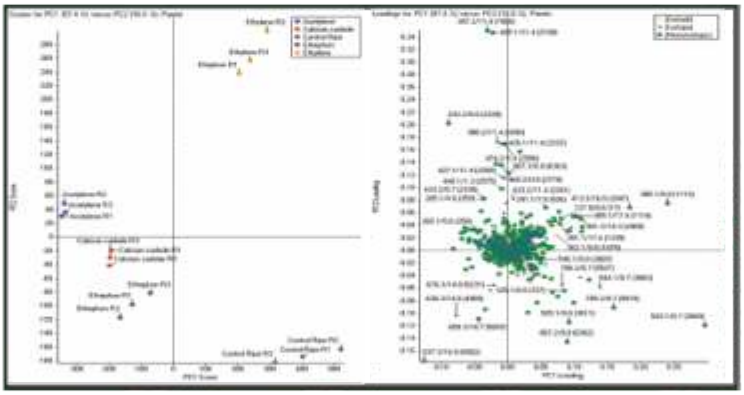


Figure 6: Principal component analysis (PCA) score (left) and loadings plots (right) revealing segregation of mango fruits ripened with different ripening elicitors.

adopted with more stringent criteria such as higher probability, metabolites' intensities, and log-fold change. The exact masses of the putative biomarkers were annotated against public domain databases such as Metlin and Massbank to tentatively identify these compounds. The formula finder algorithm in the PeakView was employed to derivatize the potential molecular formulae of the metabolites. Furthermore, the MS/MS fragmentation patterns of these metabolites were annotated against the public metabolome databases, whenever available. The potential biomarkers linked to the calcium carbide ripening in banana and mango fruit are listed in Table 1.

Salient Achievements:

1. Synchronous artificial fruit ripening was

Table 1: List of putative metabolites linked to the calcium carbide-induced ripening in banana and mango fruits.

m/z	MS/MS Fragmentation	*p*-value	Formula	Putative Identification (Metlin, Massbank)
		Banana		
188.0702	118, 146, 143, 170	<0.001	C9H21N3O	N-acetyl spermidine
191.0373	98, 110, 170, 201, 182	<0.001	C6H6O5S	3- Sulfocatechol
205.0965	118, 146, 188, 170, 159	<0.001	-	Unknown
259.1887	129, 111, 147	<0.001	C14H28O2	Tetradecanedioic acid
277.2148	121, 93, 107, 179, 259	<0.0001	C18H30O	Octadecadiynoic acid
280.2361	263, 245, 95, 149, 81, 109	<0.0001	C18H30O	2,4,6-tri tertiary butyl phenol
287.2361	153, 161, 133	<0.0001	C15H10O4	Chrysin
291.2299	93, 135, 121, 149, 119, 175	<0.001	C16H34O4	1,2,3,4- Hexadecanetetrol
292.1744	113, 215, 95	<0.0001	C13H22O6	Ethyl-4,4-diethoxy-2-(ethoxymethylene)-3-oxo butanoate
325.1622	127, 163, 145, 69	<0.01	C17H24O6	Dibutyl-2,6-dimethyl-4-oxo-4-H-pyran-3,5-dicarboxylate
366.3724	203, 349, 331, 71	<0.0001	C16H23N5O5	Isopentyl adenine-7-glucoside
555.2929	313, 155, 537, 393, 317, 401, 98	<0.0001	-	Unknown
575.3840	455	<0.0001	C15H22O4	12, 13-epoxytrichothec-9-ene-4,15-diol
663.4543	495, 551, 439, 607, 383, 327	<0.0001	-	Unknown
955.556	657	<0.0001	C64H100O4	Guanosine pentaphosphate adenosine
		Mango		
392.7713	613, 301, 144	<0.0001	-	Unknown
405.2996	89,133,396,221, 115, 388	<0.0001	C23H42O4	Oxalic acid allyl octadecyl ester
412.3084	133, 403, 177, 199, 221	<0.0001	-	Unknown
434.3219	89, 425, 133, 177, 340, 155,	<0.0001	-	Unknown
456.3356	133, 177, 448, 362, 207	<0.0001	-	Unknown
514.3945	497, 133, 177, 215, 371, 453	<0.0001	C32H48O4	Unknown
555.4084	184, 367	<0.0001	C27H48O9	3-Beta-galactopyranosyloxy-2-hydroxypropyl-9,12-octadecadienoate
572.4354	133, 177, 229, 415, 555	<0.0001	C35H54O5	Bis(2-(4-nonylphenoxy)ethyl carbonate
616.4613	89, 599, 133, 177, 229, 309, 557	<0.0001	C37H58O6	17-Hydroxy-3,11,20-trioxoprega-4-en-21-yl acetate .
762.5554	177, 133, 287, 353, 591	<0.0001	-	Unknown

achieved using ripening elicitors such as ethylene, its analogues (acetylene and propylene), ethephon and calcium carbide in banana and mango fruits.

2. The biomarker discovery following LC-QTOF-MS approach has been achieved to larger extent in banana than in mango.

3. The putative identification of some potential biomarkers has been performed using multiple approaches based on exact mass, molecular formulae, MS/MS fragmentation pattern.

4. The LC-MS/MS based method for estimation of ethephon and HEPA residues on the fruit surface has been tested and evaluated to monitor the maximum residue limits in fruits.

Future Perspectives:

1. The validation of biomarkers linked to carbide-induced ripening in banana and mango fruits will be conducted considering multiple factors.

2. The comprehensive targeted profiling of metabolites responsible for flavour and nutrition will be conducted in view of artificial fruit ripening.

2.1.2 Quality and postharvest stability of Kinnow mandarin for fresh and processing industries

Principal Investigator
Sukhvinder Pal Singh

Research Fellow
Manpreet Kaur Saini

Introduction

'Kinnow' mandarin is the most important commercial fruit crop of the Punjab state. In 2011-12, its production has increased to about 1.0 million metric tonnes from an area of about 43,000 hectares. The narrow harvest window (mid-January to mid-February) recommended for optimal fruit quality necessitates postharvest cold storage at 5°C for extended availability of fruit for fresh and processing industries. During postharvest cold storage, fruit metabolism though continues at an inhibited rate, but leads to significant metabolic changes during long-term storage affecting its nutritional, flavour, and processing quality. The analysis of metabolic events during the progression of cold storage can provide an estimate of divergence from the healthy fruit metabolome. Key metabolites responsible for these quality traits belong to different pathways associated with ripening, senescence, and postharvest cold stress. The abundance and diversity of metabolites in a biological system including that of a fruit can be best studied through metabolomics approach. Mass-spectrometry based global untargeted metabolomics has been shown to gain comprehensive coverage and insight into the fate of thousands of metabolites in the biological system. On the other hand, targeted approach to absolute quantitation of metabolites primarily responsible for flavour, nutrition and processing quality gives quantitative overview of the metabolic shifts and their consequences on perceivable juice quality parameters. Sugars, organic acids, and limonoids are the major metabolites that determine the juice quality of 'Kinnow' mandarin. The metabolic changes in these metabolites during cold storage can seriously affect the fruit quality.

Objectives

1. Explicating the metabolic shifts in the 'Kinnow' fruit metabolome in response to long-term cold storage and its correlation with juice quality.

2. Targeted profiling of metabolites responsible for flavour, nutrition and processing quality of 'Kinnow' mandarin juice.

Research Progress

To study the metabolic shifts during cold storage of 'Kinnow' mandarin, commercially mature fruit were stored at low temperature (5°C) for 8 weeks and sampling was conducted at weekly intervals, 2 weeks onward. The juice extracts were injected into an UHPLC-QTOF mass spectrometer operated in an independent data acquisition mode

enabling generation of TOF-MS and MS/MS data. Mass spectral output analysis involved peak alignment, normalization against an internal standard, and then unsupervised and supervised multivariate analyses using Analyst™, PeakView™ and MarkerView™ software packages. About 12,000 features for each data set were detected in the mass range of 100-1000 m/z. Following data pre-processing and data reduction based on different levels of confidence ($P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$), multivariate statistical analyses reflected significant metabolic shifts during cold storage for 8 weeks. Based on PCA score plots, clustering of fruits into three major groups was achieved: early-(0-2 weeks), mid- (4-6 weeks) and late- (7-8 weeks) stages of cold storage (Figure 7).

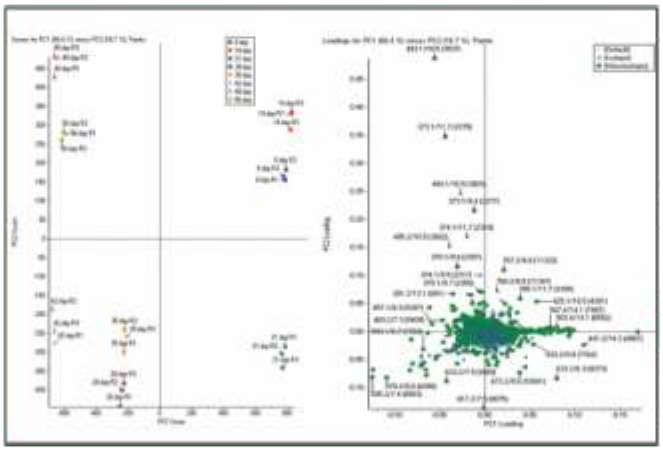


Figure 7: Principal component analysis (PCA) score (left) and loadings plots (right) depicting grouping of 'Kinnow' mandarin fruit during different stages during cold storage at 5°C for 8 weeks (56 days).

The distribution of different samples representing cold storage progression in the principal coordinates indicates a massive shift in the whole fruit metabolome during storage. The juice quality generally determined in terms of sugars to acid ratio undergoes major change in loss of organic acid during cold storage. However, the metabolomics approach underscores tremendous changes in the dynamics of small molecules representing various chemical classes. Multivariate analyses coupled with metabolite annotation using databases such as Metlin and MassBank revealed putative identification of discriminant metabolites linked to different stages of cold storage of 'Kinnow' mandarin. Therefore, LC-QTOF based metabolomics can be a powerful tool to unravel the mechanisms underlying the postharvest cold storage-induced metabolite changes ultimately leading to fruit quality.

The absolute quantitation of important

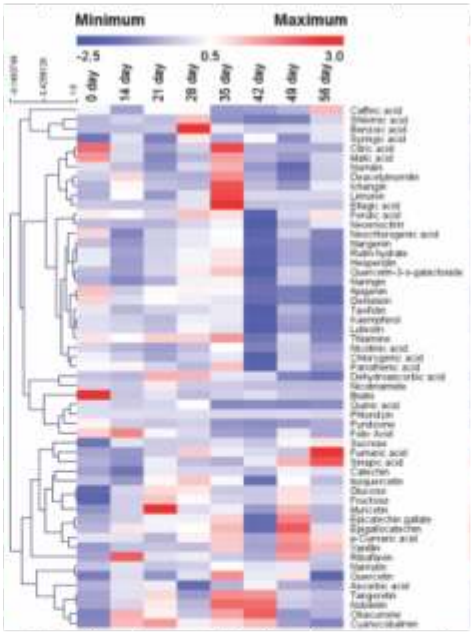


Figure 8: Heat map showing quantitative changes in the concentrations of various metabolites responsible for flavour, nutrition and processing traits in 'Kinnow' mandarin. Based on HCA, clustering pattern of metabolites is also shown.

metabolites with increased sensitivity and accuracy provides the quantitative information and also compliment the untargeted analysis for better understanding the fruit metabolome and establishing correlation with the desired quality traits. Targeted analysis of metabolites responsible for flavour, nutrition and processing quality of 'Kinnow' mandarin was conducted using high-throughput LC-MS/MS techniques. Statistical tools for pattern-recognition, such as hierarchical cluster analysis (HCA) and PCA were used for comparison, and visualization of similarities and differences between data sets by definition of clusters. The quantitative data on these metabolites were centred, and then subjected to autoscaling before HCA. The HCA was performed using MultiExperiment Viewer (MeV) software to generate a dendrogram explaining the clustering pattern of metabolites based on cold storage duration. Heat map depicting changes in levels of metabolites during different storage intervals is shown in Figure 8. A significant increase in concentrations of organic acids (citric acid, malic acid), limonoids (limonin, nomilin, deacetylnomilin, and ichangin) and phenolic acid (ellagic acid) was observed after 35 days of storage. The magnitude of increase in concentrations of these compounds resulted in clustering together in the HCA.

Salient Achievements

1. The metabolic events related to progression of cold storage of 'Kinnow' mandarin were revealed and discriminant metabolites linked to different stages of cold storage were putatively identified.
2. The targeted metabolite profiling of 'Kinnow' mandarin for flavour (sugars, organic acids and flavonoids), nutritional (water soluble vitamins, B-complex and C), and processing quality (limonoids) has been achieved, deciphering the composition of Kinnow juice for the first time.

Future Perspectives

1. GC-MS based metabolomics approach will be followed to compliment the data from LC-MS.
2. The comprehensive data from untargeted and targeted approaches using LC-MS and GC-MS will be integrated and annotated against the important metabolic pathways to discover major metabolic switches regulated by the cold storage.

The basic knowledge about these metabolites will be translated into recommendation/ practices for the juice processing industry.

BASIC BIOLOGY FOR CROP IMPROVEMENT

3.1 Designing a dominant-negative protein that inhibits the DNA binding activities of seed-specific b-ZIP family of transcription factors

Principal Investigator
Vikas Rishi

Research Fellows
Prateek Jain
Kaushik Shah

Introduction

Higher plants are characterised by the formation of seed that contains an embryo protected by maternally derived *seed* coat. After initial cell divisions and morphogenesis, embryo enters maturation phase that involves accumulation of storage products, desiccation tolerance, water loss and induction of dormancy. This phase of seed maturation (MAT) is fine-tuned and is regulated by number of genes prominent among these are seed storage protein genes (SSP), ABA3, FUS3, and LEC1. MAT genes, promoter analyses have revealed several *cis*-regulatory elements known to bind number of transcription factors (TF) including b-ZIP family of TFs. In *Arabidopsis* three b-ZIP proteins, namely b-ZIP10, b-ZIP25 and b-ZIP53 are reported to play prominent role in seed development and maturation. Studies using Arabidopsis suggested b-ZIP53 to be a key regulator of MAT genes. B-ZIP53 expression increases during seed development and localizes to the embryo and endosperm during maturation phase. b-ZIP53 binds to G-Box (CCACGTGG) as a homodimer or a heterodimer partnering with b-ZIP10 or b-ZIP25. *In vitro* studies showed that compare to homodimer, heterodimers are proficient and impart a synergistic effect on gene expression.

Interestingly b-ZIP53 knockdown plant still sets viable seeds suggesting functional biological redundancy. In absence or sub-optimal expression of b-ZIP53 protein, other b-ZIP TFs like b-ZIP10, b-ZIP25 or some hitherto unknown protein(s) may regulate seed-specific gene expression. In order to address the issue of overlapping functions we propose to design a protein that will heterodimerize with all three seed-specific b-ZIP TFs (b-ZIP53, b-ZIP10, and b-ZIP25) and inhibits their functions. Such heterodimers cannot bind to the DNA and can be used to study gene regulation. Previously this strategy has been used successfully in animal model systems.

Objectives

1. Designing a dominant-negative protein that inhibits the DNA binding of b-ZIP53, b-ZIP10, and b-ZIP25.
2. Efficacy studies of designed protein for their preferentially interaction with wild type proteins.

Research Progress

b-ZIP or basic-region leucine zipper proteins are a family of transcription factors that dimerize to form a coiled-coil and bind to DNA in a sequence specific manner. As shown in Figure 1, it has N-terminal DNA binding domain that is unstructured in absence of DNA and a c-terminal dimerization domain called leucine zipper. The name arose because leucines occur every seven amino acids in this dimerization domain. These leucines are critical for the dimerization and DNA binding of b-ZIP proteins.

There are approximately 72 b-ZIP TFs reported in *Arabidopsis*. Experiments with other b-ZIP TFs of

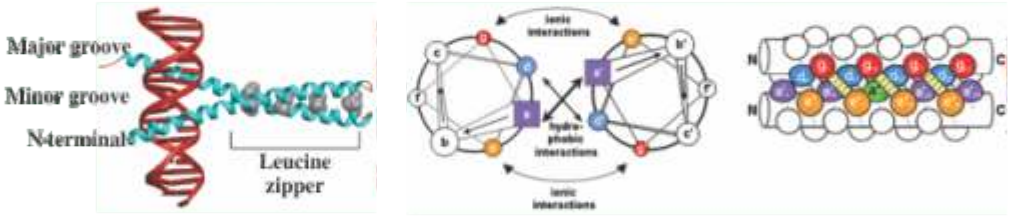


Figure 1: A) X-ray structure of the b-ZIP dimer bound to DNA. DNA is in red, the alpha-helices are in blue. The d or leucine position amino acids are shown in grey. B) An end view of the leucine zipper dimer looking from the N-terminus. The letters inside circles and squares are the standard nomenclature for the seven amino acids in a heptad (a,b,c,d,e,f,g). Amino acids at position a and d creates a hydrophobic core whereas amino acids in position e and g are involved in ionic interactions. C) Leucine zipper coiled-coil as viewed from the side.

human and Drosophila origin enabled us to predict the dimerization properties of these proteins in Arabidopsis. Some prefer to homodimerize and others prefer to heterodimerize. Previous studies have shown that amino acids in the e and g positions are critical for dimerization specificity and the amino acids in a and d positions contribute to stability of coiled-coil.

To unravel the biological function of a b-ZIP TFs, it is useful to use proteins that inhibit the DNA binding and function of individual b-ZIP genes. These types of proteins are called dominant-negatives. The term 'dominant' refers to their genetic dominance. The term 'negative' describes the inhibition of the function of cellular proteins. In its simplest form, a dominant-negative could be a truncated b-ZIP protein, which would heterodimerize with endogenous wild type proteins, producing an inactive heterodimer. However, the binding of DNA stabilizes the b-ZIP structure and this complicates the design of biologically active dominant-negative proteins. We have overcome this problem by designing dominant-negatives based on two different strategies. There are two ways a dominant-negative can be designed. First strategy is to tweak the leucine zipper motif. This can be done by changing the amino acids in e and g position. Other way is based on the concept that an amino acid sequence could mimic the properties of DNA. Knowing that the b-ZIP basic region could form an alpha helix when bound to DNA, a protein sequence was designed such that it could mimic DNA. In these dominant-negatives termed A-ZIP the designed protein sequence replaces the DNA-binding region. This sequence forms a very stable heterodimer with wild type b-ZIP protein and prevents the b-ZIP complex to bind to DNA (Figure 2).

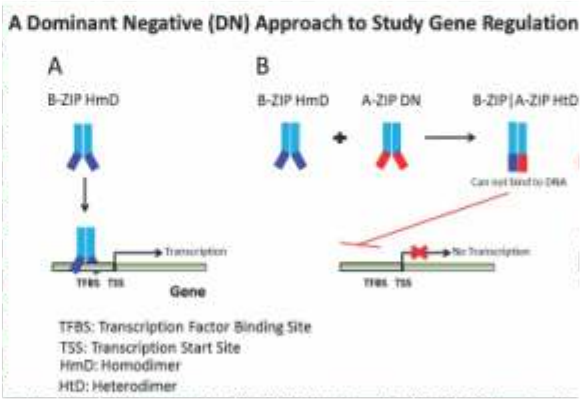


Figure 2: A schematic showing mode of action of a designed dominant-negative protein against a b-ZIP transcription factor. Dominant-negative protein here refers as A-ZIP, heterodimerizes with wild type b-ZIP. This complex is more stable than the b-ZIP bound to DNA. Once formed, a heterodimer between b-ZIP and A-ZIP cannot bind to DNA thus knocking out or bringing down the gene expression.

