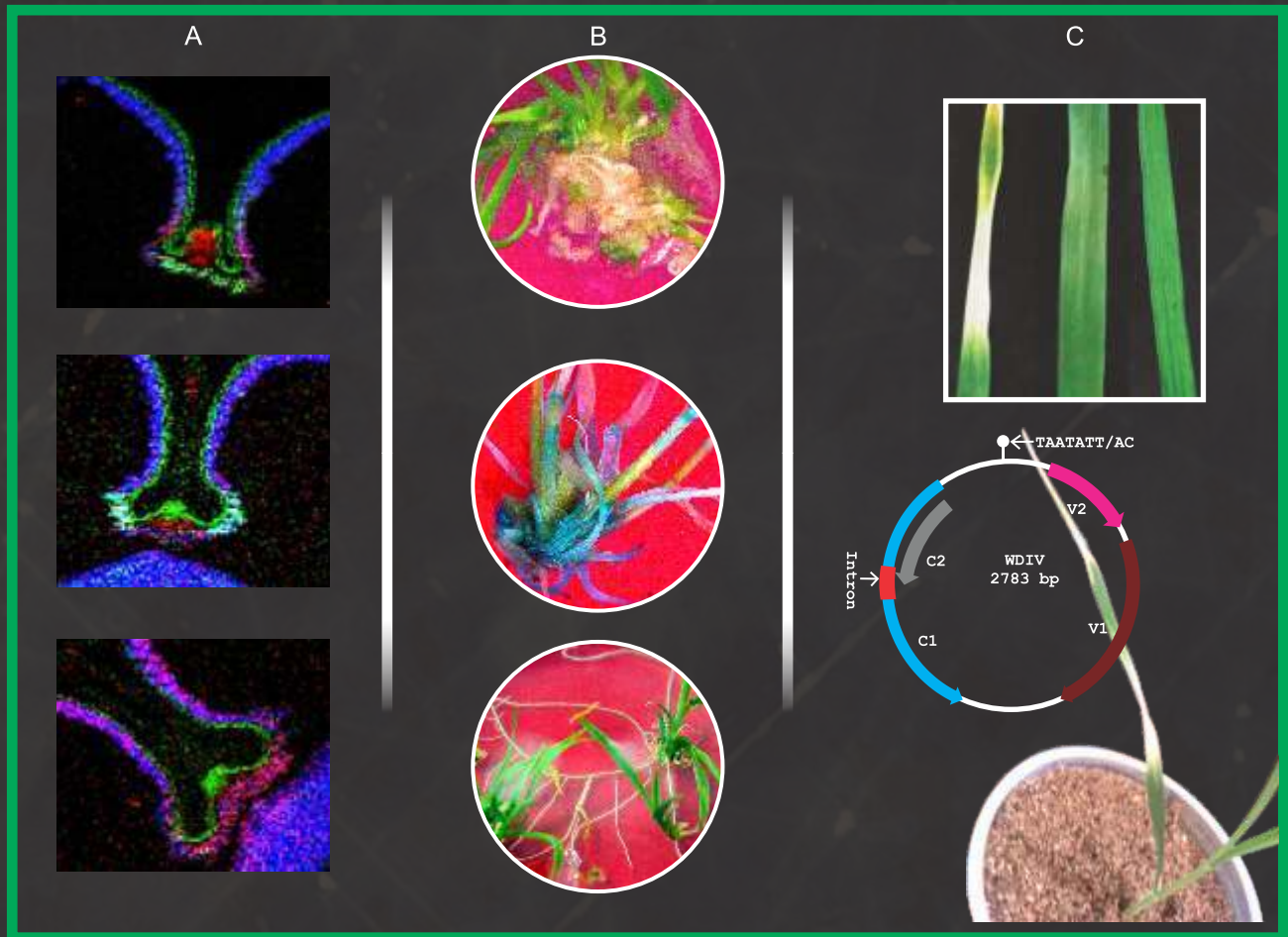


वार्षिक प्रतिवेदन ANNUAL REPORT 2011 - 2012



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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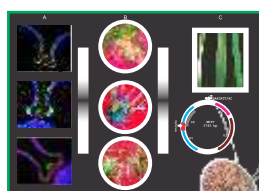
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Image on Cover Page:

The cover page portrays: (A) Iron μ -PIXE maps of cross-section of grains of *Aegilops kotschy* accession 3790 (top), *Triticum aestivum* L. IITR26 (middle) and *Triticum aestivum* cv. WH291 (bottom) (PP. 7-10). (B) *In-vitro* multiple shoot induction and elongation from wheat callus (top), GUS expression in transgenic shoot of wheat (middle), *in-vitro* root formation in regenerated wheat shoots (PP. 16-17). (C) Genome organisation of Wheat Dwarf India Virus (middle) and silencing of visual marker gene (photo bleaching upon PDS suppression: top and bottom) in wheat leaves (PP. 13-15).

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FROM THE DESK OF THE EXECUTIVE DIRECTOR

During its second year of growth, NABI began to prepare itself to function as a 'Change-Leader' who should anticipate the future needs of agri-food sector, expand knowledge, and translate it to design food plants for the future. The Governing Body constituted the first Scientific Advisory Committee (SAC) and the Programme Advisory Committee (PAC) of NABI to guide its advancement around turning points in agri-food biotechnology. Expanding the faculty and staff at NABI was the immediate need. The Programme Advisory Committee in its first meeting held on July 26, 2011 emphasized upon establishing a system for attracting bright young scholars. The Governing Body approved a number of fellowships at NABI and stipend for short term trainees. Soon, the institute developed critical mass in some areas, and began to grow.

The faculty strength grew from seven last year to twelve this year. The administration and technical staff grew from seven to thirteen. Besides faculty, four contractual scientists, fifteen research fellows and seventeen trainees energized the institute and its resources. The infrastructure at the Interim Facility expanded in genomics, tissue specific transcriptomics, metabolomics, genome informatics, animal and plant tissue culture, controlled environment plant growth facilities, food chemistry etc. An architect was appointed to design the NABI campus. Germplasm collection at experimental field became richer. The institute began to vibrate with energy.

The Governing Body and the Scientific Advisory Committee inspired NABI to develop into a world class institute on the strength of transformational innovations required for the shifting role of plants in bio-based economy. The current objective of designing plants for 'economic access to food' was shifting to designing plants differently for 'food sufficiency with health and bioeconomy'. Some of the photo synthetically most efficient plants will be designed more creatively in future to produce

specialty molecules for industry - the starches, oils, proteins and phytomolecules. A twenty first century institute must generate daring ideas in biotechnology, beyond the current GM technologies and design plants for hybrid



vigour, photosynthetic potential, bioavailable nutrients, post harvest stability, specialty products, nutrient and water utilization efficiency and endurance to biotic and abiotic stresses. Transformational inventiveness, team work, big thinking, confidence to succeed from farm to fork and societal priorities should set the research agenda for NABI.

Some of the important projects executed during the year included efforts towards the identification of molecular traits and genes that determine the processing and nutritional quality in food crops, the ingredients that enhance the value of food for consumer health, the growth and developmental pathways that determine seed formation and the quality of fruits, transformational tools appropriate to designing plants for future, and sustainable biological approaches for adding value to complete agricultural produce from seed to straw. Besides *Arabidopsis* as the model plant, the crop species in the above researches were wheat, millets, litchi, custard apple and mango. Details of individual projects are described in the chapters that follow.

The first research paper on the work 'conceived and initiated' at NABI was accepted for publication. This was a motivating news in the second year of an institute that started from nothing. The paper reported the first finding of a gemini virus infecting wheat in India. It was published in the Archives of Virology. NABI will hopefully use this virus to develop first

indigenous tools in the form of gene silencing and expression vectors for basic and applied studies on wheat. Discovery of the wheat virus in two geographically and climatically distinct locations (Mohali and Nilgiri) may be a pointer at taking this new virus seriously by the breeders and wheat pathologists in the country.

The first sponsored research project that NABI received was in the important area of information flow from root to seed. The early findings on the possibility of moving small RNA signals across the plant, can open valuable opportunities for regulating economically and agronomically important traits. The applications of mobile small RNA signals can open extraordinary possibilities for designing plants. NABI should hopefully apply this technology to develop smarter horticultural plants that are difficult to improve due to long growing seasons.

The emphasis on commitment, creativity, infrastructure, intellect and team work should kindle high ambitions in the young faculty and research fellows to keep NABI ahead of time in addressing the challenges in nutrition, food security and biobusiness. A good beginning had been made - thanks to the insights and encouragement by the members of the

Programme Advisory Committees, Scientific Advisory Committee, Governing Body, the Society of NABI; and hard work of the faculty and staff. The heritage of a rich beginning should take the institute to greater accomplishments in the years to come.

It is a continuing journey for NABI. My colleagues have done well in preparing the institute to match the high expectations in transforming plant sciences. A new institute like NABI is freer to sketch and plan its areas for investment, when the resources and details are still uncommitted, than at a later stage when the die has been cast and one must live with what the past had chosen. Through this Annual Report, I urge the readers and well wishers to guide us to the path of exceptional creativity for addressing the changing landscape and dynamics of knowledge for national good and global networks.

A handwritten signature in blue ink, reading "R. Tuli", is positioned above the name of the Executive Director.

(Dr Rakesh Tuli)

Executive Director

National Agri-Food Biotechnology Institute



VISION AND MISSION STATEMENT

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

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



RESEARCH PROGRESS AND EMERGING AREAS

1. IMPROVING CEREALS FOR NUTRITION AND PROCESSING QUALITY

1.1 Functional genomics for enhancing mineral nutrition and processing quality in wheat

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

Principal Investigator:

Rakesh Tuli

Co-Investigator:

Sudhir P. Singh

Research Fellows:

Anuradha Swami

Raja Jeet

Introduction:

In wheat grain iron is located in the outer layer, called bran (Figure 1) and is lost substantially during milling and processing. The wheat flour is almost devoid of iron. The prospect of developing wheat grain with iron-enriched endosperm is of a great interest. We are trying to understand the bottlenecks preventing iron translocation from the outer bran layer into the endosperm in wheat. Barriers to mineral transport within grain can be explored by examining the turnover rate for metals between different grain tissues in contrasting genotypes. Synchrotron radiation is a useful approach to study the spatial element distribution, element concentration, state of metals and chemical environment in different tissues of wheat grain. μ -X ray Fluorescence (μ -XRF) and μ -Proton Induced X-ray Emission (μ -

PIXE) are promising tools to study iron localization and tissue specific iron concentration in seeds of contrasting wheat genotypes. X-ray Absorption Near Edge Structure (μ -XANES) and Extended X-ray Absorption Fine Structure (EXAFS) can be used to investigate ionic state of iron and the chemical environment around iron metal in biological tissues. The aim of the project is to study the dynamics of iron distribution pattern and chemical forms of iron in the seed tissues of contrasting wheat genotypes by using synchrotron powered beam sources (XRF, PIXE, XANES and EXAFS).

Plant genome encodes families of metal transporters, which control metal distribution in plants by different expression pattern and cellular localization. It is essential to examine difference in temporal and spatial expression of proteins within the tissues of developing grains of contrasting wheat genotypes. The project aims at studying tissue specific transcriptomics by using Laser Capture Micro-dissection (LCM) facility to study the molecular biology of iron trafficking from maternal tissues to filial tissues of wheat grains.

Research Objectives:

- To study iron localization pattern and chemical forms in the grains of contrasting wheat genotypes.
- To examine differential transcriptomics in the tissues of developing wheat grains.
- Gene discovery for high accumulation of bioavailable iron in wheat.

Long Term Objective:

Development of wheat varieties with high bioavailable iron content in grains.

Research in Progress:

1. μ -XRF iron localization maps have been generated focusing on the crease region of mature grains of contrasting genotypes of

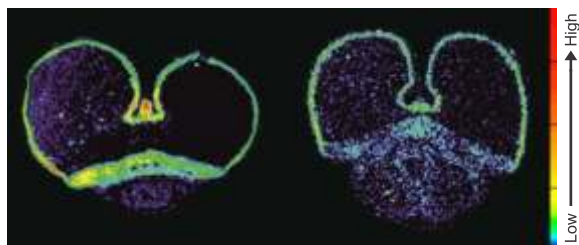


Figure 1: Iron distribution pattern in transverse section of *Aegilops kotschy* (L) and *Triticum aestivum* (R) grain by μ -PIXE (micro-Proton Induced X-ray Emission). Most of the iron is localized in outer bran layer.

wheat by using synchrotron powered beam line-VESPERS (Very Sensitive Elemental and Structural Probe Employing Radiation), at Canadian Light Source (CLS), Saskatoon, Canada. XRF images obtained of the crease region of diverse genotypes showed different iron localization pattern. The high iron genotypes (*Aegilops kotschy* acc. 3790, *Triticum aestivum* L. ITR26) showed relatively high intensity of iron near pigment strand and nucellar projection tissues of crease region (Figure 2). On the other hand, in the low iron wheat genotypes

different tissues of mature wheat grain of contrasting genotypes at University of Ljubljana and Jozef Stefan Institute, Ljubljana, Slovenia. The μ -PIXE imaging analysis of *Aegilops kotschy* acc. 3790 revealed the presence of high concentrations of iron in crease region, in particular vascular strands (Figure 3). The other iron rich tissues were nucellar projection, the crease-aleurone, aleurone and scutellum. The endosperm showed significantly low iron accumulation. Its embryo region showed only little increase in iron

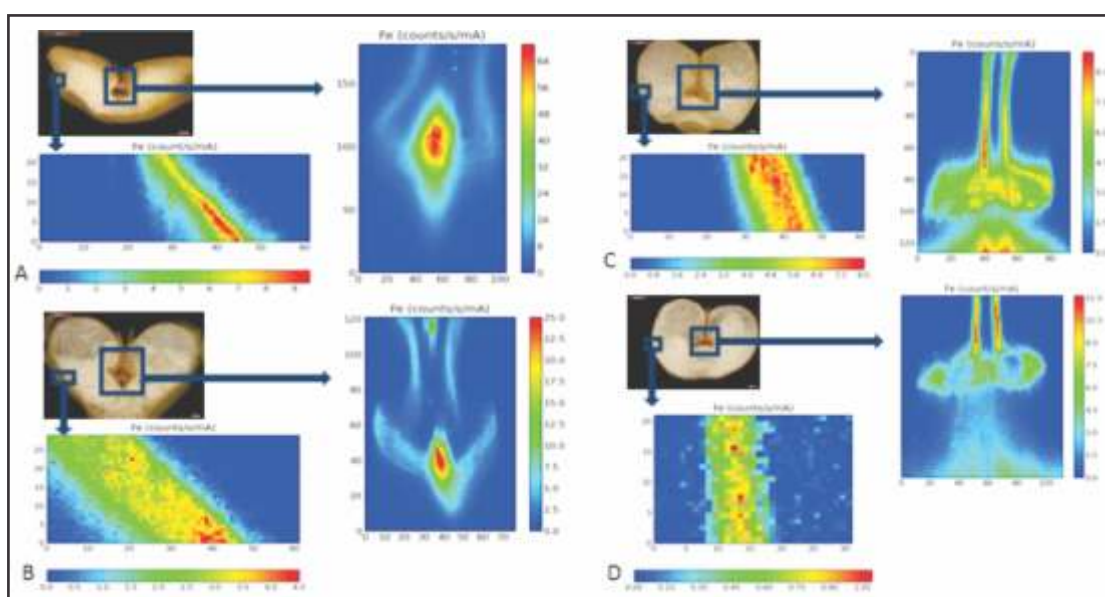


Figure 2: Iron XRF maps of transverse section of seed of (A) *Aegilops kotschy* accession 3790. (B) *Triticum aestivum* L. ITR26. (C) *Triticum aestivum* cv. WH291. (D) *Triticum aestivum* cv. WL711. The colour scale represents relative intensities of fluorescence, with blue and red corresponding to the lowest and highest iron intensity, respectively.

