वार्षिक प्रतिवेदन
ANNUAL REPORT
2015-2016

राष्ट्रीय कृषि–खाद्य जैव प्रौद्योगिकी संस्थान

National Agri-Food Biotechnology Institute

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

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Cover Page Image:

The cover page portrays development of anthocyanin rich wheat grains (page 16), low phytic acid wheat lines (page 18) and proposed mechanisms of IMOs/lycopene in diet induced obesity (page 53).

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FROM THE DESK OF EXECUTIVE DIRECTOR

National Agri-Food Biotechnology Institute (NABI) was established with the objectives of promoting and coordinating high end basic and applied research for translational perspective focussed on agri-food sector for nutrition and health gains. Presently, the institute is working in the five core areas that include, improving cereals for nutrition and processing quality; improving fruits for post-harvest quality and nutrition; basic biology for crop improvement; diet and health, and computational biology approaches for marker and gene discovery.

This year many significant achievements have been made in the above research areas. In the area of improving cereals for nutrition and process quality, our scientists developed high amylose wheat lines. Multiple genes that play important role in controling starch accumulation in wheat granule have been identified. A new initiative related to the study of diversity in celiac disease epitopes in Indian wheat cultivars has been initiated and few wheat translocation lines having reduced immunogenic epitopes have been developed. Wheat lines with high anthocyanin content have been developed and studies are in progress to characterize their traits including micronutrient content. In an approach to develop low phyate lines in wheat, a putative transporter for phytic acid was targeted and wheat lines with reduced phytate content have been developed through RNAi approach. In another collaborative project being pursued jointly with Queensland University of Technology (Australia), banana has been successfully transformed with multiple gene constructs to enhance provitamin-A content in its fruit. These transgenic plants showed promising results with respect to enhanced provitamin-A content.

In the area of basic biology for crop improvement, stability studies for AZIP53 and its six mutants were carried out. In addition, multiple wheat BZIP were identified

and cloned to study seed development. Analysis of the transcriptome data of *Annona squamosa* to identification of multiple homologous genes involved in hormone sensing, embryogenesis and ovule development. To modulate gene expression through tanslocated siRNAs, root-scion mediated silencing of one of the marker gene (INO) was demonstrated. Using this technique, seedlessness in *Arabidopsis* was observed in wild scion grafted onto the rootstock expressing siRNA against INO gene. In the area of improving fruits for post-harvest quality and nutrition, a coating film has been developed using chemically modified oat β-glucan, followed by its blending with arabinoxylan from wheat straw. This coating film showed potential for improving moisture barrier properties.

In the area of diet and health, significant progress has been made in the research areas that address overnutrition as well as undernutrition. NABI researchers observed that water extractable non-starch dietary fibre from finger millet could improve high fat diet induced derangements in mice. Studies are in progress to understand the changes in gut bacteria and dietary fibre supplementation. Further, our scientists have isolated multiple bacterial strains exhibiting antagonistic activity against common food borne pathogens. Our scientists have designed a

novel cobiotic that combines antioxidant, lycopene plus prebiotic and isomalto-oligosaccharides that was effective in preventing high fat diet induced obesity. A prototype food based on the cobiotic has been developed. As a novel approach, our scientists have shown that TRPM8 mediated cold mimicking could shift white adipose tissue from "fat storing state" to "fat burning state".In the area of micronutrient undernutrition, interaction between hepcidin and GDP was studied.

In the area of computational biology, multiple novel miRNA have been identified from wheat, rice and maize. Also, their co-expression networks have been developed to gain insight for their molecular function. A web interface for all the data generated regarding expression, conservation, similarity, correlation and differential expression (PmiRExAt) has been developed. Our scientists have developed an in-house analysis pipeline for identification of regulatory peptides from primary miRNA leading to recognition of 2092 such peptides from wheat. Using previouisly developed in-house software DIITOWAA, 40,2619 and 212 hypothetical gene models of wheat, rice and *Arabidopsis* have been annotated.

In addition to all the research achievements and progress mentioned above, the institute currently has 56 research students. The institute also provides research training to young students in its mandated research areas. In the financial year under reference, the institute hosted 13 extramural research grants from different national and international funding agencies. On the front of scientific output, the institute published more than 30 high impact research papers in various journals of international repute.

As the R & D landscape of the institute grows, it is being planned these and ensuing research activities would converge to constitute flagship program. A couple of flagship programs have been evolved after the suggestions from experts during the detailed delebrations in Scientific Adviosry Committee meetings, open house discussion with the current faculty of NABI. The same is being put-up before the governing body of the institute at its forthcoming meetings for suggestions and finalization for adoption and action plan by the institute leadership and faculty.

The above research achievements that I have narrated in this note, were made under the learned leadership of Prof. Akhilesh Kumar Tyagi as Executive Director of NABI during the financial year 2015-16. On behalf of my colleagues and on my own behalf, I express our gratefulness to him for his commendable leadership. On behalf of the institute, I express sincere thanks and gratitude to the Chairman and Members of Governing Body, Scientific Advisory Committee (SAC) and Programme Advisory Committees (PACs) of the institute for their valuable advices and suggestions to our scientists.

The institute's regular campus development has progressed at an accelerated pace during the year under report, and I am sure within the financial year 2016-17, the institute would begin to operate from its permanent campus. This would facilitate further expansion of institutional strengths of R&D personnel as well as dimensions. I thank Chairman and Members of Building Committee and Consultant Monitoring Committee for their commendable support and advices that catalyzed this pace of development of the campus at Knowledge City, Mohali.

Finally, I thank all my colleagues at NABI whose dedicated work and contributions in their respective roles form the basis of real beauty of the Annual Report of the institute for the year 2015-16.

Jai Hind!!

Dr. Rajender S. Sangwan
Executive Director

VISION & MISSION OF NABI

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

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

RESEARCH PROGRESS

IMPROVING CEREALS FOR NUTRITION AND PROCESSING QUALITY

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

Principal Investigator
Joy K Roy

Research Fellows
Anuradha Singh
Monica Sharma
Pankaj Kumar
Ankita Mishra

Introduction

Wheat flour is processed into several end- use food products, whose complex quality depends largely on biochemical composition of grains such as storage protein, starch, phenolics, lipid, etc. Starchaffects the processing, cooking, and organoleptic qualities, and digestibility of starch-based food products. It is also modified to increase amylose portionin starch (~25% of total starch), which is considered as healthy starch, i.e. resistant starch. Apart from starch, phenolic compounds (PCs) are also important biomolecules whichcontribute towards organoleptic attributes such as colour,aroma and taste of the end use food products. The knowledge of genome-wide distribution and allelic variants of genes/chromosome regionscontrolling processing and nutritional quality is important for understanding their genetics and molecular basis for improvement. The present wheat varieties require improvement in nutrition and processing quality to meet the increasing demand of consumers and baking and processing industries. In this project single nucleotide polymorphisms (SNPs) in the candidate genes and DNA-based markers such as microsatellites to be identified by sequencing will be used on a large set of the diverse wheat germplasms to identify markers showing association with processing and nutrition quality traits. Validation of the associated genes and markers will be done using biparental mapping such as backcross and TILLIN Gpopulations and/or functional genomics tools. The validated genes and markers will be used in wheat improvement through molecular breeding approaches such as marker-assisted selection.

Objectives

1. Creating repositories for germplasm and genomic resources for gene discovery for processing and nutrition quality traits in wheat.
2. Development of EMS treated mutant lines showing variation in amylose content in grain starch.
3. Identification of candidate genes through transcriptome sequencing in two diverse M4 mutant lines for amylose content and their parent variety, 'C 306'.
4. Phenotyping of processing and nutrition quality traits and genotyping using SNPs and microsatellites i.e. simple sequence repeats (SSRs) on a diverse wheat germplasm set to identify the gene and genomic regions controlling the traits through association studies.

Research Progress

Objective 1

1. A comprehensive set of wheat germplasm is being maintained at NABI. It comprises of about 500 indigenous and exotic wheat varieties and landraces and about 1,000 EMS treated M5 population. These germplasms were multiplied at NABI research farm in 2015-16. A subset of this germplasm are being used for gene and genomic region identification for processing and nutrition quality related traits.
2. A large set of transcriptome sequencing data were produced in two diverse mutant lines (low and high amylose grain starch) and the parent variety to identify candidate genes for amylose variation affecting processing and nutrition quality.
3. For transferability of starch trait (resistant starch or high amylose grain starch) into different genetic backgrounds and genetics study of resistant starch/high amylose starch, several crosses (F1 seeds) and backcrosses (BC1F1 seeds) were done in the last wheat grown season.

Objective 2

1. An amylose prediction model was developed by regressing amylose content measured by colorimeteric method on the time taken to develop blue color after dipping their half-cut seeds in a specific diluted I2:KI solution in the 1,035 M3 EMS treated mutant lines (Figure 1). The model will be validated in the M5 mutant lines.
2. A sub set of 101 M4 EMS-treated mutant lines showing variation between ~3% to 76% in amylose content in grain starch was identified by measuring the amylose content by traditional I2-KI method and were further confirmed through Concanavaline A (ConA) method except few lines.

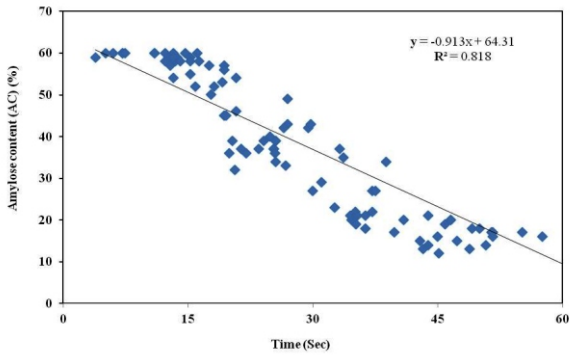

Figure 1: Regression of amylose content (%) determined by colorimetric method on the time taken to develop blue color (sec) in their half-cut seeds using five-time diluted standard Iodine-Potassium Iodide (I_2-KI) solution in 1,035 M3 EMS treated mutant lines.

3. Resistant starch was measured in the above 101 mutant lines using the modified Megazyme protocol. The mutant lines showed variation in resistant starch from 0 to 41%. Twenty-one lines showed resistant starch (RS) content at least 10% in comparison to their parent variety with <1%.
4. Statistical correlation analysis showed a poor negative correlation betweenthousand kernel weight (TKW) and amylose content (r = -0.131, p< 0.05). However, 18 out of 21 high resistant starch (at least 10% RS) lines showed better performance in TKW (range: 41 to 49 g/TKW) over the parent variety (40g/TKW).

Objective 3

1. Pair-end (2x100) transcriptome sequencing of total RNAs extracted from the developing grains at 28 DAA of high and low amylose mutant lines and the parent variety ('C 306') identified many SNPs and differentially expressed genes among them.
2. Many homozygous SNPs among high ('TAC 75') and low ('TAC 6') amylose mutant lines and the parent variety'C 306' were identified at three read depth (RD 2, RD 5, and RD 10) (Table 1).
3. The unique sets of differential expressed genes (DEGs) showing up (Figure 2A) or down (Figure 2B) expression among any two of the parent variety'C 306' high ('TAC 75') and low ('TAC 6') amylose mutant lines were identified, as shown in Figure 2.
4. Fifty-two bZIP transcription factors showing their expression in wheat seed were identified through *insilico analysis*. The differential expression analysis of the 52 TFs wereanalysed in the three seed development stages (21, 28, and 35 days after anthesis, DAA) of the high ('TAC 75') and low amylose ('TAC 6') mutant lines and the parent variety ('C 306') (Table 2). Using statistical correlation analysis of their expression data with that of five key genes and isoforms (GBSSI-4A, GBSSI-7A, GBSSI-7D, SBEIIa, and SBEIIb) involved in biosynthesis of amylose and amylopectin fractions of starch identified few candidate bZIP TFs, which were positively and negatively correlated with genes responsible for amylose biosynthesis.

Table 1: Homozygous SNPs identified through transcriptome sequencing of the parent variety, 'C 306' and two diverse mutants for amylose (high amylose: 'TAC 75' and low amylose: 'TAC 6') at three read depths (RD 2, RD 5, RD 10).

Genotypes	RD 2			RD 5			RD 10		
	'C 306'	'TAC 75'	'TAC 6'	'C 306'	'TAC 75'	'TAC 6'	'C 306'	'TAC 75'	'TAC 6'
'C 306'	0			0			0		
'TAC 75'	101	0		64	0		61	0	
'TAC 6'	280	355	0	65	156	0	32	114	0

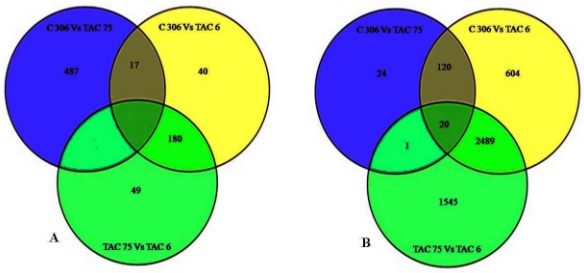

Figure 2: The unique sets of up (A) and down (B) regulation of the differentially expressed genes among three combinations of two of the parent variety ('C 306'), high amylose ('TAC 75'), and low amylose ('TAC 6') mutant lines.

Objective 4

1. A total of 69 phenolic compounds (PCs) using 93 plant PCs as reference were tentatively identified in a subset of 42 diverse wheat lines on LC-QTOF (MS/MS). Following the International Conference on Harmonization (ICH Q2(R1)] guidelines (ICH, 2005) for quantification, limit of detection (LOD) and limit of quantification (LOQ) of 11 out of 17 standards were detected based on S/N ratios of 3 and 10, respectively using the QTOF and for the reproducibility and repeatability of the method, interday and intraday precision was used and expressed by Relative standard deviation (%RSD). Their data are not given here. The 11 PCs were quantified in the 42 varieties showing variation in their content (Figure 3 for *p*-coumaric acid; Table 2).

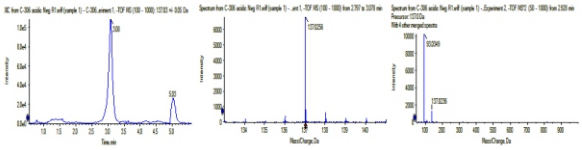

Figure 3: Chromatogram, MS and MS/MS patterns and showing retention time (RT), major peaksand their fragmentation patterns of p-coumaric acid (m/z= 163.04) identified through LC- TOF-MS/MS.

Table 2: Minimum and maximum concentration with SD of 11 PCs quantified in 42 diverse wheat varieties

	Vanillic acid	**vanillin**	**Sinapic acid**	**Hesperidin**	**Rutin**	**Ferulic acid**	**Caffeic acid**	**Salicylic acid**	**2,4-hydroxybenzoic acid**	**Nobiletin**	**4, hydroxy benzoic acid**
Maximum concentration (ng/ml)	43.2± 0.7 (K 8962)	21.7 ± 0.6 (RIL 251)	136.7 ± 0.3 (Raj 3765)	22.2 ± 0.5 (K 7410)	22.1 ±0.4 (Amylopectin)	174.5 ±1.0 (CIMMYT)	3.5±1.5 (NP 4)	547.2±0.9 (CIMMYT)	23.6±1.1 (Bobwhite)	887.1 ± 0.7 (DBW 14)	2642.2 ± 0.5 (CIMMYT)
Minimum concentration (ng/ml)	26.6±0.6 (K 7410)	1.4 ±0.3 (HI 1431)	3.2 ± 0.1 (HI 977)	2.3 ± 0.3 (PBW 550)	0.8 ±0.4 (GW 190)	0.7 ±0.1 (C 273) 1.9±0.0	(DBW 14, HD 2329, LOK 1, PBW550)	1.7± 0.2 (Raj 4083)	6.7 ±0.2 (HI 1431)	147.8 ± 0.6 (HD 2329)	248.8 ± 0.54 (DBW 16)

2. Initial association mapping analysis using 28 SSRs on 42 wheat varieties for 16 parameters related to starch physical, pasting, thermal and retrogradation properties identified one SSR marker allele each showing association with four parameters such as trough and final viscosity of starch pasting properties and onset and conclusion temperature of starch thermal properties.

Achievements

1. A set of 101 M4 EMS-treated mutant lines showing variation between ~3 to 76% in amylose content and 0 to 41% in resistant starch in grain starch were identified and advanced to M5 generation.
2. Transcriptome sequencing of total RNAs of high and low amylose mutant lines and the parent variety ('C 306') identified many SNPs and differentially expressed genes.
3. Differential expression analysis of 52 bZIP transcription factors showed variation in their expression in high and low amylose mutant lines.
4. Sixty-nine phenolic compounds were identified in 42 wheat genotypes and 11 of them were quantified on them.
5. One SSR marker allele each showing association with four parameters such as trough and final viscosity of starch pasting properties and onset and conclusion temperature of starch thermal properties were identified.

Future Objectives

1. Transcriptome sequencing will be done on additional high and low amylose mutatnt lines to identify common genes and their SNPs showing variation with amylose content in the mutant lines.
2. The mutant lines and crossing populations will be advanced to next generation.
3. Phenotyping of starch-based processing and nutrition traits and genotyping of SNPs and SSRs will be done on a larger set of wheat germplasm to identify QTL regions and genesshowing association.
4. Collaboration will be initiated with madras diabetes research foundation (MDRF), Chennai for human clinical studies of resistantstarch as they have clinical population for diabetes study.

1.2 Improvement of processing and nutritional quality in wheat

Principal Investigator
Monika Garg

Research Fellows
Rohit Kumar
Aman Kumar
Navneet Kaur

Introduction

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

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

wheat with medium protein content. The contribution of different genes/alleles to chapatti making is poorly understood. It is important to understand structure, allelic variation and interaction pattern of different seed components and transfer them to high yielding, disease resistant and locally adapted cultivars.

Objectives

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

Research Progress

1. Accelerated breeding for processing quality improvement

a.) For improvement of chapatti making quality, good chapatti making old cultivars (C306, Lok1) were crossed with high yielding recent cultivars (PBW343, PBW550 and PBW621). Near isogenic lines (NILs, $BC_3F_{7)}$from each cross have been selected. Selection is based on absence of GBSS-1B gene, background screening and morphological similarity to recipient parent. Seeds from selected lines/plants will be utilized for chapatti quality analysis. Further BC_5 based NIL preparation is in progress.

b.) For improvement of Biscuit making quality donor landrace was crossed with high yielding recent cultivars (PBW343, PBW550 and PBW621). NILsfrom each cross have been selected. Selection is based on puroindoline gene, background screening and morphological similarity to recipient parent and grain softness. Seeds from selected lines/plants will be utilized for biscuit making analysis.Further BC_5 based NIL preparation is in progress.

c.) For improvement of bread making quality, we are utilizing wild species *Ag. elongatum, Ae. longissima, Ae. searsii, Ae. geniculata* and *Ag. intermedium.* These genetic stocks are being crossed with high yielding cultivars PBW550, PBW621 and HD2967. We intend to transfer HMW-GS genes related to high grain strength from wild species to chromosome 1A of wheat. Chromosome 1A specific translocation line of *Ag. elongatum* [1EL(1AS)] with potential of bread making quality improvement was generated in the background of soft wheat cultivar Norin61 (Figure 4). Transfer of this translocation to hard wheat cultivars PBW621 has been achieved (1EL(1AS)/**5***PBW621-F_7). Replicated trials have been harvested. Bread making quality of these lines will be studied.

Achievements

1. Advanced breeding material for improvement of chapatti and biscuit and bread making quality has been generated
2. Translocation line of *Ag. elongatum* in hard wheat background 1EL(1AS)/**5***PBW621-F_7 has been created.

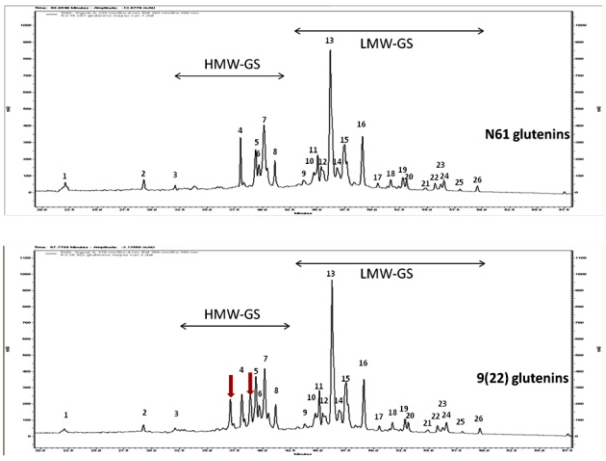

Figure 4: RP-HPLC profiles of glutenins of Norin61 and 1EL(1AS) translocation line indicating added HMW-GSs from Ag. elongatum with no change in LMW-GS pattern

Future Perspective

Generation and confirmation of breeding material with improved processing quality.

—x—

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

Principal Investigator
Monika Garg

Research Fellow
Nand Kishore

Project Assistant
Swati Missar

Introduction

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

(i) Transcriptome data screening of Indian wheat cultivars indicated differential expression of CD causing α-gliadins at different stages of seed development.

(ii) Preliminary antibody based screening of the selected translocation lines of *Hynaldia villosa* (6VS-6DL) in wheat indicated that it has lower immunogenic proteins as it replaces chromosome arm 6DS that codes most immunogenic proteins.

(iii) Marker assisted backcross based breeding to transfer 6VS-6DL translocation from exotic lines to regionally adapted cultivars is in second to third backcross stage.

Objectives

1. Comprehensive mapping of CD epitopes (peptide variants) in wheat
2. Gene targeting and evaluation by stable RNAi genetic transformation
3. Accelerated breeding to transfer non immunogenic protein from wild wheats

Research Progress

a) Sequence analysis of transcriptomic data of old cultivars

Celiac disease epitopes namely DQ2 Gliaα1, DQ2 Gliaα2, DQ2 Gliaα3 and DQ8Gliaα1 were screened in already available database (Wheat Illumina Reads) of C306 and Sonalika cultivars at 7, 14 and 28 days after anthesis (DAA). The transcriptome data for housekeeping genes (18S rRNA, ADP-ribosylation factor and Actin of C306) were also analysed for authenticity of transcriptome data. It led to following observations:

i. Very high number of transcripts (>40,000, not reported before) were observed in several cases.
ii. For all the variants of four epitopes analysed, C306 had increasing trend in number of transcripts from 7DAA to 28DAA.
iii. For 46% variants of four epitopes analysed, Sonalika had increasing trend in number of transcripts from 7DAA to 28DAA. But for 31% had decreasing trend in number of transcripts from 7DAA to 28DAA. For 6% in was stable and for 17% it first decreased and then increased. Overall there was increase in number of transcripts
iv. For DQ2Gliaα1 and α2, at 7DAA, Sonalika expressed more number of CD epitopes than C306, whereas, C306 expressed high number of epitopes at 14 DAA and 28 DAA than Sonalika. This indicates that overall accumulation of DQ2Gliaα1 and α2 CD epitopes in C306 was higher than Sonalika.
v. In contrast, DQ2Gliaα3 and DQ8Gliaα1 epitopes expression was higher in Sonalika than C306 at all stages (7DAA, 14DAA & 28DAA).
vi. Transcripts of one of the variants of DQ8Gliaα1 were not expressed in both the cultivars.
vii. Out of four, two epitopes (DQ2Gliaα1, DQ2Gliaα2) were high in C306 and other

two (DQ2Gliaα3, DQ8Gliaα1) were high in Sonalika indicating cultivar specific protein expression.

viii. Several transcripts were very low in C306 but very high in Sonalika in early stage (7DAA), again indicating cultivar specific protein expression.

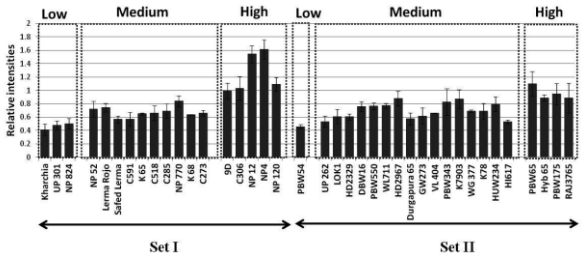

Figure 5: Antibody based quantisation of alpha gliadins from different wheat cultivars and landraces.

b) *Anti-gliadin antibody (Polyclonal) based response in different wheat varieties/landraces*

A total of 43 wheat genotypes including old wheat varieties, land races, modern wheat varieties were selected for comparative analysis of anti-gliadin antibody response. In the set I of old cultivars, three groups with low, medium and high relative intensities were separated (Figure 5). Higher number of cultivars had medium level of expression (10/18) followed by high (5/18) and low (3/18) expression. Cultivar Kharchia showed lowest response to anti-gliadin antibody. Cultivars, NP824 and UP301 also showed low level of protein expression. From the set II, large number of cultivars showed medium (16/21) response followed by high response (4/21) and low (1/21) response. Their expression varied from medium to high level. Lowest level of response was observed in PBW54. Old good chapatti making cultivars C591, C518, C273 and K78 showed medium level of alpha-gliadin expression. Highest response was observed in NP12 and NP4. Our results were in agreement with the Punjab Agricultural University, Ludhiana (personal communication). They carried out, amplification, cloning and sequencing based approach to screen the same lines as used in our study.

c) *Gene targeting and evaluation by stable RNAi genetic transformation*

Sequences corresponding to alpha-gliadins were screened in NCBI database and aligned according to genomes using genome specific reference sequences. Genome specific sequences included wheat (A-, B- and D- genome) as well as genome donors (*T. monococcum*, *Ae. speltoides* and *Ae. tauschii*).The primers were designed for A and D genome specific amplification of alpha-gliadin gene as B genome encoded alpha gliadin has been reported to be non-immunogenic. Cloning and sequencing of alpha-gliadin genes from A and D genome was carried out in pGEM®-T Easy vector. Analysis of the sequences indicated correct orientation and genome specificity. Our next target is to combine these two clones (*Gli-A* and *Gli-D*) in single vector. Restriction site analysis of pGEMT easy vector clones and final pMCG161 RNAi vector indicated that it is not possible in the current vector. So we are now using additional vector i.e. pBS SK+ vector to first insert *Gli-A1* clones and then combined it with the *Gli-D* clone. We have already inserted A genome and promoter sequences in pMCG161 RNAi vector. After combining the two inserts in pMCG161, transformation to wheat will be carried out.

d) *Accelerated breeding to transfer non immunogenic protein from wild wheats*

We have procured all the required translocation lines.Different crosses were started before commencement of the project and it is now in the next stage as mentioned below.To reduce the immunogenicity associated with alpha-gliadins encoded by chromosome 6AS and 6DS of wheat, translocation lines of *Hynaldia villosa* in wheat (6VS.6DL and 6VS.6AL) were selected. These lines were crossed with high yielding Indian cultivars. Crosses were carried out with hard wheat cultivars, soft

wheat and colored wheat lines for generation of end product specific breeding materials. Breeding experiment was carried out at NABI farm in the main season and at IIWBR Regional Station, Keylong, Lahaul Spiti, Himachal Pradesh in off season. In addition, off season cultivation, screening and selection was also carried out at NABI growth chambers. So far, BC_3F_4 and BC_2F_5crosses have been achieved (Table 4). Positive plants were screened first with 6vs-Bd6 marker for 6VS chromosome and thereafter with different 6AS and 6DS specific SSR markers.

Table 3: Antibody based quantisation of alpha gliadins from different wheat cultivars and landraces.

S.N.	Translocation lines	Wheat type	Crosses
1	6VS.6DL	Hexaploid Hard	6VS.6DLxHD2967xHD2967xHD2967 xHD2967-F_4
			6VS.6DLxPBW550 x PBW550 x PBW550 x PBW550-F_4
		Hexaploid & color	6VS.6DLxHD2967 xTA3972xHD2967xHD2967-F_4
			6VS.6DLxPBW550xTA3972xPBW550xPBW550-F_4
2	6VS.6AL	Hexaploid & Hard	6VS.6ALxPBW550xPBW550xPBW550xPBW550-F_4
			6VS.6ALxHD2967 xHD2967xHD2967xHD2967-F_4
		Hexaploid & Colored	6VS.6ALxPBW550xTA3972xHD2967xPBW550 xPBW550-F_4
			6VS.6ALxPBW550xTA3972xPBW550xPBW550 xPBW550-F_4
		Tetraploid	6VS.6ALxPDW233xPDW233xPDW233-F_4
			6VS.6ALxPDW291xPDW291xPDW291-F_4
			6VS.6ALxWHD943xWHD943xWHD943-F_4
3	6VS.6DLx6VS.6AL	Hexaploid Hard	6VS.6DLx6VS.6ALxHD2967xHD2967xHD2967-F_4
			6VS.6DLx6VS.6ALxPBW621xPBW621xHD2967-F_4
		Hexaploid Soft	6VS.6DLx6VS.6ALxI66HHH*-F_4
		Hexaploid Color	6VS.6DLx6VS.6ALxBLACK WHEATxPBW621 xPBW621xPBW621xPBW621-F_4

6VS.6DL and 6VS.6AL = Translocation lines; I66HH* = IITR67 (soft wheat) x PBW621x PBW621 x HD2967 x HD2967; PBW550, PBW621, HD2967 = High yielding recent cultivars

In this project we are getting good leads. Several useful lines have been developed that include translocation lines alone and in combination with high anthocyanin content. One line (6VS.6ALxHD2967xHD2967 xHD2967-F_5) showed improved yield potential, reduced plant height, improved disease resistance with a new source of yellow rust resistance (Yr26) and above all lowered immunogenicity (Figure 6; Table 3). We are trying to contact breeder for its introduction to national trials for subsequent release. Morphological data of this line indicated that it had longer spikes with higher number of spikelet per spike and seed number. Another important feature was lower plant height than recipient cultivars. Recently, from several years, the incidence of rain with wind has been increasing during grain filling and maturation of wheat. It leads to lodging of tall and average height cultivars. Lower height will give it special edge over other cultivars.