We have cloned the DNA binding domain and leucine zipper region of b-ZIP53, b-ZIP10, and b-ZIP25 using Arabidopsis c-DNA. Figure 3 shows the amino acid sequences of these three b-ZIP transcription factors. These are cloned in prokaryotic expression system. The cloned genes are expressed in BL21 LysE bacterial strain and purified to 95% + purity. These protein samples

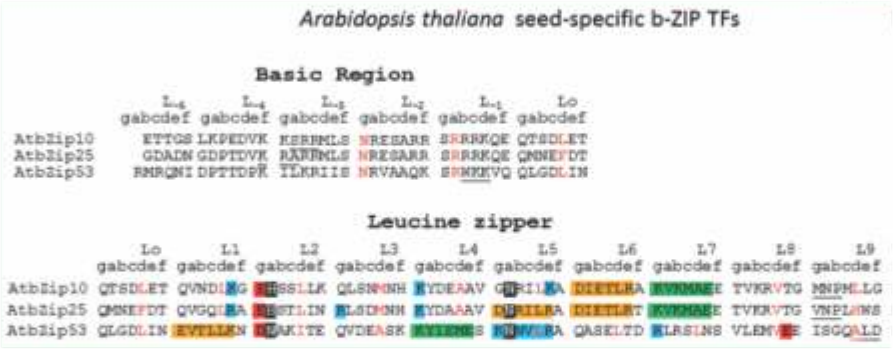


Figure 3: Amino acid sequences of three seed-specific b-ZIP transcription factors cloned from c-DNA of *Arabidopsis thaliana*. Leucine zipper amino acids are arranged in heptads where a and d positions are occupied by hydrophobic amino acids and e and g amino acids are typically charged. Each heptad and individual amino acids are color coded. Orange and green heptads depict attractive interactions due to the presence of oppositely charged amino acids at e and g positions. Repulsive interactions are depicted by blue. Amino acid is coded black if it is charged or polar and occupies a position in heptad. Individual basic amino acid in position e or g is depicted by red whereas it is blue in case of acidic amino acid.

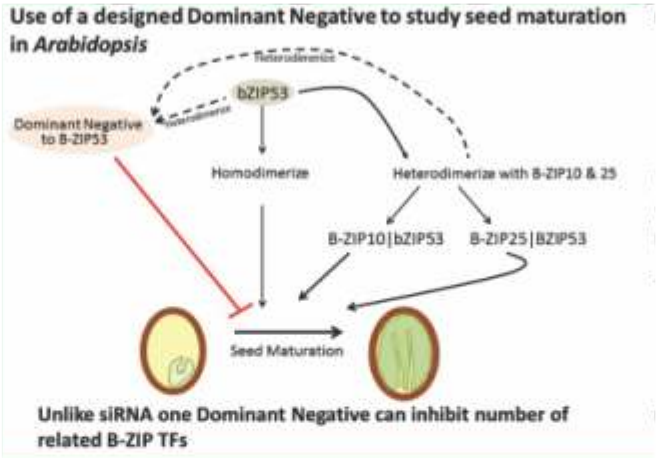


Figure 4: A schematic depicting a possible mechanism of action of our designed dominant-negative in effecting the gene expression of three seed specific b-ZIPs.

will be used for structural studies. As mentioned above we have designed a protein that we hope will interact preferentially with b-ZIP 53, 10 and 25. This will be accomplished by changing the amino acids in e and g position of b-ZIP53 leucine zipper.

Salient Achievements

1. Successful cloning and milligram expression of b-ZIP53, 10 and 25 has been achieved. They will be further studied using EMSA and circular dichroism spectroscopy.
2. Unlike other knock out techniques like siRNA one dominant negative can inhibit the function of number of transcription factors.

Future Perspectives

1. Using biochemical, biophysical and cell biology techniques an elaborate study will be carried out to study the contribution of individual amino acids in stability and specificity of heterodimers.

3.2 Biology of seed development in custard apple and litchi

Principal Investigator
Sudhir P. Singh

Co-Investigator
Shrikant Mantri

Research Fellows
Yogesh Gupta
Ashish Kumar

Introduction

The *Annona squamosa* fruit develops from the cluster of fertilized carpels, thus the aggregate fruit contains several fruitlets. Each of the carpel has a single anatropous ovule that could develop into a single seed. *A. squamosa* is an excellent model for fruit development. To investigate the molecular mechanism underlying fruit set in *A. squamosa*, the next-generation sequencing was employed to profile early-stage fruit development in two genotypes with contrasting number of seeds in fruit.

Litchi chinensis 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 cultivars. The ovule specific transcriptome is being examined in the contrasting accessions of litchi to identify genes related to small size of seeds.

Objectives

1. To examine transcriptome in developing fruits of *A. squamosa* genotypes with contrasting number of seeds in fruit.
2. To examine transcriptome in developing fruits of *L. chinensis* genotypes with contrasting size of seed in fruit.

3. To identify candidate pathways/genes/SNPs related with seedlessness in fruit crops.

Research Progress

1. Transcriptome sequencing of four RNA-seq libraries, prepared from developing fruits at 0, 4, 8, and 12 days after pollination (DAP) in the two contrasting genotypes of *A. squamosa*, Sitaphal (large number of seeds in fruit) and NMK1 (less number of seeds in fruits). The average number of reads produced for each library was 0.24 million. Average read length for each library was 650 base pairs. Contigs with more than or equal to 200 bp were selected for further analysis. Contigs were mapped to non-redundant database (Table 1).

2. The Contigs of different developmental stages were further assembled into super-contigs using CAP3, resulting 14921 and 14178 super-contigs in Sitaphal and NMK1, respectively. The super-contigs were blast against the nr database and that of closely related species such as Straberry, Grape and Peach (Table 2). This analysis revealed about 3851 and 2542 contigs as specific to Sitafal and NMK1 genotypes, respectively.

3. Transcripts related to hormones were determined in the transcriptome data by BLAST search analysis using *Arabidopsis* protein sequences as query. In both the genotypes, 240 hormonal genes were detected; few of them were genotype specific which need to be further validated (table 3).

4. The analysis of ovule specific transcriptome data in Litchi is in progress. The transcriptional profiling of genes related with seed maturation pathway is being analyzed. Up-regulation of B3 domain containing gene was noticed in the developing ovules of bold seeded genotype, in comparison to the small seeded genotype (Figure 5). Further analysis is in progress.

Table 1: Summary of RNA-seq data from developing fruits of two contrasting *A. squamosa* genotypes, Sitaphal and NMK1, at four developmental stages.

Genotype	Developmental stage (DAP=days after pollination)	Total Reads	Average Read length	Total contigs	Contigs (>200bp)	Annotated (>200bp)	Annona specific genes (>200bp)
Sitaphal	0 DAP	227,732	711	11872	10403	8176	2227
	4 DAP	198,269	637	2522	2074	1808	266
	8 DAP	219,057	695	7671	6850	6023	827
	12 DAP	292,212	650	8408	7394	6512	882
MK1	0 DAP	288,216	650	9985	8645	7401	1244
	4 DAP	287,824	650	12559	11004	9038	1966
	8 DAP	272,750	650	8008	7001	6003	998
	12 DAP	143,649	610	2500	2078	1886	192

Table 2: Statistics of NMK1 and Sitaphal unigenes with sequence matches against public protein databases

Database	Contigs (Sitaphal)	Contigs (NMK1)
NCBI nr	10169 (68.15%)	11469(80.89%)
Grape protein	9187 (61.57%)	11126 (78.47%)
Peach protin	9152 (61.33%)	11108 (78.34%)
Strawberry protein	9218 (61.77%)	11206 (79.03%)
Annona sp. specific	3851 (25.80%)	2542 (17.92 %)

Table 3: Hormonal genes detected in the contrasting genotypes *A. squamosa*, using Arabidopsis protein sequences as query

Hormone	Sitaphal specific gene	NMK 1 specific gene	Common gene	Total hormone related genes detected in *A. squamosa*	Total hormone related genes in *Arabidopsis thaliana*
Auxin	7	8	24	39	156
ABA	1	1	28	30	38
GB	3	2	17	22	33
Cytokinin	2	6	6	14	32
BR	8	4	35	47	74

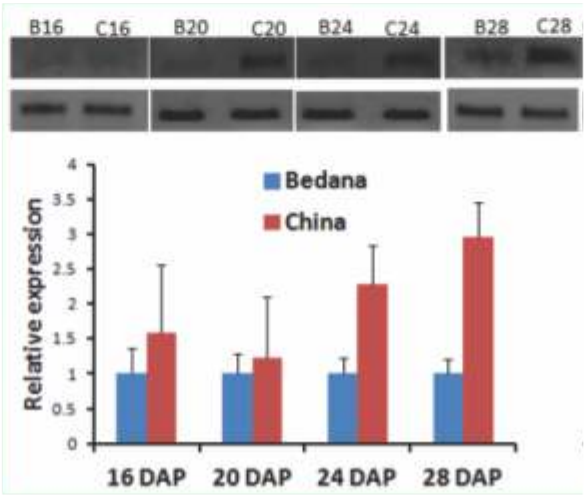


Figure 5: Quantification of the level of transcription of B3 domain containing gene in the developing ovules (16, 20, 24 and 28 days after anthesis (DAA)) of small seeded (B: bedana) *vs* bold seeded (C: china) litchi genotypes using semi-quantitative and real-time PCR.

Salient Achievements

1. Transcriptome sequencing has been completed in the developing fruits of *A. squamosa* and in the developing ovules of *L. chinensis*, in the genotypes having contrast in number of seeds and size of seeds in the fruit, respectively. Further analysis is in progress.
2. Difference has been notified in the expression pattern of B3 domain containing gene in small *vs* bold seeded Litchi. The gene is known to regulate seed maturation pathway during seed development in *Arabidopsis thaliana*. Further analysis is in progress.

Future Perspectives

1. The transcription analysis of the genes related to auxin, cytokinin, and gibberellins, and crosstalks among them during fruit and ovule development in the contrasting genotypes of *A. squamosa* and *L. chinensis*.
2. Identification of SSR microsatellite markers and SNP detection in the contrasting genotypes of *A. squamosa* and *L. chinensis*.
3. Identification of candidate pathways/genes related with seedlessness in *A. squamosa* and *L. chinensis*.

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

Principal Investigator
Sudhir P. Singh

Research Fellows
Anita Kumari

Introduction

The research project anticipates establishing long-distance transmission of mobile signals in the form of siRNAs to achieve gene silencing in flowering tissues. It is desirable to develop transgenic rootstocks for delivering silencing signals in non-transgenic scions for the modification of economically important traits, such as seedlessness. The majority of viral vectors are unable to reach the apical meristem, which limits their use in functional analysis of genes in flower tissues. The project also aims at designing a viral vector which could achieve silencing of a gene which express specifically in ovule, and thus seed related traits can be targeted in non-transgenic mode.

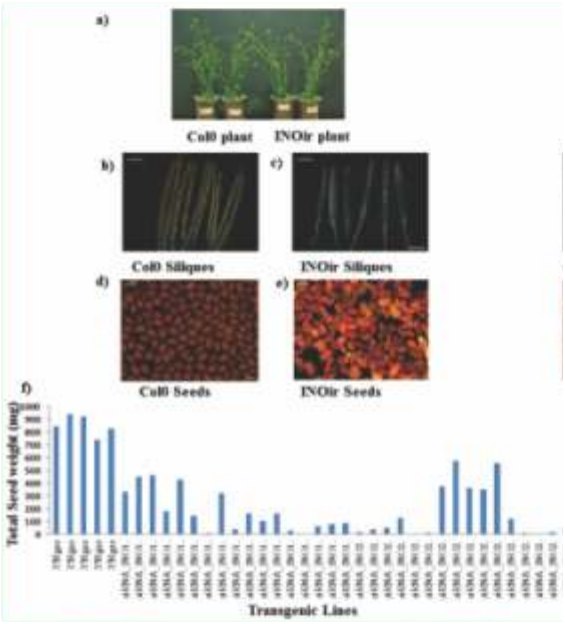


Figure 6: Transgenic lines expressing inverted repeats of *INO* gene fragment (INOir) (a) Wild Col0 plant (left) INOir expressing transgenic lines (right) (b) siliques of wild plant (c) siliques of transgenic plant expressing INOir in phloem (d) seeds of wild plant (e) seeds of transgenic plant expressing INOir in phloem (f) Total seed weight of transgenic lines expressing INOir, compared with GUS expressing transgenic plants.

Objectives

1. Establishment of gene silencing in ovule of wild scion grafted onto a root-stock which can transmit silencing signals in the scion
2. Development of a viral vector for gene silencing in ovule through leaf infiltration

Research in Progress

1. Silencing of reporter gene (*uidA*) was observed in flowering buds of scion by siRNAs delivered through rootstock.
2. Transgenic lines expressing siRNAs constitutively and phloem- specific against an integument specific gene, *INO* have been raised and phenotype of transgenic plants and seeds was observed by comparing with wild plant (Col0). Several transgenic lines, expressing dsRNA of *INO* gene were observed to produce seedless siliques (Figure 6).
3. The wild and transgenic (*uidA*) scion have been grafted on siRNA deliverable root-stock. Small RNAs have been extracted from the developing buds of the scion to examine transmission from root-stock to the ovules of wild and transgenic (*uidA*) scion.

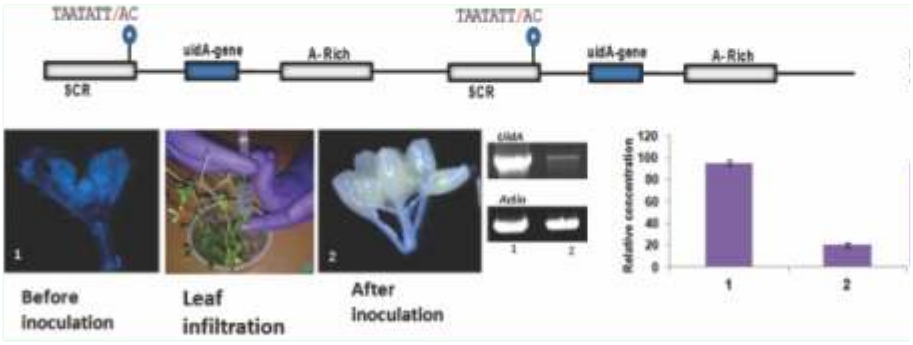


Figure 7: The leaf infiltration of the viral vector resulted transcriptional silencing of *uidA* gene in flowering tissues.

Sequencing of small RNAs has been planned.

4. A viral vector has been developed for inducing gene silencing in flowering tissues. Silencing was observed in flowering buds after leaf infiltration of the viral vector (Figure 7).

Salient Achievements

1. Silencing of reporter gene was achieved in flowering tissues by siRNAs delivered from root-stock..
2. Silencing of reporter gene was established in flowering tissues by leaf infiltration of viral vectors.

Future Perspectives

1. Silencing of ovule specific gene by silencing signals transmitted from modified root-stock.
2. Silencing of ovule specific gene by leaf infiltration of a modified viral vectors.

3.1 RNA guided genome editing in plants

Principal Investigator

Santosh K Upadhyay

Introduction

Specific and effective genome editing through non-transgenic approaches is an area of high priority research for the improvement of food crops. Several genome-editing technologies like zinc finger nucleases (ZFN) and transcription activator–like effector nucleases (TALEN) have been deployed for targeted genome modifications, but these are rather complicated in designing and need protein engineering for each target sequence. Recently, a new technology based on type II prokaryotic Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein (Cas) system has been developed as an effective tool for genome engineering. It is highly specific, inexpensive and easy to engineer. CRISPR consists of an array of repeat sequences separated by 'spacer' sequenees which belong to the targeted gene/genome. A long primary transcript transcribes from CRISPR arrays and gets processed into short CRISPR RNAs (crRNAs). crRNA consists of a conserved repeat sequence and a variable spacer sequence (guide) complementary to the target gene sequence. The ribonucleoprotein complex formed by short crRNA and Cas9 protein binds to the target sequence by base pairing and causes sequence-specific dsDNA cleavage. The presence of a conserved sequence motif (NGG) known as proto-spacer adjacent motif (PAM) at 3'downstream of target spacer sequence is also reported as essential for cleavage. CRISPR-Cas system has been demonstrated to work efficiently for genome editing in bacteria, yeast and animal systems and has been applied to plants recently. Although, some nonspecific editing has been reported, CRISPR-Cas system is very simple to design, highly effective and can be improved for specificity.

Objectives

1. To establish this system in model and crop plants.
2. To use this system for functional genomics in crop plants.
3. Validation of system for non-transgenic mode of genome editing.

Research Progress

1. We established the application of CRISPR-Cas mediated genome editing in wheat (*Triticum aestivum*) and *Nicotiana benthamiana*. Mutations in *inositol oxygenase* (*inox*) and *phytoene desaturase (pds)* genes in cell suspension culture of wheat and *pds* gene in leaves of *N. benthamiana* were achieved (Figure 8).
2. We developed a tool for the prediction of CRISPR-Cas binding site in large genome like wheat. The detection of CRISPR-Cas binding sites is simple, need the direct analysis of sequences for the presence of a specific ~23 nucleotide sequence including NGG PAM at 3'end. An online tool is available for the determination of CRISPR-Cas binding sites, but it is limited to the analysis of a very small number of sequences and cannot modify according to the users need. Further, it is web based tool which

depends upon internet connection and speed. Therefore, a simple, easy to edit and high throughput computational tool/script is required for the analysis of large data sets on the local machine (Figure 9). We developed a python based tool for high throughput detection of specific CRISPR-Cas binding site in huge nucleotide datasets. This tool is compatible with all kinds of Windows, Mac OS and Linus/Unix based operating systems, and user friendly to the peoples from non-Bioinformatics background also.

3. We analysed the frequency of CRISPR-Cas target site in available wheat ESTs. More than 90% ESTs has shown the presence of one or more probable target site, which indicated a bright scope of genome engineering in crop improvement programs.

Salient Achievements

1. CRISPR-Cas system has been established for genome editing in wheat, which can be utilized in functional genomics and crop improvement programs.
2. A high-throughput CRISPR-Cas binding site prediction tool has also been developed to analyze the probable target site.

Future Perspectives

1. Development of universal vector for CRISPR-Cas system for large scale functional genomics studies.
2. Development of non-transgenic method for crop improvement by complementing the CRISPR-Cas genome editing with Mendelian segregation.
3. Biosynthetic pathway engineering and suppression of negative regulators for crop improvements in non-transgenic mode.

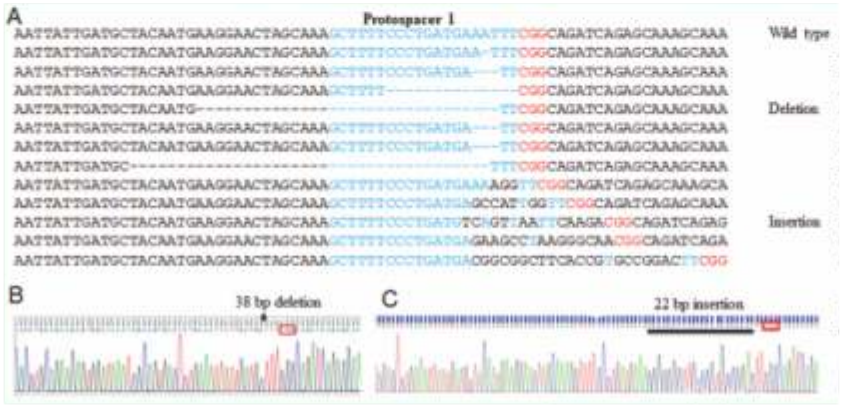


Figure 8: Editing at protospacer 1 of phytoene *desaturase (pds)* gene of *N. benthamiana* by CRISPR-Cas system. (A) Alignment of wild type and sequences with indel at protospacer 1 of *pds* gene. (B) and (C) show Sanger sequencing of selected deletion and insertion mutants, respectively.

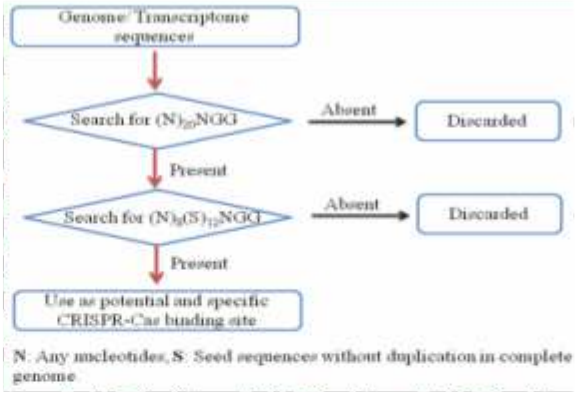


Figure 9: An outline of strategy for CRISPR-Cas binding site prediction.

3.1 Isolation and characterization of new insecticidal proteins toxic to whiteflies from lower plant diversity

Principal Investigator
Santosh K Upadhyay

Introduction

The insects that feast on crop plants cause a severe loss of yield and productivity, thereby affecting the agriculture economy of the country. Insect pests cost billions of dollars in the form of crop losses and insecticides, and the farmers face an ever present threat of insecticide resistance, fueling a continual search for alternative pest control strategies. Transgenic crops with enhanced biotic or abiotic stress tolerance have shown promising contribution in achieving greater crop productivity. Transgenic cotton expressing Cry toxin of *Bacillus thuringiensis* has tremendously increased the production as well as the societal status of farmers in our country. However, a concomitant increase in the population of minor pests like aphids, whiteflies and others has demanded certain new approaches. They create direct damage to the plant by feeding on phloem sap as well as indirect damage by the transmission of viruses. Further, they excrete very high amount of sugar on plant parts, which promotes bacterial and fungal colonization. None of the Bt-Cry proteins cause toxicity to sap sucking pests. However, other proteins like enzyme inhibitors, chitinases and lectins can provide some degree of resistance to sap sucking pests. The idea to introduce novel genes from lower plant diversity can be a powerful tool to tackle the control of sap sucking pests.