(*Triticum aestivum* cv. WH291, *Triticum aestivum* cv. WL711) the high intensity region was mobilized towards aleurone of crease (Figure 3). XRF scanning in all four genotypes showed aleurone as the iron hot spot region with very low signals for iron in the internal layers of endosperm (Figure 2).

2. μ -PIXE iron localization maps have been generated to further validate the μ -XRF and to get accurate quantification of iron in

accumulation than in endosperm. Iron distribution analysis in *Triticum aestivum* L. ITR26 showed nucellar projection as iron hot spot, carrying highest concentration of iron. The next iron rich tissues were crease-aleurone, aleurone and scutellum. In vascular strand, iron was three times lesser than in nucellar projection, indicating mobilization of iron from vascular tissue to nucellar projection.

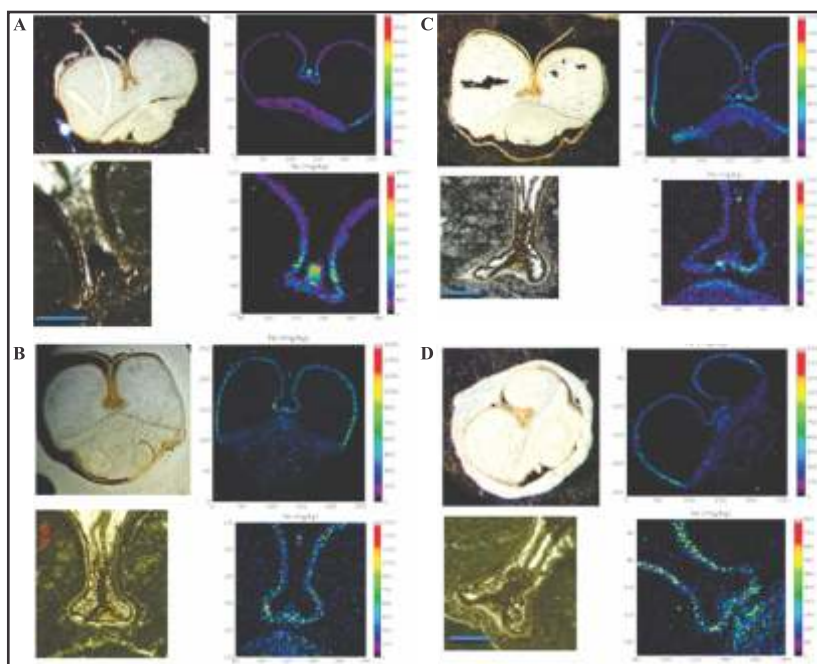


Figure 3: Iron μ -Proton induced X-ray emission (μ -PIXE) maps of cross-section of grain of (A) *Aegilops kotschy* accession 3790. (B) *Triticum aestivum* L. ITR26. (C) *Triticum aestivum* cv. WH291. (D) *Triticum aestivum* cv. WL711.

PIXE element maps of low iron genotypes *Triticum aestivum* cv. WH291 depicted crease-aleurone, nucellar projection and aleurone as major tissues for accumulation and localization of iron, followed by scutellum and vascular strand. On the other hand, in WL711, the genotype having less grain iron content, aleurone was the iron richest tissue followed by nucellar projection and scutellum. In nucellar projection, iron concentration was half of the aleurone. In vascular tissue, iron was three times lesser than that in aleurone. Thus, in contrast to the high iron genotypes,

a gradual increase in iron concentration was observed along the mineral-transport route (from vascular tissue, nucellar projection to crease-aleurone) of both of the low iron genotypes. However, significantly less relative amount of iron in endosperm of all the genotypes affirms aleurone as the major hurdle in iron mobilization towards endosperm.

3. μ -XANES has been recorded from different tissues of crease region of mature wheat grains to identify different ionic forms of iron and chemical environment around iron

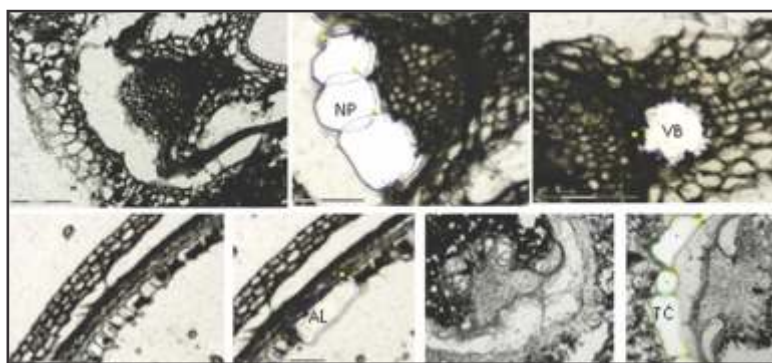


Figure 4: Laser captures micro-dissection of nucellar projection transfer cells (NP), vascular bundle (VB), aleurone (AL) and endosperm transfer cells (TC) of wheat grain at 14th days after anthesis.

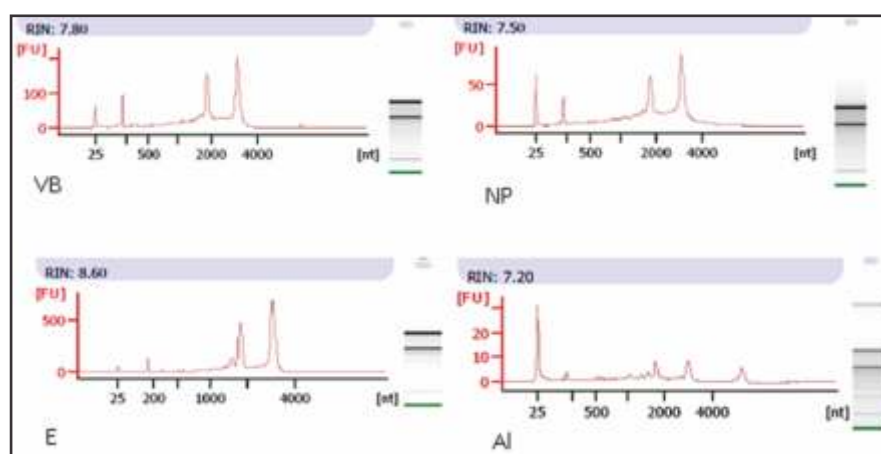


Figure 5: Electropherogram of RNA, extracted from seed tissues of WL711: VB=Vascular Bundle, NP= Nucellar Projection, E= Endosperm, Al= Aleurone, showing RIN value and RNA integrity assessed on Bio-analyser (Agilent).

metal at CLS. XANES of bulk seeds are in progress at Jozef Stefan Institute, Slovenia. Extended X-ray absorption fine structure (EXAFS; at Hamburg Synchrotron Radiation Laboratory, Hamburg, Germany.) will be performed on the mature wheat grain samples to get the chemical form in which iron is localized in the mature wheat grains.

4. To examine the tissue specific differential transcriptome in the tissues of developing grains of contrasting genotypes, LCM was applied to micro dissect tissues such as vascular bundle, nucellar projection, endosperm and aleurone (Figure 4). RNA extraction from the micro-dissected tissues has been established. The RNA Integrity Number (RIN) was above 7 in each case (Figure 5), hence suitable for tissue specific transcriptomics studies to be taken up in the future.

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

Co-ordinator:

Rakesh Tuli

Investigators:

Joy K. Roy

Shrikant Subhash Mantri

Project Scientist:

Nishima Wangoo

Research Fellows:

Anuradha Singh

Monica Sharma

Introduction:

Wheat is one of the most important staple food crops and wheat flour is processed into a wide range of baked and processed foods. Although wheat breeders have been successful in achieving high yields, but the present high yielding varieties require improvement in processing and nutrition related traits to meet the increasing demand of healthy wheat diets for consumers and better processing quality for baking and processing industries. Wheat is also a good source of dietary fibres (germplasm & bran) i.e. non-starch carbohydrates known to be helpful in the prevention of diet related health problems such as diabetes, hypertension and some types of cancers. Better understanding of genes and regulators of metabolic pathways and their interaction with environment is required for the improvement of seed quality for nutrition and processing.

Research Objectives:

- Transcriptome and small RNA sequencing and genomic expression studies using wheat microarrays to identify genes and regulators

of starch and phenolics biosynthesis pathways having significant effects on processing and nutrition quality.

- Evaluation of effects of variation in intermediates and products of the above pathways on processing and nutrition quality.
- Validation of gene function.

Long Term Objectives:

To improve the high yielding Indian wheat varieties and/or advanced breeding lines for processing and nutritional quality to meet the increasing demand of healthy wheat diets for consumers and better processing quality for baking and processing industries.

Research in Progress:

1. Four popular varieties of Indian wheat (C306, Lok1, Sonalika, WH291) were evaluated for processing and nutrition traits.
2. Genomic expression was compared at three developmental stages by using Affymetrix wheat arrays. Several genes involved in iron metabolism, starch metabolism, enzymes (cellulase, catalase, peroxidase, lipase, xylanase, peptidase, chitinase), hormone biosynthesis (auxins, gibberellic

acid, cytokinin, ethylene and abscisic acid), cell division, heat shock, cold acclimatization, storage proteins and transcription factors were examined.

3. Gene expression profiles for nicotianamine synthase, ferritin, metallothioneine and phytic acid pathway genes were identified. These can be potential candidates for the improvement of wheat for high accumulation and bioavailability of micronutrients such as iron and zinc. Based on sequence homology with the inventory database of wheat transcription factors (wDBTF), twenty three probe sets showing >10 fold changes at least at one developmental stage were identified. Among them, two transcription factors: Plant Homeo Domain (PHD) and C2C2 GATA showed 50 fold change (Figure 6).
4. Peroxidase and some homologs were up regulated 30 fold at 28 DAA, while granule bound starch synthase (mutant GBSS1, Ta.24114.7.A1_at), cold acclimatization protein (Ta.14489.1.S1_at) and cell division protease (FtsH, Ta.27445.1.S1_at) were down regulated at 28 DAA (Figure 6).

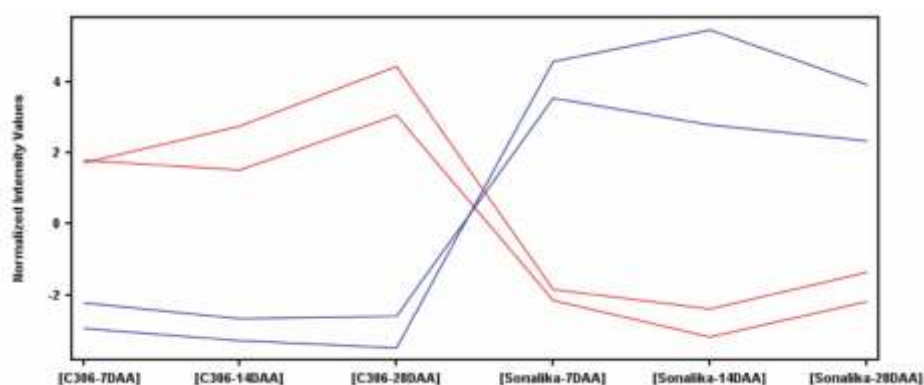


Figure 6: Differential expression of two major up-regulated genes (peroxidase and C2C2 GATA) and two major down-regulated genes (mutant GBSS1 and PHD) between good (C306) and poor (Sonalika) processing varieties at 3 developmental stages-7, 14, 28 DAA.

5. The processing quality is affected by size and distribution of starch granules. Starch granules were visualized under light

contrasting wheat varieties, C306 and Sonalika. These showed more differences in free phenolics than bound phenolics (Figure

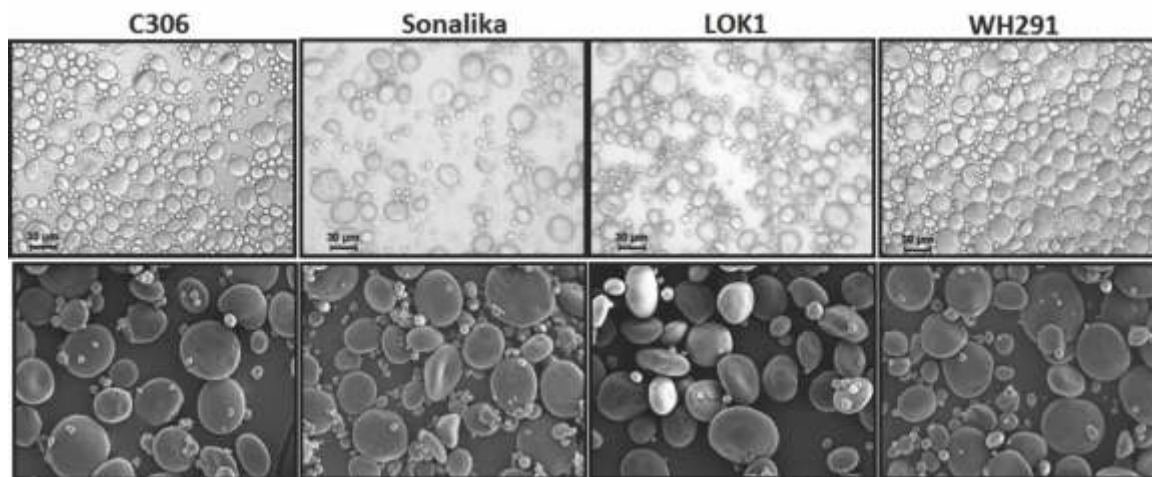


Figure 7: Starch granules of C306, Sonalika, LOK1 and WH291 at 40X magnification viewed under a light microscope (upper panel) (scale bar = 30µm) and at 1000X magnification under Hitachi VP-SEM S-3400N facility (scale bar=50 µm).

microscope and scanning electron microscope (Figure 7). The four varieties showed differences in size distribution.