Figure 6: Comparison of spikes of translocation line with recipient cultivar HD2967

Table 4: Comparison of morphological data of cultivar HD2967 and translocation line developed in this work

S.No.	Trait	6VS.6ALxHD2967xHD2967xHD2967-F5	HD2967
1	Seeds per spike	. .	61.10±1.21
2	Plant Height	91.46±0.57	104.90±2.66
3	Spike length	12.61±0.17	8.96±0.19
4	Spikelets per spike	24.35±0.24	19.40±0.49
5	Awn Length	6.12±0.07	5.86±0.24
6	Flagleaf Length	18.37±0.25	19.50±0.95
7	Flagleaf Width	1.58±0.02	1.51±0.06
8	Yellow Rust	Resistant	Susceptible
9	TKW	40	40

Achievements

Transfer of *Hynaldia villosa* (6VS-6DL) translocation with reduced immunogenic epitopes to Indian cultivar has given us good wheat lines with improved yield potential, reduced plant height, improved disease resistance with a new source of resistance (Yr26).

Future Perspective

Creation of breeding material with lowered number of immunogenic epitopes.

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

Principal Investigator

Monika Garg

Research Fellow

Saloni Sharma

Introduction

Plant phytochemicals such as anthocyanins can act as antioxidants and show anti-inflammatory, anti-cancer, anti-aging activity and prevent cardiovascular diseases and type-2 diabetes. In the present proposal, we aim to develop colored wheat lines with high anthocyanin content that could be exploited for nutraceutical applications. It has advantage over anthocyanin rich fruits and vegetables, as later has very short shelf life and cannot be stored for long. Wheat is major farmer crop, with all required machinery in place. Colored wheat can be used as novel ingredient resource for the development of value added products and functional foods. In this project, we will employ non-GMO breeding technologies to transfer of grain color from exotic germplasm to high yielding Indian wheat cultivars and chemically characterize the anthocyanins profile in the resultant varieties. Preclinical studies will be carried out to enhance outreach and commercial abilities of these products. In the later phase of project, we will develop value added and functional food products for better human health.

Objectives

1. Development and yield advancement of black, blue and purple wheat lines with high anthocyanin content
2. Chemical characterization of the anthocyanin profile in the resultant lines
3. Functional characterization (in-vitro and in-vivo model systems) of whole colored wheat for its health benefits
4. Development of value added products and functional foods

Research Progress

1. Colored wheat was non-existent in Indian germplasm. There is no publication on colored wheat from India. NABI initiated this, few years back by getting the required donor lines from USA and Japan and several advanced colored wheat lines have been developed in the due course. Yield of advanced lines is comparable to high yielding cultivars.
2. Localisation of blue color in the aleurone layer and purple color in the pericarp has been confirmed by microscopic studies. Genetic studies on blue aleurone trait indicated that blue color development in triploid aleurone required three dominant alleles derived from two dominant complementary genes of diploid plant.
3. Enhancer and suppressor genes affected the expression of the blue grain color. Higher anthocyanin content was observed in black followed by blue, purple and white wheat

lines. Similar trend was observed for antioxidant activity. Colored wheat showed Anti-inflammatory effect on LPS induced RAW264.7 macrophages and reduction in inflammatory markers. Higher reduction was observed for purple wheat, followed by black and blue wheat.

4. Different products were prepared from colored wheat and tested for antioxidant potential. All of them showed high antioxidant activity compared to normal wheat products (Figure 7 and 8).
5. In case of products highest anti-inflammatory on Macrophage cell lines was observed in case of purple wheat bread. Colored wheat lines have been multiplied in the farmer's field for possible collaboration with industry.
6. Crossing with rust resistant cultivars was initiated and is being followed.

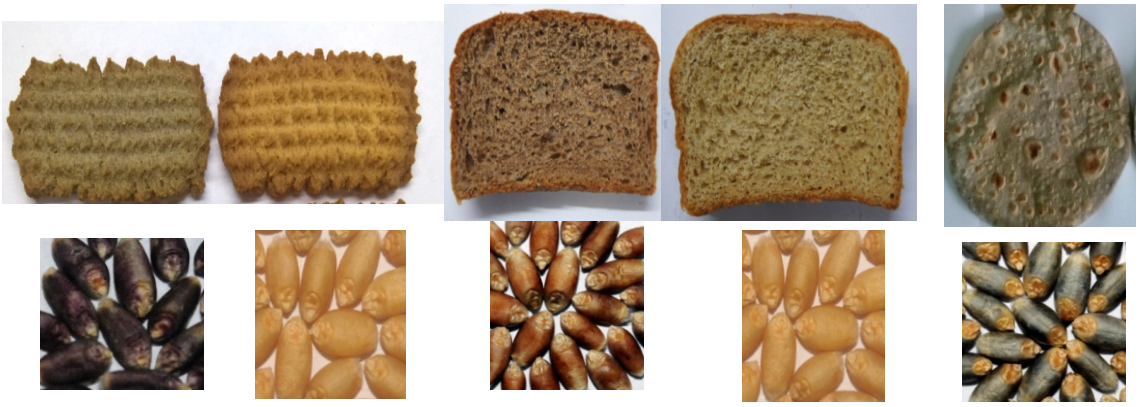

Figure 7: Different products prepared from colored wheat lines and their anti-oxidant potential (µg/mg).

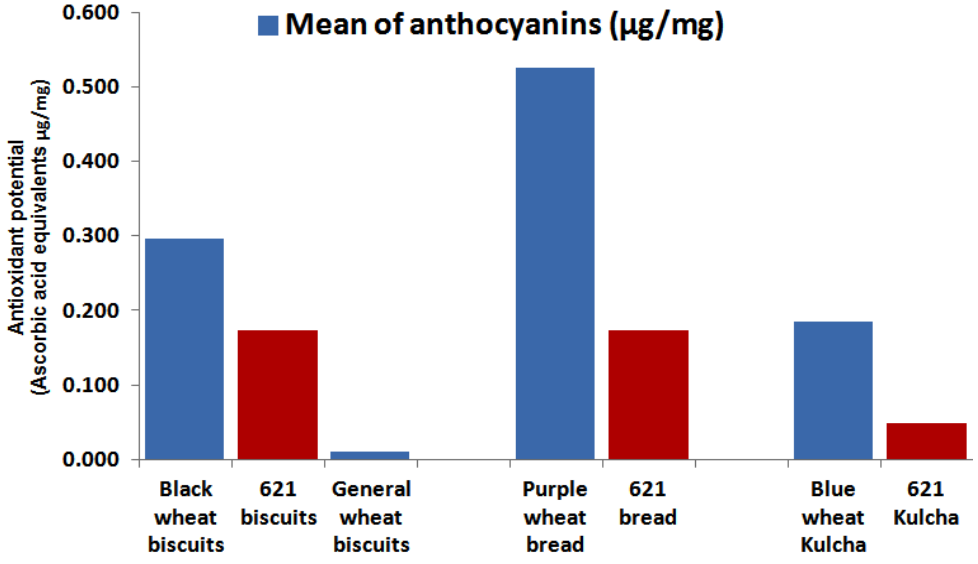

Figure 8: High antioxidant level of colored wheat products (Ascorbic acid equivalents -µg/mg)

Salient Achievements

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

Future Perspectives

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

1.5 Metabolic engineering of phytic acid pathway for improving iron bioavailability in wheat

Principal Investigator
Ajay K. Pandey

Co-Investigator
Siddharth Tiwari

Research Fellows
Kaushal K Bhati
Sipla Aggarwal

Introduction

Approaches for reducing phytic acid (PA; anti nutrients) content in seed to enhance iron bioavailability has been employed in different crops like maize, soybeans and rice. Genes involved in PA pathway are not reported from wheat. In this project we want to utilize functional genomic tool/s to address the role of phytic acid synthesis genes. Our goal is to first identify genes contributing for PA pathway and subsequently generate low PA wheat lines by targeted gene silencing. We anticipate that wheat grains with 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. To gain insight into the function of *TaABCC13* and *TaIPK1* in wheat, monocot specific RNAi silencing vector (pMCG 161) was used to target at the conserved region of the homoeologous gene sequences.
2. Nine independent putative transgenic events survived during multiple rounds of selection on BASTA, but only four of the putative transgenic plants survived the hardening procedure. Earlier, these four putative transformants were subsequently confirmed for presence of the transgene by amplifying and sequencing the *bar* and *OCS1* terminator sequences. The T_1 progenies from the third event (K3) failed to survive due to the reduced seed setting and subsequent failure of seed germination. Eventually, three independent transgenic events (K1, K2 and K4) showed healthy growth and seed germination for further analysis.
3. The lines from these three independent events were propagated to the T_4 generation, which was analysed in detail. Two transgenic plants from lines K1 (K1B4-2-5, K1A13-8-2), K2 (K2C4-6-8, K2C9-2-3) and K4 (K4G3-5-1, K4G7-10-3) selected randomly for further study.
4. The selected T_3 or T_4 transgenic plants developed from three events (K1, K2 and K4) were subjected to qRT-PCR to assess the level of gene silencing in the wheat tissues. Transcript abundance of *TaABCC13* was quantified in T_4 seeds of 14 DAA. The silencing of *TaABCC13* resulted in 20% to 60% reduction in the transcript levels in the seed tissue.
5. An altered spikelet arrangement was noticed in the developing spikes of transgenic lines (reduction in total spikelet counts). These observations were consistently observed for both the silenced lines. The occurrence of head sterility was also observed in these RNAi lines in T_2 to T_4 progenies (Figure 9). To examine the effect of silencing of *TaABCC13* in seeds, PA content was measured in the mature grains. Significant differences in the accumulation of PA were observed among the transgenic lines that ranged from 22% to 34% reduction when compared to the non-

transformed mature seeds. The maximal reduction in seed PA was observed in line K1B4-2-5 (~34%) followed by K4G7-10-3, which had a ~22% reduction in the PA level. The transgenic seedling of TaABCC13 developed short root (lateral) and these transgenic were sensitive for the presence of Cd.

6. The T_1 plants for TaIPK1 silencing are currently been raised and subsequently screened for putative transgenic.

Salient Achievements

1. Transgenic wheat with reduced PA was achieved. A reduction of 34% in total PA content was observed.
2. *TaABCC13* is also involved in lateral root formation in wheat seedlings that showed enhanced sensitivity for the presence of Cd.

Future Perspectives

1. Transgenic wheat with targeted silenc-ing of *TaIPK1*, a gene involved in last step of PA biosynthesis will be generated.
2. Bioavailability studies preformed for the low phytate wheat grains.

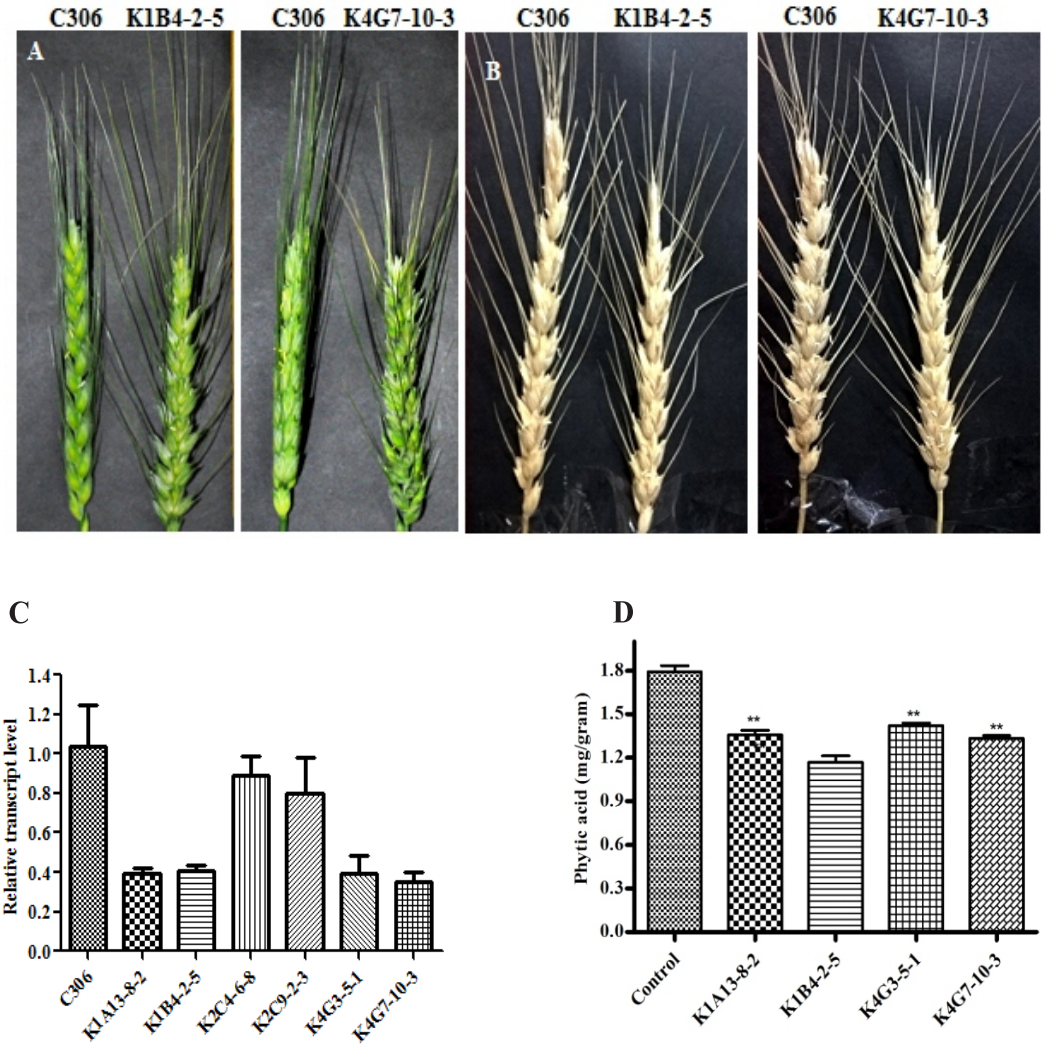

Figure 9: Phenotype of transgenic wheat spikes along with the control C306 (A and B). Representative images of developing wheat caryopsis at onset of flowering for C306 (control) and representative TaABCC13:RNAi lines. Silenced wheat spikes of C306 Silencing in seed, phytic acid estimation (C and D).

1.6 Functional Characterization and Implications of Plant Inositol Pyrophosphate Kinase

Principal Investigator
Ajay K.Pandey

Co-Investigator
Vikas Rishi

Introduction

The higher anionic forms (IP_7 and IP_8; PPx-InsPs) of phytic acid (IP_6) derived after hyper phosphorylation acts as a strong reservoir of phosphate molecule. Genes responsible for the production of the pyrophosphate derivatives are referred as inositol phosphate-6 kinase (IP_6K) and reported in yeast and human (kcs-1 and vip-1). Recently, two genes (*AtVIP1* and *AtVIP2*) were reported and characterized from *Arabidopsiswithout offering any functional importance* (Desai et al., 2014). Previous studies in yeast have suggested the role of inositol pyrophosphates in regulating the phosphate homeostasis and IP_6 synthesis. Since, wheat seeds are an important reservoir of bound and free phosphate we hypothesize that *TaVIP1 may be one of the regulators for phosphate homeostasis and other cellular function especially in seeds*. Based on this, the current research proposal will test the hypothesis that in plants VIP1 could act as a sensor/regulatory molecular for cellular homeostasis in wheat grains. The long-term goals of this project are to understand how these unique higher forms of IP_6 molecules are regulated in wheat and if they help control phosphate sensing.

Objectives

1. Molecular, biochemical and functional characterization of wheat VIPs
2. Identification and characterization of interacting partners of TaVIPs

Research Progress

1. To clone the full length of wheat VIPs, sequences from yeast and Arabidopsis were blasted against wheat ESTs. Two possible wheat VIPs were identified as two different UniGene ID. The sequence information was utilized to design the 5' and 3' RACE experiments. Subsequently, full ORF of wheat VIP1 from wheat was amplified. The wheat VIP1 encoded 3.1 kb and as both kinase as well as phosphatise.

2. The differential expression of both TaVIP1 studied (Figure 10). Our results along with gene-investigator data confirmhigh expression of TaVIP1 in different wheat tissue as compared to TaVIP2. Further, to perform the biochemical activity of TaVIP1, it was cloned in the expression vector (pET23a) and an attempt for protein expression was made.

3. For the interaction studied TaVIP1 was cloned in the BD yeast shuttle vector and

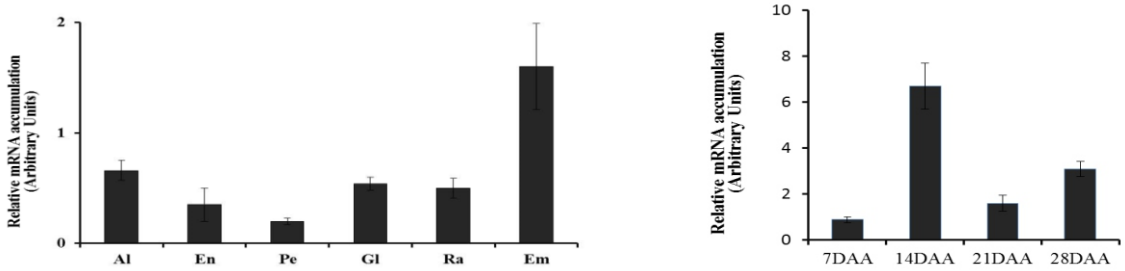

Figure 10: Expression profiles of TaVIP1 in different wheat tissue. qRT-PCR analysis was performed in the cDNA of different wheat tissue prepared from 2µg of total RNA. Ct values were normalized against wheat ARF as an internal control.(Al-aleurone, En-endosperm,Pe-pericarp,Gl-glume, Ra-rachis, Em-embryo)

library were made from seed and roots of wheat. Y2H experiments will be now subsequently performed and interacting partners will be identified and confirmed for defining the role of wheat VIPs.

4. The cloning of full length TaVIP2 is currently ongoing.

Salient Achievement

Two wheat VIPs were identified. Expression characterization revealed high expression of

Future Perspectives

1. Purified protein of TaVIP1 and TaVIP2 will be used for enzymatic assays.
2. Complementaitons and funcitonal assays of TaVIP1/TaVIP2 will be undertaken.

1.7 Identification, cloning and functional characterization of myo-inositol oxygenase (MIOX) from Wheat

Principal Investigator
Siddharth Tiwari

Research Fellows
Anshu Alok
Harsimran Kaur

Introduction

Myo-inositol oxygenase (MIOX) plays important role in *myo*-inositol catabolism, therefore, is considered as an enzyme of the physiological and nutritional interest. The alternative pathway for ascorbic acid biosynthesis directed through oxygenation of *myo*-inositol by MIOX has been of great interest. Molecular and functional characterizations of MIOX have been reported in *Arabidopsis thaliana*, *Oryza sativa* and *Glycine soja*. Recent studies have shown that plant MIOX not only involved in biosynthesis of ascorbate but also showed diverse physiological and biochemical role against abiotic stresses. Wheat being an important crop, there is a need to address the role of wheat MIOX (TaMIOX). In an attempt to understand its role in wheat, the project was funded by Science and Engineering Research Board (SERB), Department of Science and Technology (DST) under the Start Up Research Grant (Young Scientists - Life Sciences) in May 2014. In this report, biochemical and expression characterization of *TaMIOX* have been performed. The study suggested TaMIOX as a possible candidate for metabolic engineering to develop certain trait/s in wheat.

Objectives

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

Research Progress

1. The sequence alignment of the *TaMIOX* with the reported *MIOX* revealed that *TaMIOX* contains a putative open reading frame of 912 bp including 109 bp and 343 bp as 5′ and 3′ UTRs, respectively. Full-length *TaMIOX* cDNA encodes a functional polypeptide of 303 amino acid residues with a molecular mass of 35.2 kDa.
2. The homologous genes of *TaMIOX* were located on A, B and D genomes on large segments of chromosome 7. Phylogenetic analysis of TaMIOX across kingdoms confirmed the close relationship with *Triticum urartu* and *Aegilops tauschii*.
3. A predicted secondary structure of TaMIOX consists of α-helixes (42.9%), β-turns (7.26%) joined by extended strands (14.85%), and 37 random coils (34.94%) (Figure 11A). A predicted 3D structure of TaMIOX was determined using comparative modelling by applying I-TASSER simulation and suggested its close functional and structural resemblance with known MIOX (Figures 11B and C).
4. Purification and biochemical characterization of recombinant TaMIOX was performed in bacterial system. The molecular mass of purified protein was estimated approximately 35 kDa using

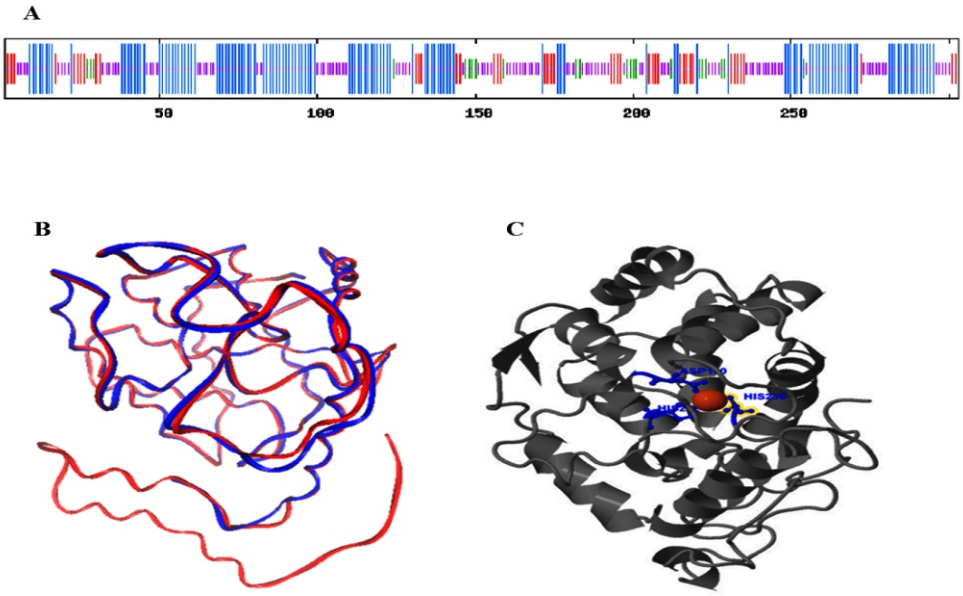

Figure 11: Structural analysis of TaMIOX. **(A)** Secondary structure of TaMIOX. Blue, red, green and violet vertical lines indicate helices, sheets, turn and coils, respectively. **(B)** The 3D structure shows superimposition of common regions of TaMIOX (red) on the mouse MIOX (blue).**(C)** Substrate binding site in TaMIOX. Blue color indicates amino acid residue whereas brown sphere for hetrogens.

SDS-PAGE (Figure 12A) and used for further enzyme activity. The recombinant TaMIOX was active in a Tris–HCl buffer pH range 7.0 to 10.0 with a maximum activity at pH 8.0 measured at A670 nm (Figure 12B) whereas decreased activity was observed at a pH range of 5.0 to 6.0 in a MES buffer. Recombinant TaMIOX was active over a temperature range from 5 °C to 45 °C with an optimum activity at 35 °C (Figure 12C). MIOX activity was completely abolished at >50 °C. The Km value and catalytic activity of the TaMIOX were determined as 5.6 mM and 3.47 µkatal, respectively from the Michaelis–Menten plot (Figure 12D).

5. Differential expression pattern of *TaMIOX* was observed in leaves, root, stem, seed and seed developmental stages. The highest expression of the *TaMIOX* was observed in leaves followed by root, stem and seed (Figure 13A). Expression analysis showed that *TaMIOX* was expressed throughout the developmental stages of grain at varying levels with highest expression at 28 DAA(Figure 13B). Tissue specific analysis of *TaMIOX* suggested higher expression in the endosperm as compared to the aleurone layer of wheat grains indicated its site of activity (Figure 13C).
6. The exogenous application of *myo*-inositol enhanced the expression of *TaMIOX* in leaves and roots suggested *myo*-inositol acts as an inducer for the *TaMIOX* expression (Figure 13D and E).

Salient Achivements

1. *In-silico*, biochemical and expression characterization of the *TaMIOX* gene was performed.
2. The work was published in Plant Gene, 4 (2015); 10-19

Future Perspectives

1. The *TaMIOX* overexpression construct will be designed and to validate its function in model plant system.
2. *TaMIOX* overexpressed wheat transgenic lines will be generated and evaluated for the desirable trait(s).

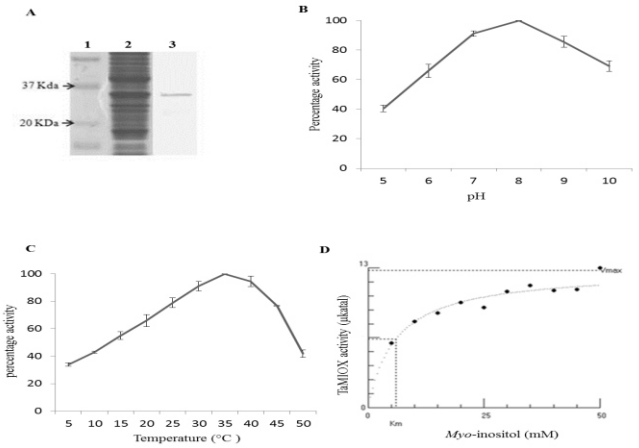

Figure 12: Recombinant expression and enzyme kinetics studies of TaMIOX. **(A)** purification of recombinant TaMIOX (Lane 1, protein molecular weight markers; Lane 2, Induced whole cell lysate and Lane 3, His-tag purified recombinant protein). **(B)** Optimum pH for the purified recombinant TaMIOX. **(C)** Optimum temperature for the purified recombinant TaMIOX. **(D)** Kinetics for the substrate *myo*-inositol inMichaelis-Menten plot follows a hyperbolic curve.Experiments (Fig. 4B and C) repeated with two biological replicates and each experiment consisting of three technical replicates.The graph shows values ± SD.

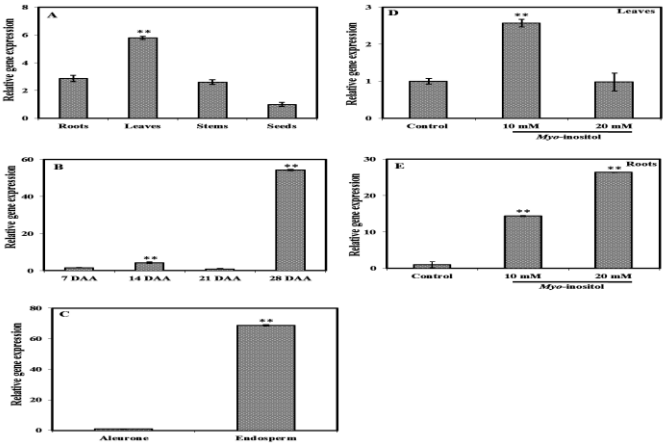

Figure 13: Tissue specific transcript expression of *TaMIOX* in wheat. **(A)** Expression analysis in different tissues of 14 DAA old wheat plant. **(B)** Expression analysis during different stages of grain filling. **(C)** Expression quantification in aleurone and endosperm. **(D)** Expression in leaves when wheat plants exposed with 10 mM and 20 mM*myo*-inositol for 24 h treatment. **(E)** Expression in roots when wheat plants exposed with 10 mM and 20 mM*myo*-inositol for 24 h treatment. The level of *TaMIOX* transcript in different tissues normalized with reference to *ARF* taken as an internal control. Bars denote relative gene expression ± SD. All experiments were repeated at least two times and each experiment consisted of three technical replicates. Statistical analysis was performed using one way ANNOVA of Origin 6.0 software (Origin Lab Corporation, MA, USA) to check the level of significance. Statistical significance was checked at $p \leq 0.01$ (highly significant) denoted as **, with respect to the expression in roots or 7 DAA or aleurone or control for the respective experiments.

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

Principal Investigators
Sudhir Pratap Singh
Shrikant Subhash Mantri

Research Fellow
Raja Jeet

Introduction

Bread wheat (*Triticum aestivum* L.) is a leading staple food crop, serving the major source of calorie, carbohydrate, and proteins, to human and livestock worldwide. Conventional breeding efforts have improved the yield potential of wheat. However, the present day cultivars have suboptimal quantities of micronutrients (such as Fe, Zn and Mn) in grains with limited variability than primitive wheat genotypes- landraces and wild relatives. The concentration of micronutrients in grains, within-grain allocation and their occurrence in different chemical forms determine bioavailability of the minerals. The localization studies on grain tissue-specific element storage patterns established that micronutrients are highly concentrated in the outer aleurone layer, whereas the starchy endosperm is devoid of micronutrients. The mineral rich aleurone layer is removed during

milling and processing of wheat flour, leading to substantial loss of minerals in the diet. In aleurone, the micronutrients are co-localized with phosphorus, which largely represents phytic acid, chelating agent that makes a strong ionic associations with the cationic elements, making them insoluble, and severely affecting their bio-availability in food. In endosperm, the micronutrients like Fe are not supposed to be complexed with phytic acid, and therefore, minerals present in whatever amount, could be more bio-available. Hence, strategies to enhance the mobilization of micronutrients from aleurone to endosperm are important for developing wheat with improved mineral bio-availability. Transcriptome analyses during grain development have revealed distinct and wide diversity of transcript abundance during grain development. It is important to investigate transcriptional profiles of aleurone and endosperm during grain development in wheat genotypes with contrasting levels of mineral accumulation in grains.