Objectives

1. Genetic resource development of whiteflies and isolation of new insecticidal proteins encoding genes from lower plants.
2. Recombinant expression, purification and characterization of insecticidal proteins.
3. Study of mechanism of insecticidal action.

Research Progress

1. **Transcriptome sequencing and characterization of whitefly:** Absence of genetic information of whitefly (*B. tabaci*) hampers several studies regarding their physiology and insect pest control programs. Therefore, we performed transcriptome sequencing to generate comprehensive genomic resource and used for characterization. About 83 million reads consisting of ~8 Gb transcriptome data were obtained using Illumina sequencing and assembled into 72716 unitigs with 592 bp of average length. A total of 21129 unitigs was annotated at stringent parameters by Blastx search against NCBI non-redundant protein database and protein sequences of *Tribolium castaneum* (considered as model insect), and *Acyrthosiphon pisum* (closest insect to *B. tabaci*). Annotated unitigs were mapped to 52847 gene ontology (GO) terms and 554 enzyme codes. These enzymes were mapped to 131 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in different combinations. Expression profiling showed vitellogenin, ribosomal proteins and NADH dehydrogenase highly expressed genes and they can be a potential targets for the control of whiteflies by RNAi.
2. **Sequence divergence and phylogenetic analysis:** Sequence divergence and phylogenetic analysis were performed using the orthologous sequences between different species of whiteflies. Orthologous sequences were aligned and aligned regions for all species were extracted and used in similarity and divergence analysis. The average similarity of H biotype was 97.2, 97.1 and 97.8% with Middle East Asia Minor-1 (MEAM1), Mediterranean (MED) and Asia II 3 species, respectively. Maximum similarity was 100% in all combinations; however the minimum similarity varied from 81 to 81.7% (Table 5). We observed that the average divergence of H biotype with MEAM1, MED and Asia II 3 were 2, 1.95 and 0.92%, respectively. To confirm the accuracy of analysis, we had also analyzed the divergence between MEAM1, MED and Asia II 3. Similar divergence between MED / MEAM1 (0.87%), MED / AsiaII3 (1.7%) and

Table 4: Summary of the whiteflies transcriptome data.

Total number of reads	83,828,866
Total number of clean reads after removing adapter sequence and poly A, T and N sequences	82,818,787
Average read length	101 bp
Total number of scaffolds after Abyss pair wise assembly	1,324,517
Total number of distinct sequences (unitigs) obtained after CAP3 assembly	72,716
Longest unitig	12135 bp
Smallest unitig	150 bp
Mean length of unitigs	591.9
Number of annotated unitigs	21129
Total GO terms obtained	52847
Total KEGG pathway mapped	131
Total Enzymes code mapped to KEGG pathway	545
Number of unitigs showed Blast hit to symbionts genome	313

MEAM1 / AsiaII3 (1.75%) was found as reported in earlier studies, which supports the accuracy of our analysis. A phylogenetic tree was also constructed using these orthologous sequences, which showed that the MED and MEAM1 were grouped together as reported earlier, however H biotype was grouped with Asia II 3. Divergence between H biotype and Asia II 3 (0.92%) was greater than the divergence between MED and MEAM1 (0.87%). Results indicated that these are independent species.

3. **Analysis of amino acid biosynthesis in whitefly:** It is well known fact that animals lack the essential amino acid biosynthesis pathways and get them from food sources. Sap sucking insects take their food from phloem sap of host plant, which contains very less quantity of essential amino acids. Therefore, these insects are mostly depending on microbial symbionts for the synthesis of essential amino acids. In return, insects provide nonessential amino acids and several other supports to symbionts. *Candidatus Portiera aleyrodidarum* (CPA) is reported as the symbiont for whitefly. We analyzed the integration of amino acid biosynthetic pathway in whitefly and its symbiont. Genome sequence analysis of CPA clearly indicated that the genes responsible for the synthesis of non-essential amino acid are completely absent, which indicated the possibility of collaboration with host insect for these amino acid. Further, we found significant expression of these genes in whitefly transcriptome data, which probably compensate the requirement of symbiont also. On the basis of mapping and expression of the symbiont and insect

Table 5: Sequence divergence between different species of whiteflies

Species 1	Species 2	Divergence (%)
H	MED	1.95
H	MEAM1	2.00
H	Asia II 3	0.92
MED	MEAM1	0.87
MED	Asia II 3	1.70
MEAM1	Asia II 3	1.75

related genes to the amino acids biosynthetic pathways, we predicted the contribution of each and proposed a hypothetical amino acids biosynthetic pathway in whitefly. Our results supported the integration of both partners and their complementation in synthesis of essential and nonessential amino acids. Since, phloem sap is very poor source of essential amino acids and insects are not completely unable to synthesize them (except threonine and methionine) as evident by the absence of related genes, symbionts remain the only source to supply these amino acids to phloem feeding insect. We found the significant expression of symbiont genes involved in synthesis of essential amino acid, which were absent in insect. Enzymes responsible for the synthesis of arginine and tryptophan were completely encoded by the symbiont, however the last enzymes for the synthesis of phenyl-alanine valine, leucine and isoleucine were absent as reported in the case of aphid symbiont. But these genes are available in insect with remarkable expression (Figure 10). Further, we observed most of the genes involve in the biosynthesis of methionine, lysine and histidine in symbiont, however, we could not find the complete biosynthetic pathway for these amino acids in transcriptome and genome data.

4. **Collection of lower plant samples, RNA isolation and cDNA synthesis:** On the basis of available literature and prior knowledge about the probability of existence of insecticidal proteins, five lower plant samples (2 bryophytes and 3 pterydophytes) were collected. RNA isolation and cDNA synthesis have been performed, which will be used for the amplification and cloning of probable insecticidal genes in the later part of the study.

Salient Achievements

1. A comprehensive transcriptome data of whitefly H biotype have been developed and characterized.
2. Comparison of orthologous sequences among different species of whiteflies

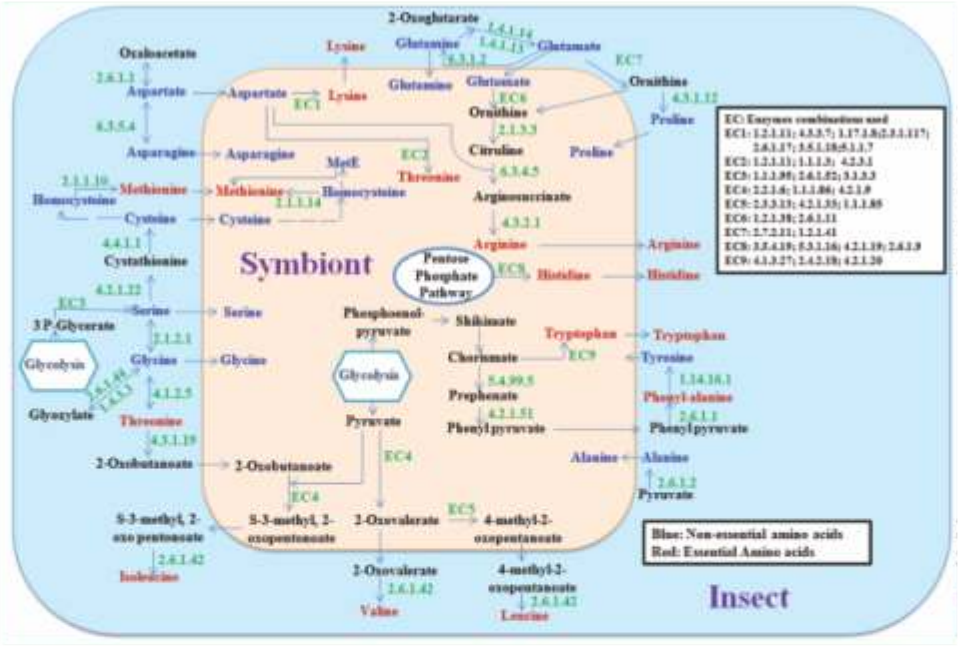


Figure 10: Association of host and symbiont genes in amino acid biosynthetic pathway in *Bemisia tabaci*. Pinkish and sky-blue circle represents symbiont and insect, respectively

indicated the significant divergence across the species. Whitefly species present in our country are significantly diverse from the species reported from another territory like China.

3. Amino acids biosynthetic pathway analysis showed the complementation between insect and its symbiont. Since, whiteflies are phloem fed, which lacks the several essential amino acids; they need an alternative source as reported in case of aphid. Our study demonstrated the contribution of host and symbiont in amino acid biosynthesis. Insect provides most of the nonessential amino acids to symbiont and in return receives essential amino acids.

Future Perspectives

1. Detail characterization of transcript data of whitefly symbiont.
2. Cloning of new insecticidal proteins encoding genes from lower plants.
3. Recombinant expression, purification and characterization of insecticidal proteins.
4. Study of mode of insecticidal action

DIET AND HEALTH

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

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

Principal Investigator
Kanthi Kiran K.

Co-Investigators
Mahendra Bishnoi
Koushik Mazumder

Research Fellows
Nida Murtaza
Siddhartha M. Sarma

Introduction

Excess calorie intake contributes for weight gain and obesity. Obesity is associated with a low grade inflammation, oxidative stress, altered adipose tissue secretome and dysbiosis of beneficial gut microflora. As a result, chronic abnormalities like atherosclerosis, diabetes and certain forms of cancer may be associated with obesity. Anti-obesity medications have been shown to pose side effects. Safe and alternate approaches are in high demand. Consumption of whole grain (WG), plant derived dietary molecules including polyphenols, non-starch dietary fibers (NSDF), prebiotics and probiotics have been shown to ameliorate obesity and associated complications. Millets come under the category of whole grains and are a rich source of dietary fibers, proteins, energy, minerals, vitamins, and antioxidant polyphenols. Single bioactive or synergistic effect of bioactives in the WG contributes for beneficial effects. In the earlier report, we have shown that NSDF from millets inhibited lipid accumulation in 3T3-L1 adipocytes. Here we report the effect of finger millet bran or whole grain consumption on high fat diet (HFD) induced changes in mice.

Objectives

1. To understand the role of non-starch dietary fibers (NSDF) and other bioactives from millets in regulating adipogenesis using 3T3-L1 cells.
2. Nutrigenomic changes associated with consumption of whole grain millet, NSDF and other bioactives in high fat diet fed mice.

Research Progress

1. **Body weight, oral glucose tolerance test (OGTT) and glucose clearance:** HFD promoted body weight gain at the end of 12 weeks as compared to normal diet (ND) fed mice. HFD-BR fed mice showed a decrease in body weight gain as compared to HFD alone fed mice whereas HFD-WG administered mice showed a non-significant decrease in body weight gain. Food consumption was not altered among different groups. Rate of glucose clearance was significantly decreased in serum in HFD fed mice whereas administration of HFD-BR significantly enhanced the clearance and no significant change was observed in HFD-WG as compared with HFD alone fed mice.

i) **Serum biochemical parameters:** Total cholesterol (TC), cholesterol esters (CE), free cholesterol (FC), free fatty acids (FFAs), LDL/VLDL-c were significantly high and HDL levels were significantly low in the HFD alone fed mice as compared to ND fed mice (Figure 1). Administration of HFD-BR significantly prevented increase in the levels of TC, CE, FC, FFA and LDL/VLDL-c and prevented the decrease in the levels of HDL-c as compared to HFD fed mice (Figure 1). HFD-WG administration significantly prevented increase in TC and CE whereas non-significantly decreased the levels of FC, LDL/VLDL-c and FFA and no significant change was observed in HDL-c levels as compared to HFD fed mice (Figure 1).

2. **Effect on visceral white adipose tissue (vWAT) gene expression:** HFD non-significantly up-regulated the expression of *DLK1, C/EBPα, PPARγ* and *PLIN1* and significantly lowered adiponectin expression as compared to ND fed mice (Figure 2). HFD-BR and HFD-WG supplementation did not alter the expression levels of *PPARγ* and *C/EBPα*. HFD-BR

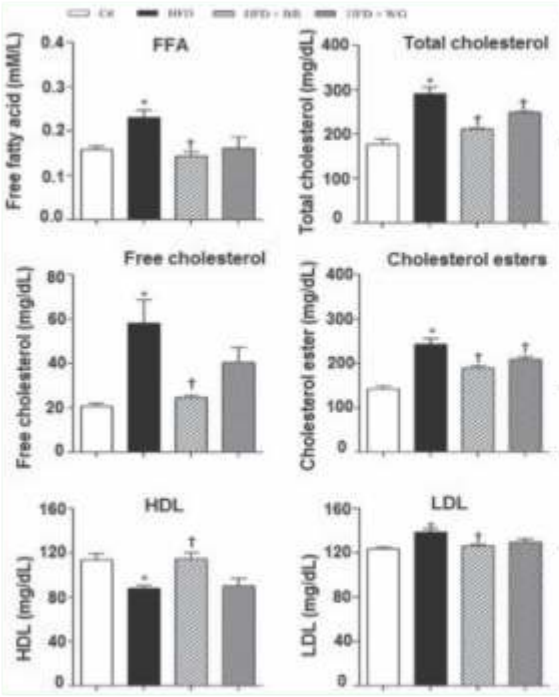


Figure 1: Effect of HFD, HFD-BR and HFD-WG on serum lipid profile. Ctl = Control, HFD = High fat diet, BR = Bran, WG = Whole Grain, ns = non-significant.

administration significantly increased the expression levels of *DLK1*, *PLIN1* and *ADIPOQ* as compared to HFD alone. HFD-WG administration significantly enhanced *DLK1* and *ADIPOQ* expression as compared to HFD fed mice (Figure 2). A non-significant increase was observed in *PLIN1* expression in HFD-WG supplemented mice as compared to HFD fed mice (Figure 2). HFD significantly down-regulated *ACC* and *GLUT4* and enhanced *FASN* expression as compared to ND fed mice (Figure 2). *GLUT4* expression was significantly up-regulated upon HFD-BR administration and *FASN* and *ACC* expression was down-regulated as compared to HFD alone fed mice (Figure 2). HFD-WG supplementation significantly down-regulated the expression of *ACC* and *FASN* whereas non-significantly increased the expression of *GLUT4* (Figure 2). *ACOX1* showed increased expression in HFD-BR and HFD-WG groups; however the increase was not significant as compared to HFD or ND fed mice. HFD feeding significantly up-regulated the expression of *TNFα* and *iNOS* whereas non-significant increase was observed in the levels of *IL6* as compared to ND fed mice. Administration of HFD-BR significantly down-regulated their expression levels whereas HFD-WG supplementation significantly down-regulated the expression of *TNFα* and *iNOS* and upregulated the expression of *IL6* as compared to HFD fed mice (Figure 2).

3. **Effect on selected gut microbial groups:** *Lactobacillus*, *Bifidobacteria*, *Roseburia*, *Akkermansia*, *Bacteroidetes* and *Bacteroides-Prevotella* abundances were significantly decreased and *Enterobacter* and *Firmicutes* abundances were increased upon HFD feeding as compared to ND (Figure 3).

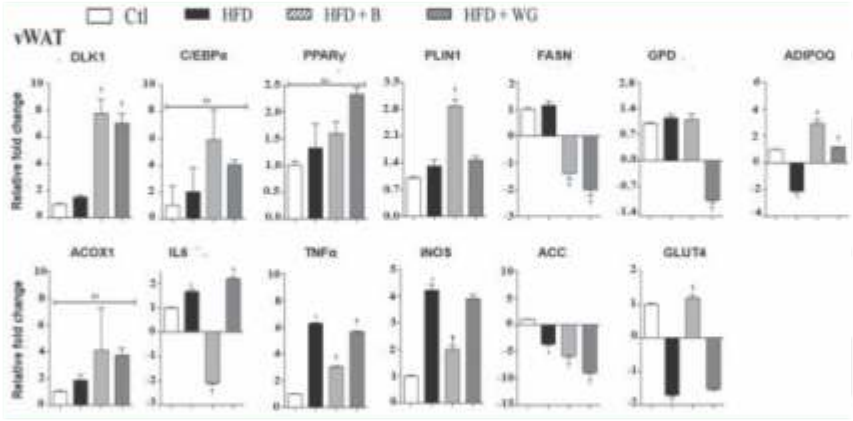


Figure 2: Effect of HFD, HFD-BR and HFD-WG consumption on gene expression in vWAT. Ctl = Control, HFD = High fat diet, BR = Bran, WG = Whole Grain

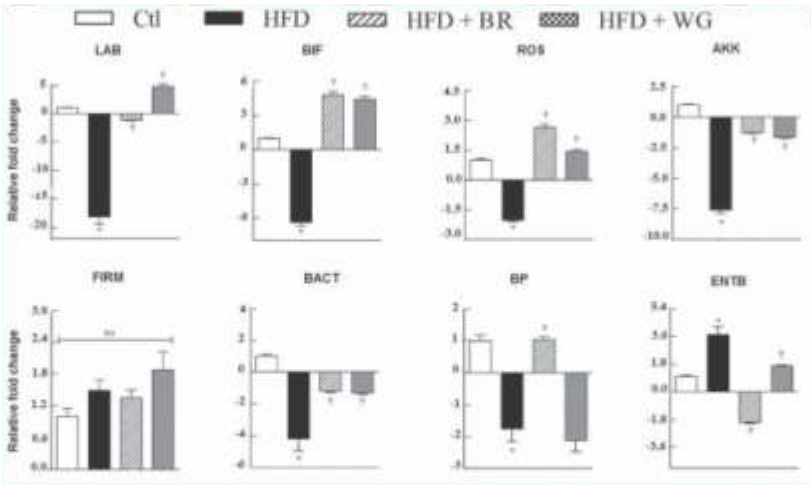


Figure 3: Effect of HFD, HFD-BR and HFD-WG consumption on relative bacterial abundance in caecum. Ctl = Control, HFD = High fat diet, BR = Bran, WG = Whole Grain, BACT = Bacteroidetes, FIRM= Firmicutes, LAB= Lactobacillus, BIF = *Bifidobacteria*, EntB= *Enterobacter*, ROS = *Roseburia*, BP = *Bacteroides Prevotella*, Akk = *Akkermansia*, ns = non-significant.

Salient Achievements

1. FM-BR prevented body weight gain; increased the HDL-c and decreased LDL/VLDL-c levels.
2. Enhanced the expression of anti-adipogenic markers in vWAT; reduced proinflammatory status and brought prebiotic alterations in the gut microflora in high fat fed mice.

Future Perspectives

1. Effect of other millets on high fat diet induced changes.
2. Role of non-starch dietary fibers (NSDF) and other bioactives from millets on adipogenesis *in vitro*.
3. Nutrigenomic effects of NSDF and other bioactives from millets using high fat diet induced obese rodent models.

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

Principal Investigator:

Mahendra Bishnoi

Co-Investigator:

Kanthi Kiran K.

Research Fellow:

Ritesh K Baboota

Introduction:

Current anti-obesity medications are pharmacological agents which can reduce or control weight by affecting one of the fundamental processes of the weight regulation in human body i.e. altering appetite, metabolism, or consumption of calories. All these medications, including orlistat, rimonabant and sibutramine, have severe side effects including depression, oily bowel movements, cardiovascular concerns and steatorrhoea. The potential side effects profile of these drugs is much more than their beneficial effects, suggesting the urgent need for alternatives. Over the years it has been seen that best and most effective options for overweight and obese individuals remain diet and physical exercise. It is important to have dietary regulations to prevent life style problems rather than to search for the treatment. Available literature suggests that sensory ion channel receptor system, Transient Receptor Potential (TRP) channels, are possible candidates to regulate energy metabolism and thermogenesis, which can lead to calorie consumption and prevention of obesity via different mechanisms. Common dietary constituents like chilli pepper, black pepper, clove, garlic, cinnamon, mint and their

constituents (capsaicin, piperine, eugenol, allicin, cinnamaldehyde, omega fatty acids, menthol etc) can modulate TRP channels. In this proposal, we will understand the role of TRP channels in adipogenesis, obesity and related complications using *in-vitro* and *in-vivo* model systems. Further, using the TRP channel receptor system we propose to come up with dietary constituents that can modulate the molecular mechanism associated with the process of adipogenesis.