6. The percentage of amylopectin in the four varieties, C306, LOK1, Sonalika and WH291 was 21.1, 23.8, 22.0 and 25.0, respectively.
7. Free phenols and bound phenols were extracted from the seeds of the two

8). The wheat variety C306 (86.9 to 104 GAE/100g) had more free phenolic content in comparison with Sonalika (63.9 to 68.8 GAE/100g).

8. To identify phenolic compounds, free phenolics were analysed on LC-MS (Figure 9). The two varieties (C306 and Sonalika) showed differences in the types of phenolic compounds.

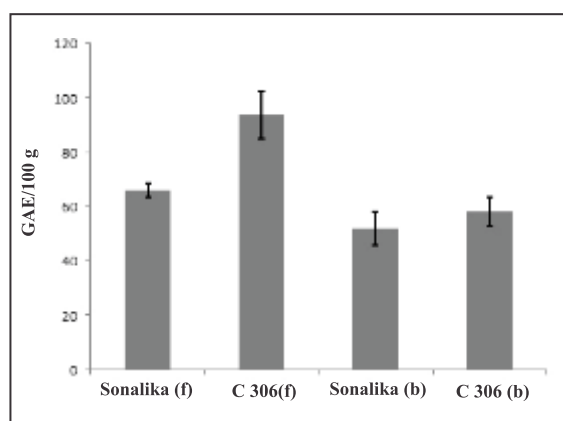


Figure 8: Free (f) and bound (b) phenolic content in two Indian wheat varieties, C306 and Sonalika, determined by FCR method (GAE=gallic acid equivalents).

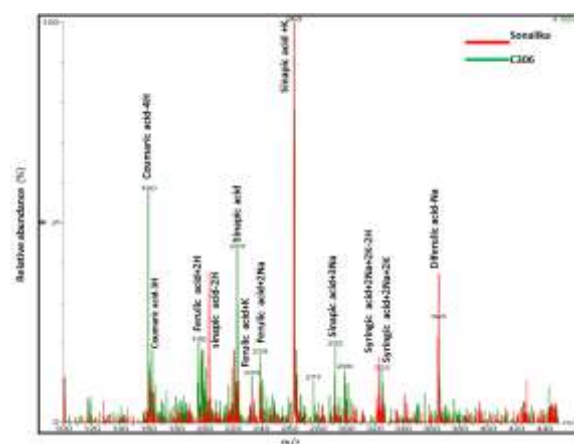


Figure 9: Different phenolic compounds identified in C306 and Sonalika using LC-MS

1.1.3 Development of Virus Induced Gene Silencing (VIGS)

Principal Investigator:

Rakesh Tuli

Research Fellows:

Jitendra Kumar

Jitesh Kumar

Kaushal Bhati

Introduction:

VIGS is an approach to facilitate the assessment of gene function in plants. A recombinant virus that infects plant tissue and spreads systemically can be very useful in the expression of small RNA and silencing of targeted endogenous genes. The target transcript is degraded by Post-Transcriptional Gene Silencing (PTGS). VIGS can validate the function of a specific gene within a single generation and obviates the need for screening large populations to identify mutations in specific genes. Being a transient method, it does not require the generation of stable transgenic plants. The project aims at developing an efficient VIGS vector for wheat.

Research Objectives:

- Detection of viruses infecting wheat in India.
- Analysis of the viral main genome and accessory satellite genomic sequences.
- Establishment of infectivity in different Indian wheat varieties by inoculating virus in the absence or presence of the accessory genomes.
- Development of VIGS vector by modifying the viral genome.
- Validation of VIGS vector using visual markers.

Long Term Objective:

Elucidation of functions of candidate genes involved in determining the processing and nutritional traits in wheat.

Research in Progress:

1. **Detection and characterisation of viruses infecting wheat in India:** The presence of virus and insect vector was investigated in



Figure 10: Difference in growth and physical appearance of healthy and WDIV infected plants. Panel on the right shows probable insect vector.

wheat growing fields and suspected samples were tested (Figure 10). About 80% of the samples tested positive for the virus in the samples collected during 2010 and 2011 (Mohali, Punjab and Wellington, Tamil Nadu). The whole genome of the virus was sequenced and nucleotide sequences of two clones were submitted to the GenBank under the accession numbers JQ361910 and JQ361911. The virus was named as Wheat Dwarf India Virus (WDIV).

2. **Detection of geminivirus associated satellites from infected wheat samples:** The wheat samples found positive for the virus were investigated, and two types of associated satellites (alpha and beta) were detected (Figure 11).



Figure 11: Amplification for a mastrevirus, beta satellite and alpha satellite.

3. **Establishment of infectivity in different Indian wheat varieties:** Infectious clones for virus and associated satellites were made and infectivity tests were done. Host range was determined by infecting different Indian wheat varieties which showed consistent infectivity (Figure 12).



Figure 12: Agroinoculated wheat plants at 40th day post inoculation. M= mock inoculated and V= virus infectious clone inoculated on different wheat varieties.

4. **Assessment of effect of alpha and beta satellites on the symptom induction by virus:** The phenotype was observed for assessing the affects of the satellites on the virus symptom induction process. The plants were observed showing more

was studied by using microarrays.

Expression analysis by using microarray revealed that, sixteen unigenes were up regulated by more than 10 fold, thirty five unigenes by more than 5 fold and about six hundred unigenes by more than 2 fold by the



Figure 13: Agroinoculated wheat plants at 42nd day post inoculation. M= mock inoculated, V= virus infectious clone and V+A+B= virus+alpha+beta satellite infectious clone inoculated wheat plants.

5. **Assessment of effect of alpha and beta satellites on virus accumulation in plants:** Semi quantitative PCR was done to assess the effect of the satellites on the virus accumulation inside the plant. The accumulation of virus was found high in the presence of the satellites (Figure 14). The effects of virus on plant at molecular level in the presence and absence of these satellites

viral infection. Eighteen different unigenes were down regulated by more than 10 fold, sixty other by more than 5 fold and seven hundred other unigenes by 2 fold at the expression level. Presence of the beta satellite supported the gene regulation pattern of the virus, however, the alpha satellite regulated different sets of unigenes. Presence of both the satellites and virus regulated more number of unigenes in the host plant (Figure 15).

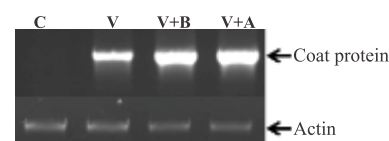


Figure 14: Accumulation of virus in the presence of satellites. V= virus, B= beta satellite and A= alpha satellite.

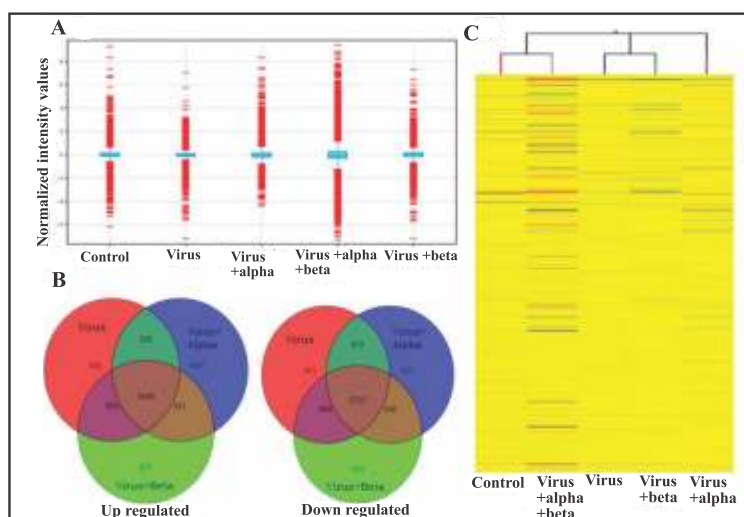


Figure 15: Gene expression analysis in wheat leaves after 21 dpi with WDIV. (A) Up and down regulation of genes. (B) Venn diagram representing the set of genes up regulated and down regulated during virus infection. (C) Hierarchical clustering and changes in gene expression in virus only or virus+satellites-infected wheat plants at 21st day post inoculation (dpi). Gene tree maps were generated using k-mean clustering at 2 fold or higher differentially regulated wheat genes.

6. **Development of VIGS vector by modifying the viral genome:** Two modifications were done in viral genome by removing a small stretch of nucleotides and inserting Multiple Cloning Sites (MCS) at the same positions. The modified viral genome was cloned in a plasmid vector for further manipulation.
7. **Validation of VIGS vector using visual markers:** A 327 bp fragment of phytoene desaturase (PDS) gene was amplified using cDNA as a template prepared from wheat (*Triticum aestivum* L.). The amplified fragment was cloned and sequenced to establish the identity. Sequence analysis showed an identity of 99% with the previously reported PDS gene (GenBank

accession number FM998042). The PDS fragment was excised from the cloning vector and was inserted into the MCS of viral genome using *SpeI* restriction endonuclease. The viral genome having PDS was termed as VIGS-PDS. In order to infect wheat for silencing of the PDS gene, head-to-tail dimerization of VIGS-PDS was done into pCambia1301 binary vector. The PDS silencing cassette (pCambia-VIGS-PDS) was transformed into *Agrobacterium tumefaciens* (strain GV3101). The empty VIGS vector was also transformed in *Agrobacterium*. The silencing cassette and empty VIGS-vector were inoculated to healthy wheat plants separately and scored for phenotype at 15 days (Figure 16 A, B and C).

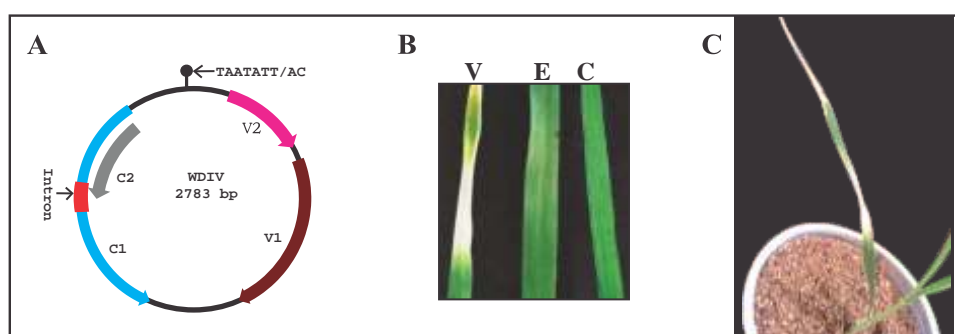


Figure 16: Modification of WDIV for functional tool. (A) Genome organization of WDIV detected from wheat in India and used for developing as VIGS-vector, C1-Rep Protein, C2-Rep-A Protein, V1-Coat Protein, V2-Movement Protein. (B and C) Leaves from silencing PDS-cassette inoculated (V), empty VIGS-vector (E) and uninoculated control (C) wheat plants. (C) Partial view of a VIGS-PDS inoculated wheat plant.

1.1.4 Efficient genetic transformation of wheat

Principal Investigator:

Siddharth Tiwari

Research Fellow:

Anshu Alok

Introduction:

Efficient regeneration and genetic transformation protocols are pre-requisite for crop improvement through genetic engineering. The protocols for callus as well as direct multiple shoot mediated *in-*

Research in Progress:

1. Establishment of *in-vitro* regeneration:

a. Callus mediated regeneration:

- i. In the four high yielding (PBW621, PBW550, HD2697 and LOK1) cultivated varieties of wheat, matured embryos cultured on MS medium supplemented with 2, 4-D showed the best response for callus induction (Figure 17 A and B).
- ii. Multiple shoot induction and elongation from callus were observed to be most efficient on MS medium supplemented with zeatin (Figure 17 C, D and E).



Figure 17: Callus induction and regeneration. (A) Explant (mature embryo). (B) Callus formation. (C-E) Stages of regenerated calli. (F) Root formation.

vitro regeneration and *Agrobacterium*-mediated genetic transformation of wheat have been optimized. Transgenic plants with reporter b-glucuronidase (GUS-Intron) gene expression have been developed and further molecular and segregation studies are in progress. The overall aim of the work undertaken is to establish an efficient *in-vitro* regeneration and genetic transformation protocols for wheat.

Research Objective:

Establishment of efficient *in-vitro* regeneration and genetic transformation protocols for wheat.

Long Term Objective:

Development of nutritionally rich and agronomically improved varieties.

- iii. Healthy roots were induced on the MS basal medium (Figure 17 F).

b. Direct multiple shoot mediated regeneration:

- i. Mature embryos of high yielding (PBW621, PBW550) cultivars of wheat showed best direct multiple shoot

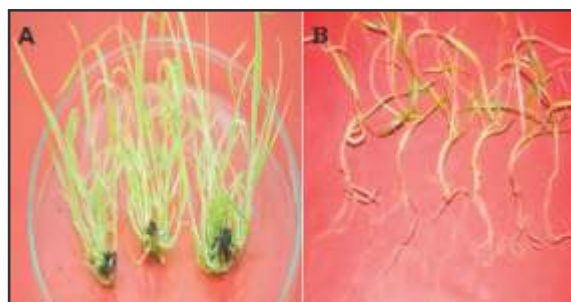


Figure 18: Direct multiple shoot regeneration of wheat plants. (A) Multiple shoots. (B) Root formation.

induction without an intermediary callus on MS media containing 6-Benzylaminopurine (BAP) and Thioridazuron (TDZ) (Figure 18A).

- ii. Culture was maintained as the continuous source of shoots.
- iii. Shoots were separated from the multiple

expression studies in transgenic wheat.