Objectives

1. To examine distribution pattern of minerals in wheat grain tissues
2. Transcriptome analysis in developing wheat grains and grain tissues
3. Identification of candidate genes for higher level, and tissue specific mineral accumulation in grains
4. Strategy to enhanced mineral bioavailability in wheat grains

Research Progress

1. Transcriptome sequencing of aleurone and endosperm tissues of developing grains (at 14 DAA) of high and low iron wheat genotypes (Figure 14) yielded a total of 2,05,159 contigs after *de novo* assembly, with an average length of 586 bp and the shortest contig of 200 bp. BLAST searches against the known sequences in public protein/EST databases on the stringent criteria of evalue<10e-5; Qcov>50%, Iden>50%; bitscore>100 identified a total of 1,01,332 contigs as annotated (Table 5)
2. The usefulness of the putative gene information was validated by analysing the expression of a few randomly selected metal relatedcontigs in aleurone and endosperm tissues of the two contrasting genotypes (Figure 15).
3. A total of 210 transcripts showed preferential expression (>2 log2 fold) in the aleurone, as compared to endosperm (Figure 16) in both the two genotypes. These putative genes could play significant aleurone specific cellular activities. The regulatory regions of the genes could be potential promoter for aleurone specific expression in wheat. However, the molecular functions of the putative genes need to be investigated in detail.
4. A total of 2089 transcripts were differentially expressed (>2 $\log_2$ fold) in the aleurone IITR26 vs WL711. Furthermore, a total of 681 transcripts were differentially expressed (>2 $\log_2$ fold) in the endosperm (IITR26 vs WL711). These transcripts could be indicative of genotype specific differences. However, the molecular functions of the contigs need to be investigated in detail.
5. The usefulness of the putative gene information was validated by analysing the expression of a few randomly selected metal relatedcontigs in aleurone and endosperm tissues of the two contrasting genotypes (Figure 17).

Salient Achievements

1. The aleurone and endosperm specific transcriptomic study identified several putative differentially expressed genes between these tissues.
2. The study would be helpful in identifying candidate genes for higher accumulation of iron in aleurone as compared to endosperm.

Future Perspectives

Candidate genes should be identified which should be targeted for the mobilization of minerals from aleurone to endosperm.

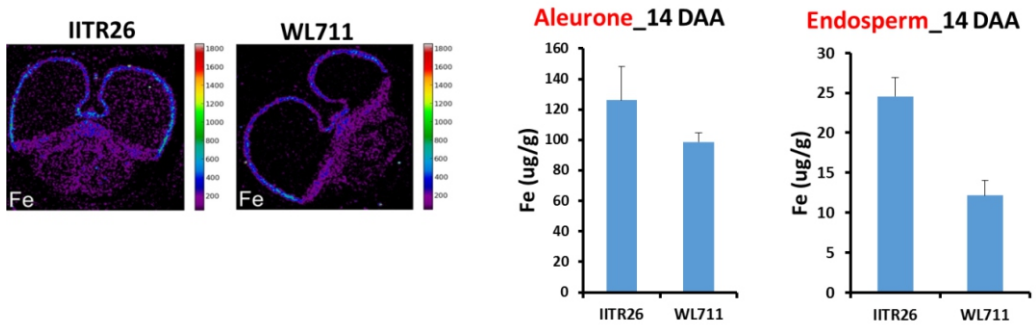

Figure 14: Contrasting pattern of iron accumulation in aleurone and endosperm of IITR26 and WL711.

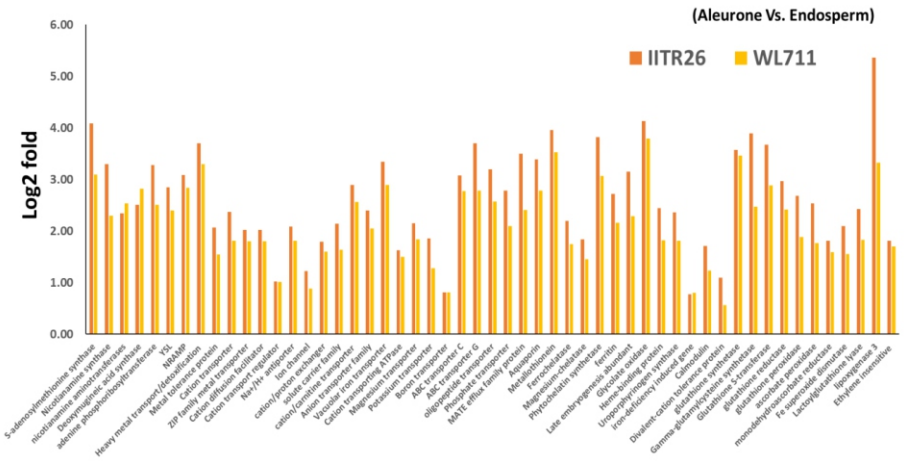

Figure 15: Comparative expression profile of metal related putative genes aleurone *vs* endosperm in high (IITR26) and low (WL711) iron wheat genotypes

Table 5: BLAST searches against public protein/EST databases

Species	Annotated contigs	Homologs to genes
Triticumaestivum(IWGSC)	46,741	34,149
Brachypodiumdistachyon	37,433	14,899
Hordeumvulgare	40,535	15,685
Oryza sativa	33,911	14,312
Zea mays	29,167	13,219
Arabidopsis thaliana	18,873	8,280
DFCI / TGI	80,622	51,890
NCBI Non-redundant	49,622	29,512
Uniprot	53139	38,026

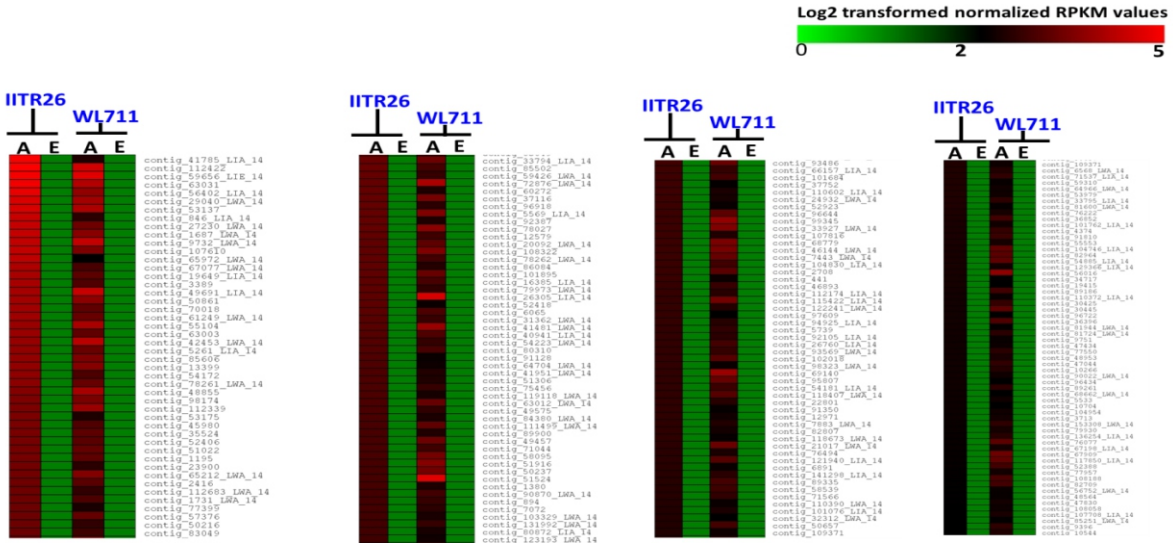

Figure16: Contigs exhibiting preferential expression in aleurone as compared to endosperm in both the contrasting wheat genotypes, IITR26 and WL711.

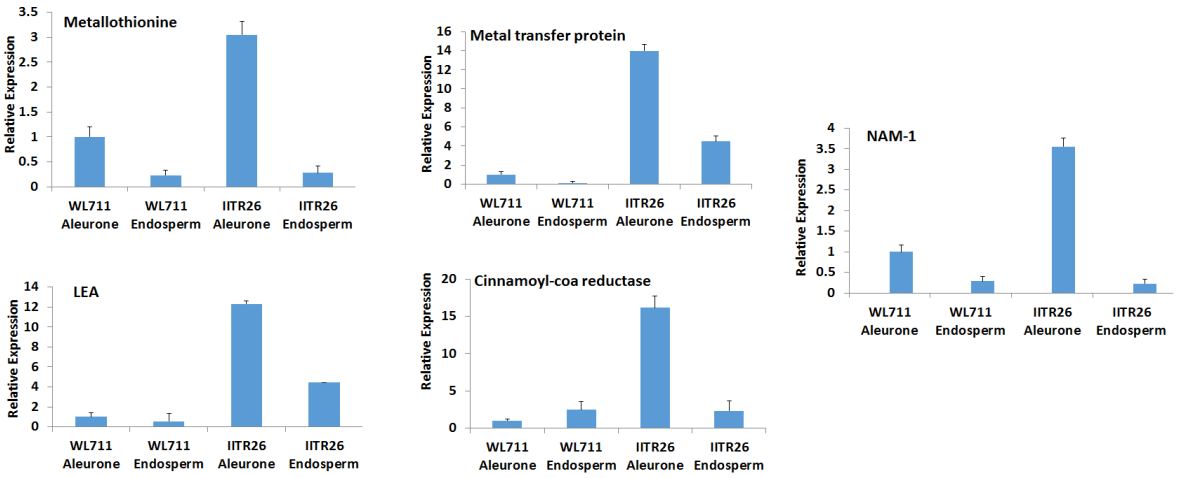

Figure 17: qRT-PCR expression analysis

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

Project funded by BIRAC for the improvement of pro-vitamin A (β-carotene) content in two Indian banana varieties cv. Grand Naine and Rasthali 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". An efficient Embryogenic Cell Suspension (ECS) culture and genetic transformation protocols for both two selected Indian banana cultivars have been optimized. At the first stage, QUT has provided the best four pro-vitamin A (PVA) Generation 2 gene constructs, containing Asupina banana derived *phytoene synthase* (*APsy2a*)gene under the control of Exp1, Ubi, ACO and BT4 promoters for the genetic transformation of Indian cultivars. Several rounds of genetic transformation experiments with four QUT gene constructs (Gen2) have been performed and desirable numbers of transgenic events have been generated for further analysis. Putative transgenic plants have been transferred into the soil-pots for acclimatization and kept under the control environment by following the DBT Biosafety Guidelines. The phenotypic observations of transgenic plants have been noted. In Sept. 2015, NABI had received Generation 3 (Gen3) constructs from QUT and distributed to Indian partner labs by following the DBT Biosafety Guidelines. We have started genetic transformation with Gen3 PVA construct at NABI.

Objectives

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

Research Progress

1. Embryogenic Cell Suspension (ECS) culture for regeneration of somatic embryos: This is round the year process to develop and maintain efficient ECS by using different lots of immature male flower buds (IMFB) for regeneration of somatic embryos (Annual Report 2013-14).
2. Genetic transformation of ECS by using reporter gene and confirmed by PCR: The efficient protocol for *Agrobacterium*-mediated genetic transformation of both banana cultivars has been optimized using pCambia1305.1/AGL1 (GUS-Intron in AGL1 strain) (Annual Report 2014-15).
3. Genetic transformation of ECS with PVA gene constructs (Gen 2) received from QUT:Desirable numbers of transgenic plants of Rasthali and Grand Naine have been developed with all four constructs. These plants are being maintained in soil-pots, rooting and germination medium. Several transformation experiments were performed to generate independent and desirable number of events (Figure 1).
4. PCR screening of putative transgenic plants (Gen2):The putative transformants generated by four constructs were randomly selected and screened by PCR. An additional primer set of *VirC* gene of *Agrobacterium tumefaciens* was also used during the PCR screening of transgenic

plants to eliminate the possibility of *Agrobacterium* contamination in the tissue sample. The selected transgenic plants have shown appropriate amplicons for promoter-gene specific primers. We did not find any *Agrobacterium* contamination in the transgenic plant samples.

5. Transgenic plants growth and phenotype observation: Several putative transgenic plants have been transferred into the soil-pots. These plants are transferred in the plant growth chambers and newly constructed climate controlled transgenic house (Figures 2 and 3). We noted normal phenotype and normal growth of most of the transgenic plants. However, some of the lines developed with construct Ubi>*APsy2a*, where ubiquitin promoter regulated the expression of *APsy2a* gene have shown golden color leaf phenotype (Figures 2 and 3). This might be due to very high constitutive expression of *APsy2a* and subsequently very high deposition of carotenoid in the leaves.
6. Genetic transformation of ECS with new PVA gene construct (Generation 3) received from QUT: Generation 3 QUT gene construct named as DC49 (MT2a>DXS + MTw2A>APsy2a) has been received from QUT in Sept. 2015. Genetic transformation of ECS of Rasthali and Grand Naine with this construct has been started from Oct. 2015. Transformed ECS lines are incubating in the regeneration medium for further growth and selection of positive events.

Salient Achivements

1. ECS culture is routinely utilized for genetic transformation with gene constructs (Gen2) received from QUT.
2. Several transformation experiments have been performed to generate desirable numbers of transgenic lines with each constructs.
3. PCR screening of selected lines have been performed.
4. Acclimatized plants are being marinated and grown in soil-pots for healthy growth.
5. Phenotypic observations of transgenic plants were noted.
6. Gen3 QUT gene constructs have been received from QUT and transformation of ECS with Gen3 PVA construct named as DC49 (MT2a>DXS + MTw2A>APsy2a) has been started to generate desirable number of events for further analysis.

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.

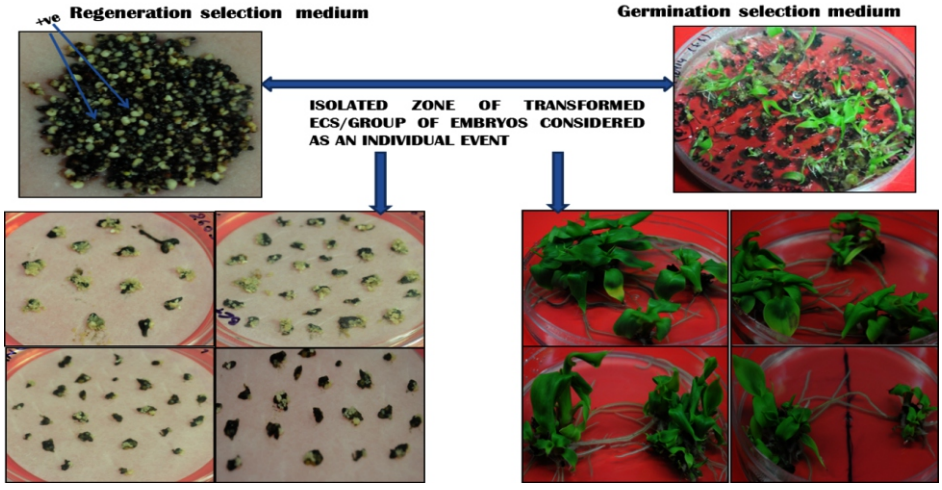

Figure 1: Visual observation of *in-vitro* regeneration, germination and root formation of transgenic banana plants on kanamycin selection medium.

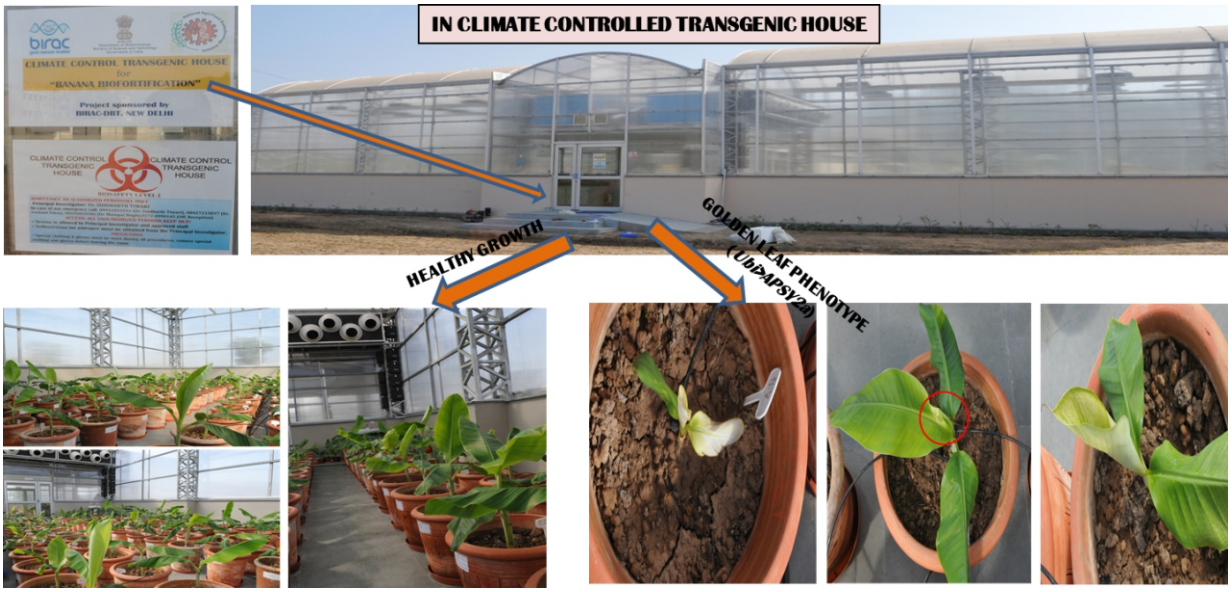

Figure 2: Acclimatization and phenotypic observation of transgenic plants in Growth Chambers.

Figure 3: Phenotypic observation of transgenic plants growing in big-soil pots under the newly constructed Climate Controlled Transgenic House at NABI.

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

Principal Investigator
Siddharth Tiwari

Project Scientist
Ashutosh Pandey

Project Fellows
Shivani
Navneet Kaur

Introduction
The scope of BIRAC funded research work is limited to the gene constructs provided by the QUT while no leads are currently available on the prospective results in terms of enhanced expression level of PVA and ultimately the bioavailability in Indian population. Thus, we initiated exploratory work with the objective to understand the regulatory mechanism of carotenoid biosynthesis in banana. The knowledge generated by these analyses would be implemented to develop improved Indian banana with enhanced content of carotenoids.

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

Research Progress
1. Suckers of around thirty established banana cultivars have been collected from different places of India and grown at NABI research field for establishing germplasm (Annual Report 2014-15).
2. Genome-wide *in-silico* analysis of MVA, MEP and carotenoid pathways genes in banana was performed. Total sixteen putative genes were identified by systematic screening from the database of the Banana Genome Hub (http://banana-enome.cirad.fr/). Phylogenetic analysis shows that banana proteins are similar with the proteins involved in the carotenoid and related biosynthesis pathways in maize, rice and arabidopsis (Annual Report 2014-15).
3. Fruit-pulp collected at the ripening stage of ten Indian banana cultivars from the field germplasm plot at the NABI, Mohali was lyophilized into dry weight (DW) and analysed for carotenoids. The highest β-CE was observed in Nendran (2143.65 µg/100g DW) while the lowest was found in Rasthali (112.53 µg/100g DW) (Table 1).
4. Carotenoid profiling during banana fruit development: The contrasting cultivars, Nendran and Rasthali cultivars were studied for changes in carotenoids in fruit-peel and -pulp as collected at unripe and ripe stages (Figure 1). Lutein, α-carotene and β-carotene were quantified, whereas lycopene and zeaxanthin were not present in detectable amounts in the tissues. In the pulp tissue, the highest content of all the carotenoids was noticed at the ripening stage of Nendran. Nearly 19-fold higher β-carotene was present in Nendran (1836.81 ± 152.76 µg/100g DW) as compared to Rasthali (97.05 ± 6.75 µg/100g DW) (Figure 1). Similarly, lutein and α-carotene were nearly 2- and 20-fold higher in Nendran as compared to Rasthali. Unripe pulp of both Nendran and Rasthali showed significantly low quantities of all carotenoids, compared to the ripe pulp (Figure 1). However, in the peel as compared to the pulp of both the cultivars, higher deposition of all carotenoids (except at the ripening stage of Nendran) was noticed (Figure 4). The greater deposition of α-carotene and β-carotene was found in ripe pulp of Nendran as compared to its ripe

peel.

5. Quantitative real-time PCR analysis of genes during fruit development: The differential fold expression of the selected 16 genes was studied in fruit-pulp (Figure 5a) and -peel (Figure 5b) at two developmental stages (unripe and ripe) to understand transcriptional regulation in the carotenogenesis in fruit tissues of two contrasting cultivars. The normalization of transcripts was carried out against *ubiquitin2* that showed most stable expression at various fruit development stages.

i. In the pulp, higher expression of *Ma-hmgr1* and *Ma-hmgr2* was observed at unripe and ripe stages of Nendran and Rasthali, respectively. The expression of *Ma-hmgr1* was not detected in the unripe and ripe pulp of Rasthali while the lower expression of *Ma-hmgr1* and *Ma-hmgr2* was found in the ripe pulp of Nendran (Figure 5a). In the peel, higher expression of *Ma-hmgr1* and *Ma-hmgr2* was noted at the ripe stage of Rasthali and Nendran, respectively. On the contrary, the lower expression of *Ma-hmgr1* and *Ma-hmgr2* was detected at the ripe and unripe stages, respectively of Nendran (Figure 5b).

ii. Three out of four *Ma-dxs* (*Ma-dxs1*, *Ma-dxs2* and *Ma-dxs4*) were expressed in fruit-pulp and -peel of both the cultivars whereas the transcript of *Ma-dxs3* was not detected. In the pulp, higher expression of *Ma*-dxs1 and *Ma*-dxs4 was noticed at the unripe stage of Nendran and the lower was recorded in ripe stage of Rasthali (Figure 5a). In the peel, higher expression of *Ma-dxs1* wasobserved at the ripe stages of Nendran and Rasthali. The higher expression of *Ma-dxs2* and *Ma-dxs4* was notedat the unripe stage of Nendran. However, the lower expression was observed in the unripe peel of Nendran (for *Ma-dxs1*)and in the ripe (for *Ma-dxs2* and *Ma-dxs4*) peel of Rasthali (Figure 5b).

iii. In the pulp, higher and lower expression of *Ma-dxr* was noted at ripe stage of Nendran and unripe stage of Rasthali, respectively (Figure 5a). In the peel, the higher and lower expression of *Ma-dxr* wasfound at unripe and ripe stages of Rasthali, respectively (Figure 5b).

iv. All four homologs of *Ma-psy* were expressed in the pulp, whereas only *Ma-psy1, Ma-psy2* and *Ma-psy4* were expressed in the peel of both the cultivars. In the pulp, higher expression of all four *Ma-psy* was observed at the ripening stage of Nendran. The relatively low expression pattern of all *Ma-psy* was found in unripe pulp of both cultivars and ripe pulp of Rasthali (Figure 5a). In the peel at the unripe stage, the higher expression of *Ma-psy4* was recorded in Nendran and that of *Ma-psy1* in Rasthali. The relative expression of all three *Ma-psy*was low in the ripe peel of both Rasthali and Nendran cultivars (Figure 5b).

v. In the pulp, the higher expression of *Ma-pds*, *Ma-zds* and *Ma-crtiso* was noticed in the ripe stage of Nendran, while these were lower at unripe stage of Rasthali (Figure 5a). In the peel at unripe stage, the higher expression of *Ma-pds* and *Ma-crtiso* was observed in Rathali and of *Ma-zds* in Nendran, while the ripe peel was recorded to have the lower expression of *Ma-zds* and *Ma-crtiso* in Rasthali and that of *Ma-pds* in Nendran (Figure 5b).

vi. In the pulp, the higher expression of *Ma-lcyb* and *Ma-lcye* was found at the ripe stage of Nendran, while the lower was observed at the unripe stage of Rasthali (Figure 5a). In the peel, the higher expression of *Ma-lcyb* and *Ma-lcye* was perceived at the ripe stage of Rasthali. On the contrary, the lower expression of *Ma-lcyb* and *Ma-lcye* was detected in unripe peel of Rasthali and Nendran, respectively (Figure 5b).

Salient Achievements

1. Banana cultivars have been collected from different places and being maintained at NABI research field for establishing germplasm.
2. *In-silico* analysis of genes involved in key enzymatic steps in MVA-, MEP- and carotenoid-biosynthetic pathways in

banana have been performed.

3. Biochemical analyses to identify contrasting cultivars were performed.
4. Carotenoid profiling in contrasting cultivars, Nendran and Rasthali were studied for changes in carotenoids in fruit-peel and -pulp as collected at unripe and ripe stages.
5. The differential fold expression of the selected 16 genes was studied in fruit-pulp and -peel at two developmental stages (unripe and ripe) to understand ranscriptional regulation in the carotenogenesis in fruit tissues of two contrasting cultivars.

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.

Table 1. Carotenoids (µg/100g DW) in ripe fruit-pulp of Indian banana cultivars.

S.No.	Musa cultivars	Genome	Fruit Type	β-Carotene	α-Carotene	β-Carotene equivalents*	Lutein	Zeaxanthin	Lycopene
1	Ney Poovan	AB	Dessert	166.56 ± 8.56	111.96 ± 2.32	222.54	272.27 ± 8.65	ND	ND
2	Grand Naine	AAA	Dessert	422.66 ± 90.02[a]	215.66 ± 16.45[b]	530.49	176.00 ± 17.64	ND	ND
3	Red Banana	AAA	Dessert	438.00 ± 14.00[a]	243.00 ± 19.00[b]	559.50	2319.00 ± 186.72[b]	ND	ND
4	Manoranjitham	AAA	Dessert	179.81 ± 16.62	54.50 ± 5.50	207.06	69.50 ± 8.50	ND	ND
5	Robusta	AAA	Dessert	192.00 ± 13.00	82.50 ±6.27	233.25	249.92 ± 9.02	ND	ND
6	Rasthali	AAB	Dessert	97.05 ± 6.75	30.96 ± 4.23	112.53	219.65 ± 17.77	ND	ND
7	Nendran	AAB	Cooking	1836.81 ± 152.76[b]	613.68 ± 55.03[b]	2143.65	482.55 ± 13.20	ND	ND
8	Poovan	AAB	Dessert	930.62 ± 38.86[b]	278.26 ± 57.43[b]	1069.75	570.57± 123.12	ND	ND
9	KachaKola	ABB	Cooking	171.84 ± 14.52	112.81 ± 2.84	228.25	222.00 ± 13.54	ND	ND
10	Karpuravalli	ABB	Dessert	134.02 ± 12.35	16.31 ± 3.48	142.18	162.76 ± 28.00	ND	ND

*β-Carotene equivalents calculated by the sum of β-carotene plus one-half of α-carotene.

Values represent mean of carotenoid content ± SD. Prism GraphPad software (GraphPad Software Inc., San Diego, CA, USA) was used to compare the mean by Dunn's *post-hoc* test. Statistical significant was checked at $p \leq 0.05$ (significant) denoted as lowercase letter [a] and $P \leq 0.01$ (highly significant) denoted as lowercase letter [b] with respect to the Rasthali. The values without letter marked are not significantly different.

DW, Dry weight; ND, Not detected

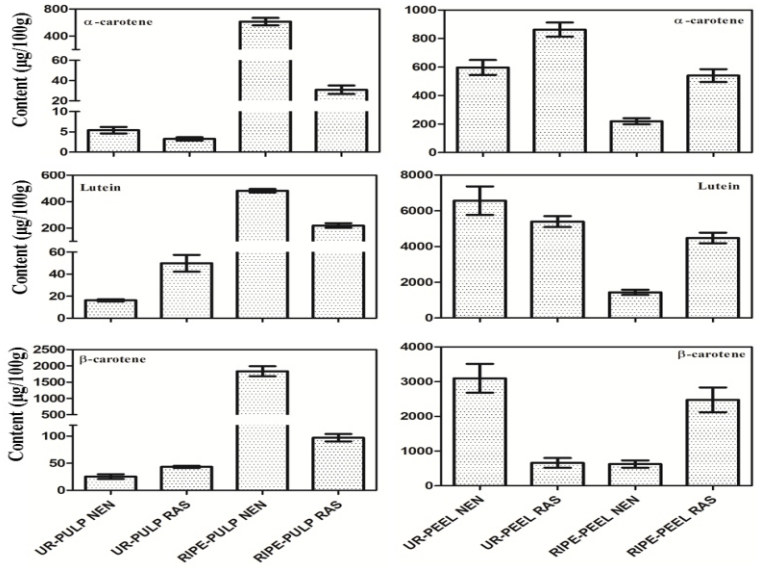

Figure 4: Quantitative assessment of carotenoids in fruit tissues of Nendran and Rasthali banana cultivars. Carotenoid profiling in ripe (RIPE) and unripe (UR) fruit tissues.Bars denote mean of carotenoid content ± SD. Statistical analysis was performed using one way ANNOVA followed by Dunn's *post-hoc* test. Statistical significance was checked at $p \leq 0.05$ (* significant) and $p \leq 0.01$ (** highly significant) with respect to the UR-PULP/UR-PEEL RAS versus UR-PULP/UR-PEEL NEN and RIPE-PULP/RIPE-PEEL RAS versus RIPE-PULP/RIPE-PEEL NEN. Compounds were quantified by separating extracts from the peel and pulp using HPLC.