Objectives

1. Determination of expression, function and significance of TRP channels in commercially available mouse preadipocytes cell lines (3T3-L1), human preadipocytes (HPAd) and adipocytes (HAd) cells.
2. *In-vitro* characterization of the molecular basis of adipogenesis and determination of effect of TRP channel modulation on adipogenesis and its associated changes.
3. To study the effect of dietary modulations of TRP channels (TRPV1: capsaicin, piperine; TRPA1: garlic, cinnamon; TRPM8: menthol; TRPC1: omega-3 fatty acids and others) on weight gain, serum biochemistry, and adipose tissue genotype in a diet (high fat) based *in vivo* mouse model of obesity.
4. Developing diets/ special dietary formulations constituted of modulating food components and study their effect on adipogenesis, obesity and related complications in human trials.

Research Progress

Previously we found out that multiple TRP channel genes are expressed in mouse 3T3-L1 preadipocytes. Further, these channels are also expressed in murine white adipose tissue (WAT) and brown adipose tissue (BAT). These channels are also present in human preadipocytes (high to moderate expression: TRPV1, TRPC6, TRPC1, TRPV4, TRPM2, TRPV3, TRPC4; low expression: TRPM7 TRPM3, TRPM5, TRPV6, 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 (Annual report 2012-2013). Taking lead into the role of TRPV1, we initiated and completed *in-vitro* (3T3L1 preadipocyte cell lines) and *in-vivo* (high fat diet (HFD)-induced weight gain model) studies for capsaicin, a TRPV1 agonist.

1. ***In-vitro* studies:** TRPV1 channels were functionally expressed in preadipocytes but not in adipocytes. Capsaicin and RTX dose-dependently increased Ca^{2+} influx in preadipocytes which was prevented by capsazepine, a TRPV1 antagonist. Capsaicin inhibited lipid accumulation at lower doses (0.1-1 µM) whereas it enhanced accumulation at (10-100 µM) higher doses. TRPV1 gene expression was also increased at lower doses and decreased at higher doses, suggesting its relation with presence of preadipocytes. Capsaicin (0.1-100 µM) promoted major pro-adipogenic gene, *PPARγ* and some of its related downstream genes. Capsaicin (1 µM) up-regulates anti-adipogenesis genes. Further, capsaicin at lower dose significantly increased brown fat cell marker genes during differentiation of 3T3-L1 preadipocytes into adipocytes. On similar note, capsaicin administration leads to increase in browning specific genes in mice WAT. Global *TRPV1* ablation (i.p. RTX administration) leads to no change in body weight and increase in locomotor activity.
2. ***In-vivo* studies:** Oral administration of capsaicin significantly prevented weight gain as compared to HFD-fed mice. Serum leptin and TNFα was significantly increased in HFD-fed mice in comparison to control mice. However, co-administration of capsaicin with HFD significantly prevented this release. HFD significantly raised the adiponectin level which was prevented by capsaicin co-administration.
3. **Effect of capsaicin on hypothalamic gene expression:** Immunofluorescence imaging showed the widespread expression of

TRPV1 immunoreactivity in hypothalamus and arcuate nucleus of mice. *TRPV1* was expressed in hypothalamus of control mice whereas its expression was significantly down- regulated in HFD-fed mice. However, capsaicin increased its expression levels comparable to control in HFD-fed mice. Anorectic genes such as *UCN*, *PYY*, *RAMP3*, *GRP*, *BDNF* and *CARTPT* were significantly down-regulated in HFD-fed mice as compared to control group whereas their expression was significantly enhanced in HFD+ capsaicin group. HFD significantly increased the expression of orexigenic genes like *CNR1*, *GALR1*, *GHRL*, *ADRA2B*, *NPY1R* and *GHSR*. However, their expression, except for *ADRA2B* and *NPY1R*, was significantly lowered in HFD+ capsaicin group. No change was observed in *NPY* expression levels in HFD group whereas its expression was significantly increased in HFD+ capsaicin group.

4. **Capsaicin supplementation on anorectic, orexigenic and energy expenditure related genes in visceral WAT:** Anorectic genes such as *BDNF*, *DRD1A*, *DRD2* and *PYY* were significantly up-regulated in HFD group as compared to control whereas significant reduction in expression level was observed in HFD+ capsaicin group. HFD significantly up-regulated the expression levels of orexigenic genes *GALR1*, *HCRT* and *NR3C1* which upon capsaicin administration were significantly decreased as compared to HFD group. No significant change was observed in expression levels of *GHRL* and *HCRTR1* in HFD group as compared to control but they were significantly lowered in HFD+ Cap group. Energy expenditure related genes such as *ADCYAP1R1*, *ADIPOQ*, *ADIPOR1*, *ADIPOR2* and *CPD* were significantly reduced in HFD group as compared to control group whereas capsaicin administration significantly raised their levels. Effect of capsaicin supplementation in HFD-induced changes in expression levels of metabolic genes *i.e. FASN*, *GPD1* and *ACOX1* was evaluated in sWAT and vWAT. HFD significantly down-regulated the expression levels of all three genes in sWAT as well as in vWAT except for *ACOX1* where no significant change was observed. Capsaicin supplementation significantly enhanced the expression levels of *GPD1* and *ACOX1* in sWAT and vWAT. *FASN* was significantly increased in HFD+ capsaicin group in sWAT whereas no significant change was observed in vWAT.

5. **Effect of capsaicin supplementation on "*browning*" genes in BAT, subcutaneous WAT and visceral WAT:** In BAT, HFD significantly increased the expression of "*browning*" genes as compared to control animals whereas their expression levels were further increased by capsaicin administration. Similarly, in vWAT these genes were significantly increased in HFD-fed mice but no significant difference was observed in capsaicin treated group as compared to control group. Different trend in expression levels of these genes was observed in sWAT. *BDNF*, *NCOA1*, *PPARα* and *PTGS2* showed similar pattern as that of BAT i.e. significant increase in expression in HFD group and further increase in HFD+Cap group whereas *UCP1*, *NRIP1*, *CIDEA*, *PGC1α*, *MPAK14* and *SIRT2* were significantly down-regulated in HFD group as compared to control whereas increased significantly in HFD+Cap group (Figure 4).

6. **Effect of capsaicin on different bacterial groups in caecum:** *Enterobacteriaceae* and *Firmicutes* abundance was significantly higher in caecal contents of HFD-fed mice as compared to control. Capsaicin supplementation significantly prevented this increase. Abundance of *Akkermansia*, *Bacteriodetes*, *Bacteroides Prevotella* was significantly lowered in HFD group in comparison to control. Significant increase in their abundance was observed in HFD+ capsaicin group. *Bifidobacteria* and *Lactobacillus* abundance was significantly increased in HFD group as compared to control. *Bifidobacteria* further showed significant decrease whereas *Lactobacillus* was found to be significantly higher in HFD+

capsaicin group as compared to HFD group.

In summary, our *in-vivo* studies suggest that in addition to its well known effects, oral administration of capsaicin (a) modulates hypothalamic satiety associated genotype, (b) alters gut-microbial composition, (c) induces "browning" genotype (BAT associated genes) in subcutaneous WAT and (d) increases expression of thermogenesis and mitochondrial biogenesis genes in BAT (Figure 5).

Salient Achievements

1. Our *in-vitro* findings suggest the bidirectional modulatory role of capsaicin in adipogenesis. Capsaicin inhibits adipogenesis in 3T3-L1 *via* TRPV1 activation and induces of brown like genotype.
2. Our *in-vivo* findings suggest that oral administration of capsaicin modulates hypothalamic satiety associated genotype, alters gut-microbial composition, induces

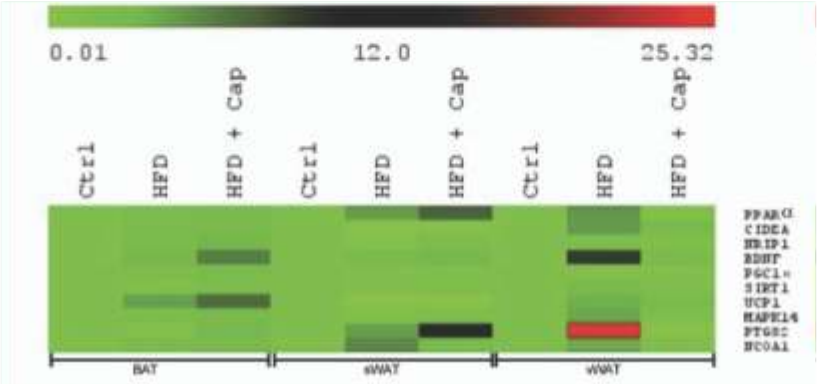


Figure 4: Effect of capsaicin on HFD-induced changes in browning genes (mitochondrial biogenesis and thermogenesis related genes) in BAT, sWAT and vWAT.

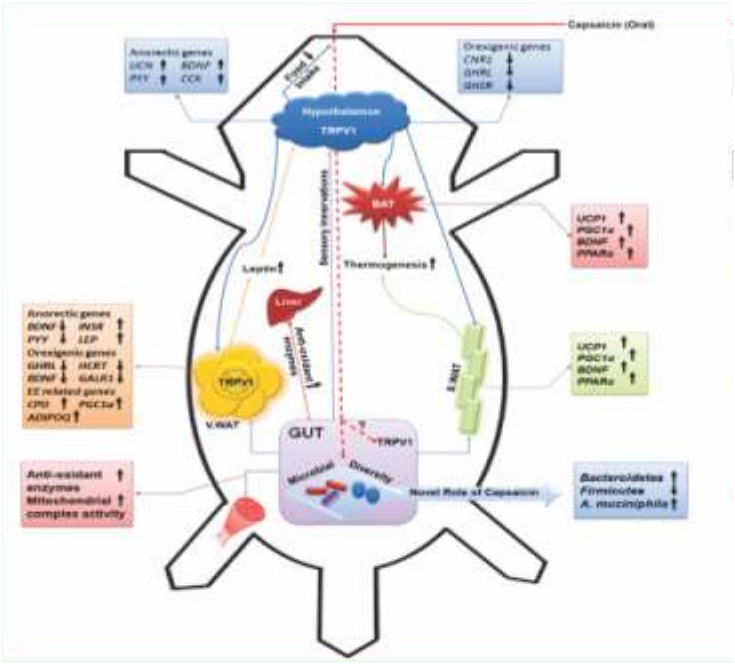


Figure 5: Schematic diagram that summarizes the proposed mode of action of capsaicin in HFD induced obese mice. Capsaicin supplementation, via gastrointestinal TRPV1 activation or vagal afferent activation induces hypothalamic TRPV1. In hypothalamus it alters anorectic and orexigenic genes. Induced hypothalamic activity induces SNS activation which might be responsible for increased thermogenesis in BAT and sWAT and decrease in obesity markers in vWAT. Capsaicin can directly affect the gene changes in vWAT and sWAT via direct absorption into blood. Unabsorbed capsaicin lead to beneficial alteration in gut microbial population.

"browning" genotype (BAT associated genes) in subcutaneous WAT and increases expression of thermogenesis and mitochondrial biogenesis genes in BAT. These findings provide evidence for novel and interesting mechanisms to explain the anti-obesity effect of capsaicin.

Future Perspectives

1. *In-vitro* studies to understand the role of other TRP channels (i.e. TRPA1, TRPM8, TRPV2 and TRPC1) in adipogenesis and its associated changes.
2. To study the effect of dietary modulations of other TRP channels (TRPA1: garlic, cinnamon; TRPM8: menthol; TRPC1: omega-3 fatty acids and others) on weight gain, serum biochemistry, and adipose tissue genotype in a diet (high fat) based *in vivo* mouse model of obesity.
3. Preclinical and clinical follow-up of capsaicin studies.

4.1.3 Structural characterization of arabino-xylans from millets and their biological activity

Principal Investigator
Koushik Mazumder

Research Fellow
Vandana Bijalwan

Introduction

Millets, the staple foods for millions of people across the world, are the chief source of soluble dietary fiber. Epidemiological studies have clearly demonstrated that increased consumption of soluble dietary fibers has been associated with a reduced risk of cardiovascular diseases and diabetes. Many of the life style disorders and chronic diseases are associated with oxidative stress which is combined with free radical formations.

Phenolic acids such as ferulic acid and other hydroxy-cinnamic acids (HCA) exhibit very strong antioxidant activity since they terminate free radical chain reactions. HCA in the bound form are esterified to cell wall arabinoxylan polysaccharides and influence their physicochemical and functional properties. More interestingly, hydroxy-cinnamic acid bound arabinoxylans (HCA-AXs) as cell wall component of the cereal grain exhibit stronger antioxidant activities than free acids. Hence in the present study, the variability in the fine structures of the hydroxy-cinnamic acid bound arabinoxylans (HCA-AXs) from finger millet (FM), kodo millet (KM), barnyard millet (BM), foxtail millet (FOXM), proso millet (PM) and their antioxidant activity will be evaluated using *in vitro* model. The present study can be exploited in preparing nutraceutical health foods based on dietary fibers enriched with HCA-AXs.

Objectives

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

Research Progress

In our studies, we have standardized the protocol of mild alkali extraction for isolation of HCA bound arabinoxylans from five Indian millets, 0.5% KOH extraction was performed to avoid the de-esterification of the phenolic acids. The total phenolics content of the extracted materials were determined colorometically using folin's method. The analysis demonstrated the total phenolics content of KM and FOXM extract as ~13% and 5% respectively whereas the other millet extracts (FM, BM and PM) showed phenolics content of ~8%-9%.

The sugar compositional analysis of the extracted materials was carried out using GC and GC-MS as alditol acetate derivatives. The analysis showed the presence of arabinose and xylose as major constituents (~60-70%) in all the extracted material together with glucose, galactose and mannose as minor constituents (Figure 6).

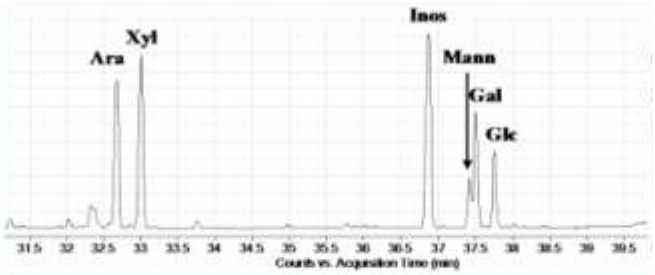


Figure 6: GC spectrum of the extracted HCA bound arabinoxylans from kodo millet. (Ara: Arabinose, Xyl: Xylose, Inos: Inositol (internal standard), Mann: Mannose, Gal: Galactose, Glc: Glucose).

The estimation of the bound phenolic acids were performed by de-esterification of the extracted HCA bound AXs, purification and followed by HPLC analysis. The HPLC analysis showed the presence of ferulic acid as major constituent phenolic acid in FM extract (relative percentage 89%) together with caffeic and para-coumaric acid as minor constituents (relative percentage 3 and 8 % respectively) whereas in KM extract all the three phenolic acids i.e., caffeic, para-coumaric and ferulic acid were present in considerable amount (relative percentage 30%, 33% and 37% respectively). Both the BM and FOXM extracts contained only para-coumaric (80%) and ferulic acid (20%), whereas PM extract contain para-coumaric and ferulic acid in the relative percentage of 56% and 44% respectively (Figure 7).

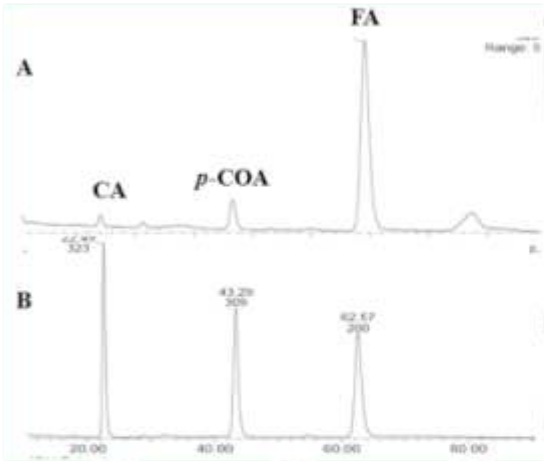


Figure 7: HPLC profile of bound hydroxy-cinnamic acids in Finger (A) and Kodo millet (B). (CA: Caffeic acid, *p*-COA: *p*-Coumaric acid, FA: Ferulic acid)

The phenolic acids bound AXs were also enzymatically hydrolyzed with endo-xylanase to generate oligosaccharides, the oligosaccharides were analyzed by MALDI-TOF MS, the presence of oligosaccharides bound to ferulic acid in finger millet (P_4FA_2Ac at *m/z* 985, P_6FAAc at *m/z* 1051) and para-coumaric acid together with ferulic acid in barnyard millet (P_4*p*-$COAAc_2$ at *m/z* 837, P_4*p*-$COAFAAc_2$ at *m/z* 1013) confirmed the covalent ester binding of the hydroxyl-cinnamic acids to the arabinoxylans (P: Pentose, *p*-COA: *p*-Coumaric acid, FA: Ferulic acid, Ac: Acetyl group).

Currently, the role of fine structures of HCA bound arabinoxylans from finger millet, foxtail millet, kodo millet, barnyard millet, proso millet and their antioxidant activity were evaluated using *in vitro* assay (DPPH assay). The kodo millet extract showed highest antioxidant activity of 94% (0.06) at a lower concentration of 100µg/ml whereas finger millet extract showed antioxidant activity of 93% (0.05) at a concentration of 400µg/ml. Proso and barnyard millet extract exhibited moderate antioxidant activity of 69% (0.11) and 63% (0.06) at concentration of 600µg/ml respectively, whereas foxtail millet extract exhibited lower antioxidant activity of 37% (0.12) at a concentration of 700µg/ml. Although the total phenolics estimation data indicated that the total phenolics content of the FM, BM and PM extract were in the range of ~8-9%, but the significant variation in antioxidant capacity was observed among FM, BM and PM extracts which suggested that the variation in the antioxidant activity is associated with the compositional and structural variability of the bound HCA and arabinoxylans.

The procedure is under progress, in depth structural analysis of the HCA bound

arabinoxylan poly and oligosaccharides will be carried out using various analytical methods (GC-MS, HPLC, MALDI-TOF-MS, ESI-MS/MS and NMR). In future, their role regulating oxidative stress will be further extended using different *in vitro* assays i.e., comet assay (SCGE) and HepG-2 cell lines.

Salient Achievements

1. The preliminary structural and *in vitro* studies showed considerable differences in the structure and antioxidant potential of HCA bound AXs from various millet varieties.
2. The detailed structural characterization and *in vitro* studies of the HCA bound arabinoxylan poly and oligosaccharides are in progress using various physico-chemical methods (GC-MS, HPLC, MALDI-TOF-MS, ESI-MS/MS and NMR) to establish their structure-function relationship with respect to antioxidant activity.

Future Perspectives

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

4.2 Iron alginate encapsulated ferric saccharate microemuslions: Synthesis, characterization and evaluation

Principal Investigator
Dr. Nitin Kumar Singhal

Co-Investigators
Dr Hariom Yadav
Dr Rajat Sandhir

Research Fellow
Kimmi Mukhija

Introductions

Iron is a vital mineral in the body which is involved in many physiological functions but primarily needed in the formation of hemoglobin. Excess of iron is stored in the liver, spleen and bone marrow as ferritin. In the blood stream, it is bound to a specific carrier protein, transferrin. Insufficient iron availability during growth phases or states of increased requirement results in iron deficiency. Iron deficiency is the most common cause of anemia in the world and continues to be the most prevalent nutritional anemia in developing countries. Iron deficiency anemia is characterized by a defect in hemoglobin synthesis, resulting in abnormally small (microcytic) RBCs which contain a decreased amount of hemoglobin.

One approach to reduce the organoleptic apparent problems and increasing the bioavailability is iron encapsulation. Microencapsulation can prevent iron from interacting with the environment before reaching the intestine. Iron salts are very reactive, so this protection can avoid oxidation of the food matrix and reduce side effects when the fortified product is ingested. Encapsulating ferric saccharate with alginate having high nutritional value not only decreases the organoleptic problems but increase the iron bioavailability as well. Alginate bio-adhesive as well as polycation ability can enhance the para cellular absorption through the intestinal membrane. Ferric saccharate complexes with high absorbance power and little organoleptic problems have been selected as core and encapsulated with alginate.

Objectives

1. To synthesize and characterize alginate encapsulated ferric saccharate micro-emulsions.
2. To evaluate and compare the bioavailability of iron in alginate encapsulated ferric saccharate emulsions using Caco-2 cell lines.
3. To compare the effect of alginate encapsulated ferric galactose microemulsion (AFGM) and ferric galactose complex (FGC) on iron bioavailability and oxidative stress in anemic animals.