- a. **Callus based regeneration of transgenic wheat:** The transient and stable expression of reporter gene was noticed as blue colour on the embryos and callus-derived shoot bud, respectively (Figure 19 A and C). No

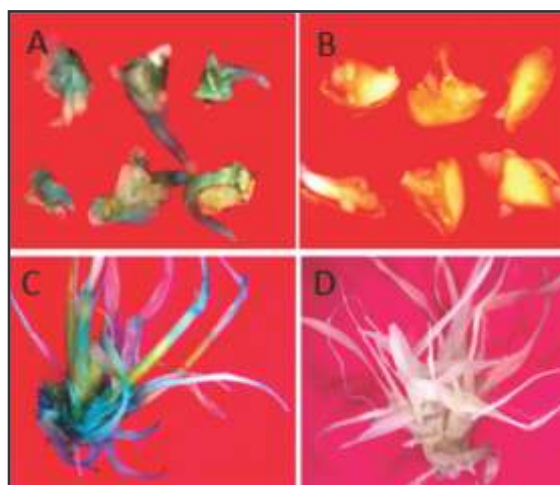


Figure 19: GUS histochemical assay. (A) Transient expression in embryos (blue color). (B) Non-transgenic embryos. (C) Stable expression in callus-derived shoot bud (blue color). (D) Non-transgenic shoot bud.

shoot clumps and rooted on MS basal medium containing Indole-3-butyric acid (IBA) and Naphthaleneacetic acid (NAA) (0.5mg/l each) (Figure 18 B).

2. **Establishment of genetic transformation:** Several factors were considered for the optimization of *Agrobacterium*-mediated genetic transformation. Mature embryo derived callus and direct multiple shoot induction based *in-vitro* regeneration was used for reporter (GUS-Intron) gene

expression of GUS was noticed in the control embryos and shoot bud (Figure 19 B and D).

● **Direct multiple shoot induction based regeneration of transgenic:**

- a. Stable expression was noticed as blue color on the shoots developed without an intermediate callus (Figure 20 A and B). No expression of GUS was noticed in control shoots (Figure 20 C).



Figure 20: GUS histochemical assay. (A and B) Transformed germinated shoots (blue color). (C) Non-transformed shoots.

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

Principal Investigators:

Ajay K. Pandey

Co-Investigator:

Siddharth Tiwari

Research Fellow:

Roohi Bansal

Introduction:

Phytic acid (myo-inositol-1, 2, 3, 4, 5, 6-hexakisphosphate; PA; InsP₆) beside been a storage form of phosphorus in seed is also a well

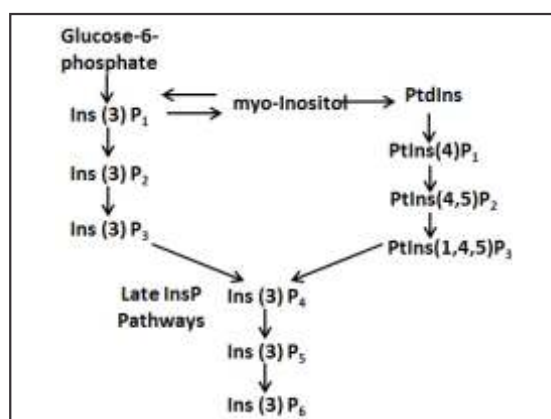


Figure 21: Schematic representation of PA pathway in plants.

know seed anti-nutrient. The chelating properties of PA in seeds contribute to reduced iron and other micronutrients bioavailability. One of the major challenges faced by researchers worldwide is how to increase the bioavailability of iron in wheat seeds. In this project we want to utilize functional genomic tool/s to address the role of phytic acid synthesis genes. Our goal is to reduce the PA content by silencing late PA pathway genes (Figure 21) in wheat grains and therefore anticipating an increasing bioavailability of total iron.

Research Objectives:

- Identification of genes involved in phytic acid pathway.
- Comparative gene expression analysis during different developmental stages of the wheat grains.
- Construction of RNAi vectors to inhibit the expression of the target genes in wheat plant.
- Stable transformation and expression (for RNAi) in wheat plants using selection markers.
- Examination of quantitative relationship between total phytate content and inorganic phosphorus in silenced wheat plants.

Long Term Objectives:

Developing wheat varieties with reduced total phytate content and enhanced bioavailable iron.

Research in Progress:

1. We analysed transcriptome data for the genes which are differentially regulated and possibly involved in phytic acid synthesis pathway. During our initial analysis of C306 and LOK1 varieties of wheat grains, we were able to identify some genes which putatively codes for inositol phosphate kinases (Table 1).
2. Blastx analysis of the corresponding gene ID suggests that they belong to inositol phosphate kinase (IPK) gene family. All the genes mentioned below belong to the late phase of the InsP synthesis pathway.
3. Wheat being a hexaploids crop (A, B and D genome), we are designing primers targeting at the conserve nucleotide region to amplifying different genes by utilizing genomic DNA as a template. Further, RNAi constructs will be developed to target these genes to assess their functional role in PA synthesis

Table 1: List of putative phytic acid biosynthesis pathway genes regulated during the anthesis in wheat plants.

Probe set	Gene Bank ID	Annotated function (based on blast homology)
Ta.27561.1.S1_at	CK215630	inositol-tetrakisphosphate 1 -kinase 1 (from <i>Zea mays</i>)
Ta.30464.1.A1_at	CK214494	inositol hexaphosphate kinase (from <i>Arabidopsis</i>)
Ta.5574.1.S1_a_at	BJ227701	1-phosphatidylinositol-3-phosphate 5 -kinase-like (from <i>Oryza sativa Japonica</i>)
TaAffx.113052.1.S1_at	CA618510	inositol-tetrakisphosphate 1 -kinase 1 (from <i>Zea mays</i>)
TaAffx.121326.1.S1_at	CA690518	inositol 1,3,4 -trisphosphate 5/6 -kinase (from <i>Oryza sativa Japonica</i>)
TaAffx.19998.1.A1_at	CD867474	inositol phosphate kinase
TaAffx.112860.1.S1_at	AF532601	multidrug resistance associated like protein : MRP2 (<i>Zea mays</i> : lpa-1 gene)

1.2 Accelerated breeding for quality improvement

Principal Investigator:

Monika Garg

Research Fellows:

Rohit Kumar

Vishal Gupta

Shaweta Arora

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 used. Indian cultivars are released based upon agro climatic zones, time of sowing and soil fertility. Validated markers are not available for a major product i.e. chapatti. Available validated markers are not being utilized. In India, there is a need of breeding cultivars, based upon their processing quality (milling and baking characteristics), marker development and utilization of validated markers.

Processing quality of wheat depends upon seeds harvested from field and its components like proteins, starch, non starch carbohydrates and lipids. Protein's contribution to processing quality

is well known. The protein content and types determine the end product quality like bread, biscuit, cake, chapatti and noodles etc. Biscuit making requires soft wheat with low protein content and specific combination of different alleles (2+12 allele of High Molecular Weight glutenin subunit gene (HMW-GS) at chromosome 1D (locus *GluD1*), *Pina-D1a*, *Pinb-D1a* alleles of puroindoline gene etc). Bread making requires hard wheat with high protein content and specific combination of different alleles (5+10 allele of *GluD1*-HMWGS, *Pina-D1b*, *Pinb-D1a/b* etc). Chapatti making requires medium strength wheat with medium protein content. The contribution of different genes/alleles to chapatti making is poorly understood.

Research Objectives:

- Generation of breeding material with improved processing quality.
- Marker discovery.
- Generation of near isogenic lines for marker discovery.
- Generation of RIL populations to validate markers.

Long Term Objectives:

- Development and validation of markers associated with different processing quality traits.
- Screening of generated breeding material for multiple traits and introduction into multinational trials with the help of Directorate of Wheat Research (DWR)/ State University scientists to design end product (bread, biscuit, chapatti) specific cultivars.

Research in Progress:

1. Marker discovery

Three traits (bread, biscuit and chapatti making) and the factors responsible for their good quality are under study. Transcriptomics studies at different developmental stages of good (C306 and LOK1) and poor chapatti making varieties (Sonalika and WH291) indicated that among the earlier reported genes affecting wheat processing quality, granule bound starch synthase 1 (*GBSS1*) was several folds down-regulated in good chapatti varieties. *GBSS1* is involved in amylose starch synthesis. Genomic variation of *GBSS1* was studied in several wheat cultivars. Allelic variation of *GBSS1* in Indian cultivars indicated that *GBSS-A1* and *GBSS-D1* genes were non-polymorphic and present in all the cultivars studied. *GBSS-B1* gene was polymorphic based on presence/absence alleles. Preliminary study on different cultivars and lines with known chapatti making quality indicated that absence of *GBSS-B1* gene was co-related with good chapatti making quality (Figure 22). Among starch pasting characteristics, breakdown in viscosity was found to be co-related with good chapatti making quality. Validation of relationship between *GBSS1* and (Rheo Visco Analyzer) RVA-breakdown of viscosity in chapatti making is in progress.

For improvement of biscuit making quality major genes (puroindoline and HMW glutenin genes) responsible for grain softness were characterised in selected

lines. PCR multiplexing for selection of grain softness genes in the backcross lines was standardised.

2. Accelerated breeding

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

- For improvement of chapatti making quality, good chapatti making old cultivars were crossed with high yielding recent cultivars (PBW343, PBW550 and PBW621). Crossed seeds were backcrossed at DWR Regional Station, Dalang Maidan, Lahaul, Himachal Pradesh during off season. One hundred and eighty nine BC₁ plants were screened for absence of *GBSS-1B* and out of them sixty plants were selected. Negative plants were backcrossed with selected cultivars and BC₂ plants have been sown at Dalang Maidan.
- For improvement of biscuit making quality, donor landraces were again crossed with high yielding recent cultivars (PBW343, PBW550 and PBW621). Crossed seeds were backcrossed at Dalang Maidan during off season. One hundred and seventy eight BC₁ plants were selected based upon presence of puroindoline gene *PinaD1a* and fifty selected plants were backcrossed with selected cultivar. BC₂ plants have been sown at Dalang Maidan.
- For improvement of bread making quality, wild species/genetic stocks of *Ag. elongatum*, *Ae. searsii* and *Ag.*

intermedium, are being utilized. We want to transfer HMW-GS genes related to high grain strength from wild species to chromosome 1A of wheat (translocation lines) as later has some alleles that contribute negatively to bread making quality. For this purpose marker assisted selection and transfer to agronomically superior cultivars is in progress. The endosperm half of seeds of the above genotypes were screened for storage proteins of interest by sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE). The remaining half seeds expressing the selected wild species HMW-GSs were raised into plants and crossed with the selected Indian wheat varieties. The above procedure was repeated one more time at NABI and the selected seeds were grown at Dalang Maidan. The seeds were reselected and sown at NABI field

1.2.1 Development of puroindoline gene database of Indian cultivars

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

Research in Progress:

1. PCR amplification of *Pina-D1* gene from five hundred and fifty one Indian germplasm indicated amplification and thus presence *Pina-D1a* functional allele in fifty three lines i.e. 9.6% of total lines.
2. Sequencing of some of the expressed *Pina-D1a* allele indicated no sequence variation in the Indian germplasm. PCR amplification of *Pinb-D1* gene from five hundred and fifty one Indian germplasm indicated amplification in all the lines studied (Figure 22).
3. Sequencing of *Pinb-D1* gene from different Indian cultivars indicated sequence variations. Out of thirty three lines analysed, twenty six lines had *Pina D1b* (null) allele and functional *Pinb-D1a* allele. These varieties included RAJ4120, HS505, VL616, K0307, WH1062, HD3002,

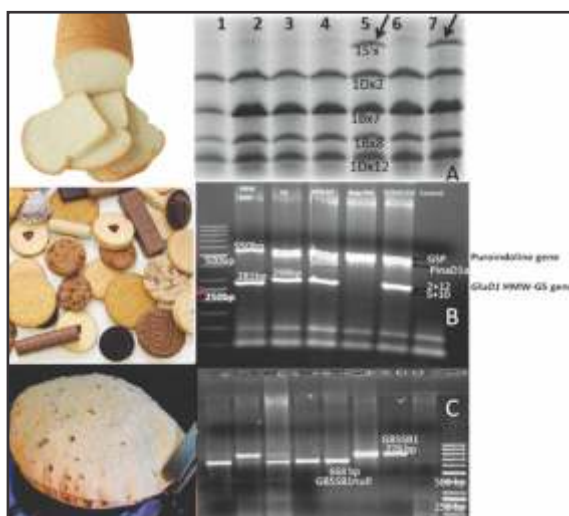


Figure 22: Marker assisted breeding for processing quality. (A) SDS-PAGE of HMW-GSs of endosperm half of seeds to find individual plants carrying addition subunit from wild species (indicated by arrow) having positive effect on bread making quality. (B) PCR multiplexing for biscuit making quality to co-amplify puroindoline gene and high molecular weight glutenin genes. (C) PCR multiplexing for chapatti making quality to co-amplify *GBSS-1B* functional and non-functional alleles.

Table 2: Indian cultivars having mutated *Pin b-D1* gene with mismatch position and changed nucleotide and /or amino acid.

S. No.	Cultivar	Allele	No. of entries	Mismatch position Nucleotide /Amino acid	Base change/ AA change	Allele
1	VL934	<i>Pin b-D1b</i>	1	223/ 46	G-----A/ G-----S	Published
2	DBW17	<i>Pin b-D1y1</i>	1	92	T-----C	New
3	WH1073	<i>Pin b-D1y2</i>	1	93/2	T-----A/V-- --A	New
4	HS511	<i>Pin b -D1y3</i>	1	256/57	T-----C /C- ---R	New
5	HP1102	<i>Pin b-D1y4</i>	1	52	T-----A	New
6	PBW613K 0710	<i>Pin b-D1y5</i>	2	53	T-----A	New

MACS6222, NP809, HS1138, K816, KSML3, NARMADA195, UP215, HUW12, HW5210, NP836VL916, VL921, HS508, VL829, HPW251, UP2771, HS295, HS512, HS513, HPW296, VL924, VL925, VL935, UP2772, HD2967, PBW343, PBW621, WH1061, PBW628, WH1081, HD3014, UAS315, K0607, DBW39, HUW612 and DBW46.

- One line VL934 had *Pina-D1a* (functional allele) and *Pinb-D1b* (non-functional allele). *Pinb-D1b* allele resulted from Guanine to Adenine nucleotide mutation leading to glycine to serine amino acid mutation at 46th position as reported in literature.
- In addition five new mutations (*Pin b-D1y1* to *Pinb-D1y5*) were identified as listed in Table 2. Allele *Pin b-D1y1*, *Pin b-D1y4* and *Pin b-D1y5* showed single nucleotide

substitution without change in amino acid. Allele *Pin b -D1y3* and *Pin b -D1y4* resulted in amino acid substitutions at positions 2nd and 57th. These mutations were not within tryptophan rich domain (34th to 47th amino acid) that leads to functional change of the *Pin b-D1* gene. 78% of Indian cultivars have wild type *Pinb-D1a* allele.