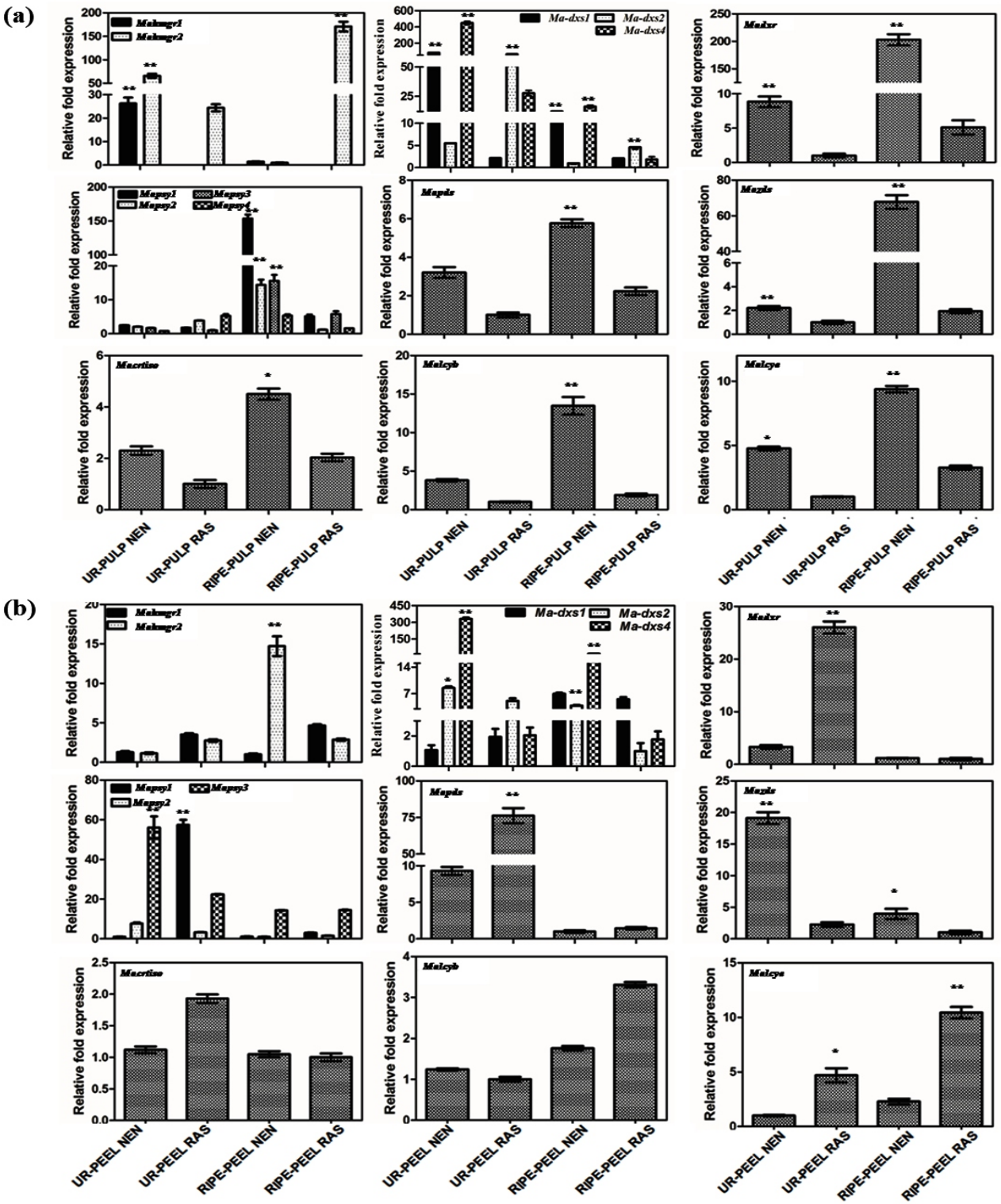

Figure 5: Spatiotemporalreal-time PCR expression analysis of 16 genes involved in the MVA-, MEP- and carotenoid-biosynthetic pathways in banana fruit.Transcript expression profiles are presented in ripe (RIPE) and unripe (UR) fruit-pulp (a) and -peel (b) of Nendran and Rasthali cultivars. The gene expression was normalized with reference to *ubiquitin2* taken as internal control. Bars denote mean fold expression as compared to the lowest expressing gene in the group of samples ± SD. Statistical analysis was performed as described in Figure 1.

2.3Post-harvest improvement in quality of produce:Application of dietary fibers as edible fruit coating materials

Principal Investigator
Koushik Mazumder

Research Fellow
Usman Ali

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

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

Research Progress
1. Arabinoxylan (AX) and β-glucan (BG) were isolated from wheat straw and oat bran with yield of 18-20% and8.5-10% respectively. The sugar compositional analysis showed AX contained arabinose and xylose as major constituent with about 90% purity, whereas BG contained glucose as major constituent with about 80% purity.
2. The BG was conjugated with various fatty acids to improve hydrophobicity and moisture barrier properties. The IR spectroscopic analysis of the conjugated BGs showed sharp stretching peak in the region of 1710-1740 cm^{-1} suggested the presence of C=O group of carboxyl ester of fatty acid moiety.
3. The films were prepared by blending AX with fatty acid conjugated BGs. The water vapour transmission rate (WVTR) analysis showed addition of fatty acid conjugated BGs to AX reduced the water vapour transmission by 1.5-5.0 folds with respect to control (AX + un-derivatized BG).
4. The SEM analysis suggested that micro-structural arrangements of film components played a more relevant role controlling the water vapour transmission properties, the films with laminate like structures comprising greater successive layers contributed more effectively to reduce the water vapour transmission.
5. The detail studies on optical, physical and mechanical properties of the coating materials are in progress.

Salient Achievements
1. The oat β-glucan chemically modified and blended with arabinoxylan from wheat straw to improve moisture barrier properties.
2. SEM analysis revealed films comprising greater successive layers contributed more effectively to reduce the water vapour transmission, thus improved the water

barrier properties.

Future Perspectives

Application of the coating materials on various fresh fruits to prolong their shelf life and development of effective coating materials.

BASIC BIOLOGY FOR CROP IMPROVEMENT

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

Principal Investigator
Vikas Rishi

Research Fellow
Prateek Jain

Introduction

BZIP family of transcription factors plays a pivotal role in all fundamental aspects of growth and development in plants including seed formation and maturation. BZIP10, BZIP25, and BZIP53 are known to regulate the expression of genes involved in embryo and seed formation and thus are valid molecular targets to understand basic biology of seed formation. Structurally all BZIPs are similar and are represented by a typical coiled coil motif. BZIPs bind to DNA either as homo-or heterodimer. It is our endeavour to understand the factors that are responsible for the stability as well as the specificity of these BZIPs transcription factors when they homo- or heterodimerize. Knowledge gained from studying the wild type BZIPs for their ability to form homo-and heterodimer has enabled us to design dominant-negative proteins that inhibit the binding of wild type transcription factors. Unlike other gene knock-out technologies, dominant-negatives can inhibit the function of all members of a family or related families.

Objectives

1. To design repertoire of dominant-negative proteins against BZIP10, BZIP25, and BZIP53, each with different heterodimeric stability and specificity.
2. To test the *in vivo* efficacy of designed dominant-negative(s) and their ability to inhibit the activities of wild type BZIP transcription factors.

Research progress

1. Previously using *in vitro* biochemical and biophysical techniques we characterized the dominant-negative protein A-ZIP53 and its ability to preferentially heterodimerizes with other B-ZIP transcription factors involved in *A. thaliana* seed development and maturation.
2. Our excruciating studies showed that A-ZIP53 heterodimerizes with BZIP53, BZIP25, and B-ZIP10 and inhibits their DNA binding activities. A-ZIP53 is specific is highlighted by the fact that it does not heterodimerizes with BZIP39 or BZIP72, the other two prominent BZIPs involved in seed formation. A series of A-ZIP53 mutants were designed and characterized for their ability to form strong heterodimers. One surprising result was the heterodimerizing properties of A-ZIP53 (A-E) mutant where alanine at *g* position of -4 heptad of DNA binding domain was substituted by a glutamic acid. A-ZIP53 (A-E) dominant negative preferentially dimerizes with BZIP25 but does not interact with either BZIP53 or BZIP10 making it specific for BZIP25 (Figure1).

3. Recently we have started to examine the efficacy of A-ZIP53 dominant negative proteins *in vivo*. *A thaliana* leaf protoplasts were used for transient expression of BZIP10/25/52, and A-ZIP53. We used two/three plasmid system to understand the functionality of A-ZIP53 *in vivo* (Figure 2). First plasmid has GUS reporter gene under the control of albumin 2S2 promoter. This promoter has a unique G-Box, a known binding site for BZIP transcription factors used in this study. Expression of all BZIPs and A-ZIP53 was under the control of CMV promoter.
4. Protoplasts were transiently transfected with GUS reporter plasmid and BZIPs (10, 25, 53) encoding plasmids. Compared to the no plasmid control GUS activity was enhanced by the expression of all three BZIPs used in this study. When cells were co-transfected with third plasmid

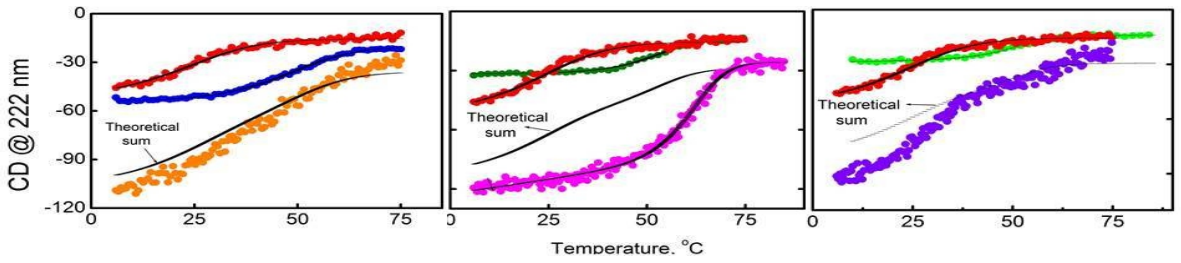

Figure 1: Dimerization studies of BZIPs with A-ZIP53 (A-E) mutant. Left panel shows the thermal denaturation of BZIP10 (●), A-ZIP53 (A-E) (●) and their equimolar mixture (●). Middle panel shows the thermal denaturation of BZIP25 (●) A-ZIP53 (A-E) (●) and their equimolar mixture (●). Right panel shows the thermal denaturation of BZIP53 (●), A-ZIP53 (A-E) (●) and their equimolar mixture. A-ZIP53 (A-E) mutant strongly interacts with BZIP25 whereas it fails to heterodimerize with BZIP10 and BZIP53.

expressing dominant-negative A-ZIP53, there was a marked decrease in GUS activity. We interpret these results as the testimony of AZIP53 ability to heterodimerizes with all three BZIPs resulting in the inhibition of their DNA binding activities (Figure 3).

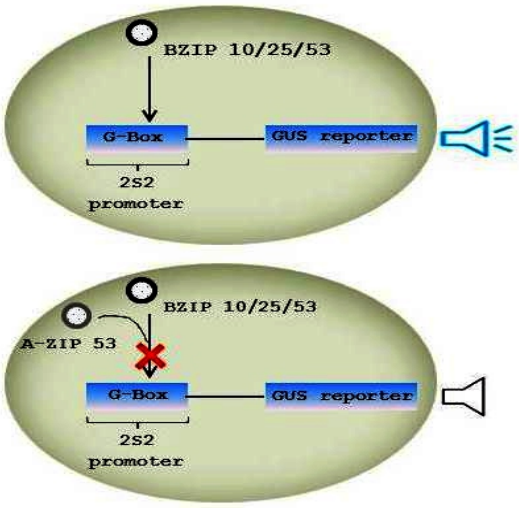

Figure 2: Schematics of transient transfections studies using *A thaliana* leaf protoplasts system. GUS reporter activity under the control of 2S2 promoter was measured by overexpressing BZIP10, BZIP25, and BZIP53 (upper panel). A-ZIP53 overexpressing plasmids were used to modulate the DNA binding activities of BZIPs (lower panel).

Salient Achievements

1. Stability of AZIP53 and its six mutants were studied. A-ZIP53 was chosen for further *in vivo* study.
2. Transient transfection studies showed the *in vivo* efficacy of A-ZIP53.

Future Perspectives

1. Transgenic Arabidopsis plants will be generated and characterized.
2. We expect a stronger seed phenotype since we are aiming to inhibit the activities of number of structurally and functionally seed-specific BZIP transcription factor.

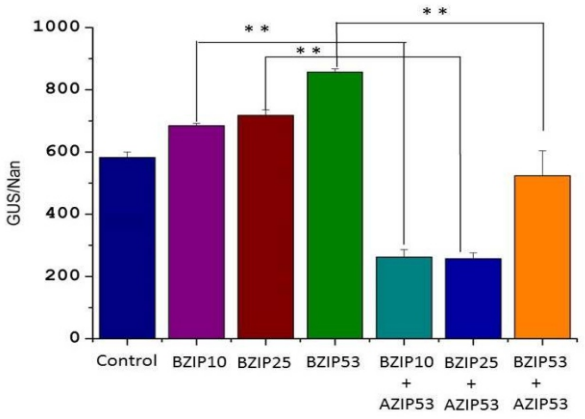

Figure 3: Transient transfections showing the *in vivo* activity of A-ZIP53. GUS reporter gene activity was used to measure the DNA binding activities of three BZIPs. Nan was used as an internal control and all values were normalized to that of Nan activity. BZIPs DNA binding was inhibited in presence of A-ZIP53. Decrease in GUS/Nan was statistically significant at $p<0.01$ (**).

3.2 Identification of bread wheat seed-specific BZIP transcription factors binding sites by genome-wide in vitro binding analysis

Principal Investigator
Vikas Rishi

Research Fellow
Koushik Shah

Introduction

Deciphering of hexaploid bread wheat (*Triticum aestivum*) genome and transcriptome has led to spurt in transcription biology and gene regulation research. Based on signature sequences bread wheat have approximately 165 BZIP transcription factors. A number of them are previously known to regulate expression of genes involved in abiotic stress, pathogen defense, and seed development. Studies using Arabidopsis and other plants revealed that seed-specific BZIP transcription factors are active in different parts of tissues at the unique time of seed development. A prerequisite to understanding how these BZIPs regulate gene expression is to know their genome-wide binding. In wheat,DNA binding sites of most of the seed-specific BZIP transcription factors are not known. Chromatin immunoprecipitation, a gold standard for deciphering genome-wide binding of a transcription factor has limited use in bread wheat because of the non-availability of anti-sera. To overcome this shortcoming we have adapted a novel approach known as "Bind-N-Seq" to get insight into genome-wide binding of a specific BZIP transcription factor. A schematic of protocol is given below (Figure 4)

Objectives

1. To design ligation-independent cloning (LIC) vectors for high-throughput cloning of wheat BZIP transcription factors.
2. *In vitro* identification of genome-wide DNA binding sites of seed-specific BZIP transcription factors.

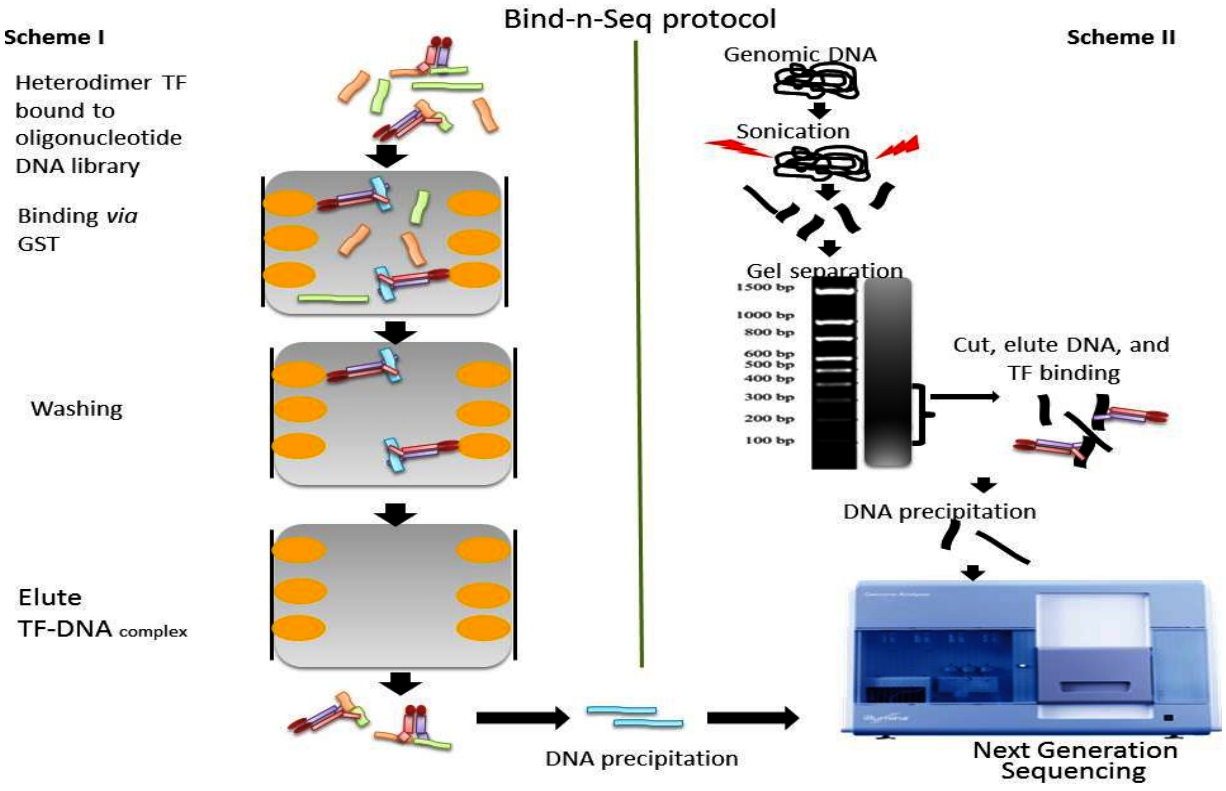

Figure 4: Schemes I, and II showing "Bind-N-Seq" protocol.

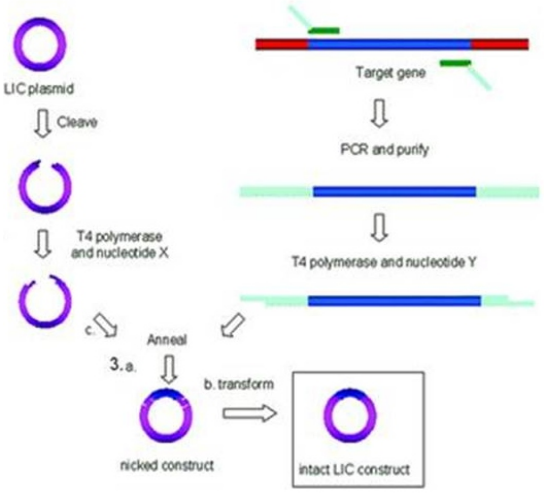

Figure 5: Cartoon showing LIC vector development.

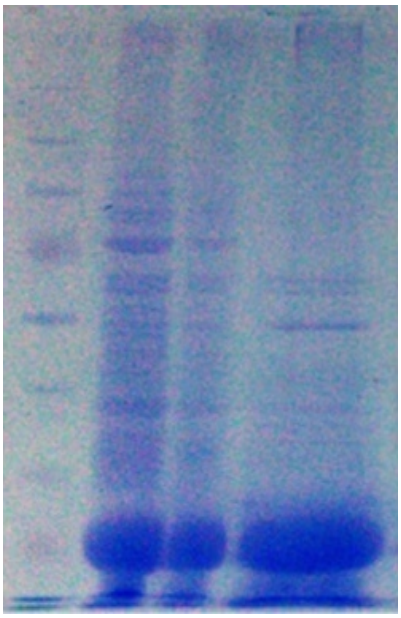

Figure 6: SDS PAGE showing the expression of conventially cloned (lane 2 and 3) and LIC cloned A-CREB protein (lane 4). Lane 1 has protein marker. Similar amount of protein is expressed using either of the vector.

Research Progress

For high-throughput cloning of wheat BZIP proteins we decided to develop ligation-independent cloning vectors usingPET based pT5 and GST vectors.Strategy to develop LIC is shown in the following figure (Figure 5).

The efficiency of LIC vectorwas tested by expressing A-CREB protein. A-CREB expression by LIC- cloned ORF was comparable to conventionally cloned gene in pT5 vector allowing us to use LIC plasmid for high-throughput cloning and expression of BZIP proteins.

We have identified seed-specific BZIP transcription factors based on their elevated expression levelsduring seed development and maturation stages. Four wheat BZIPs, HBP-1b, ABI-5, ABFBand TaBZIP1 were cloned. HY5, a seed-specific Arabidopsis BZIP was also cloned and will be used to validate "Bind-N_Seq" method.

Salient Achievements

Successful cloning of four seed-specific BZIP transcription factors in LIC vectors and subsequent expression in BL-21 Lys E stain.

Future Perspectives

HPLC ultra-pure protein samples will be used for "Bind-N-Seq". DNA sequences pulled down by specific BZIP transcription factor will be sequenced using NGS platform.

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3.3 Biology of seed development in custard apple and litchi

Principal Investigators
Sudhir Pratap Singh
Shrikant Subhash Mantri

Research Fellows
Ashish K Pathak
Yogesh Gupta

Introduction

Seedlesssness is a desirable trait in fruit crops as it avoids the presence of hard seeds of sometimes bitter taste, and may enhance the content of pulp in edible fruits. We have collected the natural accessions of fruit crops with contrasting fruit seed number (*Annona squamosa*) and seed size (*Litchi chinensis*). In order to gain molecular insight into seed development in the genotypes with contrasting seed related traits, we are studying the transcriptional pattern in the developing fruits and/or ovules of bold- and small seeded genotypes.

Objectives

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

Research progress

1. After generating the first early-stage fruit specific transcriptome sequence resource for *Annona squamosa*, deep sequencing was performed from the RNA-seq libraries prepared from the early-stage developing fruits of two *Annona squamosa* genotypes, with contrast in seed number. The differential expression analysis is in progress.
2. In case of *Litchi chinensis*, transcriptome sequencing of bold- and small-seeded ovule specific libraries at 0, 6 and 14, yielded 1,19,939 contigs after *de novo* assembly. These contigs represented 87,072 unique transcripts with possible splice variants. When compared (*E*-value 10^{-5}) against several public databases (NCBI non-redundant (NR), Swiss-Prot protein database, and Cluster of Orthologous Groups (COGs)*Citrus sinensis*, *Populus trichocarpa*, *Ricinus communis*, *Glycine max*, *Fragaria vesca, Carica papaya*, *Vitis viniferaArabidopsis thaliana*), a total of 68,988 (57.51%) contigs, including their possible splice variant, did not match with any protein. These many contigs could be specific to *Litchi chinensis*.
3. Differences in the expression pattern of transcripts, between the early-stage ovules of bold- and small-seeded genotypes, were investigated. The most distinct expression profile was noticed between the ovules of bold- (HC0) and small-seeded (HS0) genotypes at 0DAA, exhibiting 7,946 differentially expressed contigs ($\log_2$ fold change ≥2 and pvalue ≤ 0.001) (Figure 7). The results suggest the domination of maternal factors in the regulation of bold or small seed trait in litchi.
4. The BLASTx searches revealed a total of 2,331 hormone related putative genes in the transcriptome of the developing ovules. The maximum representation was of the transcripts related to abscisic acid pathways followed by auxin in the ovule transcriptome data. A total of 430, 50 and 137 transcripts were differentially expressed (HC vs HS) at 0, 6 and 14 DAA, respectively. The transcripts related to auxin and brassinosteroid biosynthesis were down-regulated in small-seeded genotype at 0 and 6 DAA (Figure 8). Down-regulation of auxin transport in embryo sac may affect megagametogenesis and embryo patterning.The putative transcripts for auxin efflux carrier family protein such as PIN1 (AT1G73590), PIN6 (AT1G77110) and

EIR1 (AT5G57090), involved in the maintaince of auxin gradient in embryo sac, were down-regulated in the developing ovules of small-seeded litchi. The auxin transporter LAX2 (AT2G21050) regulates vascular development. It was repressed in small-seeded ovules. Sterol methyltransferase 2 is involved in sterol biosynthesis and loss of function leads to defective cotyledon growth. DWARF1 (AT3G19820) is involved in the conversion of brassinosteroid precursor 24-methylenecholesterol to campesterol, and loss of function induces dwarf phenotype, sterility and seed abortion. Interestingly, we found relatively repressed expression of the putative genes for sterol methyltransferase 2 and DWARF1 in the developing ovules of small-seeded litchi.

5. Transcription factors (TFs) are key regulators of spatial, temporal and quantitative gene expression. The BLAST analysis revealed a total of 2,155 putative TFs involved in early-stage ovule development in bold- and small-seeded litchi.The MYB family's TFs were most abundant in the ovule transcriptome data of litchi. A total of 426, 33 and 125 TF related contigs were differentially expressed (bold seeded vs small seeded) at 0, 6 and 14 DAA, respectively. The expression of YABBY, WRKY, LSD, HD-ZIP and EIL family TFs was up-regulated in developing ovules of small-seeded litchi. The TF families, ARF, CPP, Dof, E2F and GRF, were down-regulated in small-seeded genotype. ARFs activate or repress genes in response to auxins. The A-class homeotic gene APETALA2 (AP2), a floral homeotic TF, is known to negatively regulate seed size but promote integument growth. AP2 was up-regulated in small-seeded genotype at 14 DAA. This is in agreement with the development of fruits with under developed seed and well developed aril of integument origin.
6. The expression pattern of several embryogenesis related genes was investigated in the two contrasting litchi genotypes. Ole e 1 allergen and extensin family protein (AT2G34700), involved in pollen tube guidance during fertilization, were down-regulated in the developing ovules of small-seeded litchi. Xyloglucan endotransglucosylase / hydrolase 23 (AT4G25810), involved in cell wall development and pollination was down-regulated in small-seeded ovules. The flavin binding monooxygenase (AT5G25620) which provides ROS tolerance during pollination was down regulated in small seeded genotype. Expression of Topless (AT3G16830) was suppressed in small-seeded genotype. In the loss of function mutant of this gene, axis establishment is severely affected. N-acetyl – glucosaminidase / CYC 1 (AT5G13690) was down-regulated at 0 and 6 DAA ovules in small-seeded genotype. Mutation in the gene encoding Shrunken Seed protein (AT2G45690), which is involved in peroxisome assembly and protein trafficking, leads to shrunken seeds was down-regulated in small-seeded genotype. Thus, unusual expression pattern of several key genes involved in early-stage embryo development leads to under-developed embryo, resulting into development of small seed size in litchi.

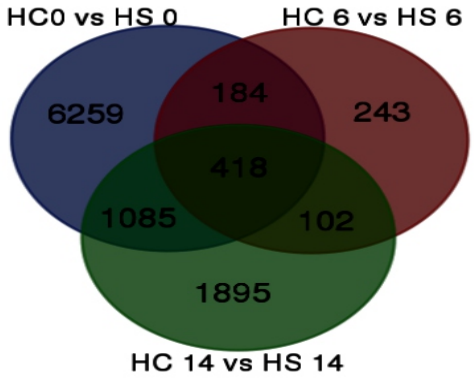

Figure 7: Venn diagram showing the total number of the differentially expressed transcripts and overlap between bold- (HC) and small- seeded (HS) ovules at 0, 6, and 14 DAA (≥ 2 $\log_2$ fold; P-value ≤0.001)

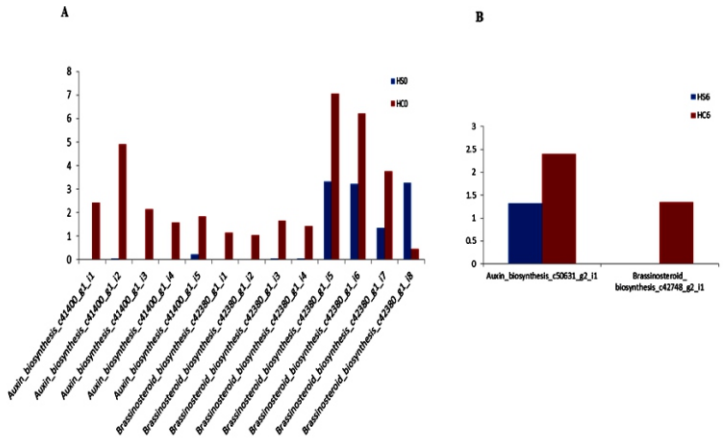

Figure 8: Histogram representing expression of differentially expressed (fold change ≥ $\log_2$ (2); Pvalue ≤0.001) hormone related putative genes in early ovule developmental stages of bold-seeded (HC) vs small-seeded (HS) litchi genotype. A) Log_2 FPKM of putative auxin and brassinosteroid biosynthesis transcripts at 0 DAA. B) Log_2 FPKM of putative auxin and brassinosteriod biosynthesis transcripts at 6 DAA.

Achievements

1. We have generated the first ovule specific transcriptome of litchi, for two cultivars contrast in terms of seed size.
2. We have identified various differentially expressed homologous genes associated with hormones, transcription factors, ovule identity determination, and embryogenesis, which could be key factors for the development of fruits with bold or small seeds in litchi.

Future Perspectives

1. Examination of transcriptional changes during mid and late phases of ovule development in litchi.
2. Differential expression analysis in early-stage developing fruits of *Annona squamosa.*
3. Identification and characterization of candidate genes for seedlessness in fruit crops

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3.4 Development of approach for the modulation of trait through long distance signalling

Principal Investigators
Sudhir Pratap Singh
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Research Fellows
Anita Kumari

Introduction
Grafting is a well-established practice to facilitate asexual propagation in horticultural and agricultural crops. This method provides a platform for studying molecular aspects of root-to-shoot and/or vice-versa signalling events. The research project involved in establishment of long-distance transmission of mobile signals (siRNAs) to achieve gene silencing in flowering tissues. It is desirable to graft the wild scion on the developed transgenic rootstocks for the delivery of the siRNAs from the rootstock for the improvement of economically important traits such as induction of seedlessness in the scion.

Objectives
To establish an approach for inducing seedlessness in the wild scion through the transmission of siRNAs from the modified rootstock.

Research Progress

1. Double transformed lines, expressing *uid*A gene and siRNAs against *uid*A gene,were developed. The scion expressing *uid*A gene was grafted onto the double transformed rootstock. Severe silencing of *uid*A gene was observed in scion by the siRNAs delivered from the rootstock (Figure 9).

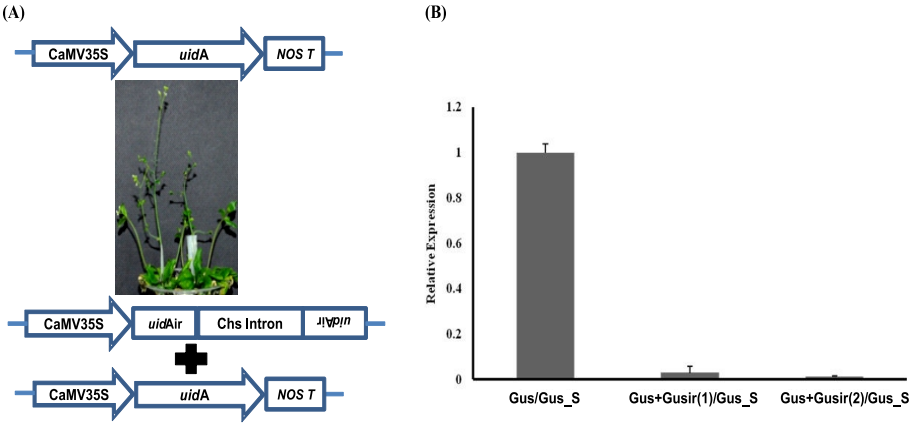

Figure 9: Silencing of *uid*A reporter gene in the developing flower buds of scion by delivery of siRNAs against *uid*A from double transformed rootstock. (A) Grafting of reporter gene (*uid*A) expressing scion onto the double transformed plant as rootstock, which expresses *uid*A gene as well as siRNA against it. (B) The bar diagram represents relative gene expression of *uid*A gene in the developing flower buds of scion by RT-PCR. GUS/GUS_S: *uid*A gene expressing scion grafted onto the rootstock expressing *uid*A gene; GUS+GUSir(1,2)/GUS_S: *uid*A gene expressing scion grafted onto the two different lines rootstock expressing *uid*A gene as well as siRNA against it.