Ressearch Progress

1. **Synthesis and Characterization of emulsions:** (C)In brief, monosaccharide (D-Glucose, D-Sorbitol, D-Fructose, D-Mannose and D-Galactose) was suspended in

methanol and sodium metal was added in small pieces with stirring, then methanolic solution of anhydrous iron (III) chloride was added to give precipitate. After 3 hours, a solid product was obtained by filtering which was as purified by methanol and acetone, dried under vacuum. These reactions resulted in formation of ferric saccharate complexes: Ferric-Glucose, Ferric-Sorbitol, Ferric-Fructose, Ferric-Mannose and Ferric-Galactose respectively. The percentage of iron present in the complexes was determined by inductively coupled plasma mass spectrophotometer (ICP-MS). All the ferric saccharate complexes were characterized by FTIR. The mean diameter and zeta potential of all microemulsions in the dispersion (with appropriate dilutions) was determined using laser diffraction technique (Mastersizer 2000, Malvern Instruments, UK followed by analyzed microscopically using TEM. FTIR spectra of the complexes are indicative of the cleavage between extensive intermolecular hydrogen bonds existing in the solid state of the parent ligands, thus resulting in a broad band $3390 \pm 10 cm^{-1}$. The TEM analysis of microemulsions showed that most of the particles were nearly spherical shaped and was present as individual uniform entities rather than agglomerates indicating their stability. Average particle size microemulsions were in the range of 800-1000 nm as measured by the laser diffraction and zeta potential of all the iron monosaccharide emulsions were in the range of -20 to -34mV, confirm the stability of microemulsions. All the characterization is for AFGM summarized in Figure 8.

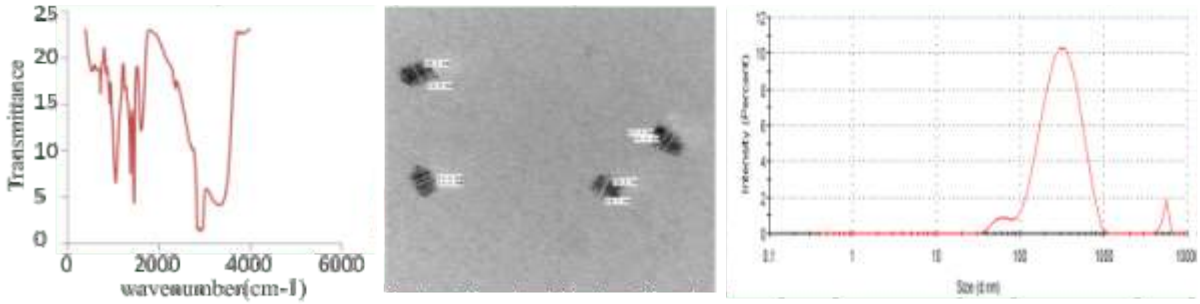


Figure 8: Characterization of Alginate encapsulated Ferric Glucose Nanoemulsion (AFGM) (A) FTIR spectra; (B) TEM; (C) Particle size distribution

2. ***In-vitro* results:** The bioavailability of iron with complexes in cells was significantly higher as compared to microemulsions may be because iron present in the complexes was in uncoated form and directly available to the cells. The MTT assay was meant for measuring the activity of living cells via mitochondrial dehydrogenases. The toxicity shown by various iron compounds has been shown in Figure 9. The toxicity of ferric

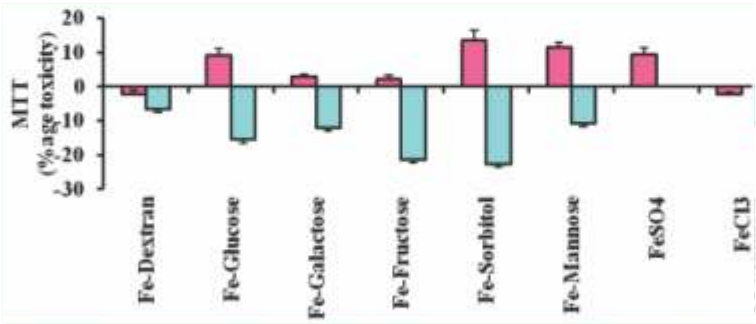


Figure 9: The effect of alginate encapsulated ferric microemulsions and ferric saccharate complexes on cell viability.

saccharate complexes was found to be very high as compared to AFGM. However among the commercially available products, iron dextran and $FeCl_3$ show minimal toxicity while $FeSO_4$ shown higher toxicity against all alginate encapsulated ferric saccharate microemulsions.

3. ***In-vivo* results**: The result of heme repletion study and RBC count is shown in the Figure 3. The hemoglobin of iron-deficient animals was found to be decreased by 26.4% as compared to control animals. AFGM treatment increased hemoglobin of anemic animals by 63.5% while FGC treatment increased hemoglobin of anemic animals only by 43.71%. The RBC count of Fe-deficient animals was found to be 14.07% less than that of control animals (Figure 10). However, after AFGM and FGC treatment, the RBC count of AFGM animals significantly increased by 82.57% while that of FGC treated animals was increased by 33.44% which indicates better bioavailability of encapsulated iron in the body.

4. **Histopathological studies:** In liver, control animals showed normal morphology with normal distribution of cells around central vein. Anemia resulted in moderate damage to the liver having inflamed cells with prominent nuclei. AFGM treated animals showed normal liver morphology while FGC treatment caused very high damage to the liver resulted in high infiltration of macrophages and the basic architect plan of the cell radiates out from central vein was completely destroyed (Figure 11).

5. **QRT PCR expression analysis:** Our gene expression data shows that AFGM carrying iron molecules delivered iron into the body that was reflected with improved gene expression profile of iron regulatory genes in iron storing cells without any adverse effects Figure 12.

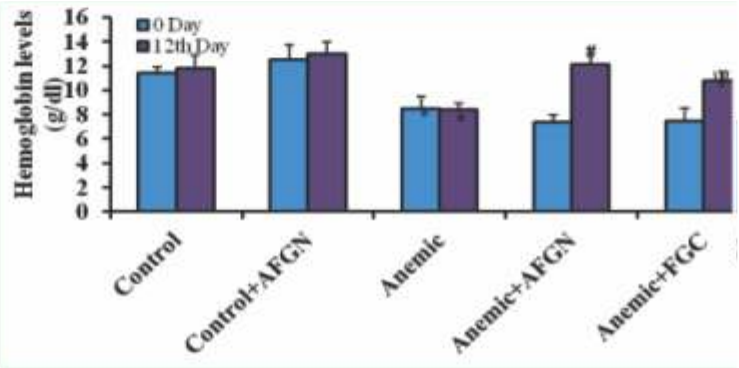


Figure 10: Effect of AFGN and FGC treatment on hemoglobin levels of anemic animals. Values are expressed as Mean±SD; n=5. *Anemic significantly different from control, $^{\#}$Anemic+AFGN significantly different from anemic and $^{\$}$Anemic +FGC significantly different from anemic $^{*/\#/\$}$ ($p<0.05$).

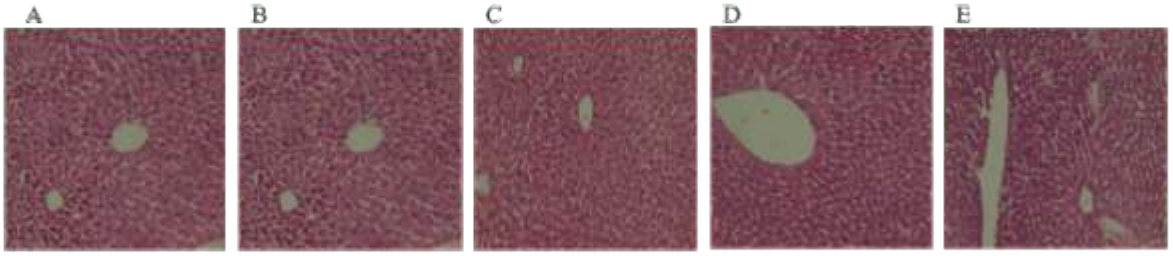


Figure 11: H&E staining of liver at 20X (A) Control; (B) Control+AFGN; (C) Anemic; (D) Anemic+AFGN & (E) Anemic+FGC.

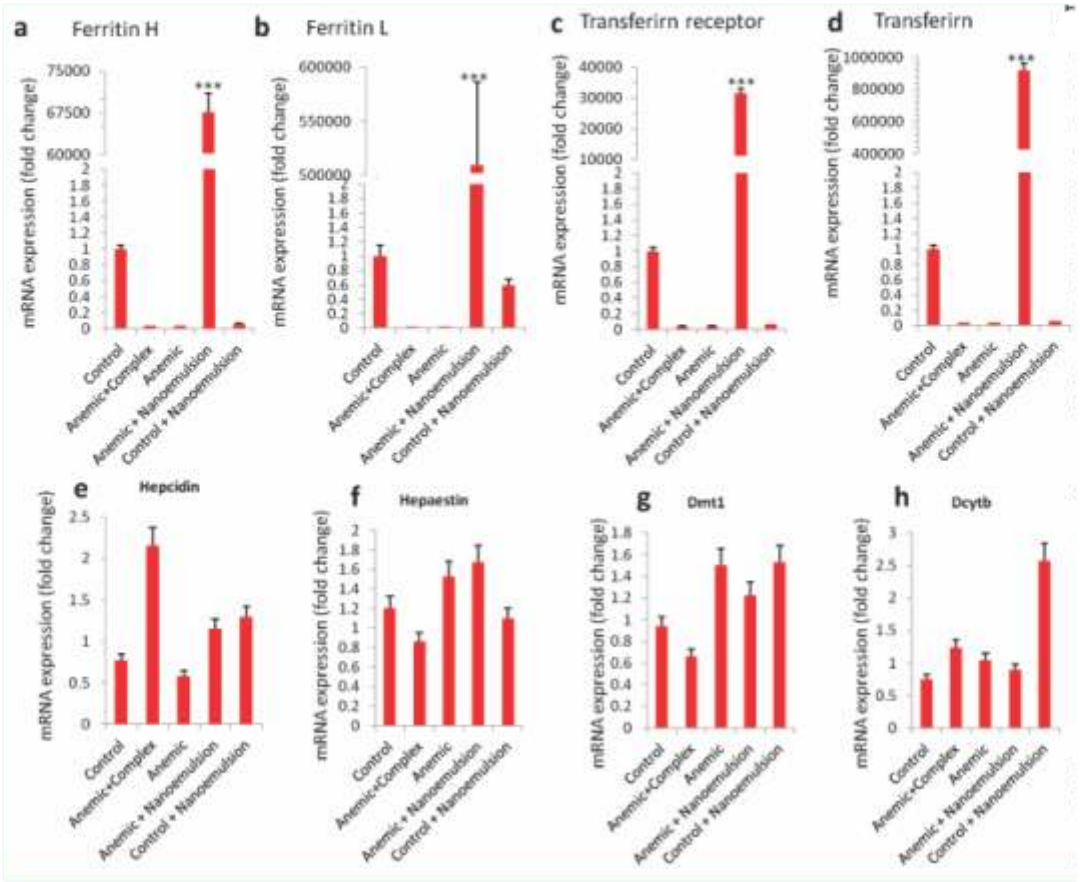


Figure 12: Effect of nano-emulsion treatment on gene expression of iron homeostasis genes in liver tissues of mice. Values are presented here mean of the values of triplicate analysis and error bars with SEM.

The results clearly indicate that microemulsions prepared were adequate in shape, size and stability with high encapsulation efficiency. These microemulsions were found to be better absorbed without any toxic effects in caco-2 cell lines. Thus, suggesting that microemulsions are more effective in increasing iron bioavailability as compared to iron in complex form. It is evident that iron is involved in haemoglobin formation which is responsible for the alterations in RBC count. The results of the present study demonstrate that iron supplementation as microemulsion was more bioavailable and effective in attenuating the alterations associated with iron deficient anemia as compared to iron saccharate complexes.

Salient Achievements

1. Our study suggested that iron supplementation as alginate miroemulsions were more bio-available and effective in attenuating the alterations associated with iron deficient anaemia.

Future Perspectives

1. Studies will be undertaken study the factors influencing iron homeostasis.
2. Responsible genes will identify for the targets of the nanoparticles used.

4.3 Investigate dietary constituents to regulate iron homeostasis and their use against iron deficiency

Principal Investigator

Hariom Yadav

Research Fellows

Stanzin Angmo

Shelley Sardul Singh

Introduction

India is one of the countries with very high prevalence of iron deficiency anemia in the world. Almost 58 per cent of pregnant women in

India are iron deficient and it is estimated that iron deficiency anemia 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 anemia in South Asia. Nutritional anemia is a major public health problem in India and is primarily due to iron deficiency. The National Family Health Survey-3 (NFHS-3) data suggests that anemia is widely prevalent among all age groups, and is particularly high among the most vulnerable – nearly 58 per cent among pregnant women, 50 per cent among non-pregnant non-lactating women, 56 per cent among adolescent girls (15–19 years), 30 per cent among adolescent boys and around 80 per cent among children under 3 years of age.

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 present in vegetarian diet (non-heme) iron is less bioavailable, therefore increasing bioavailable iron content in Indian foods will be one of my important future research aspects that my group will establish 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.

On the same time absorption of iron in human body is tightly regulated by various feedback mechanism(s) in human body. 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 (at the time of RBC formation). Hepcidin binds with Ferroportin (an iron transporter to release intracellular iron) that lead to degradation of Hepcidin-Ferroportin complex. Therefore blocking interaction of Hepcidin and Ferroportin become one of the important targets to develop strategies to combat iron deficiency. Here our group is developing strategies to block the hepcidin-ferroportin interaction using natural and/ or diet derived bioactive compounds to ameliorate iron deficient anemia.

Research Objectives

1. Analysis of hepcidin levels in different ethnic populations in India and its correlation with prevalence of iron deficiency.
2. Develop novel dietary compounds that can inhibit expression of hepcidin and improve iron absorption and release.
3. Screen and find-out novel natural compounds that can block the interaction of hepcidin and ferroportin and can exhibit beneficial effects against iron deficiency.

Research Progress

Interestingly, one of our meta-analysis study indicated that circulating hepcidin levels are dramatically increased in Indian population than other countries, suggest that along with consuming less bioavailable iron, there seems to be blockage of iron absorption in Indians through increased hepcidin levels. Therefore, my group has started the screening of new natural compounds present in food that can inhibit hepcidin expression and/or can block the action of hepcidin, and we found certain compounds that show potential action on hepcidin target (Figure 13). Interestingly, we also found that one of the selected compounds is highly beneficial to increase iron bioavailability

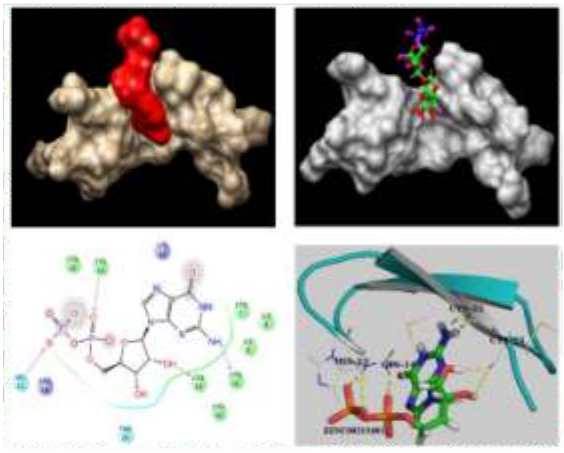


Figure13: Interaction of a selected compound with hepcidin.

in cell lines, mouse models as well as treating anemia in mice models. Hence specifically my group is targeting three strategies to increase iron absorption; 1) increase bioavailable iron content in Indian foods, 2) decrease the hepcidin expression and 3) inhibit hepcidin action using natural compounds.

My future goals in this direction is to establish the novel compounds that can inhibit hepcidin expression or block the action and incorporate these compounds in Indian foods to increase the iron absorption, that can ultimately help to combat with iron deficiency.

Salient Achievements

1. Around 70,000 natural compounds library have been screened using in-silico method for hepcidin binding potential and one top candidate have been selected for further in-vitro and in-vivo experiments.
2. Selected compound has been further investigated to ameliorate iron deficiency and established to inhibit the hepcidin action.

Future Perspectives

1. Compound(s) found from these investigations will be highly significant for potential to translate them against amelioration of iron deficiency.

4.4 Establishment of probiotic therapy against obesity and diabetes

Principal Investigator
Hariom Yadav

Project Assistant
Priyanka Chopra

Introduction

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 such 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.

Research Objectives

1. Isolation and characterization of new food/human origin probiotic strains with anti-obese/anti-diabetic potential.
2. Explore the target genes of probiotics action(s) and their correlation with gut-flora modulation.
3. Role of probiotics in prevention of childhood obesity and its mechanism of action.
4. Pre-clinical and clinical efficacy of VSL#3 in weight loss studies.

Research Progress

Overall, our previous studies show that probiotics (VSL#3) treatment plays a vital role in maintenance of glucose homeostasis by regulating the processes of insulin sensitivity and adiposity. Our long terms goals are to address following outstanding issues described as specific aims:

1. We will isolate new probiotic strains and establish them for therapeutic/preventive potential for obesity and insulin resistance in long term search for better probiotic strains. We will determine how the new selected probiotic strains can reduce the development of obesity/ diabetes and

can play an important role in maintenance of normal glucose homeostasis.

2. We expect that probiotics targets a set of target organs i.e. gut, brain and adipose tissues, and genes in these tissues whose functionality influences glucose homeostasis. At this point, though, the 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.

3. We will evaluate the pre-clinical and clinical efficacy of probiotics in weight loss studies, will give an unique opportunities to use probiotics in weight management regimens and help to fight against obesity and diabetes.

Probiotics are known for various health benefits, here we explored new role of probiotics in amelioration of obesity and diabetes. Our recent studies found that feeding of selected probiotics i.e. VSL#3 ameliorate obesity and diabetes via modulation of gut flora. Interestingly, we first time reported the mechanism of action of probiotics to ameliorate obesity. Here we found that administration of VSL#3 probiotics changes the metabolomic profile into the gut of obese mice through changing the composition of gut flora. Particularly, short chain fatty acids i.e. butyrate was significantly increased in VSL#3 fed mice compared to their control group. Interestingly, increased butyrate stimulated the glucagon-like protein-1 (GLP-1) secretion from intestinal L-cells and enhanced metabolic function to ameliorate obesity and diabetes. On the basis of these results, we isolated around 100 lactobacilli strains that can produce butyrate and can be used for designing functional foods against obesity and diabetes. Further, we established the probiotic attributes of these selected lactobacilli isolates and among them we selected two best probiotics and butyrate producer lactobacilli. After 16sRNA sequencing and characterization, we are using these lactobacilli strains to develop functional foods i.e. yogurt/ dahi. In future we will establish the anti-diabetic/obese effects of these strains as well as functional foods in mice models as well as in human studies. Developing these strains and functional foods will not only provide the alternative and complementary options for obese and diabetic people to better manage their health, but will also develop an unique model to generate revenue for the institute (NABI) and DBT.

Salient Achievements

1. In our investigations we first time established that probiotics modulated gut flora plays an important role for amelioration of obesity and diabetes.

2. In these studies we found that probiotic mediated gut flora modulation changes the gut hormone axis that regulates energy metabolism and contribute to reduce obesity and diabetes.

Future Perspectives

Developing new probiotic strains that can modulate gut-flora-metabolite-hormone axis to reduce obesity and diabetes will have high translatable potential in Indian market.

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

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

Principal Investigators
Shrikant Subhash Mantri
Joy K Roy

Project Scientist
Shailesh Sharma

Research Fellows
Anoop Kishor Singh
Sherry Bhalla

HPC application support engineer
Parichit Sharma, CDAC-Pune

Introduction

The function of newly sequenced gene can be discovered by determining sequence homology with known proteins. With the advent of Next Generation Sequencing (NGS) Technologies it has now become possible to study genes and their expression at genome wide scale. Functional annotation of all the genes is done by sequence similarity search against multiple protein databases. Such genome and transcriptome annotation task is computationally very intensive and will take time in days for getting complete results. Although many parallel mpi-enabled bioinformatics applications are available in the public domain, researchers are reluctant to use them due to lack of expertise in Linux command line and relevant programming experience. With these limitations, it becomes difficult for biologists to use supercomputers for accelerating annotation. There is extreme need to develop new tools to overcome these limitations and explore other new big data opportunities.

Objectives

1. To develop advanced algorithms, databases, tools and pipelines for accelerating data mining and discovery.
2. To utilize rich source of publically available wheat genomic data (genome, transcriptome and epigenome and smallRNA) for marker discovery and annotation of unannotated/hypothetical proteins.
3. To analyze in-house transcriptome data and its comparative analysis.
4. To provide Bioinformatics support to all major projects.