- Mutant *Pinb-D1b* allele commonly found in Australian wheat is rare in Indian cultivars. Five new alleles have been identified in Indian germplasm that may contribute more to the sources of variation. We have sequenced only thirty three cultivars for *Pinb-D1* gene. Currently work is in progress to sequence *Pina-D1* and *Pinb-D1* gene. Others genes in the pipeline are GSP and *Pinb-2D1* that are known to be involved in grain hardness.

2. IMPROVING FRUITS FOR POST HARVEST QUALITY AND NUTRITION

2.1 Genetic transformation of banana for quality improvement

Principal Investigator:

Siddharth Tiwari

Co- Investigator:

Rakesh Tuli

Research Fellow:

Anshu Alok

Introduction:

Banana and plantain are among the most important fruit crops in the world and contribute significantly towards the food security in many developing countries. India is the largest producer of banana in the world with an annual production of about 18 million tonnes. Bananas are consumed fresh (except cooking types (plantain)) without any thermal cooking and processing. The pulp of dessert banana remains unexposed to light, hence allowing minimal degradation of carotenoids in the edible flesh. Genetic improvement of banana through conventional methods is limited because most of the cultivated varieties are triploid in nature, hence sterile and provide natural barrier to cross pollination. Therefore, transgenic approaches hold high promise for developing sustainable resistance against diseases/pests and for biofortification in banana. Plant regeneration of banana has been reported from various explant sources. Immature male flowers are the most responsive material for initiating the embryogenic cultures. An Embryogenic Cell Suspension (ECS) culture is being used for *Agrobacterium tumefaciens* mediated genetic transformation in many laboratories. However, genetic transformation and regeneration frequencies are reported to be highly genotype dependent. Thus, optimization of transformation protocol for any particular type of cultivar becomes a prerequisite for genetic improvement in that cultivar.

Research Objectives:

- Development of efficient genetic transformation in Indian banana varieties viz., Grand Nain and Rasthali.
- Evaluation of fruit specific promoters and pro-vitamin A candidate genes and combinations thereof after transformation in Indian banana.
- Development of transgenic lines using constructs for pro-vitamin A biofortification.
- Initial field and nutritional analysis of fruit under containment conditions.
- Selection of lines for nutritional and bioavailability analysis.

Long Term Objectives:

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

Research in Progress:

1. Germplasm collection and plantation at NABI research field:
 - a. Suckers of established banana cultivars (Grand Nain, Robusta, Nendran, Poovan, Rasthali, Red Banana, Ney Poovan, Virupakashi, Karpuravalli, Dwarf Cavendish, Dwarf-Robusta, Udhayam and Nanjanagud Rasabale) have been collected from Tamil Nadu Agricultural University (TNAU), Coimbatore and Gandhi Krishi Vigyana Kendra (GKVK) Bangalore (Figure 1A). The collected suckers have been grown at NABI research field for establishing germplasm (Figure 1B and C).
2. Establishment of ECS culture for genetic transformation of banana:
 - a. Immature male flower buds of two cultivars (Grand Nain and Rasthali) were collected from TNAU, Coimbatore and NRCB,

- Trichy, Tamil Nadu (Figure 2 A and B).
- b. Immature male flower hands of rank 1 to 15 adjacent to the floral apex were isolated and cultured on MS medium containing several
 - d. Best response for callus formation was optimized (Figure 2 D).
 - e. Optimization of ECS has been initiated for the induction of globular embryos and



Figure 1: Establishment of Banana germplasm at NABI research field. (A) Banana suckers. (B) Banana germplasm. (C) Banana plant with fruit at NABI research field.

- combinations and concentrations of different growth regulators for the optimization of protocol (Figure 2 C).
- c. Calli were formed on the callus forming medium. However, efficiency and response for callus induction depends upon the cultivars.
 - f. ECS was used for transient transformation of reporter (GUS-Intron) gene and expression was noticed as blue colour on the ECS (Figure 3 A and B). No expression of GUS was noticed in control ECS (Figure 3C).



Figure 2: Stages of ECS and culture development for somatic embryogenesis. (A, B) Immature male flower bud. (C) Immature male flower hands of rank 1 to 15 adjacent to the floral apex. (D) Embryogenic callus induction. (E) Suspension culture.

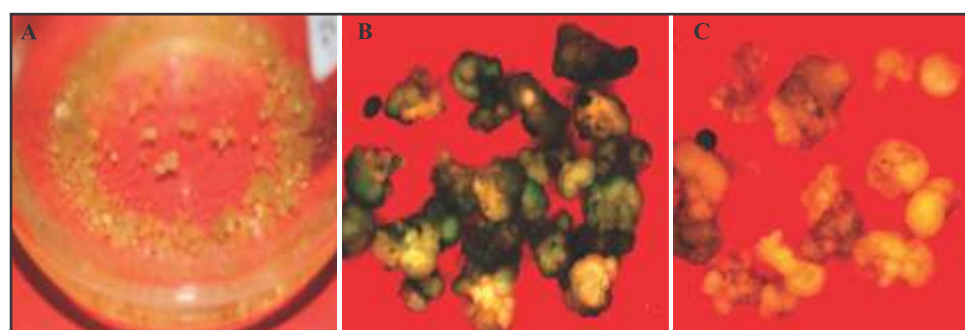


Figure 3: Transient genetic transformation of ECS. (A) ECS (B) Transformed suspension culture cells (blue clour). (C) Non-transformed suspension culture cells (no blue clour).



Figure 4: Different stages of micropropagation of banana. (A) Banana sucker as explants. (B) Sucker turned into green bud. (C) Induction of multiple shoots. (D) Shoot elongation. (E) Tissue culture raised plants.

3. Establishment of micropropagation:
 - a. Suckers of Grand Nain, Rasthali and Dwarf Cavendish cultivars were used for optimization of protocol for micro propagation (Figure 4 A).
 - b. Multiple shoots were induced and multiplied under some of the culture conditions (Figure 4 B, C and D).
 - c. Tissue culture raised acclimatized plants have been generated and transferred at NABI research field (Figure 4 E).

2.2 Quality enhancement and postharvest stability of tropical fruits

Principal Investigator:

Sukhvinder P. Singh

Research Fellow:

Manpreet Kaur

Introduction:

Qualitative and quantitative postharvest losses of fresh fruits are significant in India. Enhancing and maintaining quality through adaptive preharvest and postharvest actions is important to reduce losses, meet consumer expectations and promote domestic and international trade. The prime objective of this research program is to understand biological basis of fruit quality and generate basic knowledge to develop pre and postharvest strategies for extending the storage stability and maintaining produce quality. The research efforts are directed to expand the horizon of measurement, definition and meaning of 'quality'

for fresh fruits. The concept of global harmonization of quality standards is emerging rapidly which warrants the development of globally acceptable analytical tools for quality assessment and delivering the logical and sustainable solutions to maintain postharvest quality in the supply chain. The genetic, physiological and biochemical diversity in the fresh produce require the commodity specific approach to develop postharvest solutions.

Research Objectives:

- Understanding and explicating the complexity of biochemical and molecular factors contributing to produce quality.
- Development of preharvest and postharvest strategies to improve quality and enhance postharvest stability of farm produce.
- Translation of knowledge and innovation into commercially important products/processes/services for primary producers and industry.

Long Term Objectives:

- To develop protocols to address commodity specific quality and postharvest issues.
- To improve and maintain farm produce quality through application of Generally Recognised As Safe (GRAS) compounds, natural metabolites, growth regulators and antagonistic microorganisms.
- Generating primary data on the produce quality with special reference to flavour and

nutritional quality.

Research in Progress: Mango, citrus, litchi and guava are the targeted crops to study the quality and postharvest stability of fruits.

Mango

Mango export from India has been jeopardised by the postharvest phytosanitary requirements by several countries such as the United States of America, Japan, Australia and New Zealand. In recent years, heat-based phytosanitary treatments have received regulatory approvals from Japan, Australia and New Zealand which led to the establishment of these facilities in the major mango producing and exporting zones of the country. However, there are certain quality concerns associated with these treatments which can affect consumer acceptance of the exported fruit in the international markets. Our objective was to assess the impact of heat treatment protocols on quality and postharvest behaviour of mango fruit.

1. In 2011 season, an experiment on Vapour Heat Treatment (VHT) of 'Chausa' mango was conducted in a commercial VHT facility at Saharanpur, U.P.
2. The results showed that the VHT accelerated rate of fruit ripening, resulted in development of uniform skin colouration (Figure 5). The heat treated fruits reached ready-to-eat



Figure 5: Effect of VHT on skin colouration in 'Chausa' mangoes after 48 hours at 26°C.

stage within 4 days of treatment against the 8 days in control held at ambient conditions (26°C).

3. Post-VHT treatment with 1-methy-

lencyclopropene (1-MCP), which is an ethylene action inhibitor, was not effective to retard the process of fruit softening in both treated and control fruit. The inefficacy of 1-MCP is contrary to its proven effectiveness in other fruits.

4. The qualitative profiling of aroma volatiles in mango showed that sesquiterpenes and monoterpenes constituted about 85-90% of the total volatiles production. With the progress of fruit ripening, the abundance of sesquiterpenes decreased and that of monoterpenes and esters increased.
5. The evolution of aroma volatiles such as monoterpenes and esters was faster in VHT fruit, parallel to other changes in fruit ripening, compared to control.
6. The abundance of esters (fruity odour) declined significantly in VHT fruit after 8 days of shelf-life at 26°C. The VHT was effective to reduce the microbial on fruit surface and also helped reducing the incidence of anthracnose disease.
7. The VHT fruit stored at 10°C for 2 or 3 weeks and then held for 4 days at 26°C for simulated shelf conditions showed lower incidence of chilling injury. However, the detrimental effect of VHT in terms of faster fruit softening was not prevalent in fruit held in cold storage after treatment.

In future, the efforts will be made to minimise the detrimental effects of VHT on mango fruit quality attributes and also to retard the process of fruit ripening through better understanding of fruit physiology.

Litchi

Skin colouration in litchi is due to the presence of anthocyanin pigments. whose biosynthesis and postharvest stability can seriously affect fruit quality. Targeted metabolite profiling of litchi (cvs. 'Calcuttia', 'Dehradun' and 'Seedless') was conducted at different stages of maturation (green, colour break/pink, and red).

1. The results showed that the concentrations

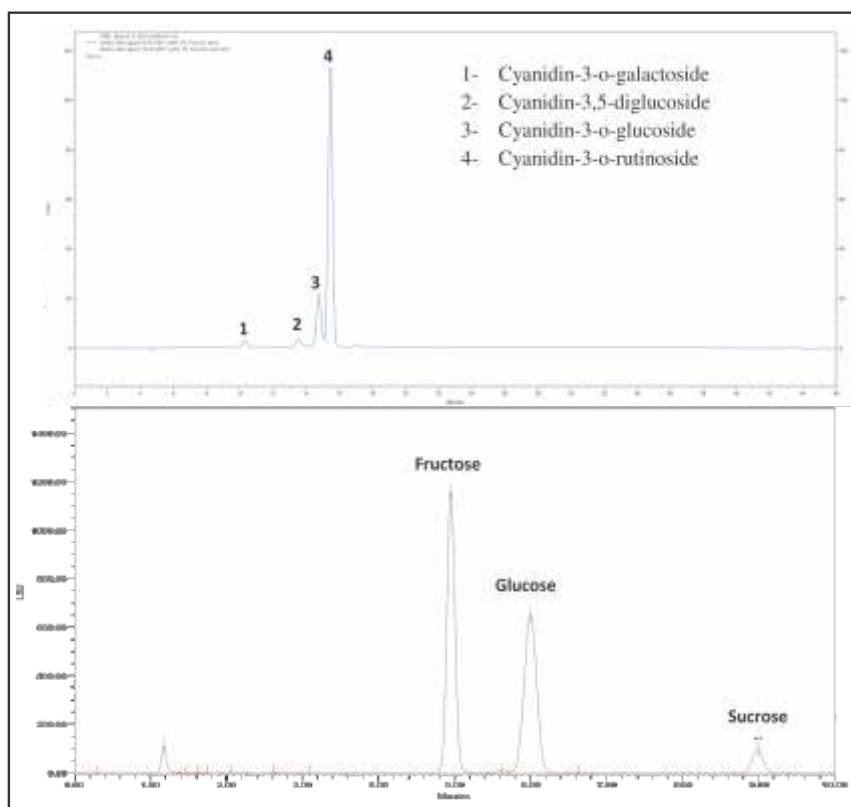


Figure 6: Chromatograms representing the anthocyanins (top) in the pericarp and sugars (bottom) in the aril of litchi fruit.

of chlorophyll a, chlorophyll b, xanthophyll and pheophytin in the pericarp tissue declined during maturation in three cultivars.