2. Wild scion was grafted on the transgenic rootstock expressing siRNAs against INO gene which plays important role in ovule development. Phenotype of developing ovules and mature seeds was examined in the scion and control plants (Figure 10).

2. Seed setting was severly affected in the wild scion grafted on the siRNA expressing rootstock against *INO* gene. We observed 40-50 % of seedlessness in the wild scion grafted onto the rootstock expressing siRNAs against *INO* gene.

Salient Achievements

1. We experienced significant silencing of reporter gene (*uid*A) gene in the scion by the delivery of siRNAs from the double transformed rootstock.

Future Perspectives

Validation of the silencing of *INO* gene in scion by the siRNAs delivery of rootstock, at molecular level.

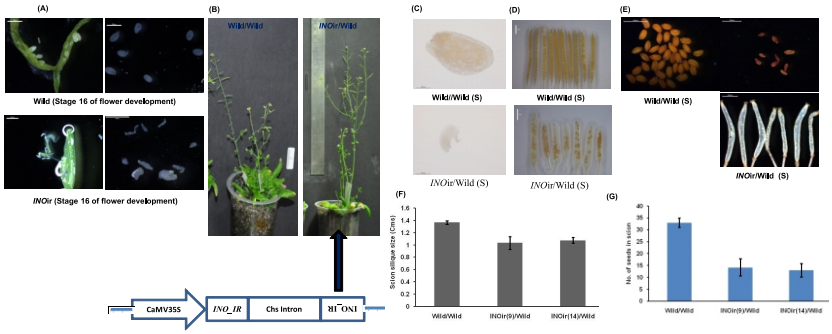

Figure 10: Induction of seedlessness in the wild scion by delivery of siRNAs against the *INO* gene expressed in rootstock. (A) Ovules of wild and *INO*ir expressing transgenic plants at stage 16 of flower development. (B) Grafting of wild scion onto wild rootstock and wild scion onto *INO*ir expressing rootstock. (C) Ovules of wild scion onto wild rootstock and wild scion onto *INO*ir expressing rootstock. (D) Seed setting in siliques of wild scion onto wild rootstock and wild scion onto *INO*ir expressing rootstock. (E) Mature seeds of wild scion onto wild rootstock (left panel) and mature seeds (right panel above) and seedless siliques with aborted ovules (below) of wild scion onto *INO*ir expressing rootstock,. (F) Representative bar diagram representing silique size in wild scion onto wild rootstock and wild scion grafted onto *INO*ir expressing rootstock. (G) Representative bar diagram representing seed number per silique in wild scion onto wild rootstock and wild scion grafted onto *INO*ir expressing rootstock.

DIET AND HEALTH

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

Principal Investigator
Kanthi Kiran

Co-Investigator
Mahendra Bishnoi

Research Fellows
Siddhartha M Sarma
Paramdeep Singh

Introduction
Obesity and associated metabolic complications (insulin resistance, type 2 diabetes, cardiovascular problems and some forms of cancers) are major health concerns worldwide. Sedentary lifestyle and excess calorie intake are the leading causes, which results in low grade inflammation, oxidative stress, and dysbiosis of beneficial gut microbiota. Current drugs are known to have side-effects highlighting the need for alternate and safe approaches. Our earlier studies suggested that millet whole grain and bran consumption could alleviate diet induced obesity. Dietary fibres isolated from small grain cereals such as millets have not been investigated for their role in regulating high fat diet induced alterations.

Objectives
To evaluate the role of non-starch dietary fiber from finger millet (FM-NSDF) in counteracting high fat diet induced alterations in mice.

Research Progress
Previously, we have reported on the beneficial effects of (a) finger millet (b) kodo millet whole grain/bran supplementation in counteracting the high-fat induced alterations in mice and (c) anti-inflammatory role of millet non-starch dietary fibre (Mi-NSDF) in regulating inflammation using *in vitro* cell culture model (Annual report 2013-14 & 14-15). Here we are reporting on the beneficial effect of FM-NSDF in counteracting the high fat induced alterations in mice.

Prevention of high fat diet induced alterations by millet non-starch dietary fibre fraction:
Age and weight matched Swiss albino mice (n=8) were fed with normal diet (ND) or high fat diet (HFD). (FM-NSDF) was administered through oral gavage in two different doses (0.5 and 1.0g/kg BW) to mice fed with HFD (60% energy derived from fat) for 10 weeks. Blood, different tissues and caecum contents were collected for analysis.

Body weight, Oral Glucose Tolerance Test (OGTT) and glucose clearance:
Oral administration of FM-NSDF decreased the body weight gain in the mice relative to HFD counterparts (Figure 1A & 1B). FM-NSDF administration improved body mass index (BMI) and Lee's index (Figure 1D & 1E). No difference was observed in the fasting blood glucose levels among the experimental groups however FM-NSDF supplementation improved the oral glucose tolerance with a significant change in glucose clearance rate relative to HFD fed mice (Figure 1F-1H).

Serum lipid parameters and lipid indices:
High fat diet administration increased serum lipids in mice such as triglycerides, cholesterol, non-esterified fatty acids, HDL-c, LDL-c and VLDL-c. FM-NSDF administration at both doses reduced serum triacylglycerols, NEFA, cholesterol, cholesterol esters, HDL-c, LDL-c and VLDL-c relative to HFD fed group.

Selected gut bacterial abundances in caecum:
FM-NSDF at 1.0g/kg body weight improved abundances of *Lactobacillus sp., Bifidobacterium sp.,Roseburia sp.,* and Bactero-idetes significantly while restoring the abundances of Firmicutes to the level similar to that of the control group. Interestingly, *per se* administration of FM-NSDF at 1.0g/kg bodyweight promoted the growth of *Bifidobacterium sp.* and *Roseburia sp.* suggesting its prebiotic effect.

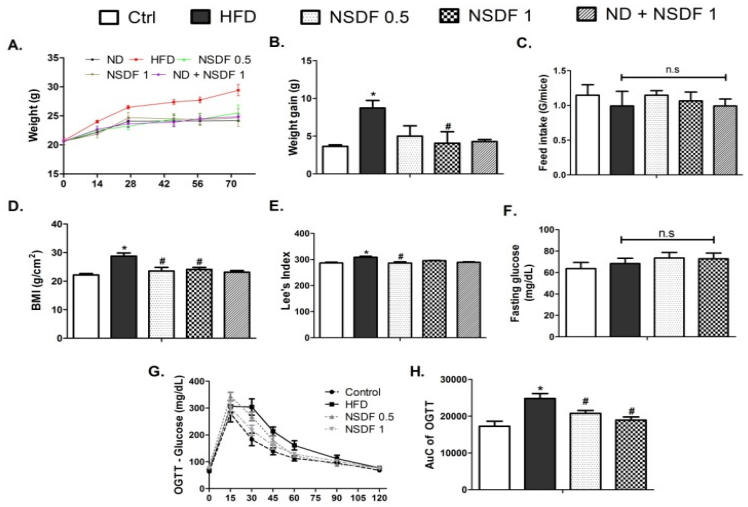

Figure 1: Effect of FM-NSDF supplementation on (A) Body weight (B) Weight gain, (C) Feed intake, (D) Body mass index, (E) Lee's index, (F) fasting glucose (G) Oral glucose tolerance test (H) Area under the curve of OGTT. Values are represented as Means ± SEM. One-way ANOVA with Tukey's post hoc test has been used for assessing statistical significance ($p \leq 0.05$). * Represents significance vs ND group, # represents significance vs HFD fed group. (n=8 for morpho-metric measurements; n=6 for Fasting glucose and OGTT assays)

Salient Achievements

Water extractable non-starch dietary fibre from finger millet was shown to improve high fat diet induced alterations in mice.

Future Perspectives

Gene expression and histological changes in liver, white adipose tissue and intestinal tissues and short chain fatty acid levels in the caecal contents will be studied.

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4.2 Development of synbiotics for the prevention of chronic diseases (diabetes/obesity)

Principal Investigator
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Co-Investigator
Mahendra Bishnoi

Research Fellows
Shashank Singh
Paramdeep Singh

Introduction

Dysbiosis in gut microbiota has been observed in various clinical conditions including metabolic disorders such as diabetes, obesity, and cardiovascular diseases as well as in mental illnesses. Hence restoration to normal gut microflora composition using probiotics and prebiotics has gained tremendous potential as a preventive or therapeutic approach. Probiotics are safe viable microorganisms administered in optimal amounts to improve microbial balance in gastrointestinal tract. Prebiotics are non-digestible carbohydrates that not only promote the growth of beneficial bacteria in the gut but also preserve the viability of exogenously administered probiotics during gastrointestinal passage and during food processing. Based on research gaps the following objectives are being set.

Objectives

1. Isolation, biochemical characterization and molecular identification of potential lactic acid bacterial strains.
2. Probiotic characterization based on DBT-ICMR guidelines
3. Development of probiotics and synbiotics for combating metabolic diseases

Research Progress

1. Forty five Gram positive and catalase negative lactic acid bacteria have been isolated from local fermented food products, human infants and adults.
2. Strains have been identified to the species level using 16S rRNA gene sequencing followed by phylogenetic determination using Blast programme. All the strains have been evaluated based on the DBT-ICMR

selection criteria for probiotic strains. Biochemical characteristics have been studied.

3. Carbohydrate utilization pattern using API carbohydrate kits have been established. Antibiotic sensitivity patterns have been established and majority of them are sensitive to common antibiotics. Majority of the strains exhibited antimicrobial activity against common food borne pathogens such as *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*.
4. Some of the strains showed significant binding to colonic epithelial cells, porcine type III gastric mucin and exhibited cholesterol lowering properties under *in vitro* conditions. Further studies are ongoing.

Salient Achievements

Based on the *in vitro* studies, lactic acid bacterial strains exhibiting antagonistic activity against common food borne pathogens, colonization efficiency and cholesterol lowering property have been prioritized for further studies.

Future Perspectives

Efficacy of the candidate probiotic strains will be evaluated in rodent models for metabolic diseases.

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

Principal Investigator

Mahendra Bishnoi

Co-Investigator

Kanthi Kiran

Research Fellows

Ritesh K. Baboota
Dhirendra Pratap Singh
Pragyanshu Khare

Introduction

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

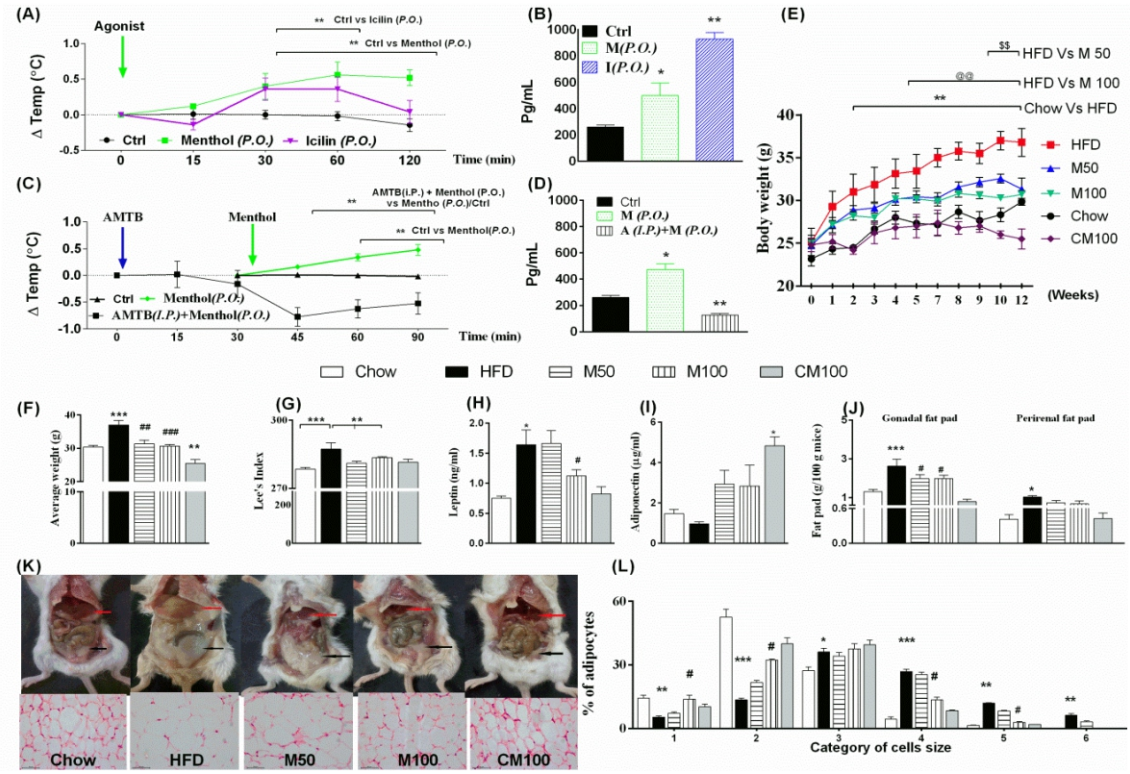

Figure 2: TRPM8 activation modulates adaptive thermogenesis, serum glucagon level, and prevented HFD induced weight gain. (A-D)*Acute effect of TRPM8 agonists; menthol and icilin on* (A) Change in rectal temperature for 120 min and (B) Serum glucagon levels 120 minutes after the menthol and icilin (C) pre-treatment of TRPM8 antagonist (AMTB) (D) serum glucagon was measured 120 minutes after menthol administration. Ctrl- Vehicle treated animal; Icilin (*P.O.*)-Single dose of icilin (per oral; 20 mg/kg); Menthol (*P.O.*)- single dose of menthol (per oral; 200 mg/kg); AMTB(*I.P.*)+Menthol(P.O.)- Single dose of AMTB (*i.p.;* 4mg/kg) followed by menthol (per oral; 200 mg/kg) 30 min. after AMTB. (E-L). Chronic effect of menthol administration *(p.o.)for 12 weeks* on (E) Weekly change in body weight, (F) Average body weight at the end of 12 week study (G) Lees's index, (H) Serum leptin levels by ELISA (I) Serum adiponectin levels by ELISA (J) Adipose tissue weight (K) Representative mouse showing gonadal fat pad and their H&E stained sections and (L) Frequency distribution Analysis of adipocyte cell size after 12 weeks of experiments. Chow- Normal rodent chow fed; HFD- High fat diet fed, M50- HFD fed plus daily administration of 50 mg/kg menthol (*P.O.*), M100- HFD fed plus daily administration of 100 mg/kg menthol (*P.O.*), CM100- Normal rodent chow fed plus daily administration of 100 mg/kg menthol (*P.O.*).

All the values are expressed as mean ± S.E.M; * $p < 0.05$, ** $p < 0.01$ versus Ctrl group by (A-B) two way ANOVA or (C-D) one way ANOVA, (E) **, @@, and $$ $p < 0.01$ by two way ANOVA, (F-L) * $p < 0.05$, ** $p < 0.01$,*** $p < 0.001$ vs Chow fed group and # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs HFD fed group by one way ANOVA. All ANOVA tests were followed by Tukey's multiple comparisons.

Research Progress

Previously we found out that many TRP channel genes are expressed in mouse 3T3-L1 preadipocytes, differentiated adipocytes, murine white adipose tissue (WAT), brown adipose tissue (BAT) and human preadipocytes and adipocytes (Annual report 2012-2013). Taking lead into the role of TRPV1 and TRPA1, we initiated and completed *in-vitro* (3T3L1 preadipocyte cell lines) and *in-vivo* (high fat diet (HFD)-induced weight gain model) studies for capsaicin and cinnamaldehyde, a TRPV1 and TRPA1 agonist respectively. We found out significant role of these channels and agents in preventing weight gain and related complications via different mechanisms (Annual report 2013-2014; Annual report 2014-2015). During the last year, we focused to understand the mechanistic role of TRPM8 and dietary constituents modulating these channels in adipogenesis, high fat diet (HFD) induced obesity and related complications.

TRPM8-dependent pharmacological cold mimicking is central to its therapeutic implications in obesity and associated complications:

Single dose oral administration of TRPM8 agonists, icilin and menthol, significantly increased whole body temperature and serum glucagon concentration which were prevented by pre-treatment with TRPM8 antagonist, N-(3-Aminopropyl) -2-[(3-methylphenyl) methoxy] -N-(2-thienylmethyl) benzamide hydrochloride (AMTB) (Figure 2). Chronic administration of menthol for 12 weeks prevented weight gain, corrected related complications like insulin resistance & ectopic fat accumulation, activated glucagon machinery, increased glycogenolysis, gluconeogenesis, "*browning*" of white adipose tissue, and enhanced activity of brown adipose tissue (Figure 2). Interestingly and importantly, no functional presence of TRPM8 receptor was found in white adipose tissue *in-vitro*. In conclusion, the present work strongly indicates that activation of TRPM8 plays a significant role in energy regulation *via* glucagon dependent pathways.

Salient Achievements

1. We have provided evidence that TRPM8 mediated cold mimicking causes global shift to "fat burning state" (glucose utilization, gluconeogenesis in the liver; "browning", mitochondrial activation in adipose tissues) from "fat storing state". This may serve as a novel therapeutic approach for prevention of obesity and related complications.

Future Perspectives

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

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4.4 Development of cobiotics (anti-oxidants/anti-inflammatory and pre-biotics) for the prevention of obesity

Principal Investigator

Mahendra Bishnoi
Kanthi Kiran

Research Fellow

Dhirendra Pratap Singh

Introduction

NCDs, especially those related to metabolism, diabetes and obesity are growing at an alarming rate in India, foreboding severe life threatening health complications for an average Indian adult in the coming decades.

The change in the metabolic status of our population is mainly due to replacement of traditional dietary habits with energy dense westernized diets, which leads to dysbiosis in gastrointestinal tract microflora which has been causally related to metabolic disorders in recent years. Attempts have been made over the years via drug and artificial sweetener development but with limited success. Hence, there is a need to find alternate diet based strategies to address the metabolism related issues in Indian population.Through this proposal, we will develop and functionally characterize low calorie sugars (prebiotics, oligosaccharides). Further, we will add these in different food matrices to develop novel concept based functional foods replacing refined sugars for the management of the metabolic health of people of different age groups.

Objectives

1. To study the synergistic/combinatorial effects of oligosaccharides with antioxidant/anti-inflammatory agents in rodent models *per se* and disease state
2. Prototype functional food product development using oligosaccharides as sugar alternative and its functional evaluation

Research Progress

Isomalto-oligosaccharides, lycopene and their combination (cobiotic) protects from high fat diet-induced alterations in mice:

Lycopene, IMOs and their combination prevented weight gain, adiposity, improved adipose tissue fat mobilisation and reduced insulin resistance. Hypothalamic orexigenic and anorectic genes have also been modulated by these treatments. Dietary interventions prevented NAFL type symptoms and improved glucose homeostasis. Improved gut microflora and SCFAs profile along with reduced systemic inflammation, metabolic endotoxemia and improved ileal and colonic health were observed in mice supplemented with lycopene, IMOs and their combination.

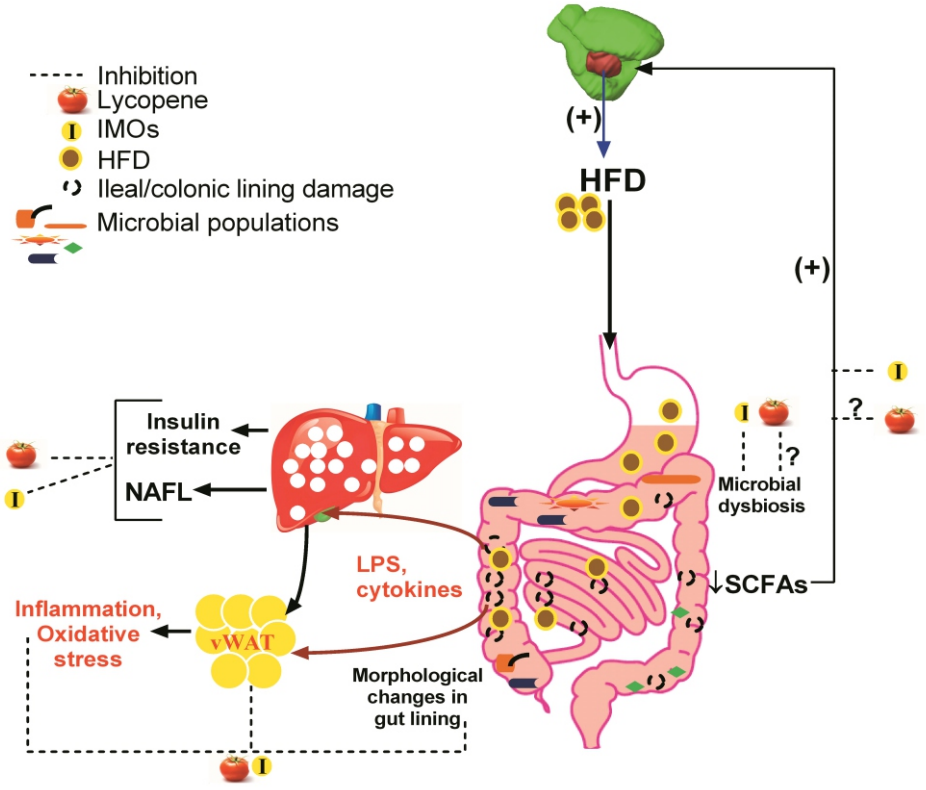

Figure 3: Proposed mechanisms of action of IMOs/lycopene or their cobiotic combination in HFD induced alterations.

Interestingly, cobiotic combination synergistically improved many of the HFD induced alterations. The proposed mechanism of action of lycopene, IMOs and their combination is graphically summarized in Figure 3.

Salient Achievements

1. This is the first elaborative scientific evidence for beneficial effect of new approach based on cobiotic combination (antioxidant plus prebiotic).
2. Cobiotic combination (antioxidant, lycopene + prebiotic, IMOs) effectively controlled HFD induced adiposity, low grade systemic inflammation, insulin sensitivity and gut microbial dysbiosis.

Future Perspectives

Prototype functional food product development and its functional evaluation.

—x—

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

Principal Investigator
Koushik Mazumder

Research Fellow
Vandana Bijalwan

Introduction

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

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

Objectives

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

Research Progress

1. *In vitro* antioxidant activity is influenced by various factors and no single method is sufficient to estimate antioxidant activity of potential antioxidant substances. Therefore, combination of three methods (DPPH, FRAP and β-carotene linoleate emulsion assay) were used in our study to evaluate the antioxidant potential of millet HCA-AXs in relation to their structural characteristics.
2. Our earlier studies using DPPH and FRAP assays revealed higher antioxidant potential of KM-HCA-AX compared to other millet HCA-AXs. In further study using β-carotene linoleate emulsion assay (Figure 4), KM-HCA-AX exhibited higher antioxidant activity coefficient (AAC) of 50.6 (±1.6) at initial concentration of 100 µg/ml which gradually reached to 85.5

(±0.9) at higher concentration of 1000 µg/ml. FM-HCA-AX showed comparatively lower antioxidant activity than KM-HCA-AX, exhibited AAC of 22.1 (±1.1) and 34.7 (±1.1) at concentration of 100 and 1000 µg/ml respectively. The other three millet HCA-AXs (PM, BM, FOXM) showed AAC in the range of 15.6 (±0.2) to 26.7 (±0.3) at concentration of 1000 µg/ml.

3. The results from three *in vitro* antioxidant assays (FRAP, DPPH, β-carotene linoleate emulsion assay) suggested that KM-HCA-AX exhibited highest antioxidant activity, whereas FM-HCA-AX showed comparatively good antioxidant activity next to KM-HCA-AX. The antioxidant potential of the millet HCA-AXs decreased in the order, KM-HCA-AX > FM-HCA-AX > PM-HCA-AX> BM-HCA-AX ≈ FOXM-HCA-AX.
4. The detailed linkage analysis by GC-MS showed that KM-HCA-AX had higher molar ratio of un-substitute to mono and di-substituted xylopyanaosyl (Xyl*p*) residues (2.61:1.0) which suggested the presence of comparatively low branched arabinoxylan in KM-HCA-AX, whereas the linkage analysis of FM-HCA-AX suggested the presence of comparatively medium-branched arabinoxylan having un-substituted to mono and di-substituted molar ratio of 1.67:1.0. Furthermore, the higher uronic acid content (8.4-9.3%) of low and medium-branched KM and FM-HCA-AXs might also be responsible for their good antioxidant activity compared to other three millet (PM, BM, FOXM) HCA-AXs.
5. Furthermore, in order to evaluate the cytotoxic effects of two good antioxidants (KM and FM-HCA-AXs) on cell growth, MTT cell viability assays were performed on various cell lines (3T3 murine embryonic fibroblast, HepG2, CaCo-2, MCF7 and HeLa). It was observed that both KM and FM-HCA-AXs had no significant growth inhibition effects against cell lines in the concentration range of 100-2000 µg/ml. The results suggested that both HCA-AXs had no significant cytotoxic effects in the concentration and range produced highest *in vitro* antioxidant activity.
6. In further, the role of KM and FM-HCA-AXs regulating oxidative stress was evaluated using HepG2, CaCo-2, and HeLa cell lines; the preliminary results suggested both KM and FM-HCA-AXs exhibited protective effects against tertiary butyl hydro-peroxide induced oxidative damage in the concentration range produced highest antioxidant activity. The detailed studies on protective effects of millet HCA-AXs against oxidative stress using various cell

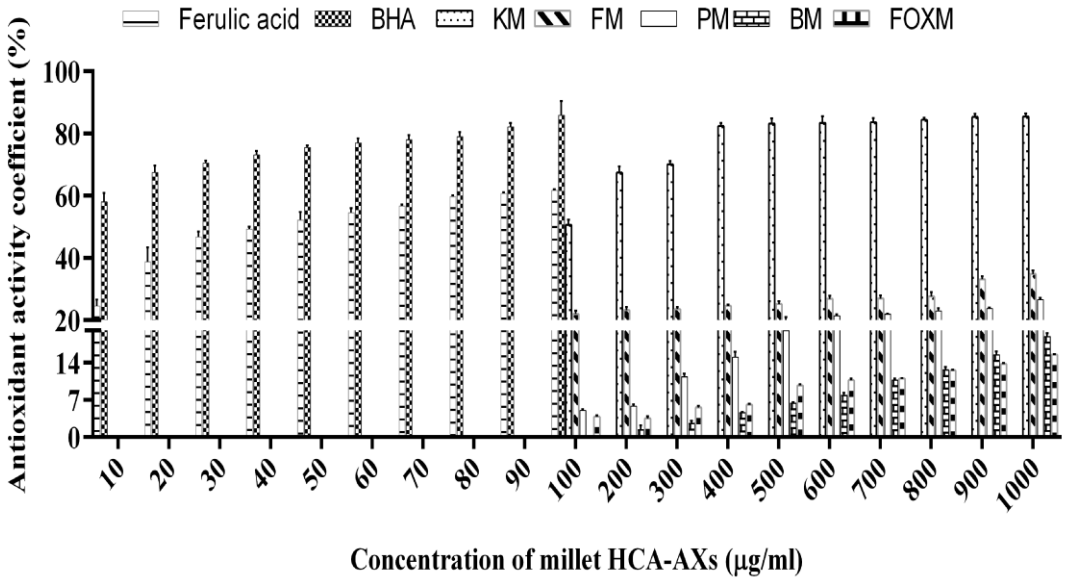

Figure 4: Antioxidant activity of different concentration of millet AXs

lines are in progress.

Salient Achievements

1. The antioxidant activity of millet HCA-AXs were evaluated using three *in vitro* assay methods which suggested both phenolic acid composition and structural characteristics of arabinoxylans (ratio of un-substitute to mono and di-substituted Xyl*p* residues and uronic acid content) could be correlated to their antioxidant potential.
2. Cell viability assay results showed that KM-HCA-AX exhibited no significant cytotoxicity on various cell lines (3T3, HepG2, CaCo-2, MCF7 and HeLa) in the concentration range produced highest *in vitro* antioxidant activity.

Future Perspectives

1. Determination of protective effects of millet HCA-AXs against oxidative stress using various cell lines and *in vivo* models.
2. Develop functional foods and nutraceuticals with free radical scavenging and immuno-enhancing additives against various lifestyle diseases.

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4.6 In vitro and In vivo evaluation of novel liposome-based nanocarrier as a hepcidin antagonist to ameliorate anemia of inflammation

Principal Investigator
Nitin Singhal

Research Fellow
Stanzin Angmo

Introduction

Hepcidin, a peptide hormone, is a key regulator in mammalian iron homeostasis. Elevated hepcidin causes iron restriction in inflammatory conditions including autoimmune disease, infection, cancers, and chronic kidney disease. Therefore, inhibiting hepcidin mediated ferroportin degradation can be an important strategy to ameliorate anemia of inflammation. The present study was aimed to develop novel liposome based nanoencapsulated formulation to ameliorate anemia of inflammation. The results obtained in this study paved a path for the design of antagonist molecule that could serve as a template for the development of drug candidates to overcome the inflammation induced anemia.

Objectives

1. Investigation of encapsulated GDP on hepcidin expression and iron regulated gene on Caco-2 and HepG2 cell line model.
2. Role of encapsulated GDP on regulating systemic iron absorption via BMP/IL-6 pathway and LPS induced anemia of inflammation in mice model.