Research Progress

1. We have developed WImpiBLAST, a user friendly open source web interface for parallel blast searches. It is implemented in Struts-1.3 using Java backbone and runs atop open source Apache Tomcat Server. WImpiBLAST supports script creation and job submission features and also provides a robust job management interface for system administrators. It combines script creation, modification features with job monitoring and management through Torque resource manager on Linux based HPC cluster Figure1.

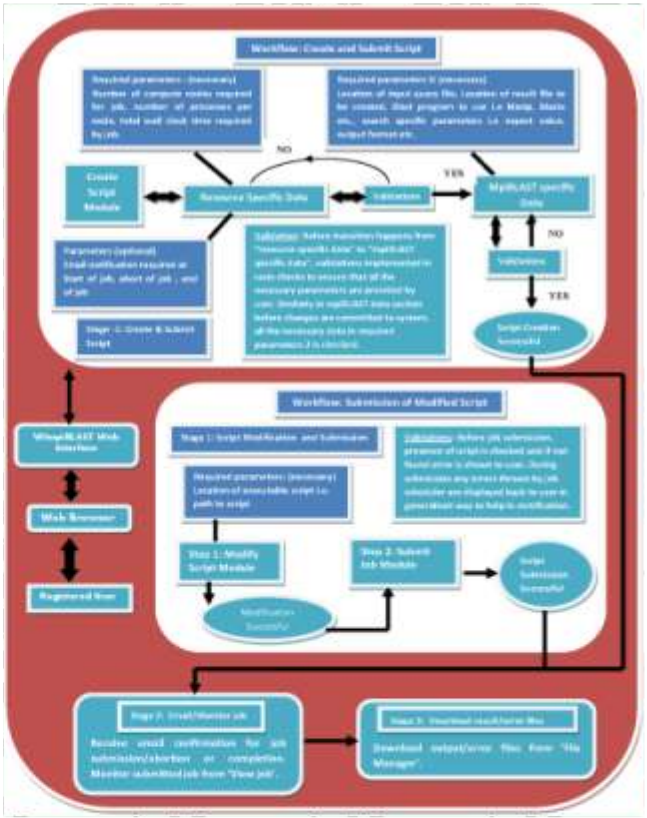


Figure 1: Procedural workflow in WImpiBLAST showing initiation of mpiBLAST job, input required at each stage and validations done between stages to prevent invalid data from entering into successive stages.

The WImpiBLAST web interface will help biologists in HPC based large scale annotation.

2. Comparative meta transcriptome analysis of published wheat transcriptomes. Database matrix of meta expression have been developed using published wheat transcriptomes. Use Case: Data Mining for Transcription Factors and Enzymes is completed and expression heatmap of clustered enzyme is show in Figure 2. This exploration of massive amount of RNA-seq data will help in identifying interesting gene regulation patterns and also help in formulating the hypothesis which can be followed up by detailed analysis either experimentally or computationally.

3. Small RNA regulation study: mature miRNA digital expression profiling in developing seed is being studied. The raw sequencing data of small RNA reads from Wheat, Rice and Maize were collected from SRA database available from NCBI website (http://www.ncbi.nlm.nih.gov/). Using commandline BLAST, PERL and shell script, transcripts per million values were computed for perfect match miRNA from databases. The expression visualization of tissue specific and species specific mature miRNA was done by developing heat maps (Figure 3) using R-language.

Salient Achievements

1. WImpiBLAST: A web interface developed for mpiBLAST to help biologist in high performance computing based large scale annotation.

2. Integrated global wheat transcriptome database developed using publicly available transcriptomes for meta-analysis of expression.

3. In-house transcriptome data analysis and database development. Sequenceservers for Wheat, Litchi and Annona Transcriptome have been developed and released for in-house data mining.

4. Small RNA regulation study: mature miRNA digital expression profiling in developing seed is studied.

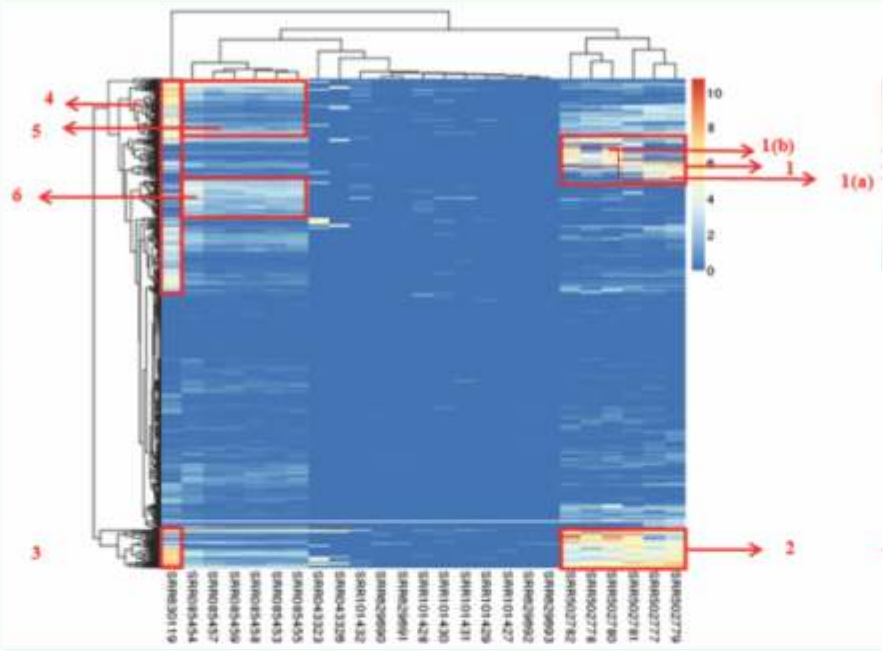


Figure 2: Expression heatmap regions1 and 2 show the enzymes expressed in seed (aleurone and endosperm). 1(a) and 1(b) differentiates between the set of enzymes highly expressed in aleurone (samples SRR502777, SRR502779, SRR502781) and endosperm(SRR502778, SRR502780, SRR502782). Regions 3 and 4 show the enzymes expressed in roots. Regions 5 and 6 shows enzymes specifically expressed in flag leaf.

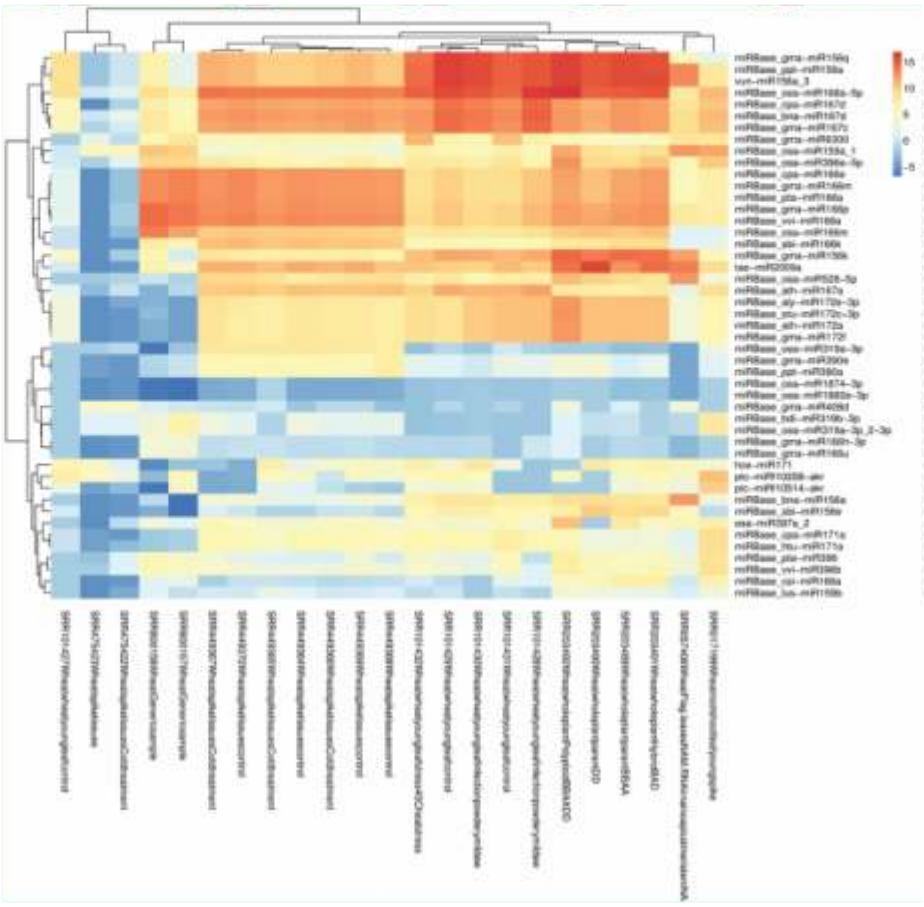


Figure 3: Highly expressing wheat miRNA having homology with published miRNA.

Future Perspectives

1. Porting more mpi enabled application on WImpiBLAST Framework. Analysis other than annotation viz. Assembly, polymorphism detection and digital expression analysis would be accelerated using HPC.
2. Development of comprehensive database of in-house transcriptome and other high throughput studies.

1.2 Development of pipeline for annotation of hypothetical proteins

Principal Investigator
Shrikant S. Mantri

Co-Investigator
Shailesh Sharma

Introduction

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

Objective

To develop a high-throughput assembly and annotation pipeline specially for *Triticum aestivum*.

Research Progress

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

DIIYOWAA is composed of steps that are executed in the specified order and each step is a Bioinformatic application which will analyze sequences and produce output. Complete algorithm is of DIIYOWAA is shown in Figure 4.

Salient Achievements

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

Future Perspectives

1. DIIYOWAA will be launched for the research community through NABI's site.
2. We will use DIYOWAA to study and annotate genomes of other food crops in high trough put way.

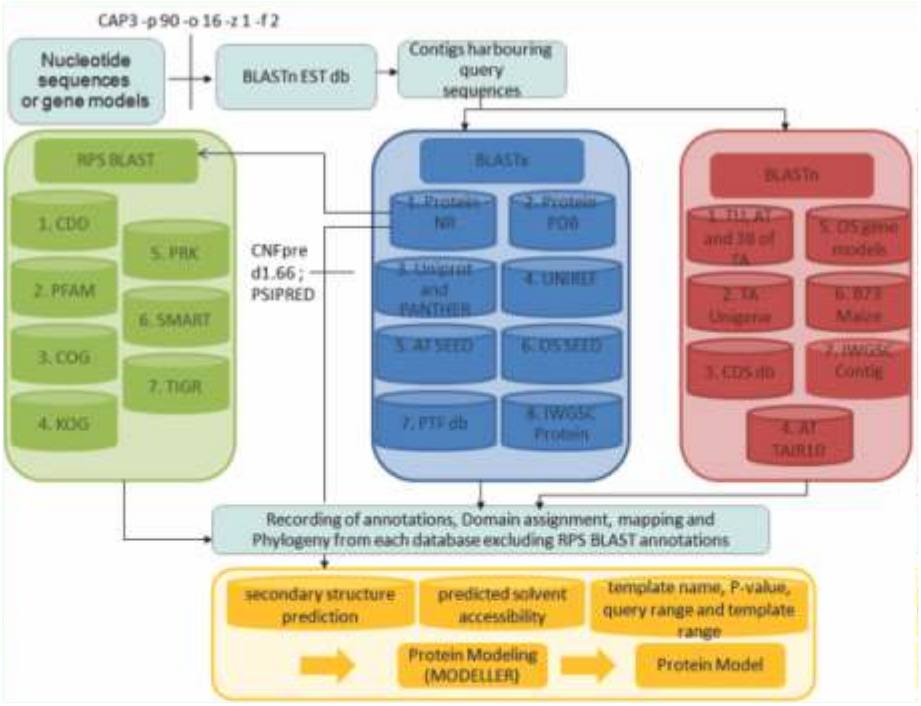


Figure 4: Pictorial representation of DIIYOWAA algorithm.

EMERGING AREAS

1. Identification of celiac disease epitopes in Indian wheat cultivars and their modulation by RNAi and breeding approaches

Investigator: Monika Garg

CD is a T-cell mediated autoimmune enteropathy caused by permanent intolerance to gluten fraction of wheat or the homologous proteins from barley or rye. Severe, immune-mediated damage to the jejuna mucosa (subtotal villous atrophy), induces symptoms of diarrhoea, vomiting, abdominal pain, abdominal distension, failure to thrive, weight loss, muscle wasting and mal-absorption. CD has prevalence between 0.3 and 2% in world populations as well as north Indian population. The only available treatment for this disease is the adherence to a strict life-long gluten free diet. Following a strict gluten-free diet is very demanding, because gluten is a ubiquitous additive in diverse foods. An approach that can help in minimizing such risks is by the development of wheat varieties with reduced gluten-toxicity profiles. Within the frame work of this project we will be working on

i. **Comprehensive mapping of CD epitopes (peptide variants) in wheat:** We will identify the non-immunogenic and immunogenic peptide variants on different genomes of wheat. Transcriptomics studies and database search and antibody based screening of wheat cultivars will tell us about new variants of immunogenic epitopes, their chromosome location and level of immunogenicity associated with them.

ii. **Elimination of the selected peptides by RNAi and accelerated breeding approaches:** Silencing of selected immunogenic peptides will be carried out using RNAi approach. Accelerated breeding will help in transfer of desired variants from old wheat cultivars and wild species in minimum possible time.

2. Transfer and characterization of anthocyanins from blue, purple and black grain coloured germplasm to high yielding Indian wheat cultivars

Investigator: Monika Garg

The existence of interesting genotypes of wheat with a red, purple, blue and white bran color has been reported in many scientific contributions. Red and purple colors are due to catechin-tennin and anthocyanins (mainly cyaniding-3-glycoside) respectively in the diploid pericarp layer. Blue color of the wheat grain is due to anthocyanins (mainly delphinidin-3-glycoside) in the triploid aleuron layer. Plant anthocyanins and phytochemicals can act as antioxidants and show anti-inflammatory and anti-cancer, antiaging activity and prevent cardiovascular diseases and type 2 diabetes. There is potential to use black, purple and blue colored wheats as novel ingredient resources for the development of value added products. Based on the potential of colored grains, several functional foods have been developed from these wheats, including purple wheat bran muffins and antho-beer made from purple grain wheat, soy sauce, vinegar, breakfast cereal and instant noodles produced from black grained wheat, and fine dried noodles made from blue grained wheat. Within the frame work of this project we will be working on

i. **Transfer of grain color from exotic germplasm to high yielding Indian wheat**

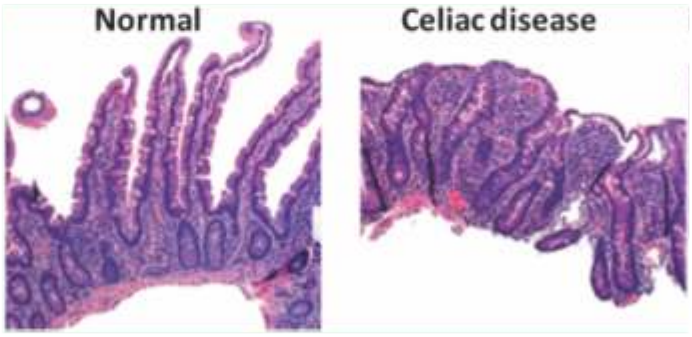


Figure 1: Biopsy of small bowel showing celiac disease manifested by blunting of villi.

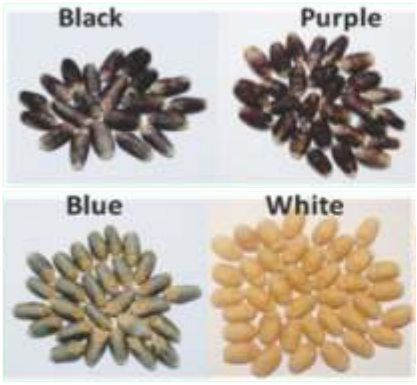


Figure 2: Different grain colored wheat seeds.

cultivars: We are utilizing different exotic germplasm to transfer blue, purple and black grain color to high yielding Indian wheat cultivars PBW550, PBW621 and HD2967.

ii. **Characterisation of different anthocyanins present in colored wheat:** We are isolating and characterising different anthocyanins from exotic germplasm and breeding lines by high resolution mass spectroscopy.

iii. Study of affect of colored wheats on human health and development of different products from colored wheat.

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

Investigator: Siddharth Tiwari

Introduction

In the proposal we intend to understand the function of gene/s in wheat which may enhance ascorbic acid biosynthesis and also decrease the concentration of phytic acid. *Myo*-inositol is known as precursor for variety of low molecular weight compounds including cell wall, phytic acid and ascorbic acid biosynthesis. The whole process for biosynthesis of cell wall components, phytic acid and ascorbic acid is flux dependent. We hypothesize, that if *myo*-inositol resources channelize towards ascorbic acid biosynthesis pathway might be reduced total phytic acid and enhance ascorbic acid biosynthesis in wheat (Figure 3). The targeted gene *myo*-inositol oxygenase (*MIOX*) is a key enzyme in L-ascorbic acid biosynthesis, associated with MIOX pathway. Upon completion of the project we anticipate transgenic wheat lines with low phytic acid and high ascorbic acid contents that might have an increase iron absorption and bioavailability. Within the frame work of this project we will be working on:

i. Identification and functional characterization of wheat *MIOX*.

ii. Over-expression studies of *TaMIOX* in wheat for trait development.

4. Exploratory work with applied objectives: Metabolic engineering for enhanced biosynthesis of provitamin-A in Indian banana fruit

Investigator: Siddharth Tiwari

Introduction

DBT-BIRAC has funded a multi-institutional project entitled "Development and Transfer of Technology from Queensland University of Technology (QUT), Australia to India for

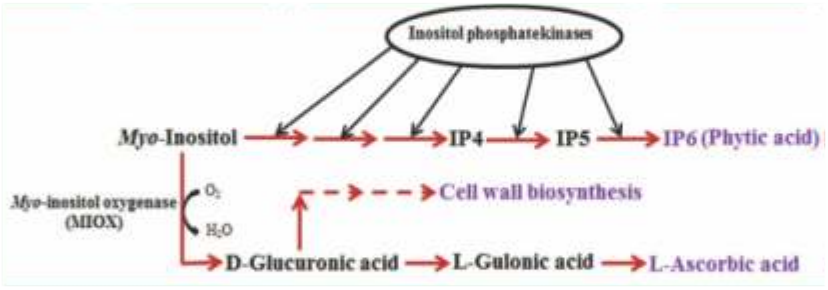


Figure 3: Proposed hypothesis where *MIOX* gene will be overexpressed and it could lead to biosynthesis of ascorbic acid. Since we anticipate more flux of *myo*-inositol towards glucoronic acid synthesis, this might leads to lowering phytic acid concentration.

Biofortification and Disease Resistance in Banana". Under this project, NABI is undertaking research on genetic transformation of two commercial cultivars of Indian banana with provitamin A gene constructs, provided by the QUT, Australia. However, the scope of research work is limited to the gene constructs provided by the QUT and transformation of only two dessert cultivars while no leads are currently available on the prospective results in terms of enhanced expression level of PVA and ultimately the bioavailability in Indian population. Therefore, there is a need to initiate exploratory research to further discover genes and promoters to enhance the carotenoid biosynthesis in the fruit pulp. In this proposal we will explore the possibility of Indian germplasm screening for the identification, isolation and characterisation of genes and promoters involved in high carotenoid biosynthesis in banana. The efficacy of biofortified banana supplementation in reducing vitamin A deficiency in cell line/animal model will be established. The successful achievement of milestones of this project will be translated into a product in the form of provitamin A rich biofortified Indian banana. Within the frame work of this project we will be working on:

1. Exploration of carotenoid-rich banana cultivar/species considering the carotenoid profile in Indian germplasm.
2. Identification, isolation and characterization of carotenoid biosynthesis pathway genes (viz., phytoene synthase, lycopene β-cyclase, DOXP synthase etc.) and fruit specific promoters from high PVA containing Indian variety.
3. Development of transgenic lines using construct/s for pro-vitamin A biofortification. Expression analysis in plant and ratoon crops of selected lines
4. The selected biofortified lines will be evaluated for agronomical practice by conducting multi-location field trials.
5. The promotion of GM banana for adaptation by farmers will be taken up in the final stage.