2. Figure 6 represents the chromatograms of various targeted metabolites such as anthocyanins and sugars. Cyanidin-3-o-rutinoside (Cyn-3-o-rut) was identified and quantified as the major anthocyanin pigment in all three cultivars and was detected during colour break/pink stage and showed increasing trend during maturation. The concentration of Cyn-3-o-rut was maximum in 'Dehradun' cultivar followed by 'Calcuttia' and 'Seedless'.
3. The other anthocyanin pigments were identified as cyanidin-3-o-glucoside, cyanidin-3,5-diglucoside and cyanidin-3-o-galactoside. The sugar profiling of fruit aril

tissue showed that the concentrations of fructose, glucose and sucrose increased significantly during maturation. Fructose was found to be the principal sugar followed by glucose and sucrose in all cultivars. 'Seedless' cultivar showed the highest concentration of sucrose at the red-ripe stage compared to other cultivars, 'Dehradun' and 'Calcuttia'. Among organic acids, malic acid was the predominant acid in all three cultivars. The other organic acids included citric acid, succinic acid, tartaric acid and shikimic acid. The concentration of malic acid and other minor organic acids declined with the progress of maturation of aril tissue. The protocols developed for identification and quantification of these metabolites will be used for objective assessment of the effects of various postharvest treatments on litchi fruits

3. BASIC BIOLOGY FOR CROP IMPROVEMENT

3.1 Biology of seed development in custard apple and litchi

Principal Investigator:

Rakesh Tuli

Co-Investigator:

Sudhir P. Singh

Research Fellow:

Yogesh Gupta

Introduction:

Seeds in many fruit crops like custard apple, litchi, guava, orange, mango and grape are a hindrance to fruit processing and fresh fruit consumption. Sugar (or Custard) apple, a popular fruit throughout the tropics, belongs to genus *Annona*. The genus *Annona* contains about one hundred and twenty species, out of which six species produce edible fruits; *Annona squamosa* (Sugar apple or Sarifa or Sitafal), *Annona reticulata* (Custard apple or Bullock's heart or Ramphal), *Annona cherimola* (Cherimoya or Hanumanfal), *Annona muricata* (Sour sop), *Annona atemoya* (Laxmanphal, a hybrid between *Annona cherimola* and *Annona squamosa*), *Annona diversifolia* (Ilama) (Gandhi and Gopalkrishna, 1957). *Annona squamosa* is the most popular species in India. The *Annona* species flowers comprise of a cluster of stamens (>200) and

carpels (>100). Each carpel has a single anatropous ovule that may develop into a single seed. The *Annona* fruit develops from the cluster of fertilized carpels, thus the aggregate fruit contains several fruitlets. Out of the multiple fruitlets a few fruitlets develop naturally, without seeds. *Annona squamosa* produces fruits with greater number of seed fruitlets, sixty-eighty seeds per fruit. A contrasting genotype, named NMK-1, has been identified having fewer numbers of seeded fruitlets and many of the fruitlets are seedless (Figure 1). The variety selected from the seedling population of *Annona squamosa* by a farmer, Shri N. M. Kasbate of Madhuban nursery, Solapur, Maharashtra.

The aim of the project is to understand the molecular basis of the development of seeded and seedless fruitlets in the same fruit of *Annona squamosa*. The major research objective is to do tissue specific differential transcriptomics in developing fruitlets of *Annona* species for identifying genes involved in seed development.

Litchi is another crop where seedlessness is a desirable trait. The inflorescence is composed of several panicles bearing three types of flowers which open in succession with the same panicle. The three types of flowers are (type 1) male flower which has functional anthers but lacks pistil, (type 2) hermaphrodite female flower which has completely developed pistil but the anthers do not dehisce and contain very less number of viable pollen. The ovary is bicarpellary, each containing an ovule, (type 3) hermaphrodite male flower is a functional male, which has pistil but lacks stigma and thus pollen fail to enter. The litchi fruit develops from type 2 flowers. Generally only one carpel of type 2 flower gets developed into fruit after pollination. Botanically the fruit is a drupe. The edible part of the fruit is called as aril (or pulp) which is an outgrowth of the outer integument. Some litchi accessions, popularly

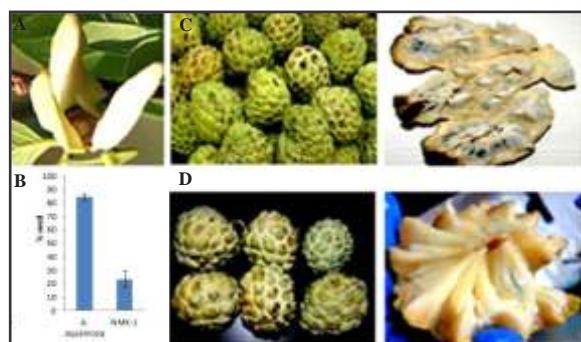


Figure 1: Seed percentage in the contrasting cultivars of custard apple. (A) Flower of *Annona squamosa* with multiple stamens and pistils. (B) Seed percentage in the fruits of *Annona squamosa* and NMK-1. (C) Fruits of contrasting genotypes of *Annona squamosa* and (D) NMK-1.

Research Objectives:

- ### Long Term Objectives:

- To identify genes responsible for the development of fruits and seeds.
- To develop different varieties of seedless fruit crops.

Research in Progress:

1. A genotype, NMK-1, of *Annona* has been identified with more number of seedless fruitlets at Madhuban Nursery, Solapur, Maharashtra.
2. Self-pollination and collection of fertilized carpels (at different days after pollination) of contrasting genotypes, NMK-1 and common custard apple (*Annona squamosa*) is in progress at Solapur, Maharashtra.
3. Litchi contrasting genotypes, showing differences in seed development and seed size are being screened for candidate genes to develop molecular approaches to seedlessness. Polymorphism in nucleotide sequences of a candidate gene for seed development has been identified (Figure 2). Further study on molecular details is in progress.

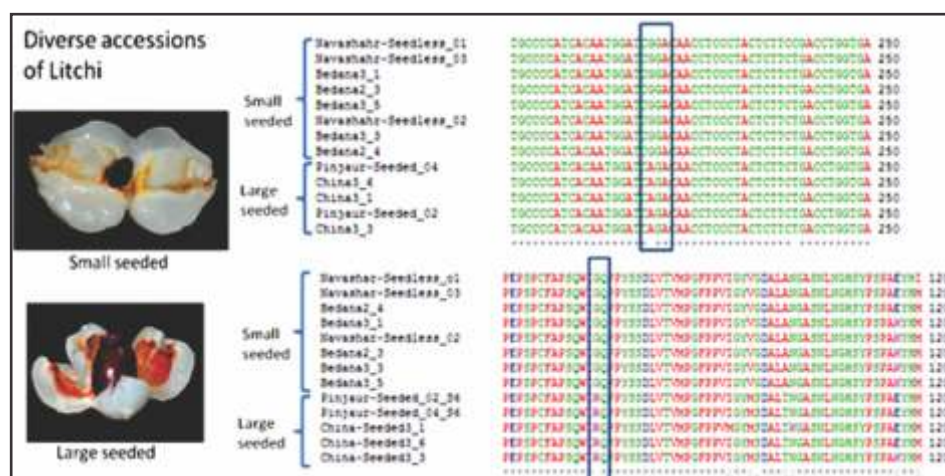


Figure 2: Polymorphism in nucleotide sequence of a gene related to seedlessness in diverse accessions of litchi

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

Principal Investigator:

Rakesh Tuli

Co-Investigator:

Sudhir P. Singh

Research Fellow:

Anita Kumari

Introduction:

Fruit crops are mostly propagated through asexual reproduction by grafting. In grafting, one plant is selected for its roots and is called rootstock. The other plant is selected for its stems, leaves, flowers



Figure 3: Stem grafting in *Arabidopsis thaliana*.

or fruits and is called the scion. Scion–rootstock interaction affects the physiology and thus phenotype of the scion plant. For example, to induce dwarfing in apple trees, scions are grafted on to dwarf root stock to give smaller canopy, so that it becomes suitable for planting the trees at higher density. In recent years, evidence suggestive of long-distance transport of signals through vascular system have been reported appears to be increasing. Such signals can influence various developmental and physiological processes such as flowering, tuberization, nodulation, leaf development, shoot branching and disease resistance.

The research project anticipates establishment of a technology which can lead to the development of a new approach to use transgenic rootstocks for delivering specific siRNAs in non-transgenic scions for the modification of economically important traits. In the present case, the principle will possibly lead to the application of the

technology to prevent the development of seeds in fruit crops, where seed may not be desirable by food processing industry. Prevention of seed development may also lead to enhancing the productivity and biomass. The objective of the present proposal is to examine if siRNA based signaling can be applied to develop rootstocks, which may influence seed development in the scion, grafted on to the stock. The rootstock would transmit siRNAs, targeted for silencing of specific genes involved in the development of embryo and endosperm. Effect of silencing on the development of fruits and seeds on the grafted scion will be studied, using the model plant *Arabidopsis thaliana* (Figure 3). If successful, the study will be applied to a suitable horticultural crop plant.

Research Objectives:

- To develop various transgenic plants harbouring RNAi constructs targeting the gene expression in ovule involved in seed development.
- To examine the movement of RNAi delivered from root-stock to scion affecting expression of genes in *Arabidopsis*.

Long Term Objective:

- Development of improved rootstock for the delivery of long distance signals into the scion.

Research in Progress:

1. Transgenic *Arabidopsis* lines expressing reporter gene (GUS) have been developed. Breeding of transgenic plants to obtain homozygous lines is in progress.
2. Development of transgenic *Arabidopsis* lines expressing reporter gene in ovule is in progress.
3. Transgenic *Arabidopsis* lines expressing (constitutive and phloem specific) double stranded RNA- hairpin homologue of reporter gene have been raised. Breeding of transgenic plants to obtain homozygous lines is in progress.
4. Transgenic *Arabidopsis* lines expressing (constitutive and phloem specific) double stranded RNA- hairpin homologue of candidate gene of seed development have been raised. Breeding of transgenic plants to obtain homozygous lines is in progress

4. DIET AND HEALTH

4.1 Effect of dietary constituents on adipogenesis

4.1.1 Role of arabinoxylans from millets in regulating adipogenesis: A nutrigenomic study

Principal Investigator:

Kanthi K. Kiran

Co-Investigator:

Mahendra Bishnoi

Introduction:

Obesity is a worldwide epidemic and has a significant negative impact on health, mortality and related costs. Diet and genetic factors including gene mutations are closely related to the development of obesity. Untreated, it may lead to chronic diseases, such as diabetes (type II), hypertension, cardiovascular disease and certain type of cancers. Present anti-obesity medications are shown to be associated with side effects upon long term usage. Phytochemicals such as polyphenols and non-starch polysaccharides have tremendous health benefits. Arabinoxylans (AXs) are non-starch hemicellulosic polysaccharides, major constituents in the cell walls of cereals and millets, are reported to have potential health benefits in alleviating disease symptoms such as diabetes, atherosclerosis and colon cancer. Wheat AXs help in regulating the weight gain and obesity by controlling the release of gut hormones like peptide YY and Glucagon-Like Peptide-1 (GLP1). The potential beneficial effects of AXs from finger millet and kodo millet in regulating adipogenesis and their metabolic fate in animal models will be studied, which is expected to provide the insight on mechanism of action of AXs in adipogenesis regulation and help in designing functional foods with enriched AXs.

Research Objectives:

- Mechanistic studies on the role of AXs on adipogenesis using mouse 3T3-L1 cell lines *in-vitro*.
- Modulating adipogenesis and obesity using

AXs in high fat diet induced obese mouse model through molecular approaches.

Long Term Objectives:

- To study the role of high dietary fiber consumption and the role of gut microbiota in alleviating weight gain and obesity.
- Designing AXs enriched functional food models that can modulate metabolic disorders.

Research in Progress:

Currently, we are standardizing the culturing of preadipocytes and differentiation into adipocytes. Primers for important transcriptional factors, enzymes in the fatty acid pathway and key adipokines are designed. Chemo-enzymatic extraction of AXs from finger millet grains is under progress.

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

Principal Investigator:

Mahendra Bishnoi

Co-Investigator:

Kanthi K. Kiran

Introduction:

Current anti-obesity medications are pharmacological agents which can reduce or control weight. These drugs affect one of the fundamental processes of the weight regulation in human body i.e. altering appetite, metabolism or consumption of calories. There is only one anti-obesity medication, Orlistat, approved by FDA. It acts through inhibition of pancreatic lipase enzyme. Rimonabant, acting through blockade of the endocannabinoid receptor and Sibutramine, acting through brain by inhibiting neurotransmitter metabolism were previously approved drugs which have been withdrawn from the market in several countries and regions

including India (Banned Medicines, press release), Ministry of Health and Family Welfare. February 10th, 2011). The potential side effects (increased cardiovascular concerns, stroke, suicidality and depression) of these drugs are much more than their beneficial effects. Over the years it has been seen that the best and most effective options available for overweight and obese individuals are dieting and physical exercise. It is important to have dietary regulations to prevent life style problems rather than to search for the treatment. Available literature suggests that Transient Receptor Potential (Transient Receptor Potential Vanilloid (TRPV1), Transient Receptor Potential Ankyrin (TRPA1), Transient Receptor Potential Metastatin (TRPM8) channels are possible candidates to regulate energy metabolism and thermogenesis, which can lead to calorie consumption and prevention of obesity. Common dietary spices like chilli pepper, black pepper, clove, garlic, cinnamon and their constituents (capsaicin, piperine, eugenol, allicin, cinnamaldehyde, menthol) can activate the TRP channels. In this proposal, using the TRP channel receptor system we intend to come up with dietary constituents that can modulate the molecular mechanism associated with the process of adipogenesis, adipose tissue related hormonal secretion and release of pro-inflammatory mediators and lipolysis.

Research Objectives:

- Determination of expression and function of TRP channels in commercially available mouse preadipocytes cell lines (3T3-L1), human preadipocytes (HPAd) and adipocytes (HAd) cells.
- *In-vitro* characterization of the molecular basis of adipogenesis using mouse/human gene microarray chip, mouse/human adipogenesis and obesity PCR arrays. Determination of effect of TRP channel activation on adipogenesis process in mouse/human adipocytes cells.

- To study the effect of dietary modulations of TRP channels (TRPV1: capsaicin, capsiate, piperine; TRPA1: garlic, cinnamon; TRPM8: menthol) in a diet (high fat) based *in vivo* mouse model of obesity.

Long Term Objectives:

- Developing diets constituted of modulating food components and study their effect on adipogenesis, obesity and related complications.
- Special dietary formulations will be made using TRP channel modulating dietary agents alone or in combination with other nutrients.
- Undertake clinical trial using human volunteers for establishing role of TRP channel modulating dietary agents in obesity.

Research in Progress:

Based on our PCR data multiple TRP channel genes are expressed in mouse 3T3-L1 preadipocytes (high to moderate expression: TRPP2 (PKD2), TRPC1, TRPV2, TRPM2, TRPV4 TRPV1, TRPV3, TRPV6, TRPC4; low expression: TRPA1, TRPC6, and TRPM8) and adipocytes (high to moderate expression: TRPP2 (PKD2), TRPV2, TRPC1, TRPV4, TRPM2; low expression: TRPA1, TRPV3, TRPV6, TRPC4, TRPV1, TRPC6, and TRPM8) (Table 1). TRPV1, TRPV3, TRPM8, TRPC4, TRPC6 were differentially expressed (multiple fold higher in preadipocytes than adipocytes) (Figure 2). Further, these channels are also expressed in murine white adipose tissue (WAT) (high to moderate expression: TRPP2 (PKD2), TRPV2, TRPC6, TRPC1, TRPV4, TRPM2, TRPV3, TRPC4; low expression: TRPV1, TRPV6, TRPM8 and TRPA1] and brown adipose tissue (BAT) [high to moderate expression: TRPP2 (PKD2), TRPV2, TRPC6, TRPC1, TRPV4, TRPM2, TRPV3, TRPC4, TRPV6; low expression: TRPV1, TRPM8, and TRPA1) (Table 1). TRPV4, TRPV6 and TRPC6 were differentially expressed in murine WAT and BAT (Figure 2).