Research Progress

In this study we treated HepG2 and Caco-2 cell line model with LPS (Lipopolysaccharide) to induce anemia of inflammation. Encapsulated GDP exhibits stable binding with hepcidin that resulted in inhibition of hepcidin-FPN interaction, explaining the prevention of FPN-degradation.

1. LPS addition dramatically increased the hepcidin, ferritin H and L and IL-6 mRNA expression causing cellular iron retention, whereas addition of encapsulated GDP and GDP respectively reversed this effect by inhibiting the hepcidin action
2. Protein expression data of ferroportin and ferritin showed that encapsulated GDP prevents hepcidin induced FPN degradation with decrease in cellular iron export in both Caco-2 and HepG2 cells.(Figure 5 A-B-C)
3. Further we aimed to study the effect of encapsulated GDP LPS induced anemic

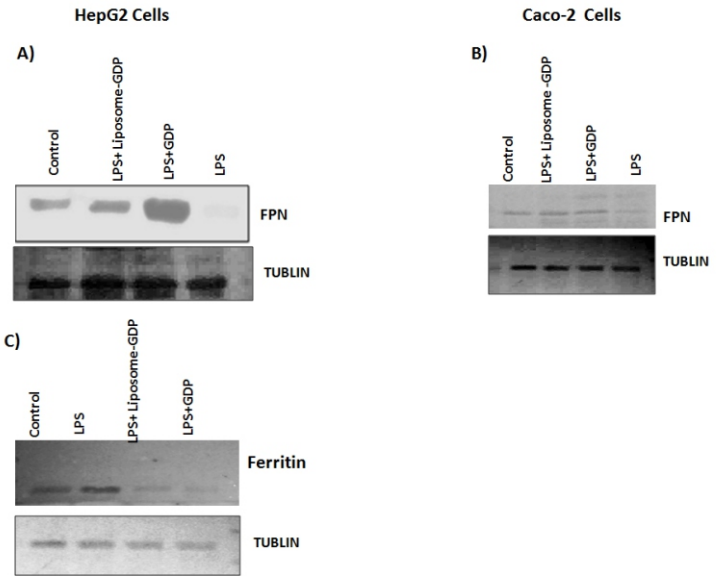

Figure 5: Western blot analysis on HepG2 and Caco2 cell line model: A-B) LPS induces hepcidin formation that causes degradation of ferroportin, whereas encapsulated GDP and GDP prevents hepcidin mediated ferroportin degradation.C) Protein expression analysis showed decreased iron absorption in encapsulated GDP and GDP whereas, LPS cause internalization of ferroportin with increase in iron restore.

mouse model. We will investigate the iron responsive gene expression and haematological parameter in relevance to encapsulated GDP and GDP respectively. We will also determine the BMP/IL-6 pathway that induce the hepcidin transcription along with treatment to LPS induced anemic mouse model with encapsulated GDP and GDP.

Salient Achievements

1. GDP in comparison to encapsulated GDP increases iron bioavaibility and iron intake in both HepG2 and Caco-2 cells.
2. Encapsulated GDP showed increase in cellular iron absorption with enhanced cell surface ferroportin retention.

Future Perspectives

1. Role of Encapsulated GDP for prevention of Iron related disorder (anemia of inflammation) and to restrict the hepcidin mediated pathway.
2. Encapsulated GDP in the form of drug to treat anemia of inflammation.

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4.7 Metallic/bimetallic magnetic nanoparticle functionalization for immobilization of α-amylase for enhanced reusability in bio-catalytic processes

Principal Investigator
Nitin Singhal

Research Fellows
Vishal Singh
Shweta Rathee

Introduction

α-amylase (1, 4-α-D-glucan-glucanhydrolase, EC. 3.2.1.1) catalyses hydrolysis of internal α-1, 4-glycosidic linkages of starch resulting in formation of low molecular weight products such as maltotriose and maltose from amylose, or maltose, glucose and "limit dextrins" from amylopectin. The free α-amylase in bioprocessing has many shortcomings that include instability, poor recovery, and poor reusability there by increasing bioprocessing costs while immobilization of α-amylase lessen these problems.

In the present study, we aimed to synthesize

different iron oxide magnetic nanoparticles that are magnetite (Fe_3O_4), hematite (Fe_2O_3), gold coated (Fe_2O_3@Au) and silica coated (Fe_3O_4@Si) magnetic nanoparticles, then covalently immobilized α-amylase on these nanoparticles by carbodiimide (EDC) chemistry. Further probe the kinetic parameters, reusability profile, activity at varying pH and temperature of free α-amylase in comparison with the immobilized counterpart.

Objectives

1. Synthesis and functionalization of magnetic nanoparticles i.e. magnetite (Fe_3O_4), gold coatedMNPs (Fe_2O_3@Au) and silica coated MNPs (Fe_3O_4@Si) with amylase enzyme.
2. Kinetic parameters, reusability profile, recovery contour, activity at varying pH and temperature of free α-amylase compare with their immobilized counterpart.

Research Progress

We successfully synthesized magnetite (Fe_3O_4), hematite (Fe_2O_3), bimetallic gold (Fe_2O_3@Au) and silica coated magnetic nanoparticles (Fe_3O_4@Si).The characterization of these nanoparticles carried out using FT-IR spectroscopy, TEM, HRTEM, FESEM, EDX spectra, UV-Visible spectroscopy and DLS. Functionalization of different metallic / bimetallic magnetic nanoparticles with 3-PPA and then immobilization of α-amylase onto these functionalized nano-particles that was effective. To confirm it, FT-IR spectroscopy and XRD was performed.

1. Relative activities of the immobilized and free amylase was measured at the varying temperatures from 30-100 °C to profile comparative performance and we, found that α-amylase exhibited optimum enzyme activity at 50 °C and this enzyme activity decreased at higher temperatures due to denaturation of the catalytic protein. However, the enzyme activity temperature optima were observed to be shifted to higher temperature in case of immobilized enzyme.
2. The optimum pH of free as well as immobilized amylase was found to be same, being around 7.0. α-amylase immobilized onto Fe_3O_4@Si and Fe_2O_3@Au proved to be able to stay

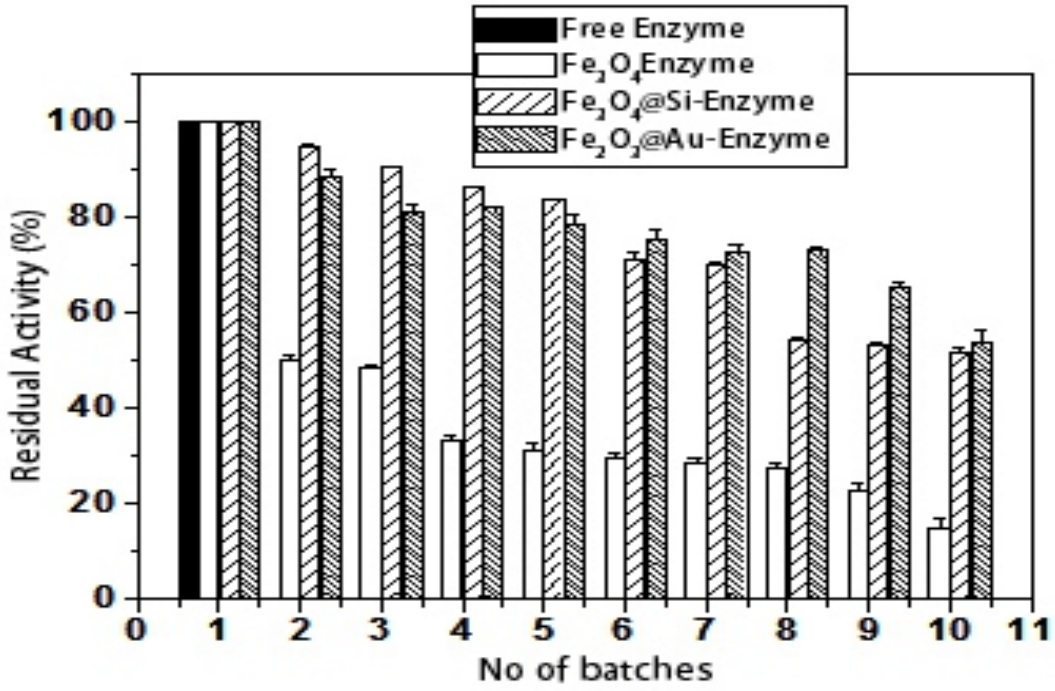

Figure 6: Operational activity of immobilized amylase in subsequent 10 cycles of reaction (error bar represent SD, n=3).

active at wider range of pH as compared to the enzyme immobilized on Fe_3O_4 confirming the role of metal coating in enabling retention of catalytically active confirmation of enzyme.

3. The reusability results showed that the immobilized enzyme retained its activity up to 25%, 55% and 60% in case of Fe_3O_4-amylase, Fe_3O_4@Si-amylase, and Fe_2O_3@Au –amylase, respectively after 10 duty cycles (Figure 6).

   V_{max}, K_m, K_{cat} and K_{cat}/K_m (catalytic efficiency) so obtained are summarized and these results showed that the affinity of amylase against starch was partially compromised by adsorption of the enzyme to the nanoparticles. The differences in V_{max} of free and immobilized enzyme were meager suggesting low level of diffusion limitations due to absorption on the magnetic nanoparticles. The K_{cat} values of free enzyme and immobilized eznymehas nearly the same value as that of free enzyme implying that the turn over number of enzyme was not affected by immobilization. The catalytic efficiency i.e. K_{cat}/K_m of free enzyme was found to be slightly higher as compared to the immobilized enzyme. This may be because of the less affinity of enzyme towards the substrate in immobilized form than the free form.

Salient Progress

1. The enzyme immobilization onto the magnetic nanoparticles in an irreversible manner led to significant gains in certain desirable catalytic traits of the enzyme as reusability, reproducibility, and the factors that are important for economizing enzyme bioprocessing.
2. The study suggests the potential applications of the approach and process in addressing certain bottlenecks in usage of biocatalysts compared to their chemical counterparts.

Future Perspectives

1. Co-immobilization of glucose isomerase and chimeric D-psicose 3-epimerase with improved operational stability and kinetic properties onto magnetic nanoparticles.
2. Application of above immobilized enzyme to obtain high fructose syrup for commercial purpose.

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

Principal Investigator
Nitin Singhal

Research Fellow
Shimayali Kaushal

Introduction

Food borne bacterial species have been identified as the major pathogens in most of the severe pathogen-related diseases among humans. These diseases represent a worldwide cause of deaths among the human society. Conventional methods for detecting bacteria are time consuming, laborious and require specialized instruments. Conventional techniques include microscopy, nucleic acid probes, immunoassay based techniques (ELISA). Nanotechnology has emerged as a great field in case of rapid detection of contaminants in recent years. Among the several nanomaterial based biosensor the gold nanorod based nanobiosensor has achieved a great interest. The gold nanorod material has good electro-optical properties because it has a larger light absorption band and scattering in surface plasmon resonance wavelength regions. The surface plasmon greatly enhances electromagnetic fields on the gold nanorods making them useful as good sensing devices. Our present study will contribute in the development of new multiplexed food borne pathogen biosensor and can have an applied

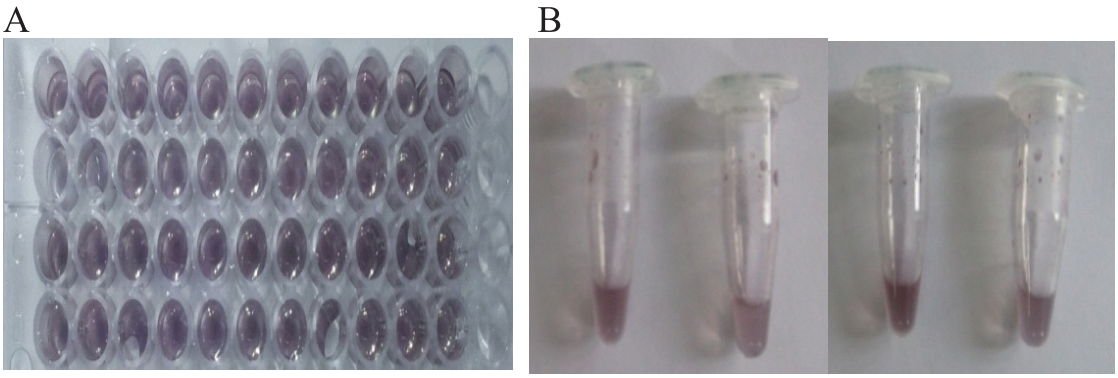


Figure 7: (A) Aggregation test lectins ConA with AuNRs functionalized with (i) Control, (ii) mannose, (iii) glucose, and (iv) galactose.(B)Aggregation test of mannose functionalized AuNRs with (a) Control (b) E.coli. Aggregation test of mannose functionalized AuNRs with (a) Control (b) Salmonella.

impact by offering a promising solution for food quality monitoring by a time effective and economical way.

Objectives

1. Modification of PEG-AuNRs with different types of sugars.
2. Characterization of sugar functionalized AuNRs.
3. Detection of different bacterial strains with these sugar functionalized AuNRs.

Research Progress

In this Work, we take advantage of the fact that a high percentage of microorganisms have both carbohydrate and lectin binding pockets at their surface. We demonstrate here for the first time that a carbohydrate labelled nanorod in combination with lectin-bacterial O-antigen recognition can be used for detection bacterial targets with remarkably high sensitivity and enhanced specificity. Three different types of simple sugars with different lectin specificity were selected, namely, 4-aminophenyl α-D-mannopyranoside, 4-aminophenyl β-D-glucopyranoside and 4-aminophenyl β-D-galactopyranoside to functionalize the PEG-AuNRs. The amount of immobilized sugar on Au NRs was determined by Anthrone reagent test. A functional mannose nanorod in combination with lectin concanavalin A (Con A) was used as molecular recognition elements (Figure 7).

These sugars conjugated AuNRs were tested with different strains of bacteria. Bacterial strains of *E. coli* (10^9 cfu/mL, MTCC no.443) and *Salmonella enterica* (10^9 cfu/mL, MTCC no.3232) were tested with mannose functionalized AuNRs. Mannose functionalized AuNRs was aggregate around both the bacterial surface as both the bacteria have mannose receptors.

Salient Achievements

1. Immobilization of sugar was done successfully which was tested by different set of lectins in aggregation test.
2. Sugar functionalized AuNRs showed aggregation on bacterial surface as compared to control.

Future Perspectives

1. To functionalize the pegylated AuNRs with different types of sugars / glycoconjugates.
2. To check the effect of using sugars / glycoconjugates functionalized AuNRs in a bacterial sample.
3. To use this sugar functionalized AuNRs to detect food borne pathogens in food samples.

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 pipelines for data mining and comparative analysis of food crop genomes, transcriptome and small RNA based regulation

Principal Investigator
Shrikant Subhash Mantri

Research Fellows
Anoop Kishor Singh Gurjar
Rajinder Gupta

HPC Application Support Engineer
Abhijeet Singh Panwar

I.Augmenting the pathway knowledge by adding identified biological entities

Introduction

Bio-molecular relationships have been the essence of the biological systems and scientific literature being the primary source of all information. With enormous growth in the amount of scientific literature it has become totally unrealistic to think of ways to extract relevant information from them through manual intervention alone. Many literature mining tools so far developed have limited precision and recall so needs manual curation at one or more steps. A much needed reform is awaited to restructure how we submit our biological relationship findings. A universal bio-molecular relationship database which should house all entities in a biological system needs to be designed and developed with community efforts. Journals need to encourage researchers to submit their relationship findings to the universal bio-molecular relationship database. More efforts from the research folk are required to curate the literature data published before norms are established for online relationship data submission and are universally accepted.

Objectives

1. To develop a utility to identify the bio-entities (genes, proteins, drugs, RNA etc.) in text
2. To develop a Universal Bio-molecular relationship database
3. To propose a standard biological relationship submission layout that can be adapted by journals and is made an inevitable part of scientific publication
4. To provide with a relationship data submission forms where a researcher can add information for biological relationships manually.

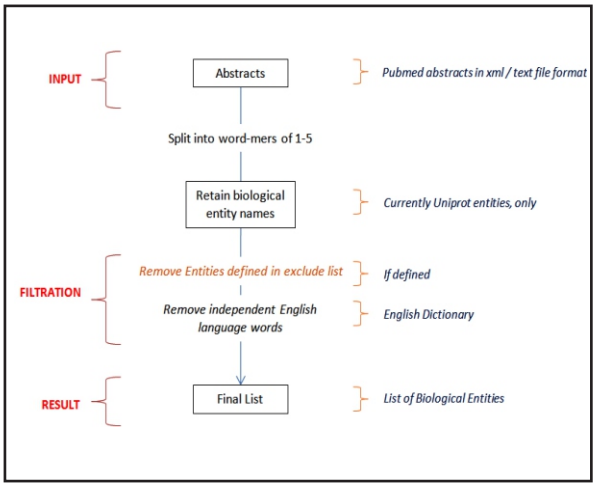


Figure 1: Utility work flow

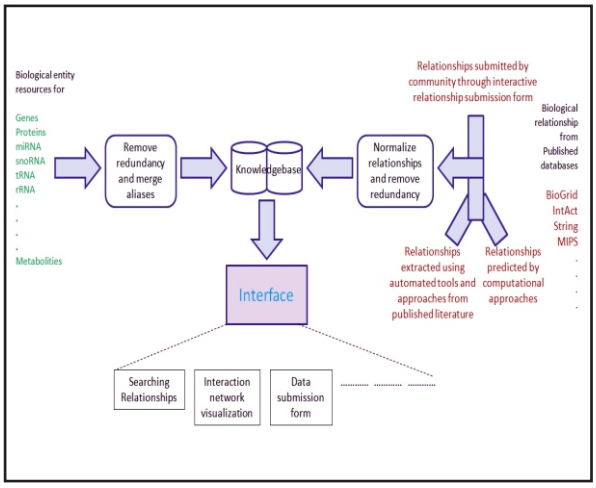


Figure 2 : Overview of the Universal Bio-molecular Relationship database

Research Progress

1. Programs and scripts to generate a non-redundant list of proteins and genes taken from Uniprot and populating them to a MySQL database.
2. Structure of the relationship data submission standard to be incorporated by the journals is prepared.
3. Designing and development of online relationship data submission forms has been completed.
4. Implementation of text mining utility to identify biological entities in literature. See Figure 1 for workflow.
5. Using the Text mining utility, ~49K Wheat abstracts taken from PubMed were parsed and it resulted in identification of ~22K bio-entities.
6. Database design to store biological relationship data has been completed (Figure2).

Salient Achievements

Published an opinion article in Frontiers in Genetics "Bio-molecular Relationships Discovered from Biological Labyrinth and Lost in Ocean of Literature: Community Efforts Can Rescue Until Automated Artificial Intelligence Takes Over".

Future Perspective

1. The current version of the utility identifies gene and protein names; we will add the option to search for any type of biological entity in it.
2. Improvements in the data submission form and Journal adopted data storage format for biological data.
3. Covering an entirety of biological published literature to look for biological relationships.

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

Introduction

Plant miRNA play important role in regulating multiple networks and development pathways. Huge amount of data and knowledge about expression of plant miRNA is getting available that can be explored to understand complex traits. We have developed a plant miRNA expression atlas database and web interface that is now accessible at http://pmirexat.nabi.res.in. This interface also avail the facility to profile new miRNA and new datasets. Recently micro RNA encoded peptides (miPEP) were reported in plants which are produced from primary transcripts of miRNA and these miPEPs regulate accumulation of respective miRNA. Accumulation of such miRNA causes more target regulation as compared to normally expressing miRNA. Currently we are mining several crop plant genomes to identify novel miPEPs.

Objectives

1. Identification of new and novel mature miRNA sequences in plant species.
2. Species specificity and tissues specificity analysis of miRNA.
3. Identification of miPEP in crop plants.
4. Addition of other plant species miRNA expression profile and new analysis features into the PmiRExAt.

Research Progress

1. Putative novel miRNA: 18 wheat, 22 rice and 14 maize highly expression putative novel miRNA were identified.
2. Highly expressing miRNA: We found 31 miRNA in wheat, 57 miRNA in rice and 17 miRNA of maize showing high expression.
3. Regulatory peptides (miPEP) from primary miRNA: In-house analysis pipeline was developed for identification of miPEP (Figure 1). 2092 ORF were predicted for wheat from 108 NR Unigenes. 5067 ORF were predicted for rice from 128 NR Unigenes. 1031 ORFs were predicted for maize from 55 Unigenes. 3717 ORF were predicted for arabidopsis from 164 NR Unigenes (Figure 3).
4. Micro RNA population similarity in different tissues: Arabidopsis leaf-seedling was showing minimum Jaccard index (0.10) and maximum between leaf-flower (0.79), next between root-shoot (0.72).

Rice anther and embryo were showing minimum Jaccard similarity index (0.43) and maximum Jaccard index between root-leaf (0.78), embryo-endosperm (0.77) and leaf-seedling (0.78). Maize root, leaf and seedling were showing minimum Jaccard similarity index (0.47) with anther and leaf-coleoptile were showing maximum Jaccard index (0.83). The Jaccard index between leaf-spike tissues of wheat was found 0.77, while correlation between them was 0.60.

5. Co-expression network: Wheat 1859 miRNA formed 1716 nodes and 318654 edges with average connectivity with approximately 371. Rice 2330 miRNA formed 1967 nodes and 372179 edges with average connectivity with approximate 378. Maize 283 miRNA formed 200 nodes and 1522 edges with average connectivity approximate 15.
6. Development of web interface for all the data generated regarding expression, conservation, similarity, correlation and differential expression (PmiRExAt).
7. Characterization of methylation in promoter region of arabidopsis miRNA genes and establishment of co-relation with mature miRNA expression.

Salient Achievements

1. A non-redundant miRNA database was developed for wheat, rice, maize and arabidopsis.
2. Development of database and web interface (PmiRExAt) for plant miRNA with multiple functionalities.
3. Establishment of correlation between mature miRNA expression and methylation in promoter region.

Future Perspectives

1. Experimental validation of identified putative novel miRNA.
2. Deciphering small RNA regulatory network in plants.
3. Validation of miPEP and their regulatory roles in plants.
4. New species data addition into PmiRExAt.

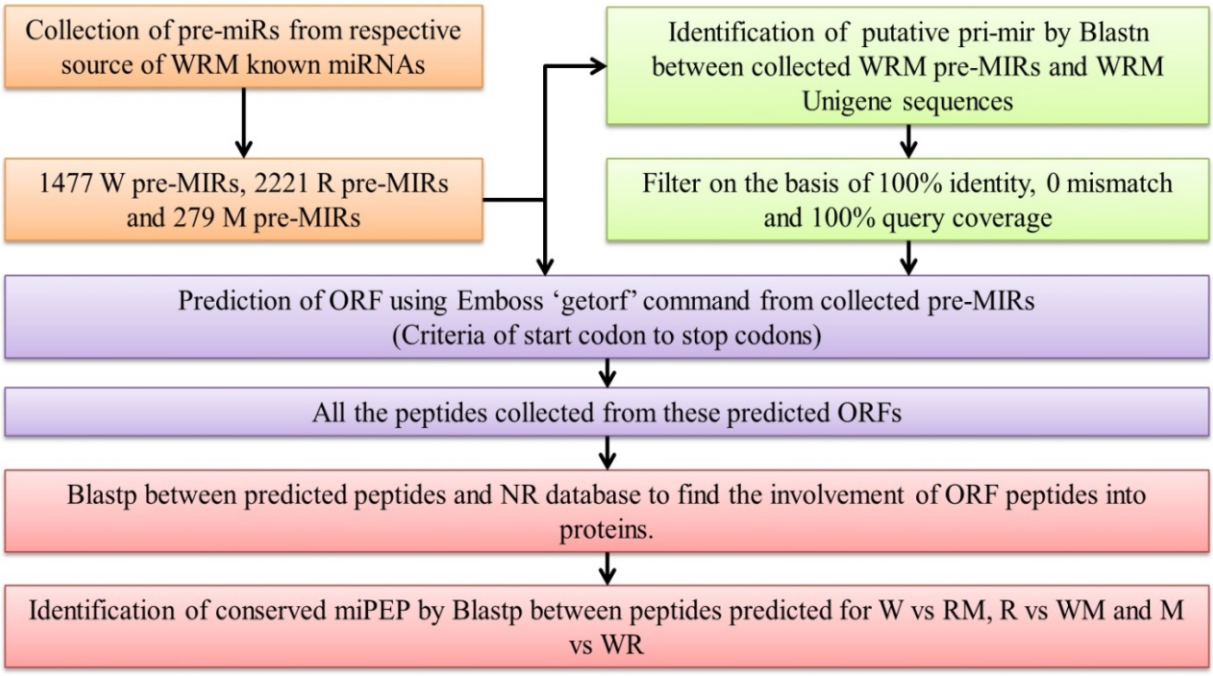


Figure 3: Pipeline for predicting putative miPEP of wheat (W), rice (R), maize (M) from pre-MIRs and Unigenes.

5.2 Molecular interaction studies and predictions for the function and designing of foods: Development of high throughput genome annotation pipeline for food crops

Principal Investigator
Shrikant S. Mantri

Co-Investigator
Shailesh Sharma

Introduction

Hypothetical, annotated, uncharacterized proteins comprise a substantial part of the genome in general. To solve this problem functional annotation is an effective approach. Functional annotation allows categorization of genes in functional classes, which can be very useful to understand the physiological meaning of large amounts of genes. Hypothetical/Unaanotated/Unknown transcript sequences were used as an input dataset for annotation.

We develop an automatic assembly, annotation and protein structure modelling pipeline. The name of our home made pipeline is DIIYOWAA which is "do it in your own way assembler and annotator". DIYOWAA is configurable open source annotation pipeline software, written in Python, used for rapid assembly and annotation of genome sequences. Currently it is used to take protein coding transcripts as input either in the form of DNA consensus sequences or gene models, and produces an annotated table in a text format which can be opened in Microsoft Excel easily.

Algorithm: EST sequences were downloaded and BLASTn search was performed against the EST database at evalue 10.00. All BLASTn hits along with the query sequence were used for sequence assembly by CAP3. The tool was designed in such a way that users could easily input the assembly parameters which will be displayed on the monitor. Contigs harbouring transcript sequences or identical to the transcript sequences were only selected and at this step user is provided a list of the databases from which One can easily select the desired database and can leave the undesired one. If in case user selects all databases, then the contig sequence will be searched against NCBI-nr protein sequence database and annotation was recorded with the protein sequence. At the second step BLASTx search was performed using the NCBI-nr protein sequence against PDB, UNIPROT, SWISSPROT, PANTHER db, AT, OS seed proteins database and UNIREF db. RPSBLAST search was performed using NCBI-nr protein sequence

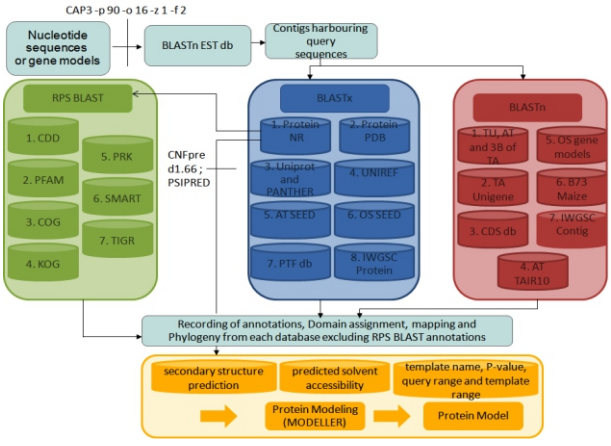


Figure 4: Pictorial representation of DIIYOWAA algorithm

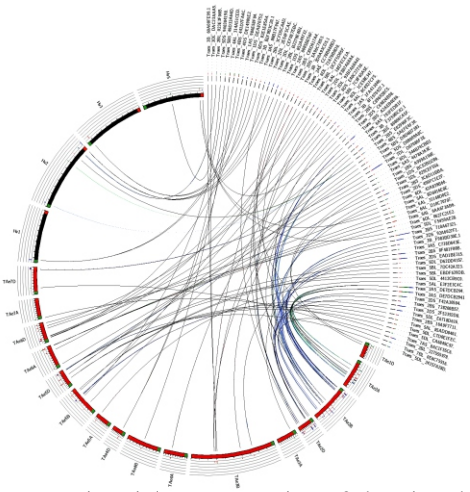


Figure 5: Pictorial representation of showing the distribution of Lectins in TaLRKs in*Hordeum vulgare* and *Triticum aestivum* genome.

against CDD, PFAM, COG, KOG, PRK, SMART, TIGR DBs for annotation. BLASTn search was performed against *Triticum urartu, Aegilops tauschii,* 3B of *Triticum aestivum, Arabidopsis thaliana* (ATtranscriptTAIR10), *Triticum aestivum* UniGene, *Oryza sativa* gene model databases. Annotation was recorded from the topmost BLAST search of every database. The user could easily go for protein structure modelling using same NCBI-nr protein sequence. Protein model will be in the .pdb format.

Research Progress

This pipeline is implemented on hypothetical/uncharacterised / unknown annotated 212 and 46 gene models in *Arabidopsis thaliana* (ATtranscriptTAIR10) database and coding (CDS) sequence of all high-confidence (HCS) gene models of *Triticum aestivum* (also known as bread wheat) respectively. We performed the genome wide identification, characterization and expression analysis of these genes in *T. aestivum* (TaLRK). In-total 263 TaLRK genes were identified, which were further classified into three groups based on the nature of lectin domain. We identified, two TaLRKs consisted of calcium-dependent lectin (C-LRK), while 84 legume-lectin (L-LRK) and 177 bulb-lectin (B-LRK) domains. The L-LRK and B-LRK genes were distributed throughout the genome of T. aestivum. Most of the TaLRKs were clustered as homologs, which were distributed either in proximity on same chromosome or on homoeologous chromosomes of A, B and D sub-genomes. A total of 9 and 58 duplication events were also predicted in L-LRK and B-LRK, respectively. Phylogenetic analysis indicated conserved evolutionary relationship of homologous and orthologous genes from multiple plant species. Gene ontology analysis indicated TaLRKs role in binding, signaling and receptor activities. Most of the TaLRKs consisted of a trans-membrane domain and predicted to be localized in the plasma-membrane. A diverse expression pattern of TaLRKgenes was found in various developmental stages and stress conditions. Some TaLRKs were found to be highly affected during a particular stress, which indicated a specialized role of each LRK gene in a specific stress condition. These results described various characteristic feature and expression pattern of TaLRK genes, which will pave the way for functional characterization in wheat.