5. Application of dietary fibers as edible fruit coating materials

Investigator: Koushik Mazumder

Introduction

Water soluble polysaccharides provide thickening effect can be used as alternative to the synthetic coating materials to extend the shelf life of fruits and menataining the sensory quality and safety of several food products. Currenly, only few carbohydrate based coating materilas are availbale mainly from cellulose and chitosan, but due to their poor misture barrier property these coating materilas are not much effective. Therefore in the present study, a novel staegy to the structuraly modify carbohydrates (polysacharuides) by deravatization to improve their physical, physicochemical properties such as vicosity, misture barrier property will be adopted, so it can be used as effective coating materials for fresh fruits. Within the frame work of this project we will be working on:

1. Chemical modification of polysaccharides (xylans, galactomannans) to their corresponding derivatives such as carboxymethylated, acetylated and fatty acid esterified derivatives using various chemical reactions.
2. Determination of physical properties of the modified carbohydrates such as viseosity, moisture barrier property and film forming ability using various physico-chemical methods.
3. Application of the derivatized carbohydrates on fresh fruits to extend their shelf life.

COLLABORATIONS & NETWORKING

1. NABI and Central University of Punjab, Bathinda signed a MOU on March 28th, 2013 for the promotion of quality research and high end research programmes between two institutes.

2. A MOU was signed with National Institute of Pharmaceutical Education and Research (Mohali), Indian Institute of Scientific Education and Research (Mohali), Post Graduate Institute of Medical and Education Research (Chandigarh), Panjab University (Chandigarh), Central Scientific Instruments Organization (Chandigarh), Indian Institute of Technology (Ropar) and Punjab Agriculture University (Ludhiana) on November 26th, 2012 to establish a Bioscience Cluster at Mohali.

3. NABI and Punjab Technical University, Jalandhar signed a MOU on October 19th, 2012 to promote academic and research interactions in the areas of science & technology to intensify the high priority programmes.

4. NABI and National Research Centre for Litchi (NRCL), Muzaffarpur, Bihar signed a MOU on September 16th, 2012 to share R&D facilities and carry out joint research projects.

5. NABI and Punjab Agricultural University, Ludhiana signed a MOU on August 14th, 2012 to jointly carry out research in the areas of agriculture and allied sciences.

6. NABI and NIPER signed a MOU on February 2nd, 2012 to undertake joint research work in the area of mutual interest besides imparting training to staff, students and technical personnel within the area of cooperation.

7. The following MOUs were signed with two Universities in neighbourhood to catalyse networking, R&D collaborations, human resource development and award of degree to students who pursue Ph.D research at NABI.

   (I) MOU with Punjab University, Chandigarh on May 27th, 2011.

   (ii) MOU with Guru Jambeshwar University of Science & Technology, Hissar on March 29th, 2011.

8. The following three MOUs were signed with Canadian institutes, for co-operation in S&T on November 24th, 2010.

   (I) MOU with National Research Council, Plant Biotechnology Institute, Saskatoon.

   (ii) MOU with University of Saskatchewan, Saskatoon.

   (iii) MOU with Genome Prairie, Saskatoon.

EXTRAMURAL GRANTS AND FUNDINGS

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

PROGRESS OF INFRASTRUCTURE AT MAIN CAMPUS

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

Model of NABI-CIAB upcoming campus in Sector -81, Mohali.

Participation in National/International Conference/Workshops:

1. Dr. Santosh K. Upadhyay was nominated to present his research work at Indian National Science Academy, New Delhi, on April 25th, 2013 for INSA Young Scientist Award 2013.

2. Dr. Sukhvinder P. Singh, was invited to deliver a talk on "Curtailing postharvest losses of fresh horticultural produce for feeding the processing industry" at National Conference on Linking Growth Drivers of Food Processing Industries; Market, Retails, Quality with Food Safety and Skills organized by the ASSOCHAM India on June 24th 2013, Chandigarh.

3. Dr. Monika Garg was invited to deliver a talk on "Experiences in using green houses" at National Workshop on "Climate Controlled Greenhouse for Agricultural Research" organized by CDAC Mohali (Punjab) on July 11th, 2013.

4. Dr. Siddharth Tiwari, was invited for a National Workshop on "Climate Controlled Greenhouse for Agricultural Research" organized by CDAC Mohali (Punjab) on July 11th, 2013. The title of the talk was "Management of Transgenic and Tissue Culture Raised Plants in Green House".

5. Dr. Sukhvinder P. Singh, was invited to deliver a talk on "Stakeholder-Driven Postharvest Research and Outreach in India" at National Conference on Postharvest Management of Horticultural Produce in India organized by Amity University, Noida, UP on August 30th 2013.

6. Dr. Sukhvinder P. Singh, was invited to deliver a talk on "Phytosanitary Requirements for Fresh Mango Fruit: Opportunities and Challenges for India in High Value Markets" at the VI International Conference on managing quality in chains (MQUIC-2013) organized by the Cranfield University and International Society for Horticultural Science (ISHS) at Bedford, United Kingdom (UK) during September 2nd – 5th, 2013.

7. Dr. Siddharth Tiwari, organized and attendant International Workshop on Banana transformation and tracker software under the BIRAC-QUT Banana biofortification project from 15th - 20th September, 2013 at NABI.

8. Dr. Sukhvinder P. Singh attended a "Master Class on Bio-Entrepreneurship-Accelerating Innovations to Marketplace" at National Institute of Immunology (NII), Delhi during September 20th -23rd, 2013.

9. Dr. Joy K Roy and Dr. Monika Garg, attended the 11th International Symposium on Rice Functional Genomics (ISRFG) held during November 20th -23rd, 2013at New Delhi.

10. Dr. Sukhvinder P. Singh, delivered a talk on "Metabolomics Applications in Quality and Safety of Fresh Fruits" at workshop on Metabolomics using high resolution mass spectrometer organized by the ABSciex at Centre of Excellence, ABSciex India, Gurgaon, India during November 25th -26th, 2013.

11. Sh. Shrikant Mantri, attended the first annual ICT meet for autonomous institute of DBT held on November 29th -30th, 2013, at NIBMG, Kalyani, West Bengal.

12. Dr. Mahendra Bishnoi, attended and made an oral presentation at XX World Congress on Parkinson's Disease and Related Disorders held at Geneva, Switzerland during December 8th -13th, 2013.

13. Dr. Shailesh Sharma, was invited to present his topic on "Emerging Trends in Agri Bioinformatics (ETAB)", organized by the Directorate of Wheat Research, Karnal during December 16th -17th, 2013.

14. Dr. Santosh K. Upadhyay, visited Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow during December 26th -28th, 2013, to participate in INSA anniversary meeting and receive INSA Young Scientist Award 2013.

15. Dr. Mahendra Bishnoi attended and chaired a session at 11th Annual Conference of International Society of Heart Research (Indian Section) held at National Institute of Pharmaceutical Education and Research (NIPER), Mohali during February 8th-9th, 2014.

16. Dr. Joy K Roy, participated and delivered a lecture in a national conference on "The Science of Omics for Agricultural Productivity: Future Perspectives" held at G. B. Pant University of Agriculture & Technology, Pantnagar, during March 4th -6th, 2014.

17. Dr. Sudhir P. Singh, was invited to deliver a talk in a National Conference on "The Science of Omics for Agricultural Productivity: Future Perspectives", organized at GB Pant University of Agriculture & Technology, Pantnagar, during March 4th -6th, 2014. The title of the talk was "Iron accumulation in wheat grains and molecular studies to enhance its bio-availability.

18. Dr. Sukhvinder P. Singh, Dr. Kanthi Kiran K. and Dr. Siddharth Tiwari, participated in a "BIRAC Roadshow & Workshop for Promotion of Bio-Science Industry in Punjab" organized by Punjab State Council for Science & Technology (PSCST) in collaboration with BIRAC and Indian School of Business (ISB) from March 5th - 6th, 2014 at ISB, Knowledge City, Sector 81, Mohali.

19. Dr. Sukhvinder P. Singh, was invited to deliver a talk on "Future Trends in Postharvest Science: 'Omics' Applications" during invited lectures series on 'Recent Developments and Advancements in Fruit Science, organized by the Department of Fruit Science, Punjab Agricultural University, Ludhiana on March 28th, 2014.

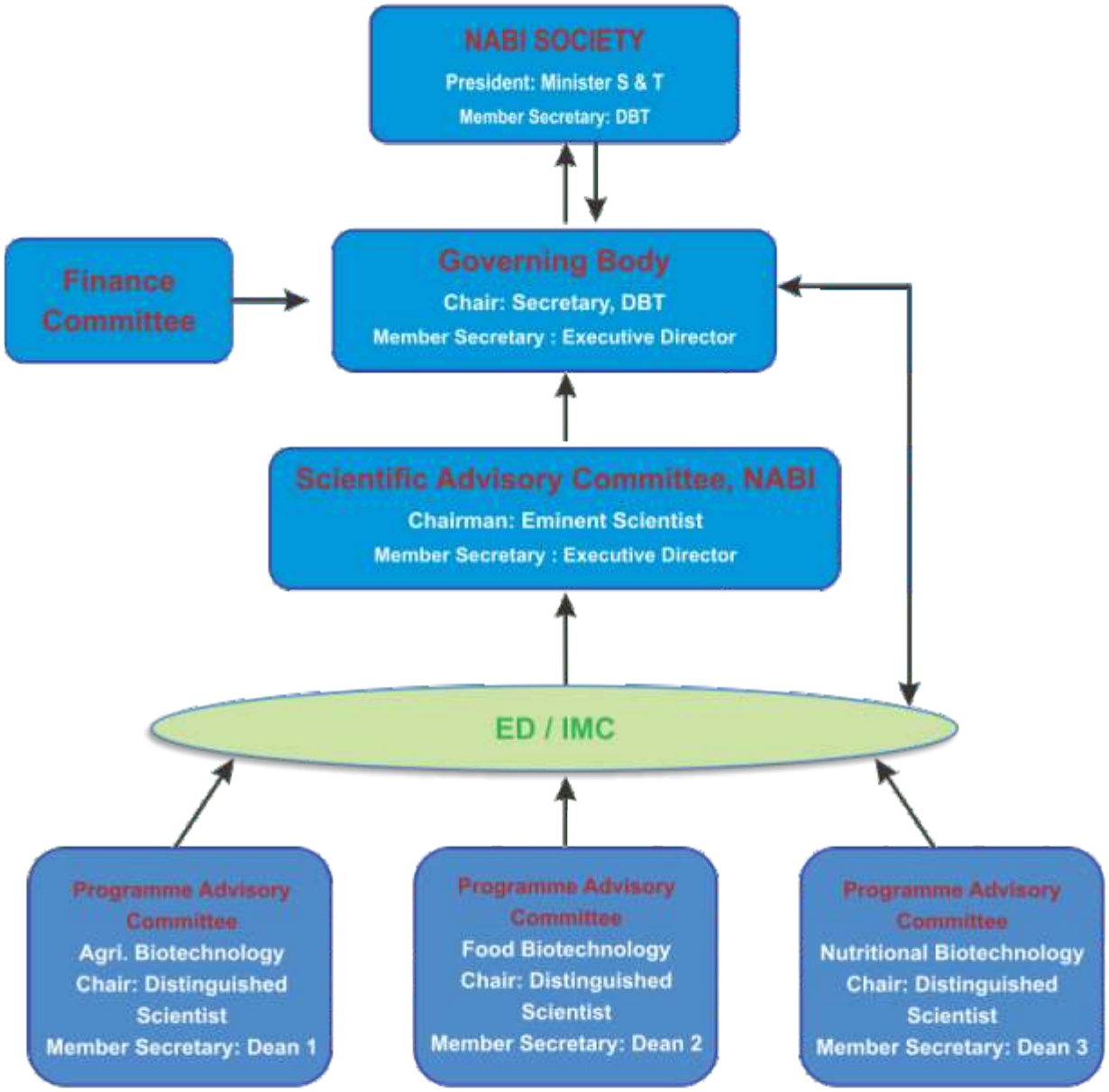
International visitors to NABI

1. An European delegation comprising of representatives from three major Agri-Food biotech clusters of Europe i.e. Parco Technologico Padano (Lombardy, Italy), Agropolis International (Languedoc – Roussillon, France) and Oost NV (Food Valley Wageninghen, Netherlands) visited NABI on May 27th, 2013 to explore the possibilities of future collaboration.

2. A delegation from the USA comprising of representatives of Postharvest Education Foundation (Drs. Lisa Kitinoja, Patrick Brown, and Lizanne Wheeler) and Agribusiness Associates, Inc. (Gurbinder Gill) visited NABI on August 0th, 2013 to explore the opportunities of future collaborations.

3. An international workshop on banana transformation & tracker software was organised, during September 15th -20th, 2013 under the BIRAC, India - QUT, Australia Banana Biofortification Project. The meeting and workshop was attended by the participants of BIRAC, New Delhi; QUT, Australia; NABI, Mohali; BARC, Mumbai; NRCB, Trichy; IIHR, Bangalore and TNAU, Coimbatore.

GOVERNANCE


NABI SOCIETY
President: Minister S & T
Member Secretary: DBT
Finance Committee
Governing Body
Chair: Secretary, DBT
Member Secretary : Executive Director
Scientific Advisory Committee, NABI
Chairman: Eminent Scientist
Member Secretary : Executive Director
ED / IMC
Programme Advisory Committee
Agri. Biotechnology
Chair: Distinguished Scientist
Member Secretary: Dean 1
Programme Advisory Committee
Food Biotechnology
Chair: Distinguished Scientist
Member Secretary: Dean 2
Programme Advisory Committee
Nutritional Biotechnology
Chair: Distinguished Scientist
Member Secretary: Dean 3

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)

Dr. K. VijayRaghavan
Secretary,
Department of Biotechnology,
Ministry of Science & Technology,
New Delhi
(Chairman)

Ms Anuradha Mitra
Financial Advisor,
Council of Scientific and Industrial Research,
New Delhi

Dr. N. Sathyamurthy
Director,
Indian Institute of Science & Education Research,
Mohali

Dr. V. Prakash
Former Director, CFTRI
Distinguished Scientist,
Council of Scientific and Industrial Research,
Mysore

Dr. B. Sesikeran
Former Director,
National Institute of Nutrition,
Hyderabad

Dr. S. Nagarajan
Former Chairperson,
Protection of Plant Varieties and Farmers' Rights Authority,
New Delhi

Dr. Rajesh Kapur
Advisor,
Department of Biotechnology,
New Delhi

Dr. Rakesh Tuli
Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(Member Secretary)
(8th Feb'10 to 30th Sept' 13)

Prof. Akhilesh Kumar Tyagi
Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(Member Secretary)
(1st October'13 to till date)

B. Governing Body

Dr. K. VijayRaghavan
Secretary,
Department of Biotechnology,
Ministry of Science & Technology,
New Delhi
(Chairman)

Ms Anuradha Mitra
Financial Advisor,
Council of Scientific and Industrial Research,
New Delhi

Dr. Manju Sharma
(Former Secretary, DBT)
President & Executive Director,
Indian Institute of Advanced Research,
Gujarat

Dr. C.R. Bhatia
Former Secretary,
Department of Biotechnology,
New Delhi

Dr. Ashok D.B. Vaidya
Research Director,
Kasturba Health Society Medical & Research Centre,
Mumbai

Dr. B. Sesikeran
Former Director,
National Institute of Nutrition,
Hyderabad

Dr. N. Sathyamurthy
Director,
Indian Institute of Science & Education Research,
Mohali

Dr. B. Siva Kumar
Former Director
National Institute of Nutrition,
Secunderabad

Dr. S. Nagarajan
Former Chairperson,
Protection of Plant Varieties and Farmers' Rights Authority,
New Delhi

Dr. R.S. Paroda
(Former Director General – ICAR)
Trust for Advancement of Agricultural Sciences,
New Delhi

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

Dr. J.S. Pai
(Former Director- UICT)
Executive Director,
Protein Foods & Nutrition Development Association of India ,
Mumbai

Dr. V. Prakash
Former Director, CFTRI
Distinguished Scientist,
Council of Scientific and Industrial Research,
Mysore

Dr. Rajesh Kapur
Advisor,
Department of Biotechnology,
Ministry of Science & Technology,
New Delhi

Dr. Joy K. Roy
Scientist- D,
National Agri-Food Biotechnology Institute,
Mohali

Dr. Sukhvinder P. Singh
Scientist- C,
National Agri-Food Biotechnology Institute,
Mohali

Dr. Vikas Rishi
Scientist- E,
National Agri-Food Biotechnology Institute,
Mohali

Dr. Rakesh Tuli
Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(Member Secretary)
(8th Feb'10 to 30th Sept' 13)

Prof. Akhilesh Kumar Tyagi
Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(Member Secretary)
(1st October'13 to till date)

C. Finance Committee

Dr. K. VijayRaghavan
Secretary,
Department of Biotechnology,
Ministry of Science & Technology,
New Delhi
(Chairman)

Ms Anuradha Mitra
Financial Advisor,
Council of Scientific and Industrial Research,
New Delhi

Dr. Rakesh Tuli
Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(8th Feb'10 to 30th Sept' 13)

Prof. Akhilesh Kumar Tyagi
Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(1st October'13 to till date)

Dr. Rajesh Kapur
Advisor,
Department of Biotechnology,
Ministry of Science & Technology,
New Delhi

Dr. R.S Sangwan
Chief Executive Officer
Centre for Innovative and Applied
Bioprocessing
Mohali

Dr. Vikas Rishi
Scientist- E,
National Agri-Food Biotechnology Institute,
Mohali

Dr. Joy K. Roy
Scientist- D,
National Agri-Food Biotechnology Institute,
Mohali1

Sh. Shrikant Subhash Mantri
Scientist- C,
National Agri-Food Biotechnology Institute,
Mohali

Sh. Suneet Verma
Finance Officer,
National Agri-Food Biotechnology Institute,
Mohali
(Non-Member Secretary)

D. Scientific Advisory Committee (SAC)

Dr. R.S. Paroda
(Former Director General – ICAR)
Trust for Advancement of Agricultural Sciences,
New Delhi
(Chairman)

D. C.R. Bhatia
Former secretary,
Department of Biotechnology,
New Delhi

Dr. Deepak Pental
Former Vice Chancellor,
University of Delhi,
New Delhi

Dr. B. Siva Kumar
Former Director,
National Institute of Nutrition,
Secunderabad

Dr. V. Prakash
Former Director, CFTRI
Distinguished Scientist,
Council of Scientific and Industrial Research,
Mysore

Dr. Imran Siddiqi
Scientist,
Centre for Cellular & Molecular Biology,
Hyderabad

Dr. Akshay Kumar Pradhan
Professor,
Department of Genetics,
University of Delhi,
New Delhi

Dr. Anura V. Kurpad
Dean,
St. John's Medical College,
Bangaluru

Dr. H.P.S. Sachdev
Senior Consultant (Paediatrics),
Sitaram Bhartia Institute of Science & Research,
New Delhi

Dr. G. Venkateshwara Rao
Former Director,
Central Food Technological Research Institute,
Mysore

Dr. Arun Sharma
Outstanding Scientist (Food Technology),
Bhabha Atomic Research Centre,
Mumbai

Dr. Rajesh Kapur
Advisor,
Department of Biotechnology,
Ministry of Science & Technology,
New Delhi

Dr. Rakesh Tuli
Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(8th Feb'10 to 30th Sept' 13)

Prof. Akhilesh Kumar Tyagi
Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(1st October'13 to till date)

E. Programe Advisory Committee (PAC): Agri-Biotechnology

Dr. C.R. Bhatia
Former secretary,
Department of Biotechnology,
New Delhi
(Chairman)

Dr. Kailash Chander Bansal
Director,
National Bureau of Plant Genetics Resources,
New Delhi

Dr. G.K. Garg
Director, ITR
Krishidhan Research Foundation Pvt. Ltd,
Aurangabad Road,
Jalna

Dr. Sunil K. Mukherjee
Scientist,
International Centre for Genetic Engineering & Biotechnology,
New Delhi

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

Dr. T. Mohapatra
Director,
Central Rice Research Institute,
Cuttack

Dr. Ramesh Sonti
Deputy Director,
Centre for Cellular & Molecular Biology,
Hyderabad

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

Dr. Rajesh Kapur
Advisor,
Department of Biotechnology,
Ministry of Science & Technology,
New Delhi

Dr. Rakesh Tuli
Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(8th Feb'10 to 30th Sept' 13)

Prof. Akhilesh Kumar Tyagi
Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(1st October'13 to till date)

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

Dr. V. Prakash
Former Director, CFTRI
Distinguished Scientist,
Centre Food Technological Research Institute,
Mysore
(Chairman – Food Biotechnology)

Dr. B. Siva Kumar
Former Director,
National Institute of Nutrition,
Secunderabad
(Chariman – Nutrition Biotechnology)

Dr. Appu Rao
Scientist,
Central Food Technology Research Institute,
Mysore

Dr. V.K. Batish
Emeritus Scientist,
Molecular Biology Unit,
National Dairy Research Institute,
Karnal

Dr. K. Madhavan Nair
Deputy Director,
National Institute of Nutrition,
Hyderabad

Dr. S.K. Roy
Emeritus Professor & Consultant FAO
Indian Agricultural Research Institute,
New Delhi

Dr. H.P.S. Sachdev
Senior Consultant (Paediatrics),
Sitaram Bhartia Institute of Science & Research,
New Delhi

Dr. H.N. Mishra
Professor,
Agriculture & Food Engineering Department,
Indian Institute of Technology,
Kharagpur

Dr. Bhupendar Khatkar
Chairman,
Deptartment of Food Technology,
Guru Janbheshwar University of Science & Technology,
Hisar

Dr. M.C. Varadraj
Chief Scientist,
Central Food Technological Research Institute,
Mysore

Dr. Rakesh Tuli
Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(8th Feb'10 to 30th Sept' 13)

Prof. Akhilesh Kumar Tyagi
Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(1st October'13 to till date)

G. Building Committee

Dr. V.S. Chauhan
Director,
International Centre for Genetic Engineering and Biotechnology,
New Delhi
(Chairman)

Dr. Rakesh Tuli
Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(8th Feb'10 to 30th Sept' 13)

Prof. Akhilesh Kumar Tyagi
Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(1st October'13 to till date)

Dr. R.S. Sangwan
CEO,
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

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

Dr. K.K. Kaul
Former Chief Town Planner,
Greater Mohali Area Development Authority,
Chandigarh

Dr. A. Vamsi Krishna
Scientist - C,
Department of Biotechnology,
New Delhi

Sh. Virendra K. Banerjee
Administrative Officer,
National Agri-Food Biotechnology Institute,
Mohali

RESEARCH PUBLICATIONS

2014

1. Ambalam P, Kondepudi KK, Nilsson I, Wadström T and Ljungh A (2014). Bile enhances cell surface hydrophobicity and biofilm formation of bifidobacteria. Appl Biochem Biotechnol. 172(4): 1970-81.