Table 1: Summary of TRP channel expression in (A) undifferentiated (preadipocytes) and differentiated (adipocytes) 3T3-L1 cells (B) mouse white (WAT) and brown (BAT) adipose tissue

TRP Gene	Mouse 3T3-L1 cell line		Mouse adipose tissue	
	Preadipocyte	Adipocyte	WAT	BAT
V1	++	+	++	++
V2	++	++	+++	+++
V3	++	+	++	++
V4	++	++	+++	++
V6	++	+	+++	++
M2	++	++	++	++
M8	++	+	+	+
A1	++	+	+	+
C1	+++	++	++	++
C4	++	+	++	++
C6	+	+	+++	+++
P2	+++	+++	+++	+++
GAPDH	++++	++++	++++	++++

(++++ = very high expression (ct values= <20), +++= high expression (ct values= 21-25), ++=moderate expression (ct values= 26-30), += low expression (ct values= > 30).

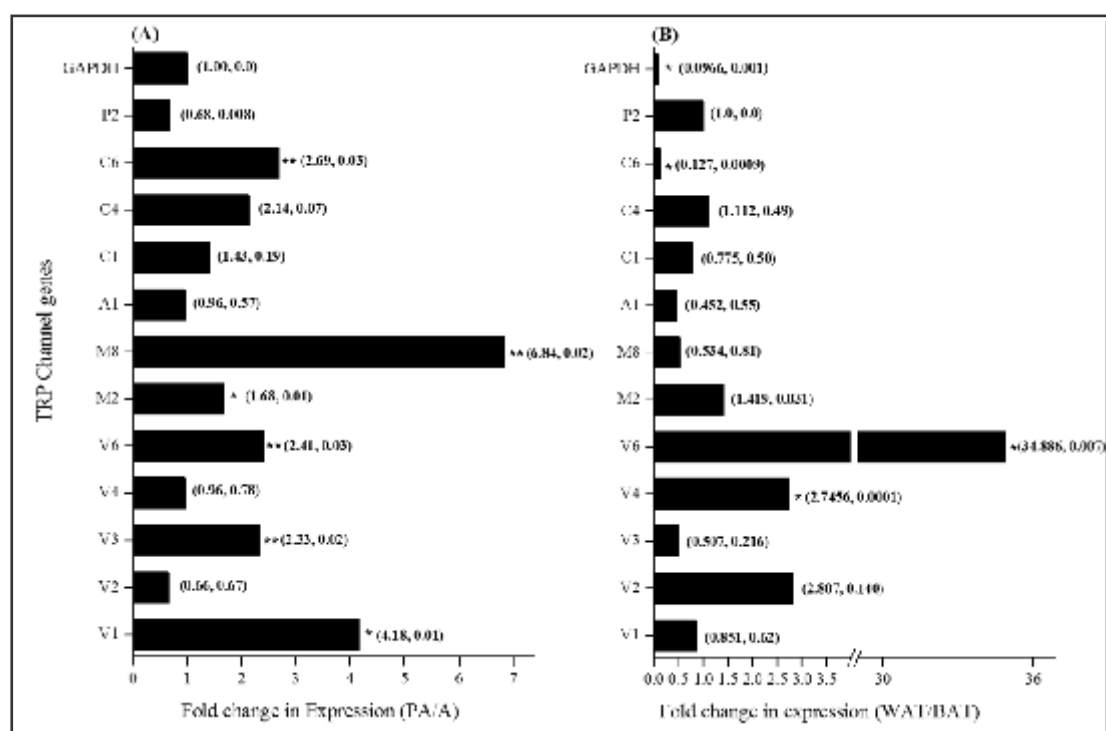


Figure 1: Differential expression of TRP channel in (A) undifferentiated (preadipocytes) and differentiated (adipocytes) 3T3-L1 cells relative to GAPDH (House Keeping Gene, HKG) (B) mouse white (WAT) and brown adipose tissue (BAT) relative to TRPP2 (HKG). * significant with p value = < 0.01; ** significant with p value = < 0.03.

4.1.3 Characterization of arabinoxylans in cereal grains

Principal Investigator:

Koushik Mazumder

Introduction:

Cereals, the staple foods for millions of people across the world, are the chief source of soluble dietary fibers. Several epidemiological studies have clearly demonstrated that increased consumption of whole grain cereals and soluble dietary fibers has been associated with a reduced risk of many life style disorders such as type-2 diabetes and obesity. In cereal grains, AXs are the major non-starchy polysaccharides which constitute the cell walls residues in cereal grains and make up an important physicochemical and physiological property beneficial to the health of consumers and for this reason, their use as food ingredients has increased rapidly. Although AXs share the same basic chemical structure, but the pattern and degree of substitution of arabinose along the xylan backbone vary with cereal sources. Therefore, the AXs exhibits a great deal of structural heterogeneity and variability with respect to molecular weight mass, xylose to arabinose ratio and branching pattern, distribution of arabinose residues and substitution with glucuronic acid/4-O-methyl glucuronic acids.

There is no documented report about the comparative study of arabinoxylan poly and oligo-saccharides from Indian millet varieties such as finger millet, kodo millet, branyard millet and foxtail millet. Hence in the present study, the variability in the structures of the AXs from various Indian millet varieties and their role in

regulating their biological activities will be evaluated using *in-vitro* and *in-vivo* model, which will further allow us to understand the structure-function relationship of the AXs in Indian millet varieties with respect to their biological activities.

Research Objectives:

The present study aims to elucidate comparative statement profile of AXs from Indian millet varieties and its effect on their biological activities.

- Isolation and purification of the arabinoxylan polysaccharides and oligosaccharides from the bran of Indian variety millets.
- Compositional sugar analysis and structural characterization of the arabinoxylan polysaccharides and oligosaccharides using various chemical and modern analytical methods (GC-MS, HPLC, MALDI-TOF and ESI-MS/MS) to establish structure-function relationship.

Long Term Objective:

Development of nutraceutical health foods based on dietary fibers enriched with Axs.

Research in Progress:

In our preliminary studies, we have standardized the extraction protocol for the isolation of AXs from Finger millet bran and the compositional analysis of the polysaccharides have been carried out (GC, GC-MS analysis) as monomeric alditol acetate derivative with inositol as internal standard (Figure 2). The compositional analysis showed the presence of arabinoxylan as major constituent (~90%) together with starch and galactomannan as minor component.

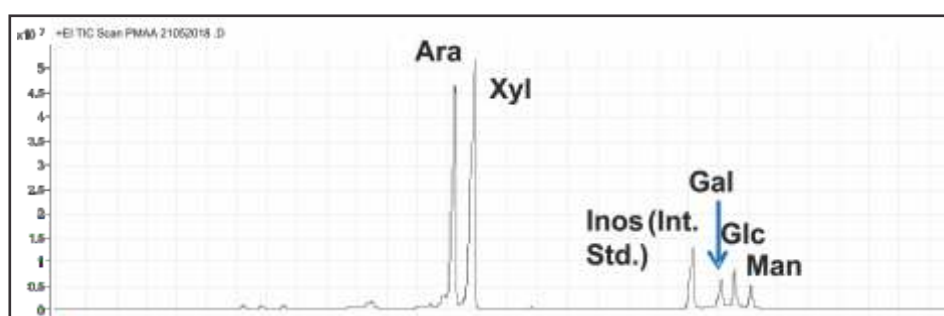


Figure 2: GC chromatogram of the monomeric sugar residues of extracted arbinoxylan fraction from finger millet bran. (Ara: Arabinose, Xyl: Xylose, Inos: Inositol, Gal: Galactose, Glc: Glucose, Man: Mannose).

5. COMPUTATIONAL BIOLOGY APPROACHES FOR MARKER AND GENE DISCOVERY FOR NUTRITION AND PROCESSING TRAITS IN GENOMES OF FOOD CROPS

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

Coordinator:

Rakesh Tuli

Investigators:

Shrikant Subhash Mantri

Joy K. Roy

Project Scientists:

Shailesh Sharma

Vandana Mishra

Research Fellows:

Gourab Das

Anuradha Singh

Introduction:

Bread wheat, *Triticum aestivum*, is an allohexaploid species. Its genome is composed of the three distinct ancestral genomes A, B and D. The polyploid nature of the wheat genome together with its large size and high percentage of repeat elements makes it extremely complex and computationally intensive to analyze ever increasing transcriptome and genome data. With the advent of next-generation sequencing technology it is now possible to generate whole genome/transcriptome sequences for a number of wheat genome. We are interested to harness the public domain data of wheat for different projects on crop improvement in terms of nutritional and processing quality. Additionally, NABI has initiated experiments to generate transcriptome sequences for Indian wheat varieties showing contrasting characters. Bioinformatics Laboratory which has the high performance computing infrastructure is a gateway for NABI scientists to use computational methods and databases to deepen and integrate their research.

Research Objectives:

- Transcriptome sequencing, assembly and annotation for contrasting wheat varieties.
- Digital expression profiling and microarray datamining for identification of Single Feature Polymorphism (SFP) in Indian cultivars and annotation of hypothetical proteins.
- SNP database development in wheat.
- Comparative genomics and related data mining.
- Small RNA analysis pipeline development.
- Microarray datamining for SFP.
- Repository of germplasm.

Long Term Objectives:

- Framework map development for wheat genome (assembly and annotation).
- Identification of genes involved in high accumulation of micronutrients.
- Genome wide association analysis for co-relating quality and processing related traits with the identified SNP's.

5.1.1 SNP database development in hexaploid wheat

Research in Progress:

Datamining of non-synonymous mutations or Single Amino acid Polymorphisms (SAPs) from ESTs and homoeologous SNPs from chromosome 1 of *Triticum aestivum*, resulted into the identification putative 35395 and 1817 SNPs respectively.

nsSNP mining in ESTs from dbEST:

Non-synonymous deleterious mutations are fairly common in crop genomes. Statistical analysis of homologous sequence from multiple genomes can

identify amino acid changes that are likely to be disadvantageous. Figure 1 shows a hypothetical alignment of coding sequence from multiple grass species. The conserved nature of the histidine

amino acid across species suggests that the non-synonymous change (indicated by the red 'G') observed in maize is likely to be deleterious. Synonymous changes are shown in black.

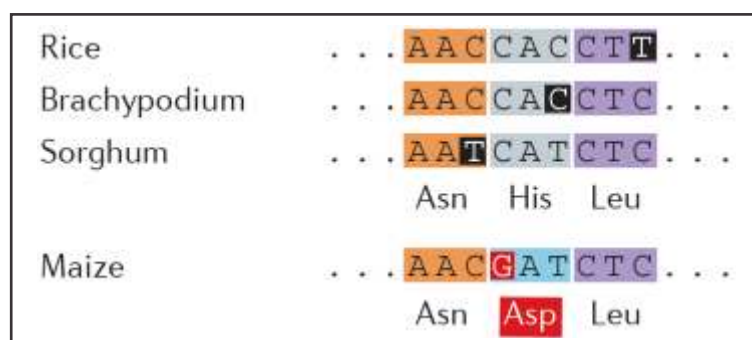


Figure 1: Hypothetical alignment of coding sequence from multiple grass species. Non-synonymous change indicated in red, synonymous change indicated in black.

Highly putative nsSNPs in hexaploid wheat ESTs were found through the following steps (Table 1-7):

1. dbEST records were parsed using custom perl script and multifasta containing all EST sequences was generated.

Table 1: dbEST summary (updated February 1, 2012).

No. of <i>Triticum aestivum</i> ESTs	No. of cultivars	No. of tissues	No. of developmental stages
1073845	101	108	132

2. These EST sequences were clustered using BLAST algorithm.

Table 2: EST clustering results. Current release March 19, 2012 contains 36692 unigenes.

No. of <i>Triticum aestivum</i> probe sets	No. of unigenes covered	No. of probe sets having no cluster info	No. of probe sets having cluster depth > 5 sequences
61115	24697	123	46372

3. SNPs were determined using autosnpV2 script.

Table 3: Overall SNPs summary.

No. of Probe sets	SNPs	Synonymous SNPs	Synonymous SNPs in CDS	Synonymous SNPs in flanking	Coding region protein substitutions	SNPs in domains
20699	161188	94654	67478	27176	35395	2486

4. nsSNPs were extracted from the overall SNP result.

Table 4: nsSNP statistics.

Total no. of nsSNPs	No. of probe sets involved	Involved probe sets with annotation	No. of nsSNPs with annotation
35395	12441	12409	35233

5. SNPs in transcription factors were identified.

Table 5: Summary of SNPs in Transcription Factors.

No. of probe sets	Synonymous SNPs	No. of nsSNPs	SNPs in domains
188	776	335	106

6. SNPs in pathways were also determined.

Table 6: Summary of mutations in pathways.

No. of pathways	No. of probe sets involved	No. of enzymes	No. of synonymous SNPs	No. of non-synonymous substitutions
147	2157	521	13442	4556

7. SNPs in starch & sucrose metabolism pathway.

Table 7: SNP summary in Starch Metabolism Pathway.

Pathway ID	No. of enzymes involved	No. of probe sets involved	No. of synonymous SNPs	No. of non-synonymous substitutions
Path: map00500	42	214	1840	525

nsSNPs in remaining 11995 unigenes from Unigene database of NCBI is being detected.

Homoeologous SNPs in chromosome1: According to SNP nomenclature, a homoeologous SNP exists if there is a difference between the three homoeologous genomes (A, B and D) in a

single individual. Varieties 1 and 2 possess the same homoeologous SNP and have the characteristic of a single individual.

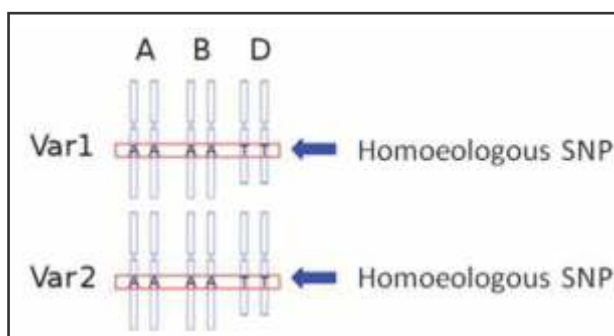


Figure 2: Depiction of homoeologus variation between A, B and D genome in wheat.