In the future we plan to integrate IIYOWAA into a virtual appliance for easy deployment across everything from laboratory work-stations to cloud computing facilities.

In the future we plan to integrate DIIYOWAA into a virtual appliance for easy deployment across everything from laboratory workstations to cloud computing facilities.

Salient Achievements

With DIIYOWAA we annotated following:

1. 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 DIYOWAA to study and annotate genomes of other food crops in high-troughput way.

EXISTING MOU FOR COLLABORATIONS & NETWORKING

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

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

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

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

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

6. NABI and NIPER signed a MOU on February 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	Status
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	Completed
2.	Dr. Siddharth Tiwari	Transfer and evaluat ion 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	Ongoing
3.	Dr. Ajay K. Pandey	Metabolic engineering of phtytic acid pathway to enhance iron bioavailability in wheat.	Department of Biotechnology, Govt. of India	Ongoing
4.	Dr. Kanthi Kiran	Effects of finger millet and kodo millet arabinoxylan on adipogenesis and associated inflammatory markers- a nutrigenomic Study	Department of Biotechnology, Govt. of India	Completed
5.	Dr. Kanthi Kiran	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	Completed
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	Completed
7	Dr. Mahendra Bishnoi - PI Dr. Kanthi Kiran - Co-PI	Nutrigenomic approach to underst and the role of TRP channel activating food components in adipose tissue inflammation.	Department of Biotechnology, Govt. of India	Completed
8	Dr. Koushik Mazumder	Variability in the fine structures of feruloyl arabinoxylans from Indian millet varietie s and their consequence on anti-oxidant activity.	SERB, DST, Govt. of India	Completed
9	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	Ongoing
10	Dr. Monika Garg	Chromosome specific wide hybridization for improvement of bread making quality of wheat.	SERB, DST, Govt. of India	Ongoing
11	Dr. Siddharth Tiwari	Identification, cloning and functional characterization of myo-inositol oxygenase (MIOX) from wheat.	SERB, DST, Govt. of India	Ongoing
12	Dr. Nitin Kumar Singhal	Developing glycoconjugates capped multifunctional gold nanorod based nanobiosensor for detection of multiple food borne bacteria	Department of Biotechnology, Govt. of India	Ongoing
13	Dr. Monika Garg	A genomics-assisted synthetic hexaploid wheat gene isolation and pre -breeding platform for improved heat tolerance and sustainable production	DBT-BBSRC, UK	Ongoing

PROGRESS OF INFRASTRUCTURE AT MAIN CAMPUS

NABI laboratory building

NABI-CIAB staff residential apartments

NABI-CIAB student hostel

Director, NABI & CEO, CIAB residence

NABI-CIAB guest house

Campus internal development

PARTICIPATION IN NATIONAL/INTERNATIONALCONFERENCES/ WORKSHOPS:

1. **Dr. Siddharth Tiwari** monitored regulatory aspect session on GM crops, one day brain storming symposium on "Regulatory Aspects on GM Crops, Food and Nutrition"held on 27th April, 2015 at NABI.
2. **Dr. Siddharth Tiwari** was invited to deliver a talk on "Innovations in Biotechnology and Importance of Bio-Safety Programme" as a guest speaker in Regional Workshop on "Communicating Science and Biosafety" for media professionals covering science, agriculture and biosafety. Workshop was organized by Indian Institute of Mass Communication (IIMC), New Delhi in collaboration with the Ministry of Environment, Forest and Climate Change and Panjab University Chandigarh during 28th – 29th July, 2015 at Panjab University, Chandigarh.
3. **Dr. Kanthi Kiran** Chaired a session in "National Conference on Emerging Challenges in Biotechnology Perspective & Prospective" held during 21st to 22nd August, 2015 at Dept of Biotechnology, Chandigarh Group of Colleges, CGC Landran, Mohali, Punjab.
4. **Dr. Kanthi Kiran** delivered a key note lecture on "Probiotics: An Indian perspective and industrial relevance" at University of Kashmir during two day national symposium on "Biotechnological Interventions for upgradation of food products of India (BIUFPI-2016) held during 9th to 10th September, 2015.
5. **Dr. Mahendra Bishnoi** attended and presented his work at TRP 2015: Third Leuven TRP Symposium held at Leuven, Belgium during 16th to 18th September, 2015. He was sponsored by DST-SERB International travel grant scheme.
6. **Sh. Shrikant Mantri** delivered an invited talk on "Exploration of Plant micro RNA expression networks" in National Conference on Bioinformatics Panorama in Agriculture and Health (NCBPAH) held during 5th to 6th October, 2015 at SHIATS, Allahabad.
7. **Dr. Kanthi Kiran** delivered a lecture on "Biotechnology: Applications and opportunities". INSPIRE 2015 at ISF College of Pharmacy, Moga, Punjab held on 15th October, 2015.
8. **Dr. Mahendra Bishnoi** delivered a lecture at DST sponsored INSPIRE science camp for school students from Punjab held at ISF College of Moga, Punjab held on 15th October, 2015.
9. **Dr. Siddharth Tiwari** attended the third series of Stewardship training at Queensland University of Technology (QUT), Brisbane, Australia (Stewardship Stage 2) held during 12th – 17th October, 2015. This training was performed under the collaborative International project on "Development and Transfer of Technology from Queensland University of Technology (QUT), Australia to India for Biofortification and Disease Resistance in Banana", externally funded by Biotechnology Industry Research Assistance Council (BIRAC), Government of India.
10. **Dr. Ashutosh Pandey** attended the third series of Stewardship training at Queensland University of Technology (QUT), Brisbane, Australia (Stewardship Stage 2) held during 12th – 17th October, 2015. This training was performed under the collaborative International project on "Development and Transfer of Technology from Queensland University of Technology (QUT), Australia to India for Biofortification and Disease Resistance in Banana", externally funded by Biotechnology Industry Research Assistance Council (BIRAC), Government of India.

11. **Dr. Siddharth Tiwari** was invited to deliver a talk on "Motivational Contact Programme for Talented School Students of Punjab" held on 2nd November, 2015 at NABI, Mohali. Programme was organized by Punjab State Council for Science & Technology (PSCST) in collaboration with NABI, INST and CIAB.
12. **Dr. Siddharth Tiwari** was invited as a member of poster evaluation committee during poster making competition organized by CIAB on 3rd November, 2015.
13. **Dr. Kanthi Kiran** delivered a talk on "Biotechnology: Applications and opportunities" at Motivational Contact Programme for talented school students of Punjab held during 2nd to 6th November, 2015 at National Agri-Food Biotechnology Institute, Mohali.
14. **Sh. Shrikant Mantri** was invited to deliver a talk on "Unlocking Genomics Potential for Increased Nutrition and Productivity" at Institute of Microbial Technology, Chandigarh on 6th November, 2015 during Bioinformatics Symposium on Current trends in Bioinformatic.
15. **Dr. Joy K. Roy** attended the 3rd International Plant Physiology Congress: Challenges and Strategies in Plant Biology Research" held during 11th to 14th December, 2015 at Convention Centre, JNU, New Delhi
16. **Ms. Navneet Kaur** participated in National Conference on Frontiers in Applied Biotechnology held during 22nd to 23rd December, 2015 at Chandigarh University, Gharuan, Mohali and received first prize in poster presentation competition on "Phytoene synthase: Role in regulation of carotenogenesis in banana (Musa spp.)".
17. **Dr. Joy K. Roy & Dr. Kanthi Kiran** attended the 10th Biotechnology for Food & Agriculture Sectional Committee, FAD 23 held on 22nd January, 2016.
18. **Dr. Joy K. Roy & Dr. Kanthi Kiran** attended DBT "Global Biotechnology Summit" held during 5th to 6th February, 2016 at Vigyan Bhawan, New Delhi.
19. **Dr. Kanthi Kiran** delivered a talk on "Biomass to (PRO, PRE, SYN, CO)Biotics" at Biomass to Biovalue Summit (BBS-1) held during 11th to 12th Feburary, 2016 at CIAB, Mohali.
20. **Sh. Shrikant Mantri** delivered an invited talk on "Unlocking Genomics Potential for Increased Nutrition and Productivity: Exploration of cereals and fruits" at Institute of Microbial Technology, Chandigarh held on 11th February,2016 during National Workshop on "Bioinformatics Approaches on Genome Resources, Assembly and Annotation"
21. **Dr. Kanthi Kiran** was invited for a talk on "Carbohydrates from Cereals and Agricultural By-products: Application as Functional Food and Edible Fruit Coating Material" during the Biomass to Biovalue Summit (BBS-2016) held on 11th to 12th February, 2016 at Center of Innovative & Applied Bioprocessing (CIAB), Mohali, Punjab.
22. **Dr. Kaushik Mazumder** was invited for a talk on "Carbohydrates from Cereals and Agricultural By-products: Application as Functional Food and Edible Fruit Coating Material" during the Biomass to Biovalue Summit (BBS-2016) held on 11th to 12th February, 2016 at Center of Innovative & Applied Bioprocessing (CIAB), Mohali, Punjab.
23. **Dr. Siddharth Tiwari** delivered a talk on "Towards Provitamin A Biofortification of Banana by Metabolic Engineering" in International BITS Conference on Gene and Genome Regulation (BCGGR-16)". Conference was organized by the Birla Institute of Technology and Science, Pilani, Rajasthan, India from 18th -20th February, 2016.
24. **Ms. Navneet Kaur** participated in

"International BITS Conference on Gene and Genome Regulation (BCGGR-16)" held during 18th to 20th February, 2016 at the Birla Institute of Technology and Science, Pilani, Rajasthan, India and received first prize in poster presentation competition on "Identification and characterization of genes involved in carotenoid biosynthesis in banana".

25. **Ms. Shivani Sharma** participated in "International BITS Conference on Gene and Genome Regulation (BCGGR-16)" held during 18th to 20th February, 2016 at the Birla Institute of Technology and Science, Pilani, Rajasthan, India and presented poster on "Identification of transcription factors involved in somatic embryogenesis in banana (Musa spp. AAA group)".

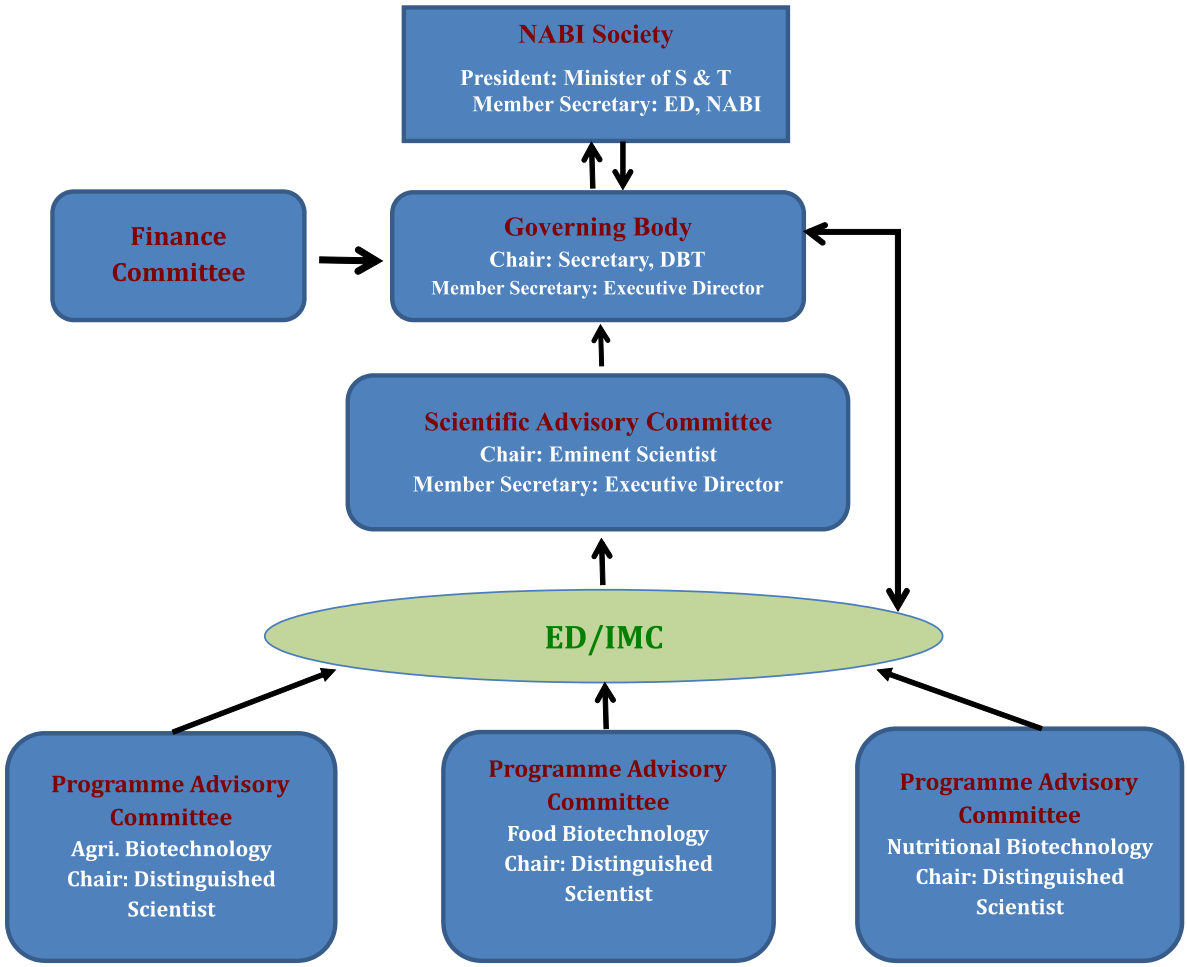
26. **Dr. Ajay K Pandey,** presented his work entitled "Biofortification in cereal grains and phytic acid reduction" during National Conference on "Food Processing: Current status and Future Prospects (NCFPT-2016)" held on 25th February, 2016 at Shoolini University, Solan, Himachal Pradesh.

27. **Sh. Shrikant Mantri** participated in workshop on "Advanced Techniques in Protein Design and Engineering" organised by CPSDE,IISER-Mohali during 15th to 19th March, 2016.

28. **Sh. Shrikant Mantri** participated in National conference on "Arabidopsis 2016" organised by IISER and NABI during 20th to 22nd March 2016.

29. **Mr. Shashank Singh** presented poster on' Prebiotic preferences and *in vitro* anti inflammatory efficacy of Iactic acid bacteria isolated from human infants and adults. 3rd Biennial Conference and International Symposium on " Symposium on " Stress, Microbiome and Probiotics", NISER, Bhubaneswar, March 11-13,2016.

GOVERNANCE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RESEARCH PUBLICATIONS OF FACULTY AT NABI 2015-2016

1 Khare P, Jagtap S, Jain Y, Baboota RK, Mangal P, Boparai RK, Bhutani KK, Sharma SS, Premkumar LS, Kondepudi KK, Chopra K & Bishnoi M (2016). Cinnamaldehyde supplementation prevents fasting induced hyperphagia, lipid accumulation and inflammation in high fat diet fed mice. Bio Factors. doi: 10.1002/biof.1265.

2 Goel R, Pandey A, Trivedi PK and Asif MH (2016). Genome-wide analysis of the Musa WRKY gene family: Evolution and differential expression during development and stress. Frontiers in Plant Science. DOI:10.3389/fpls.2016.00299.

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HUMAN RESOURCE

(as on March 31st, 2016)

I. Research Faculty

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

II. Technnical and Engineering Support

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

III. Administration

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

IV. Human Resource Development

(i) Research Scholars:

S. No	Name	Area of Research	Awarding University/Institute
Ph.D. degree awarded / Thesis submitted :			
1	Dr. 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
2	Sh. Yogesh Gupta	Gene discovery for seedlessness in *Annona* species	Panjab University, Chandigarh, Punjab
3	Ms. Anuradha Singh	Expression analysis of starch biosy nthesis 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
Students enrolled for Ph.D. degree:			
1	Sh. Anshu Alok	Cloning and functional characterization of myo - inositol oxygenase (MIOX) from wheat *(Triticum aestivum)*	Barkatullah University, Bhopal, MP
2	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
3	Ms. Monica Sharma	Genomic characterization & bi ochemical analysis of genes involved in phenylpropanoid pathway & their effect on nutritional & processing qualities of wheat.	Panjab University, Chandigarh, Punjab
4	Sh. Ritesh Kumar Baboota	Studies on modulation of adipogenesis, obesity and related complications by capsaicin	UIET Punjab University, Chandigarh
5	Sh. Jitesh Kumar	Virus induced gene silencing for functional genomics of iron nutrition related gene in wheat *(Triticum aestivum)*	Panjab University, Chandigarh, Punjab
6	Ms. Manpreet Kaur Saini	Metabolomics approach to study factors affecting quality and post -harvest stability of 'Kinnow' mandurim	Panjab University, Chandigarh, Punjab
7	Sh. Kaushal Kumar Bhati	Isolation and functional characterization of ABCC - MRO genes from wheat (Tritiu m aestivum L.) involved in Phytic acid transport	Panjab University, Chandigarh, Punjab
8	Sh. Raja Jeet	Transcriptional profiling and functional characterization of gene (s) related to iron enrichment in wheat grains	Panjab University, Chandigarh, Punjab
9	Sh. Ashish Kumar Pathak	The transcriptional changes during seed development in Litchi (Litchi Chinesis)	Panjab University, Chandigarh, Punjab
10	Ms. Sipla Aggarwal	Identification, characterization and functional analysis of genes contributing for phytic acid biosynthesis in T.aestivum L.	Panjab University, Chandigarh, Punjab
11	Sh. Prateek Jain	Experimental identification of H eterodimeriging basics – leucine zipper (B -ZIP) transcription factor families involved in seed maturation in Arabidopsi s: Use of a designed dominant negative.	Panjab University, Chandigarh, Punjab
12	Ms. Stanzin Angmo	Guanosine 5' diphosphate increases iron absorption in mice by inhibiting hepcidin – ferroportin interaction	Panjab University, Chandigarh, Punjab

Sno.	Name	Designation	Date of Joining
1	Ms. Shivani Sharma	Senior Research Fellow	12-02-2013
2	Sh. Shashank Singh	Senior Research Fellow	22-02-2013
3	Sh. Vishnu Shukla	Senior Research Fellow	25-02-2013
4	Ms. Mandeep Kaur	Senior Research Fellow	18-03-2013
5	Sh. Anoop Kishore Singh Gurjar	Senior Research Fellow	05-08-2013
6	Sh. Aman Kumar	Senior Research Fellow	05-08-2013
7	Sh. Koushik Shah	Senior Research Fellow	05-09-2013
8	Sh. Dhirendra Pratap	Senior Research Fellow	11-09-2013
9	Sh. Pragyanshu Khare	Senior Research Fellow	23-09-2013
10	Sh. Siddhartha M. Sharma	Senior Research Fellow	25-09-2013
11	Ms. Vandana	Senior Research Fellow	14-10-2013
12	Sh. Nand Kishore Sharma	Senior Research Fellow	29-01-2014
13	Sh. Pankaj Kumar	Senior Research Fellow	25-02-2014
14	Sh. Usman Ali	Senior Research Fellow	13-03-2014
15	Ms. Shivani	Project Fellow	11-05-2013
16	Ms. Shelley Sardul Singh	Junior Research Fellow	16-07-2013
17	Ms. Parul Upadhayay	Junior Research Fellow	01-08-2013
18	Ms. Harsimran Kaur	Junior Research Fellow	26-09-2013
19	Ms. Navneet Kaur	Project Fellow	30-08-2013
20	Ms. Navneet Kaur	Junior Research Fellow	28-01-2014
21	Ms. Flowerika	Junior Research Fellow	04-04-2014
22	Ms. Diksha Sharma	Junior Research Fellow	03-09-2014
23	Sh. Anil Kumar	Junior Research Fellow	06-09-2014
24	Sh. Rajinder Gupta	Junior Research Fellow	15-09-2014
25	Sh. Venkatesh Chunduri	Junior Research Fellow	25-09-2014
26	Ms. Saloni Sharma	Junior Research Fellow	30-09-2014
27	Ms. Preeti	Junior Research Fellow	10-02-2015
28	Ms. Ankita Mishra	Junior Research Fellow	13-02-2015
29	Sh. Rajesh Kumar	Junior Research Fellow	13-07-2015
30	Sh. Jay Hind Nishad	Junior Research Fellow	19-08-2015
31	Ms. Shweta Rathee	Junior Research Fellow	31-08-2015
32	Ms. Nistha Sharma	Junior Research Fellow	01-09-2015
33	Sh. Pramamdeep Singh	Junior Research Fellow	02-09-2015
34	Ms. Shimayali Kaushal	Junior Research Fellow	21-01-2016
35	Sh. Vishal Singh	Junior Research Fellow	23-02-2016
36	Ms. Neha Thakur	Junior Research Fellow	16-03-2016
37	Sh. Vijay Kumar	Junior Research Fellow	22-03-2016

(ii) Project Assistants

S.No.	Name	Designation	Date of Joining
1.	Sh. Vikrant Sharma	Project Assistant	01-04-2013
2.	Sh. Prateek Kumar	Project Assistant	16-09-2013
3.	Ms. Meenakshi Chawla	Project Assistant	28-01-2014
4.	Ms. Jaspreet Kaur	Project Assistant	10-02-2015
5.	Ms. Priya Arora	Project Assistant – II	15-06-2015
6.	Ms. Swati Misser	Project Assistant – II	31-08-2015
7.	Sh. Mohd. Saba Rahim	Project Assistant – II	07-09-2015

(ii) Trainees:

S. No	Name	Designation	Date of Joining
1	Ms. Kiran	Trainee	01-01-2016
2	Ms. Ashu Gupta	Trainee	01-01-2016
3	Ms. Deepti Pandey	Trainee	01-01-2016
4	Sh. Venus	Trainee	01-01-2016
5	Sh. Kanak Rakshit	Trainee	01-01-2016
6	Sh. Ankit Singh	Trainee	01-01-2016
7	Ms. Ramandeep Kaur	Trainee	01-01-2016
8	Ms. Shreya Gupta	Trainee	01-01-2016
9	Ms. Jyoti Sethi	Trainee	01-01-2016
10	Ms. Urvashi Dave	Trainee	01-01-2016
11	Ms. Banita Kumari	Trainee	01-01-2016
12	Ms. Alpana Bedi	Trainee	21-01-2016

PHOTO GALLERY OF IMPORTANT EVENTS

Celebration of Independence Day: August 15th, 2015

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

Independence Day celebrations at the NABI Interim Facility.

Hindi Pakhwada Celebration –September 1st to 15th, 2015

Hindi pakhwada was organized in the institute during September 1st -15th, 2015.

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

Visit of Hon'ble Minister of Science & Technology – October 18th, 2015

Dr. Harsh Vardhan, Hon'ble Minister of Science & Technology & Earth Sciences; interacting with research students.

Dr. Harsh Vardhan, Hon'ble Minister of Science & Technology & Earth Sciences; along with NABI & CIAB faculty and staff.

Dr. Harsh Vardhan, Hon'ble Minister of Science & Technology & Earth Sciences; during his visit to NABI laboratory building at the main Campus, Sector – 81, Mohali.

Dr. Harsh Vardhan, Hon'ble Minister of Science & Technology & Earth Sciences; planted 'Shami Vriksh' and 'Pride of India' at the main campus of the institute in Knowledge City, Sector -81, Mohali.

Republic Day Celebrations at NABI: January 26th, 2015

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

NABI staff, celebrating the Republic day with their family members Interim Facility

Sixth Foundation Day: March 9th, 2016

On the dais (from left) – Prof. Akhilesh K. Tyagi, Executive Director, NABI; Prof. Sunit C. Singhi, Head, Dept. of Pediatrics, M.M Institute of Medical Science & Research and Dr. R.S Sangwan, CEO, CIAB.

Prof. Sunit C. Singhi, Chief Guest lighting the lamp.

Dr. R.S Sangwan lighting the lamp.

Prof. Akhilesh Tyagi addressing the gathering

Prof. Akhilesh K. Tyagi presenting a shawl to Prof. Sunit C. Singhi.

Dr. R.S Sangwan felicitating the memento to Prof. Sunit C. Singhi.

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 2016 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 it appears from our examination of the Books and Record adequate for the purpose 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 Institute's affairs as at 31st March 2016.and
ii) In the case of income and expenditure accounts of the Deficit of the above named Institution for the year ended 31st March 2016.

for U.K. Mehta & Associates
Chartered Accountants

U K Mehta & Associates * Chandigarh * Chartered Accountants

(U.K.Mehta)
F.C.A.
M. No. 092639
FRN: 013381N

Dated: 24.05.2016
Place: Chandigarh

FORM OF FINANCIAL STATEMENTS (NON PROFIT ORGANIZATION)

NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE

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

BALANCE SHEET AS ON 31st MARCH 2016

(Amount in Rs.)

CORPUS/ CAPITAL FUND AND LIABILITIES	Schedule	Current Year	Previous Year
Corpus/Capital Fund	1	1,01,14,02,751	77,80,36,197
Reserves and Surplus	2	-	-
Earmarked / Endowment Funds	3	1,39,73,530	1,36,66,356
Secured Loans and Borrowings	4	-	-
Unsecured Loans and Borrowings	5	-	-
Deferred Credit Liabilities	6	-	
Current Liabilities and Provisions	7	84,43,008	69,23,985
TOTAL		**1,03,38,19,289**	**79,86,26,538**
ASSETS			
Fixed Assets	8	19,37,30,892	22,67,73,188
Capital Work in Progress	8	67,71,82,113	41,53,54,248
Investments- from Earmarked/Endowment funds	9	1,12,91,159	1,08,19,176
Investments - Others	10	-	-
Current Assets, Loans & Advances etc.	11	15,16,15,125	14,56,79,926
TOTAL		**1,03,38,19,289**	**79,86,26,538**
Significant Accounting Policies	24		
Contingent liabilities and notes on accounts	25		

As per our separate report of even date attached

For National Agri-Food Biotechnology Institute

Finance Officer

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

Executive Director

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

Dated: 24 MAY 2016
Place: Mohali

For U.K. Mehta & Associates
Chartered Accountants

U K Mehta & Associates
Chartered Accountants
Chandigarh

(U.K. Mehta), FCA

FORM OF FINANCIAL STATEMENTS (NON-PROFIT ORGANISTIONS)
NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE
C-127, INDUSTRIAL AREA, PHASE-8, S.A.S. NAGAR, MOHALI
INCOME AND EXPENDITURE ACCOUNT
FOR THE YEAR ENDED 31st MARCH 2016

(Amount in Rs.)

INCOME	Schedule	Current Year	Previous Year
Income from Sales/Services	12	-	-
Grants in aid /subsidies	13	11,00,00,000	9,90,00,000
Fees/subscriptions	14	-	-
Income from Investments (Income on investment from earmarked/endowment funds transferred to funds)	15	-	-
Income from Royalty, Publication etc.	16	-	-
Interest Earned	17	49,24,624	1,78,47,784
Other Income	18	7,75,934	21,13,416
Increase/decrease in stock of finished goods & work- in -progress	19		-
TOTAL(A)		**11,57,00,558**	**11,89,61,200**
EXPENDITURE			
Establishment Expenses	20	2,20,35,244	2,10,85,227
Other Administrative Expenses	21	4,36,51,335	3,61,65,297
Research & Development Expenditure (Incl. Grants, Subsidies etc)	22	2,20,25,262	2,32,16,983
Interest	23	-	-
Depreciation (net total at the year end-corresponding to schedule 8)		3,46,22,172	4,22,61,578
TOTAL(B)		**12,23,34,013**	**12,27,29,085**
Balance being surplus/ (deficit) carried to Capital Fund (A-B)		-66,33,455	-37,67,885
Significant Accounting Policies	24		
Contingent liabilities and notes on accounts	25		

As per our separate report of even date attached

For National Agri-Food Biotechnology Institute

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

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

Dated: 24 MAY 2016
Place: Mohali

For U.K. Mehta & Associates
Chartered Accountants
(U.K. Mehta), FCA

U K Mehta & Associates * Chartered Accountants * Chandigarh

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

NATIONAL AGRI FOOD BIOTECHNOLOGY INSTITUTE

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

RECEIPTS AND PAYMENTS ACCOUNT FOR THE PERIOD/YEAR ENDED ON 31.03.2016

(Amounts in Rs.)