2. Baboota RK, Murtaza N, Jagtap S, Singh DP, Kaur J, Boparai RK, Premkumar LS, Kondepudi KK and Bishnoi M (2014). Capsaicin-induced transcriptional changes in hypothalamus and alterations in gut microbial count in high fat diet fed mice. Journal of Nutritional Biochemistry *(in press)*.

3. Bishnoi M, Khare P, Kondepudi KK and Premkumar LS (2014). Role of TRPV1 in acquired diseases: therapeutic potential of TRPV1 modulators. Invited Book Chapter *(in press)*.

4. Garg M, Yanaka M, Tanaka H and Tsujimoto H (2014). Introgression of useful genes from *Thinopyrum intermedium* to wheat for improvement of bread-making quality. Breeding Science. 133:327-324.

5. Kondepudi KK, Bishnoi M, Podili K, Ambalam P, Mazumder K, Murtaza N, Baboota RK and Boparai RK (2014). Dietary polysaccharides for the modulation of obesity via beneficial gut microbial manipulation. CRC Publications. *(in Press)*.

6. Kumar J, Gunapati S, Kumar J, Kumari A, Kumar A, Tuli R and Singh SP (2014). Virus induced gene silencing using a modified betasatellite: a potential candidate for functional genomics of crops. Archives of Virology. DOI 10.1007/s00705-014-2039-x.

7. Kumar J, Kumar J, Singh SP and Tuli R (2014). Association of satellites with a mastrevirus in natural infection: complexity of Wheat dwarf India virus disease. Journal of Virology. 88(12):7093-7104.

8. Sharma S and Upadhyay SK (2014). Functional characterization of expressed sequence tags of bread wheat (*Triticum aestivum*) and analysis of CRISPR binding sites for targeted genome editing. American Journal of Bioinformatics Research (*in Press*).

9. Singh SP and Saini MK (2014). Postharvest vapour heat treatment as a phytosanitary measure influences the aroma volatiles profile of mango fruit. Food Chemistry *(in press)*.

10. Singh SP, Saini MK, Singh J, Pongener A and Sidhu GS (2014). Preharvest application of abscisic acid promotes anthocyanins accumulation in pericarp of litchi fruit without adversely affecting postharvest quality. Postharvest Biology and Technology *(in press)*.

11. Singh A, Mantri S, Sharma M, Chaudhury A, Tuli R and Roy J (2014). Genome-wide transcriptome study in wheat identified candidate genes related to processing quality, majority of them showing interaction (quality x development) and having temporal and spatial distributions. BMC Genomics. 15:29.

12. Thakur N, Upadhyay SK, Chandrashekar K, Verma PC, Singh PK and Tuli R (2014). Enhanced whitefly resistance in transgenic tobacco plants expressing dsRNA of *v-ATPaseA* gene. PLoS ONE 9(3): e87235.

2013

1. Dixit S, Upadhyay SK, Singh H, Verma PC and Chandrashekar K (2013). Enhanced methanol production in plants provides broad spectrum insect resistance. PLoS ONE, 8(11): e79664.

2. Kumar J, Singh SP, Kumar A, Khan JA and Tuli R (2013). Recombination study using Radish leaf curl virus isolates. African Journal of Microbiology Research. 7: 3542-3546.

3. Khan JA, Kumar J, Thakur PD, Handa A and Jarial K (2013). First report of a '*Candidatus* Phytoplasma ziziphi'-related strain associated with peach decline disease in India. Journal of Plant Pathology. 95(S4): 76.

4. Kumar J, Gunapati S, Singh SP, Gadre R, Sharma NC and Tuli R (2013). Molecular characterization and pathogenicity of a carrot (*Daucus carota*) infecting begomovirus and associated betasatellite from India. Virus Research. 178: 478-485.

5. Srivastava R, Rai KM, Srivastava M, Kumar V, Pandey B, Singh SP, Bag SK, Singh BD, Tuli R and Sawant SV (2013). Distinct role of core promoter architecture in regulation of light mediated responses in plant genes. Molecular Plant DOI: 10.1093/mp/sst146.

6. Singh SP and Terry LA (2013). Recent advances in storage technologies for fresh fruits. Advances in Food Science and Nutrition. Vol. 2, Wiley, MA, USA 391-412.

7. Upadhyay SK, Dixit S, Sharma S, Singh H, Kumar J, Verma PC and Chandrashekar K (2013). siRNA machinery in whitefly (*Bemisia tabaci*). PLoS ONE 8 (12): e83692.

8. Upadhyay SK, Kumar J, Alok A and Tuli R (2013). RNA guided genome editing for multiple target gene mutations in wheat. Genes Genomes Genetics. 3: 2233-2238.

HUMAN RESOURCE

I. Research Faculty

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

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 Technical Assistant	22-02-2011
3	Sh. Jagdeep Singh	Senior Technical Assistant	01-03-2011
4	Sh. Sukhjinder Singh	Senior Technical Assistant (Computers)	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 Technical Assistant	24-12-2012
8	Sh. Atul Kesarwani	Senior Technical Assistant	21-01-2013
9	Sh. Kamalendra	Senior Technical Assistant	18-03-2013
10	Sh. Pankaj Pandey	Senior Technical Assistant	29-04-2013

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. Virendra K. Banerjee	Administrative Officer	21-02-2013
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
Students enrolled for Ph.D degrees:			
1	Sh. Jitendra Kumar	Development of virus induced gene silencing vector and its application in studying gene function in wheat (*Triticum aestivum l.*)	Barkatullah University, Bhopal, MP (Thesis submitted)
2	Sh. Yogesh Gupta	Gene discovery for seedlessness in *Annona* 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
5	Sh. Anshu Alok	Cloning and functional characterization of myo-inositol oxygenase (MIOX) from wheat *(Triticum aestivum)*	Barkatullah University, Bhopal, MP
6	Ms. Anita Kumari	Modulation of scion through graft transmissible signals from rootstock, using *Arabidopsis thaliana* as a model system	Guru Jambheshwar University of Science & Technology, Hisar, Haryana
7	Ms. Monica Sharma	Genomic characterization & biochemical analysis of genes involved in phenylpropanoid pathway & their effect on nutritional & processing qualities of wheat.	Panjab University, Chandigarh, Punjab
8	Sh. Ritesh Kumar Baboota	Studies on modulation of adipogenesis, obesity and related complications by capsaicin	UIET Panjab University, Chandigarh

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

ii. Project Assistants

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

iii. Trainees

S.No	Name	Designation	Date of Joining
1	Ms. Gurpreet Kaur	Trainee	05-07-2013
2	Ms. Pankhuri Mittal	Trainee	05-07-2013
3	Sh. Chopade Devidas. V	Trainee	05-07-2013
4	Ms. Harmanjit Kaur	Trainee	05-07-2013
5	Ms. Achla Sharma	Trainee	05-07-2013
6	Ms. Shefali Roy	Trainee	05-07-2013
7	Ms. Ankita Singla	Trainee	05-07-2013
8	Ms. Priyanka Kumari	Trainee	05-07-2013

PHOTO GALLERY OF IMPORTANT EVENTS

Celebration of Independence Day: August 15th, 2013

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.

Visitors to the Institute (European Delegation) – May 27th, 2013

European delegation comprising of representatives from three major Agri-Food biotech clusters of Europe i.e. Parco Technologico Padano (Lombardy, Italy), Agropolis International (Languedoc – Roussillon, France) and Oost NV (Food Valley Wageninghen, Netherlands) visited NABI to explore the possibilities of future collaboration.

Event/Workshop Organized at Institute

The brainstorming session entitled "Postharvest Stability of Farm Produce" was organized by National Agri-Food Biotechnology Institute (NABI), Mohali on May 8th, 2013. The session was chaired by Prof K.L. Chadha, President, The Horticultural Society of India and Former-DDG (Hort), ICAR. The session was attended by highly accomplished researchers, academia and industry representatives from across the country. The eminent participants including Dr. C.R. Bhatia, Former Secretary, DBT; Dr. P.V. Sane, Former Director NBRI & advisor to Jain Irrigation Pvt Ltd; Dr. Rajesh Kapoor, Advisor, DBT; Dr. R.K. Pal, Director ICAR-NRC on Pomegranate, and Dr. Rekha Singhal, Head, ICT, Mumbai attended the session.

An international workshop on banana transformation & tracker software was organised, during September 15th -20th, 2013 under the BIRAC, India - QUT, Australia Banana Biofortification Project. The meeting and workshop was attended by the participants of BIRAC, New Delhi; QUT, Australia; NABI, Mohali; BARC, Mumbai; NRCB, Trichy; IIHR, Bangalore and TNAU, Coimbatore.

Republic Day Celebrations at NABI: January 26th, 2014

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

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

Fourth Foundation Day: February 20th, 2014

First row from left: Dr. R.S Sangwan, CEO, CIAB; Prof. Akhilesh K. Tyagi, Executive Director, NABI;
Dr. Dinakar M. Salunke, Director, RCB, Dr. Rakesh Tuli, Former Executive Director, NABI and Dr. Vikas Rishi, Sct-E, NABI
Dr. Dinakar M. Salunke, was the Chief Guest on the occasion.

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

Third row from left: Dr. Vikas Rishi, giving the vote of thanks.
Guests visiting the lab after the function

FINANCIALS

UK Mehta & Associates
Chartered Accountants
904, Sector 40-A, Chandigarh - 160036
Phone: 0172 - 2629622, 9814301213
E – mail : ukmehtas@gmail.com

AUDITOR'S REPORT

We have examined the Balance Sheet of NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE, Mohali as on 31st March 2014 and the Income & Expenditure Account for the year ended on that date, which are in agreement with the books of Accounts maintained by the said Institution.

We have obtained all the Information and Explanation which to the best of our knowledge and belief were necessary for the purpose for our Audit. In our opinion, proper books of account have been kept at the head office at Mohali so far as appears from our examination of the books and other record adequate for the purposes of our audit, subject to the comments given below:

---Nil---

In our opinion and to the best of our information and according to the explanations given to us, the said accounts give a true and fair view subject to the following:

i) In the case of Balance Sheet of the state of the above named Institution's affairs as at 31st March 2014.and

ii) In the case of income and expenditure accounts of the Surplus of the above named Institution for the year ended 31st March 2014.

The prescribed particulars are annexed hereto.

for U.K. Mehta & Associates
Chartered Accountants

(U.K.Mehta) FCA
M. No. – 092639
FRN - 013381N

Dated: 08.07.2014
Place: Chandigarh

FORM OF FINANCIAL STATEMENTS (NON PROFIT ORGANIZATION)

NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE

C-127 INDUSTRIAL AREA PHASE-7 S.A.S. NAGAR, MOHALI

BALANCE SHEET AS ON 31st MARCH 2014

(Amount In Rs.)

CORPUS/ CAPITAL FUND AND LIABILITIES	SCHEDULE	Current Year	Previous Year
CORPUS/CAPITAL FUND	1	52,18,04,081	43,85,56,250
RESERVES AND SURPLUS	2	-	-
EARMARKED/ENDOWMENT FUNDS	3	1,56,86,198	1,70,63,097
SECURED LOANS & BORROWINGS	4	-	-
UNSECURED LOANS & BORROWINGS	5	-	-
DEFERRED CREDIT LIABILITIES	6	-	-
CURRENT LIABILITIES & PROVISIONS	7	78,12,307	1,00,80,443
TOTAL		**54,53,02,586**	**46,56,99,790**
ASSETS			
FIXED ASSETS	8	26,70,98,974	31,35,37,217
CAPITAL WORK IN PROGRESS	8	6,46,01,217	1,70,14,470
INVESTMENTS -FROM EARMARKED/ENDOWMENT FUNDS	9	1,01,29,167	1,02,65,524
INVESTMENTS -OTHERS	10	-	-
CURRENT ASSETS,LOANS & ADVANCES ETC	11	20,34,73,228	12,48,82,579
MISCELLANEOUS EXPENDITURE (to the extent not written off or adjusted)			-
TOTAL		**54,53,02,586**	**46,56,99,790**
SIGNIFICANT ACCOUNTING POLICIES	24		
CONTIGENT LIABILITIES & NOTES ON ACCOUNTS	25		

As per our separate report of even date attached

For National Agri-Food Biotechnology Institute

Finance Officer

Executive Director

Dated: 08 JUL 2014

Place: Mohali

For U.K. Mehta & Associates

Chartered Accountants

(U.K. Mehta), FCA

FORM OF FINANCIAL STATEMENTS (NON-PROFIT ORGANISTIONS)
NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE
C-127 INDUSTRIAL AREA PHASE-7 S.A.S. NAGAR, MOHALI
INCOME AND EXPENDITURE ACCOUNT
FOR THE YEAR ENDED 31st MARCH 2014

(Amount In Rs.)

	SCHEDULE	Current Year	Previous Year
INCOME			
Income from Sales/Services	12	-	-
Grants/subsidies	13	5,10,00,000	7,74,94,400
Fees/subscriptions	14	-	-
Income from Investments (Income on investment from earmarked/endowment funds transferred to funds)	15	-	-
Income from royalty, publication	16	-	-
Interest earned	17	79,60,094	93,55,364
Other Income	18	53,66,152	76,95,349
Increase/decrease in stock of finished goods & work- in -progress	19	-	-
TOTAL(A)		**6,43,26,246**	**9,45,45,113**
EXPENDITURE			
Establishment Expenses	20	2,26,03,635	2,16,96,572
Other Administrative Expenses	21	3,73,77,666	5,68,58,685
Research & Development Expenditure (Incl. grants, subsidies etc)	22	1,90,24,350	1,95,90,053
Interest	23	-	-
Depreciation (net total at the year end-corresponding to schedule 8)		5,20,72,764	4,87,31,258
TOTAL(B)		**13,10,78,415**	**14,68,76,568**
Balance being excess of expenditure over income(B-A)		6,67,52,169	5,23,31,455
Transfer to special reserve (specify each)			
Transfer to/ from General Reserve			
BALANCE BEING SURPLUS/(DEFICIT)CARRIED TO CORPUS/CAPITAL FUND			
SIGNIFICANT ACCOUNTING POLICIES	24		
CONTIGENT LIABILITIES AND NOTES ON ACCOUNTS	25		

As per our separate report of even date attached

For National Agri-Food Biotechnology Institute

Finance Officer

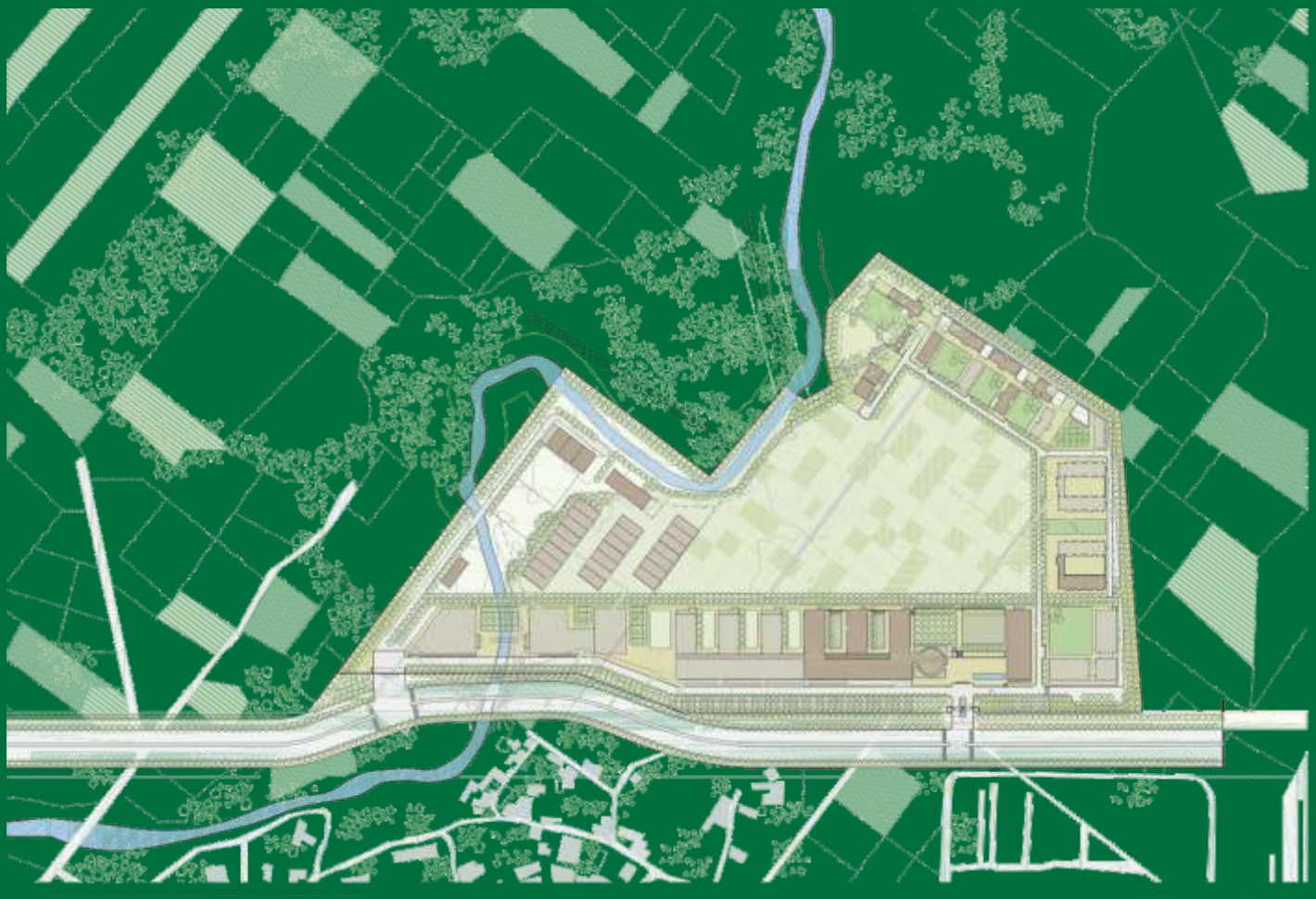
Executive Director

Dated: 08 JUL 2014
Place: Mohali

For U.K. Mehta & Associates
Chartered Accountants

(U.K. Mehta), FCA

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



PROPOSED MASTER PLAN OF NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE, SECTOR 81, MOHALI.

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

C-127, Industrial Area, Phase 8, Ajitgarh (Mohali), Punjab, India - 160071
EPABX: +91-172-2290100, Fax: 0172-4604888
Website : www.nabi.res.in

Azad Offset Printers, 144, Press Site, Indl. Area-1, Chd.
0172-4611489, 2656144, 2657144, www.azadoffsetprinters.com