RECEIPT	Current Year	Previous Year	PAYMENT	Current Year	Previous Year
Plan Grants			**Expenditure**		
(A) Opening Balance			**(A) Establishment Expenses**		
a) Cash in Hand			1. Manpower Salaries and Fellowships	2,17,65,439	2,08,97,314
b) Bank Balances			2. Staff Welfare Exp./Seminars	-	17,932
i) In current accounts					
ii) In deposit Accounts	4,13,50,830	7,18,36,045	**(B) Other Administrative Expenses**		
iii) In Savings Accounts	42,24,369	22,72,358	1. Cartrage & Carriage inward	-	800
			2. Allowances & Bonus (Honorarium)	2,57,410	5,32,964
(B) Grant-in-Aid			3. Electricity & Diesel Charges	95,55,504	68,95,461
(a) Grant from DBT	35,00,00,000	35,90,00,000	4. Rent	1,78,76,055	1,49,05,894
			5. Vehicles Running & maintenance	17,602	41,448
(C) Interest Incomes			6. Postage,Telephone & Comm charges	5,39,978	5,55,439
(a) Interest Income	42,94,964	1,81,17,447	7. Printing & stationery	3,83,826	4,79,995
			8. Travelling & conveyance expenses	18,07,060	15,57,012
(D) Other Incomes			9. Outsourcing	86,99,127	75,64,130
			10. Professional Charges	20,517	70,793
(a) Misc. Income	1,81,376	22,623	11. Advertisement	10,93,924	2,17,500
(b) Tender Fees	17,500	66,000	12. Repair & Maintenance	23,56,066	13,91,434
(c) Guest House Income	51,800	68,450	13. Office and Admn Expenses	3,95,637	3,77,504
(d) RTI Fee	40	30	14. Guest House Expenditure	61,918	37,916
(e) Project Income	4,36,026	3,71,865	15. Library Books & Periodicals	3,247	4,45,182
(E) Other Projects Receipt	3,75,77,853	1,75,71,762	**(C) Research & Deveoopment Expenditure**		
			1. Chemicals & Consumables	1,53,33,637	1,70,97,795
(F) Other Receipt			2. Computer Software & Accessories	11,68,326	12,50,801
(a) Security Deposit	2,01,361		3. Research Work Expenses	2,60,939	46,050
(b) Earnest Money Deposit	1,06,000		4. Field Expenses	1,81,412	73,439
(c) Advance for goods/Securities	27,676		5. Fellowships	50,52,434	35,29,977
(d) TDS Refund	2,24,850				
			(D) Non-Recurring Expenditures		
			1. Development of Main Campus	23,99,07,600	32,39,12,085
			2. Scientific Equip & Research Acce	14,34,735	8,25,522
			3. Computers & Books	6,600	6,43,784
			4. Furniture & Fixture	49,281	19,701
			5. Office Equipment	-	28,085
			(E) Other Payments		
			(a) External Project Expenses	2,72,95,505	1,94,50,158
			(b) Expenses Payable		
			(c) TDS Receivable		10,822
			(d) Earnest Money Deposit Paid		3,84,556
			(e) Refund of Security Deposits		3,11,209
			(F) Loan & Advances		
			(a) Advance to RITES Ltd.		
			(b) Advance to NIPER	1,357	
			(c) Advance to Employees		1,76,779
			(d) Advance to NCCS, Pune		1,500
			(e) M/s Gurukirpa Refrigeration		400
			(f) PSPCL	44,581	
			(G) Closing Balance		
			a) Cash in Hand		
			b) Bank Balances		
			i) In Current Accounts		
			ii) In Deposit Accounts	7,27,42,633	4,13,50,830
			iii) In Savings Accounts	3,82,295	42,24,369
Grand Total	**42,86,94,645**	**46,93,26,580**	**Grand Total**	**42,86,94,645**	**46,93,26,580**

In terms of separate report of even date attached

Dated: 24 MAY 2016
Place: Mohali

For National Agri-Food Biotechnology Institute

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

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

For U.K. Mehta & Associates
Chartered Accountants

U.K. Mehta & Associates Chartered Accountants Chandigarh

U.K. Mehta, FCA

FORM OF FINANCIAL STATEMENTS (NON-PROFIT ORGANISTIONS)

NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE

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

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31.03.2016

SCHEDULE-1
CORPUS/CAPITAL FUND

(Amount In Rs.)

Particulars	Current Year	Previous Year
Balance as at the beginning of the year	77,80,36,197	52,18,04,081
Add : Contributions towards corpus/capital fund	24,00,00,000	26,00,00,000
Add : Fixed Assets Created out of Project Grants	10	-
Less/(Deduct) : Expenditure over Income transferred from the income & expenditure A/c	-66,33,455	-37,67,885
BALANCE AS AT THE YEAR -END	**1,01,14,02,751**	**77,80,36,197**

SCHEDULE-2
RESERVES AND SURPLUS

Particulars		Current Year
1.Capital Reserves:		-
2.Revaluation Reserve		-
3.Special Reserve		-
4.General Reserve		
TOTAL		-

for National Agri-Food Biotechnology Institute

Dated: 24 MAY 2016

Place:Mohali

Finance Officer

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

Executive Director

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

For U.K. Mehta & Associates
Chartered Accountants

U.K. Mehta & Associates Chartered Accountants Chandigarh

U.K. Mehta, FCA

SCHEDULE 03-EARMARKED/ENDOWMENT FUNDS		Additions				Utilisation /expenditure								
							ii) Revenue Expenditure							
Sr . No.	Project Name	a) Opening balance of the Fund	b) Additions during the Year	c) Accrued Interest / Interest Recd. on Investment	TOTAL (a+b+c)	i) Capital Expenditure	Fellowships	Chemical & Consumable	Contingenty Exp/Travel etc	Overhead Exp	TOTAL	TOTAL EXP	REFUND	NET BALANCE AT THE YEAR END
1	A Novel strategy for developing scion plants of desired phenotype (e.g. seedless, early flowering) by using an RNAi delivering rootstock (GAP 01)	73,548	3,50,000	1,346	4,24,894	-		63,002		57,391	1,20,393	1,20,393	3,04,501	-
2	Development and Transfer of Technology from Queensland University of Technology, Australia to India for Bio-fortification and Disease Resistane in Banana (GAP 02)	1,44,16,834	9,67,741	3,95,084	1,57,79,659	86,14,077	11,49,891	5,65,062	11,74,640		28,89,593	1,15,03,670		42,75,989
3	Metabolic Engineering of Phytic Acid Pathway for Improving Iron Bioavalability in Wheat (GAP 03)	50,136	13,71,060	20,631	14,41,827	-	8,09,607	5,25,468	8,004		13,43,079	13,43,079		98,748
4	Effect of Finger Millet and Kodo Millet (GAP 04)	2,09,828	11,00,292	17,294	13,27,414		4,23,429	7,53,216	40,830		12,17,475	12,17,475		1,09,939
5	A Nutrigenomic study to access the role of polyphenols constituents (GAP 05)	1,55,011	4,00,000	6,609	5,61,620			4,79,113		57,500	5,36,613	5,36,613		25,007
6	Studies on transient receptor potential (TRP) cgannel medicated modulation (GAP 06)	37,041	5,00,000	5,439	5,42,480			4,33,954		81,123	5,15,077	5,15,077		27,403
7	Nutrigenomic approach to understand the role of TRP channel activating food components in adipose Tissue inflammation (GAP 08)	1,32,281	10,30,919	17,307	11,80,507		4,47,360	6,12,608	39,816		10,99,784	10,99,784		80,723
8	Variability in the fine structure of feruloyl arabinoxylans from Indian Millet varieties and thein consquence on anti-oxidant activity (GAP 09)	1,35,951	8,00,000	17,663	9,53,614		4,46,210			88,298	5,34,508	5,34,508		4,19,106
9	Identification of celiac disease epitopes in indian wheat cultivars and their modulation by RNAi and breeding approach (GAP 11)	47,563	3,28,035	2,485	3,78,083		1,94,105	1,97,271	7,478		3,98,854	3,98,854		-20,771
10	Chromosome specific wide hybridization for improvement of bread making quality of wheat (GAP 12)	85,440	6,00,000	5,223	6,90,663		3,63,833	2,33,029		67,630	6,64,492	6,64,492		26,171
11	Identification ,cloning and Functional characterization of MIOX from Wheet (GAP 13)	1,97,891	5,00,000	6,630	7,04,521	1,35,000		3,50,097		84,084	4,34,181	5,69,181		1,35,340
12	Developing glycoconjugates capped multifunctional gold nanorod based nanobiosensor for detection of multiple food borne bacteria (GAP 14)	2,23,445	7,20,000	21,182	9,64,627	1,83,974	1,53,871	2,93,848	2,762		4,50,481	6,34,455		3,30,172
13	A genomics-assisted synthetic hexaploid wheat gene isolation and pre-breeding platform for improved heat tolerance and sustainable production (GAP 15)		1,11,16,000	6,29,987	1,17,45,987		5,77,558	5,26,811	2,81,733		13,86,102	13,86,102		1,03,59,885
14	Department of Biotechnology (DBT) JRF/SRF fellowships	-16,47,351	45,90,152		29,42,801		35,23,033				35,23,033	35,23,033		-5,80,232
15	Indian Council of Medical Research (ICMR) JRF/SRF Fellowships	-2,66,342	18,90,646		16,24,304		15,83,099				15,83,099	15,83,099		41,205
16	UGC Fellowhsip	4,200	3,63,240		3,67,440		3,93,720				3,93,720	3,93,720		-26,280
17	Council of Scientific & Industrial Research (CSIR) JRF/SRF Fellowships	-1,89,120			-1,89,120		7,32,612				7,32,612	7,32,612		-9,21,732
18	DST INSPIRE Fellowship				-		4,07,143				4,07,143	4,07,143		-4,07,143
	Total	1,36,66,356	2,66,28,085	11,46,880	4,14,41,321	89,33,051	1,12,05,471	50,33,479	15,55,263	4,36,026	1,82,30,239	2,71,63,290	3,04,501	1,39,73,530

For National Agri-Food Biotechnology Institute

Dated: 24 MAY 2016
Place: Mohali

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

Executive Director
Prof. Anilesh KumarTyagi
कार्यकारी निदेशक/Executive Director
राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान
National Agri-Food Biotechnology Institute
जैव प्रौद्योगिकी विभाग, भारत सरकार
मोहाली - 160071 पंजाब, भारत

For U.K. Mehta & Associates
Chartered Accountants

U K Mehta & Associates
Chartered Accountants
Chandigarh

(U.K. Mehta), FCA

SCHEDULE-4
SECURED LOANS & BORROWINGS

(Amount in Rs.)

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

SCHEDULE-5
UNSECURED LOANS & BORROWINGS

(Amount in Rs.)

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

SCHEDULE-6
DEFERRED CREDIT LIABILITIES

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Acceptances secured by hypothecation of capital equipment		-
2. Others		-
TOTAL	-	-

for National Agri-Food Biotechnology Institute

Dated: 24 MAY 2016

Place:Mohali

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

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

For U.K. Mehta & Associates
Chartered Accountants

U K Mehta & Associates Chartered Accountants Chandigarh

U.K. Mehta, FCA

SCHEDULE-7
CURRENT LIABILITIES & PROVISIONS

(Amount in Rs.)

Particulars	Current Year	Previous Year
A)CURRENT LIABILITIES		
1. Sundry Creditors		
a) For goods/Equipment	18,55,336	18,29,560
b) For Securities	3,16,914	1,15,553
c) Earnest Money Deposit	7,28,381	6,22,381
2. Advances received from External Projects		
3. Interest accrued but not due on:		
a) Secured Loans/Borrowings		-
b) Unsecured Loans/Borrowings		-
4. Statutory Liabilities		
a) Overdue		-
5. Other Current Liabilities		
a) Manpower (Salary) Payable	16,12,471	19,34,581
b) Other Expenses Payable	17,70,385	12,10,075
c) TDS Payable	8,89,673	4,58,235
d) Fellowship Payable	12,69,848	7,53,600
TOTAL(A)	**84,43,008**	**69,23,985**
B) PROVISIONS		
1. Gratuity		
2. Superannuation/Pension		-
3. Leave Encashment		-
TOTAL(B)	0	-
TOTAL(A+B)	**84,43,008**	**69,23,985**

for National Agri-Food Biotechnology Institute

Dated: 24 MAY 2016

Place:Mohali

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

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

For U.K. Mehta & Associates
Chartered Accountants

U K Mehta & Associates Chartered Accountants Chandigarh

U.K. Mehta, FCA

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

Sl.No.	Description		GROSS BLOCK					DEPRECIATION			NET BLOCK	
Sl.No.		Depreciation Rate	Cost/Valuation as at beginning of the year	Additions during the year	Additions during the year	Deduction during the year	Cost/Valuation at the year end	As at the beginning of the year	Depreciation during the year	Total at the year end	As at the Current Year End	As at the Previous Year End
			1st April 2015	UPTO 30.09.15	AFTER 30.09.15	2015-16	31st March 2016	1st April 2015	2015-16	31st March 2016	31st March 2016	31st March 2015
A	FIXED ASSETS											
I	LAND											
	a)Free Hold	0.00%	-		-	-	-	-	-	-	-	-
	b)Lease Hold	0.00%	-		-	-	-	-	-	-	-	-
II	BUILDINGS											
	a)On Freehold Land	10.00%	83,57,674	-	-	-	83,57,674	22,64,930	6,09,274	28,74,204	54,83,470.00	60,92,744
	b)On Leasehold Land	10.00%	-	-	-	-	-	-	-	-	-	-
	c)Ownership Premises	10.00%	-	-	-	-	-	-	-	-	-	-
	d)Other Superstructures	10.00%	-		-	-	-	-	-	-	-	-
III	PLANT, MACHINERY & EQUIPMENT						-					
	EQUIPMENTS	15.00%	35,85,18,731	61,399	14,59,214	-	36,00,39,344	14,55,23,176	3,20,67,985	17,75,91,161	18,24,48,183.00	21,29,95,555
IV	VEHICLES	15.00%	6,62,497			-	6,62,497	3,45,101	47,610	3,92,711	2,69,787.00	3,17,397
V	FURNITURE & FIXTURES	10.00%	35,76,894	14,991	34,290	-	36,26,175	12,22,110	2,38,692	14,60,802	21,65,373.00	23,54,784
VI	COMPUTER/PERIPHERALS	60.00%	2,09,72,765	6,600		-	2,09,79,365	1,86,72,750	13,83,969	2,00,56,719	9,22,646.00	23,00,015
VII	LIBRARY BOOKS	100.00%	4,77,188		3,373	-	4,80,561	4,77,188	3,373	4,80,561	-	-
VIII	OFFICE EQUIPMENT	10.00%	38,96,111			-	38,96,111	11,83,418	2,71,269	14,54,687	24,41,423.00	27,12,692
	TOTAL OF CURRENT YEAR (A)		39,64,61,860	82,990	14,96,877	-	39,80,41,727	16,96,88,674	3,46,22,172	20,43,10,846	19,37,30,882.00	22,67,73,187
B	Fixed Asset Created from Projects Grants:											
	EQUIPMENTS				6		6				6.00	-
	COMPUTER/PERIPHERALS				4		4				4.00	-
	TOTAL OF FIXED ASSETS PROCURRED FROM PROJECTS (B)		-	-	10		10	-	-	-	10.00	-
	TOTAL (A+B)		39,64,61,860	82,990	14,96,887	-	39,80,41,737	16,96,88,674	3,46,22,172	20,43,10,846	19,37,30,892	22,67,73,187
XI	PREVIOUS YEAR											
	a) Expenditure on Assets/Fixed Assets		-	-	-	-	-	-	-	-	-	-
	b) Expenditure on Plan Activities		-	-	-	-	-	-	-	-	-	-
	TOTAL OF PREVIOUS YEAR		-		-	-	-	-	-	-	-	-
XII	CAPITAL WORK-IN-PROGRESS											
	a) Main Campus At Sec 81		41,52,68,375	11,92,39,800	14,72,10,198	45,56,926	67,71,61,447	-	-	-	67,71,61,447.00	41,52,68,375
			-	-	-	-	-	-	-	-	-	-
	d) Equipment		85,873		47,470	1,12,677	20,666	-	-	-	20,666.00	85,873
	TOTAL OF CURRENT YEAR (CWIP) (C)		41,53,54,248	11,92,39,800	14,72,57,668	46,69,603	67,71,82,113	-	-	-	67,71,82,113.00	41,53,54,248
	GRAND TOTAL (A+B+C)		81,18,16,108	11,93,22,790	14,87,54,555	46,69,603	1,07,52,23,850	16,96,88,674	3,46,22,172	20,43,10,846	87,09,13,005.00	64,21,27,435

For National Agri-Food Biotechnology Institute

Dated: 24 MAY 2016
Place: Mohali

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

Executive Director
डॉ. अखिलेश कुमार त्यागी
Dr. Akhilesh Kumar Tyagi
कार्यकारी निदेशक/Executive Director
राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान

For U.K. Mehta & Associates
Chartered Accountants

U K Mehta & Associates
Chartered Accountants
Chandigarh

(U.K. Mehta), FCA

SCHEDULE-9
INVESTMENTS FROM EARMARKED/ENDOWMENT FUNDS

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. In Government Securities		-
2. Other approved securities		-
3. Shares		-
4. Debentures & Bonds		-
5. Subsidiaries & Joint Ventures		-
6. Others Fixed Deposits (to be specified)	1,12,91,159	1,08,19,176
TOTAL	**1,12,91,159**	**1,08,19,176**

SCHEDULE-10
OTHER INVESTMENTS

(Amount in Rs.)

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

for National Agri-Food Biotechnology Institute

Dated: 24 MAY 2016
Place:Mohali

Finance Officer

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

Executive Director

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

For U.K. Mehta & Associates
Chartered Accountants

U K Mehta & Associates Chartered Accountants Chandigarh

U.K. Mehta, FCA

SCHEDULE-11

CURRENT ASSETS, LOANS & ADVANCES

(Amount in Rs.)

Particulars	Current Year	Previous Year
A) CURRENT ASSETS		
1. Inventories		
a) Stores & Spares		
b) Loose Tools		
c) Stock-in-trade		
2. Sundry Debtors		
3. Cash balances in hand		
4. Bank balances:		
a) With Scheduled Banks:		
-On Current accounts		
-On Fixed Deposit accounts	6,14,51,474	3,05,31,654
-On Savings accounts		
(i) State Bank of India A/c	3,82,295	42,24,369
TOTAL(A)	**6,18,33,769**	**3,47,56,023**
B) LOANS, ADVANCES AND OTHER ASSETS		
1. Loans		
2. Advances and other amounts recoverable		
a) On Capital Account		
b) Advances		
(i) Deposite with M/s RITES Ltd	8,63,78,592	10,79,57,152
(ii) Advance to CFTRI	375	375
(iii) NCCS Pune		1,500
c) Recoupable form Govt. Agencies		
(i) Director NIPER	1,972	615
(ii) DBT (Brain Storming Project)	2,21,904	2,21,904
(iii) Advance to CDAC Pune	-	-
d) Advance to Employees	41,828	2,51,775
e) Others(specify)		
(i) Security for Rent	50,000	50,000
(ii) Deposit with PSPCL	44,581	
(iii) TDS Receivable	11,423	2,36,272
(v) PSEB Elelct Security for Main Campus	11,12,090	11,12,090
(vi) Electricity Security of Interim facility	7,41,200	7,41,200
(vii) M/s Gurukripa Refrigration		400
3.Income accrued:		
a) on investments from earmarked/endowment funds		
b) Interest On Saving and Fixed Deposits	9,80,279	1,86,304
c) on loans & advances		
d) others(Accrued Interest from GAPs)	1,97,112	1,64,316
4. Claims Receivable		
TOTAL(B)	**8,97,81,356**	**11,09,23,903**
TOTAL(A+B)	**15,16,15,125**	**14,56,79,926**

for National Agri-Food Biotechnology Institute

Dated: 24 MAY 2016

Place:Mohali

Finance Officer

Suneet Verma / सुनीत वर्मा
Finance Officer / वित्त अधिकारी
National Agri-Food Biotechnology Institute
Govt. of India / भारत सरकार
Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग
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Executive Director

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Prof. Akhilesh KumarTyagi
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मोहाली - 160071 पंजाब, भारत
Department of Biotechnology, Govt. of India

For U.K. Mehta & Associates
Chartered Accountants

U K Mehta & Associates Chartered Accountants Chandigarh

U.K. Mehta, FCA

SCHEDULE-12
INCOME FROM SALES/SERVICES

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Income from sales		
2. Income from services		-
TOTAL		-

SCHEDULE-13
GRANTS/SUBSIDIES

(Amount in Rs.)

Particulars	Current Year	Previous Year
(Irrevocable Grants & subsidies received)		
1. Central Government	11,00,00,000	9,90,00,000
2. State Government		-
3. Government Agencies		-
4. Institutional /welfare bodies		-
5. International Organisations		-
6. Others (to be specified)		-
TOTAL	**11,00,00,000**	**9,90,00,000**

SCHEDULE-14
FEES/SUBSCRIPTIONS

(Amount in Rs.)

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

SCHEDULE-15
INCOME FROM INVESTMENTS

(Amount in Rs.)

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

Dated: 24 MAY 2016
Place:Mohali

for National Agri-Food Biotechnology Institute

Finance Officer

Suneet Verma / सुनीत वर्मा
Finance Officer / वित्त अधिकारी
National Agri-Food Biotechnology Institute
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Mohali, Punjab / मोहाली, पंजाब-160071

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मोहाली - पंजाब, भारत
Department of Biotechnology, Govt. of India

For U.K. Mehta & Associates
Chartered Accountants

U K Mehta & Associates * Chartered Accountants * Chandigarh

U.K. Mehta, FCA

SCHEDULE-16
INCOME FROM ROYALTY/PUBLICATIONS. ETC.

(Amount in Rs.)

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

SCHEDULE-17
INTEREST EARNED

(Amount in Rs.)

Particulars	Current Year	Previous Year
1)On Term Deposits		
a)With Scheduled Banks:		
i) Actual Received	37,77,384	47,35,303
ii) Accrued as on 31.03.2016	9,80,279	1,86,304
b)With Non-Scheduled Banks:		
2)On Savings Accounts:		
a)With Scheduled Banks:	1,46,581	1,91,825
b)With Non-Scheduled Banks:		
3)On Loans		
a)Employees/staff		
b) Interest on Mobilisation Advnace/Escrow Acc		1,27,34,352
4)Interest on Debtors & other Receivables		
a) Interest on refund of Income Tax	20,380	
TOTAL	**49,24,624**	**1,78,47,784**

SCHEDULE-18
OTHER INCOME

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Profit on sale/disposal of assets		
a) Owned Assets		
b) Assets acquired out of grants,or received free of		
2. Export Incentives realized		
3. Fee for Miscellaneous Services (Overhead Extenal Projects)	4,36,026	3,71,865
4. Miscellaneous Income		
a) Tender Fees	17,500	66,000
b) Misc Income	1,81,376	22,623
c) Guest House (Income)	51,800	68,450
d) RTI Fee	40	30
e) LD Charges	89,192	93,258
f) Recovery-Land Lord Interim Facility	-	14,91,190
TOTAL	**7,75,934**	**21,13,416**

for National Agri-Food Biotechnology Institute

Dated: 24 MAY 2016
Place:Mohali

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

For U.K. Mehta & Associates
Chartered Accountants

U K Mehta & Associates Chartered Accountants Chandigarh

U.K. Mehta, FCA

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

(Amount in Rs.)

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

SCHEDULE-20
ESTABLISHMENT EXPENSES

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Manpower	2,20,35,244	2,10,67,295
2. Allowances & Bonus (Honorarium)		
3. Contribution to provident fund		
4. Staff welfare expenses/seminar	-	17,932
5. Contribution to other fund(specify)		
6. Expenses on Employees Retirement & terminal benefits		
7. Others(specify) (Outsourcing)		
TOTAL	**2,20,35,244**	**2,10,85,227**

for National Agri-Food Biotechnology Institute

Dated: 24 MAY 2016
Place:Mohali

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

For U.K. Mehta & Associates
Chartered Accountants

U K Mehta & Associates Chartered Accountants Chandigarh

U.K. Mehta, FCA

SCHEDULE-21
OTHER ADMINISTRATIVE EXPENSES

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Cartage & Carriage inward	-	800
2. Allowances & Bonus (Honorarium)	2,57,410	5,32,964
3. Electricity, power and Water charges	99,68,669	68,54,407
4. Rent of Interim Facility and Guest House	1,78,67,040	1,64,08,790
5. Vehicles Running & maintenance	17,602	41,448
6. Postage,Telephone & communication charges	5,35,048	5,55,974
7. Printing & stationery	3,83,876	4,79,995
8. Travelling & conveyance expenses	18,22,571	15,92,076
9. Outsourcing Manpower Exp	88,34,402	75,98,898
10. Legel & Professional charges	20,672	70,793
11. Advt. & publicity	11,01,572	2,16,810
12. Repair & Maintenance Building	23,57,089	13,95,197
13. Office & Admn Expenses	4,23,364	3,78,466
14. Guest House Expenditure	62,020	38,679
TOTAL	**4,36,51,335**	**3,61,65,297**

SCHEDULE-22
RESEARCH & DEVELOPMENT EXPENDITURE (INCL. GRANTS, SUBSIDIES ETC.)

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Chemical & Consumables	1,53,98,687	1,72,98,449
2. Fellowship	50,40,651	35,29,977
3. Computer Software & Accessories	11,43,446	22,73,580
4. Research Work Expenses	2,61,066	41,538
5. Field Expenses (Ploughing, RM & Other Job work)	1,81,412	73,439
TOTAL	**2,20,25,262**	**2,32,16,983**

SCHEDULE-23
INTEREST

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. On Fixed loans		
2. On Other Loans		
3. Others (Specify)		
TOTAL		

for National Agri-Food Biotechnology Institute

Dated: 24 MAY 2016

Place:Mohali

Finance Officer

Suneet Verma / सुनीत वर्मा
Finance Officer / वित्त अधिकारी
National Agri-Food Biotechnology Institute
Govt. of India / भारत सरकार
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Executive Director

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मोहाली - 160071 पंजाब, भारत
Department of Biotechnology, Govt. of India
Mohali-160071 Punjab INDIA

For U.K. Mehta & Associates
Chartered Accountants

U.K. Mehta & Associates Chartered Accountants Chandigarh

U.K. Mehta, FCA

FORM OF FINANCIAL STATEMENTS

NATIONAL AGRI FOOD BIOTECHNOLOGY INSTITUTE

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

SCHEDULE 24

SIGNIFICANT ACCOUNTING POLICIES

A) ACCOUNTING CONVENTION

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

B) INVENTORY VALUATION

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

C) INVESTMENTS

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

D) FIXED ASSETS

Fixed assets are valued at cost of acquisition inclusive of inward freight, duties and taxes and incidental and direct expenses related to acquisition, however, the value Fixed Assets created out of the completed /closed external funded projects have been taken at the nominal value of Rupee one for each article.

E) DEPRECIATION

Depreciation on fixed assets has been charged as per the rate prescribed in the Income Tax Act-1961 on written down value method, however, no depreciation has been charged on the Fixed Assets created out of the completed /closed external funded projects as their value has been taken at the nominal amount.

F) MISCELLANEOUS EXPENDITURE

There is no deferred revenue expenditure during 2015-16

G) ACCOUNTING FOR SALES

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

U K Mehta & Associates Chartered Accountants Chandigarh

H) GOVERNMENT GRANTS/ SUBSIDIES

As the Institute is funded by the Department of Biotechnology (DBT) , Ministry of Science and Technology, (Govt. of India) and the grants are treated as irrevocable, the same has been accounted for

on receipt basis. During the FY 2015-16 , recurring grants amounting to Rs. 11,00,00,000/- has been received for the purpose as shown in schedule-13. Non-recurring Grants amounting to Rs. 24,00,00,000/- received from DBT have been shown as addition to Corpus/ Capital Fund (schedule-I).

I) Expenses payable up to 31st March, 2016 pertaining to FY 2015-16 have been shown under expenses payable. Any expenditure which has not been claimed or for which bill has not been received pertaining to any expenditure relevant to the FY 2015-16, the same will be accounted for in the year of claim.

J) RETIREMENT BENEFITS

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

K) FOREIGN CURRENCY TRANSACTIONS

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

For National Agri-food Biotechnology Institute

Finance Officer

Dated: 24 MAY 2016

Place: Mohali

Sunesh Verma / सुनीत वर्मा
Finance Officer / वित्त अधिकारी
National Agri-Food Biotechnology Institute
Govt. of India / भारत सरकार
Deptt. of Biotechnology / जैवप्रौद्योगिकी विभाग
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Executive Director

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Department of Biotechnology, Govt. of India
Mohali-160071 Punjab, INDIA

U. K. Mehta & Associates
Chartered Accountants

U K Mehta & Associates Chartered Accountants Chandigarh

(U.K. Mehta), FCA

FORM OF FINANCIAL STATEMENTS

NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE

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

SCHEDULE 25

NOTES ON ACCOUNTS

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

1. Receipt and Payment Accounts

The Receipt & Payment Account carries the figures of actual receipts & actual payments of the Institute during the financial year 2015-16. It is virtually a copy of cash book / Institute's accounts. The total receipt as shown in receipt & payment account comes to Rs. 38,31,19,446/- which include Rs. 35,00,00,000/- as Recurring and Non-recurring grants from DBT, grant of Rs. 2,75,77,853/- for externally funded projects and Rs. 55,41,593/- rest from other receipts. An amount of Rs. 35,55,69,717/- has been released as payments .

2. The Income and Expenditure Account

The Income and Expenditure accounts are prepared on accrual basis. The total income is Rs. 11,57,00,558/- out of which includes Rs. 11,00,00,000/- Recurring Grant from DBT and rest is from Interest & Other Resources.

Total expenditure (before depreciation) comes to Rs. 8,77,11,841/- and depreciation of Rs. 3,46,22,172/- has been charged in the current FY 2015-16. A sum of Rs. 66,33,455/- being excess of expenditure over income has been transferred to Corpus/ Capital Fund (Schedule-1).

3. Fixed Assets

Fixed assets are valued at cost of acquisition inclusive of inward freight, duties and taxes and incidental and direct expenses related to acquisition, however, the value of Fixed Assets created out of the completed /closed external funded projects have been taken at the nominal value of Rupee one for each article and corresponding accounting effect has given to the Corpus/ Capital Fund (Schedule-1) up to that extent .

During the FY 2015-16, a sum of Rs. 45,56,926/- has been earned as interest on deposits with RITES, which has been reduced from capital work-in-progress at main campus (Schedule-8) as advised by the JS & FA, DBT, Govt. of India in the 11th Finance Committee meeting held on 08-10-2015.

4. Depreciation

Depreciation on fixed assets has been charged as per the rate prescribed in the Income Tax Act-1961 on written down value method, however, no depreciation has been charged on the Fixed Assets created out of the completed / closed external funded projects as their value has been taken at the nominal amount.

U K Mehta & Associates Chartered Accountants Chandigarh

5. Current Assets, Loans and Advances

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

6. Land

The Government of Punjab has provided approx. 35 acres of land in Knowledge City at Sector-81, Mohali to the Institute, free of cost, for setting up of NABI Campus.

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

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

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

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

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

For National Agri-food Biotechnology Institute

Finance Officer

Dated: 24 MAY 2016

Place: Mohali

Sunee Verma/सुनीत वर्मा
Finance Officer/वित्त अधिकारी
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U. K. Mehta & Associates
Chartered Accountants

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

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