Homoeologous SNP mining was carried out through the following steps (Table 8-11):

- 1) SRA reads were retrieved from NCBI SRA database and multifasta was generated.
- 2) Reads were assembled by CLC Assembly Cell software with default parameters.
- 3) Resultant contigs were filtered to get the
- 4) SNPs in the regulatory region were identified using cis regulatory motifs in PLACE database.

homoeologs using cut-off value of minimum 5 reads from each of the 3 genomes (A,B,D) producing 105 homoeologs out of which 90 homoeologs have the polymorphism which is determined by autosnpV1 script.

Table 8: Assembly results of hexaploid wheat chromosome

CLC version	No. of SRA reads	Singleton	Assemble reads	Total no. of contigs
4 beta	1072765	6126635	4601040	885431
3.2	1072765	2850260	7877415	821609

Table 9: Assembled contig summary.

Total no. of contigs	No. of contigs with reads from 1 genome	No. of contigs with reads from 2 genomes	No. of contigs having reads from all genomes	No. of contigs with no read information
885431	284159	71530	47205	482537
821609	443337	130377	81295	166600

Both 3.2 and 4 beta versions were used to compare the number of identified SNPs.

Table 10: Homoeologous SNPs summary.

No. of contigs	Reads from A genome	Reads from B genome	Reads from D genome	Total no. of SNPs
90	4254	4810	1182	1817

Table 11: PLACE database screening report.

No. of contigs	Total no. of regulatory SNPs	No. of contigs with no regulatory SNPs	No. of annotated contigs with regulatory SNPs	No. of annotated regulatory SNPs
90	369	31	40	250

- 5) Calculation of GC content for all the genomes from chromosome 1 (Table 12).

Table 12: Nucleotide base summary and GC contents of 3 genomes.

Features	Genome A	Genome B	Genome D
GC content	0.4317	0.4368	0.4430

5.1.2 Wheat genechip microarray data mining for identification of SFPs in Indian cultivars and annotation of hypothetical proteins

Introduction:

SNPs and insertion or deletions of one or more nucleotides (indels) are DNA polymorphisms that can affect hybridization of DNA or cRNA to a probe on an array. The Affymetrix Gene Chip arrays are suitable to detect such variations because each gene is represented by a set of eleven 25-bp probes that are sensitive to target mismatch owing to their short sequence. A target sequence that matches the sequence of a probe binds with much greater affinity than one with a mismatch sequence. The resulting difference in hybridization intensity between two genotypes for an individual probe is called a SFP, where a feature refers to a probe in the array. A SFP may be caused by a SNP, a multiple nucleotide polymorphism, or an indel.

Research Objective:

To predict SFPs in the genomes of the following wheat varieties: C-306, LOK1, Sonalika and WH291, using microarray data.

Research in Progress:

1. An algorithm was written for predicting SFPs using the normalized expression signal values of the four wheat varieties.
2. Standard deviation values were computed using the normalized signal intensity values of perfect match probes of a probeset for all the four wheat varieties 7 DAA, 14 DAA and 28 DAA.
3. Four average standard deviation values were used as reference values to predict SFP. SFP is predicted if the standard deviation values of a probe of a probeset are less than or equal to the minimum of the four average standard deviation values.
4. Python script was written based on the above written statistical logic for predicting SFPs

out of the 61127 probe sets of wheat. Optimization of script is under progress.

5.1.3 Development of small RNA analysis pipeline

Introduction:

miRNAs are small and endogenous RNAs that regulate endogenous genes or gene expression. miRNAs are important for implementing developmental programs in animals and plants. Our research focus is on plant miRNA prediction and its target prediction (miRNA). In plants, these ~18-24 nt RNAs are processed from stem-loop regions of long primary transcripts by a dicer-like enzyme and are loaded into silencing complexes, where they generally direct cleavage of complementary mRNAs. NABI has initiated experiments to generate small RNA sequences using Illumina platform for RNA isolated from contrasting samples of wheat, custard apple, litchi and mango to understand small RNA regulation and identify novel regulatory small RNA. Long term goal under this component is to reconstruct gene regulatory networks using multi-dimension data. Small RNA prediction and its target prediction will be done using in-house developed pipeline of open source prediction tools and customized scripts. Bench marking of different softwares (in the pipeline) is in progress using datasets available in public domain.

Research Objectives:

- Bench-marking of miRNA prediction and miRNA-target prediction tools.
- Development of small RNA analysis Pipeline.
- Development of in-house small RNA database.
- Comparative analysis of small RNA regulation.
- Reconstruction of gene regulatory networks.

EMERGING AREAS

1. Probing B-ZIP transcription factors role in root and seed development in plants: Use of a designed dominant negative protein

Investigator: Vikas Rishi

There are approximately 72 B-ZIP transcription factors in *Arabidopsis* and a number of these are involved in root and seed development. The B-ZIP motif is a long bipartite α -helix. The C-terminal half is an amphipathic α -helix that dimerizes to form a parallel dimeric coiled-coil termed as

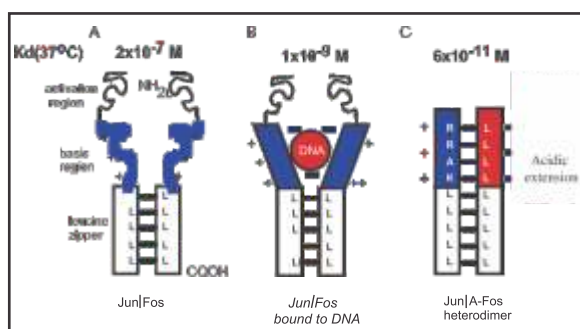


Figure 1: Design of dominant negative to B-ZIP: A-ZIP.

leucine zipper. The N-terminal half is a basic region that binds sequence specific DNA. A dominant negative (DN) protein called A-ZIP in which the basic DNA binding region of a B-ZIP transcription factor (TF) is replaced by a rationally designed acidic extension has been used successfully in mammalian systems to study the role of these TFs in growth, development and differentiation (Figure 1). These DN proteins heterodimerize with the wild type proteins and inhibit their function by not allowing them to bind to promoter of a gene. DN is biologically active reagent because a heterodimer is more stable than a B-ZIP homodimer bound to DNA. This strategy will be used to study root and seed development in *Arabidopsis*. Use of dominant negative for silencing genes has advantage over other RNA based technologies like siRNA and miRNA. These designed dominant negatives can inhibit the functions of all the TFs belonging to the same family, thus overcoming the problem of redundancy, a phenomenon common in biological systems.

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

Investigator: Nitin K. Singhal

Micronutrient deficiency such as iron deficiency may arise from inadequate intake and impaired absorption leading to anaemia, reduce cognitive development and lower work capacity. Although iron deficiency therapy with oral ferrous salts is widespread, the bioavailability of iron from such salts is poor. Anti-nutrient components, such as phytate in whole grain and bran, oxalic acid in spinach, phosphates in practically all foods, and tannins from black tea and coffee may decrease the bioavailability of ferrous iron Fe^{+2} by forming insoluble complexes.

$FeSO_4$ is water soluble compound, but is easily oxidized to low soluble Fe^{+3} form after contact with oxygen. This may often cause unacceptable change in the color and taste of foods. Moreover, Fe^{+2} frequently causes gastrointestinal side effects. It is highly desirable to find a better alternative iron source that can overcome the gastrointestinal side effects of iron. Iron oxide/hydroxide nanoparticles with neutral and hydrophilic carbohydrate nanosize shell can be used as alternatives to ferrous salts. In these formulations gastrointestinal side effects are rare because hundreds of Fe^{3+} atoms are safely packed in nanoscaled cores surrounded by the solubilizing shell. There are some evidences that particles in the range of nanometre show different properties as compared to larger particles. This and the fact that very large surface area of nanoparticles allows iron to be much more bioavailable, suggesting the potential of designing and producing particles of nano dimension. Nanoparticle based delivery systems can boost iron bioavailability and may play a vital role in food applications in the near future for treating micronutrient deficiencies.

SYNERGY THROUGH COLLABORATIONS AND NETWORKING

1. NABI and NIPER signed a MOU on 18.02.2012 to undertake joint research work in the area of mutual interest besides imparting training to staff, students and technical personnel within the areas of cooperation.
2. NABI is in advanced stage of signing MOUs with the following Institutes/Universities:
 - i. Punjab Agricultural University, Ludhiana.
 - ii. Punjab Technical University, Kapurthala
 - iii. Birla Institute of Technology, Pilani.



NEWLY JOINED FACULTY

(BRIEF PROFILES)

Name: Dr. Vikas Rishi

Date of Joining: 01-03-2012

Designation: Scientist E

Area of Research Interest: Epigenetics, DNA methylation and gene regulation, transcription factors dimerization and DNA binding specificity, protein engineering in deciphering biological functions of B-ZIP transcription factors.



Past Appointments:

1. **Visiting Scientist (2001-2006):** National Cancer Institute, National Institutes of Health, Bethesda, USA.
2. **Research Fellow (FTE position) (2006-2009):** National Cancer Institute, National Institutes of Health, Bethesda, USA.

Major Publications:

1. Warren CL, Zhao J, Glass K, **Rishi V**, Ansari AZ, Vinson C (2012). Fabrication of duplex DNA microarrays incorporating methyl-5-cytosine. Lab Chip. 12 (2): 376-80.
2. Eric RD, Chatterjee R, Zhao J, **Rishi V**, Vinson C (2012). Dominant negative induces degradation of B-ZIP proteins. Biochem Biophys Res Commun. (in press).
3. **Rishi V**, Bhattacharya P, Chatterjee R, Rozenberg J, Zhao J, Glass K, Fitzgerald P and Vinson C (2010). CpG methylation of half-CRE sequences creates C/EBP alpha binding sites that activate some tissue-specific genes. Proc Natl Acad Sci U S A. 107 (47): 20311-6.
4. **Rishi V**, Oh WJ, Heyerdahl SL, Zhao J, Scudiero D, Shoemaker RH and Vinson C (2010). Arylstibonic acids that inhibit the DNA binding of five B-ZIP dimers. J Struct Biol. 170 (2): 216-25.
5. **Rishi V**, Gal J, Krylov D, Fridriksson J, Boysen MS, Mandrup S, Vinson C (2004). SREBP-1 dimerization specificity maps to both the helix-loop-helix and leucine zipper domains: use of a dominant negative. J Biol Chem. 279 (12): 11863-74.

Name: Dr. Ajay K. Pandey

Date of Joining: 14-11-2011

Designation: Scientist D

Area of Research Interest: Functional genomics approaches in wheat and legumes crop plants to understand the linkage between the gene function and trait development. Metabolic engineering for trait development.



Past Appointments:

1. **Post Doctoral Research Associate (2008-2011):** Department of Plant Pathology, 351 Bessey Hall, Iowa State University, Ames, Iowa 50011.
2. **Post Doctoral Fellow (2004-2008):** Department of Biological Sciences, University of Alabama, Huntsville, AL-35899.
3. **Post Doctoral Fellow (2003-2004):** Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan.

Major Publications:

1. **Pandey AK**, Yang C, Zhang C, Graham MA, Hill JH, Pedley KF and Whitham S (2011). Functional analysis of the genes that contribute to *Rpp2*-mediated defense against Asian soybean rust. *Molecular Plant Microbe Interactions*. 24: 196-204.
2. **Pandey AK**, Jain P, Podila GK, Tudzynski B and Davis MR (2009). Cold induced *Botrytis cinerea* enolase (*BcEnol-I*) functions as a transcriptional regulator and is controlled by CAMP. *Molecular Genetics and Genomics*. 281: 135-146.
3. Cseke LJ, Ravinder N, **Pandey AK** and Podila GK (2007). Identification of PTM5 protein interaction partners, a MADS-box gene involved in aspen tree vegetative development. *Gene*. 391: 209-222.
4. Huang HEn, Ger MJ, Ying CC, **Pandey AK**, Yip MK, Lin MK, Chou HK and Feng TY (2007). Disease resistance to bacterial pathogens affected by the amount of ferredoxin-I protein in plants. *Molecular Plant Pathology*. 8: 129-137.
5. **Pandey AK**, Ger MJ, Huang HEn, Yip MK, Lin MK, Zeng J and Feng TY (2005). Expression of the hypersensitive response-assisting protein in Arabidopsis results in harpin dependent hypersensitive cell death in response to *Erwinia carotovora*. *Plant Molecular Biology*. 59: 771-780.

Name: Dr. Kanthi K. Kiran

Date of Joining: 02-09-2011

Designation: Scientist C

Area of Research Interest: Dietary constituents for the regulation of metabolic syndrome- nutrigenomics study, functional foods (probiotics, prebiotics and synbiotics), bacterial exopolysaccharides.



Past Appointments:

1. **Post Doctoral Fellow (2009-2011):** Lund University, Lund, Sweden
2. **Lecturer (2002-2003):** TSR & TBK Degree College and P.G Centre, Gajuwaka, Visakhapatnam

Major Publications:

1. **Kondepudi KK**, Ambalam P, Wadström T and Ljungh A (2012). Prebiotic-non-digestible oligosaccharides preference of probiotic bifidobacteria and antimicrobial activity against *clostridium difficile*. *Anaerobe*. 18 (5): 489-97.
2. Ambalam P, **Kondepudi KK**, Wadström T and Ljungh A (2012). Bile stimulates cell surface hydrophobicity, congo red binding and biofilm formation of *Lactobacillus* strains. *FEMS Microbiol Lett*. 333 (1): 10–19.
3. **Kondepudi KK** and Chandra TS (2011). Isolation of a moderately halophilic and amylolytic bacterium from saline soil of a salt manufacturing industry. *J. Pure Appl Microbiol*. 5 (2): 545-552.
4. **Kondepudi KK** and Chandra TS (2011). Identification of osmolytes from a moderately halophilic amylolytic *Bacillus* sp. strain *TSCVKK*. *Eur. J. Expt. Biol*. 1 (1): 113-121.
5. **Kondepudi KK** and Chandra TS (2008). Production of surfactant and detergent-stable, halophilic and alkalitolerant alpha-amylase by a moderately halophilic *Bacillus* sp. Strain *TSCVKK*. *Appl. Microbiol. Biotechnol*. 77: 1023-1031.

Name: Dr. Mahendra Bishnoi

Date of Joining: 16-12-2011

Designation: Scientist C

Area of Research Interest: Receptor pharmacology (metabolic disorders and nervous system disorders)



Past Appointments:

1. **Research Associate (2009-2011):** Department of Pharmacology, Southern Illinois University- School of Medicine, Springfield, Illinois, USA.
2. **Post Doctoral Associate (2009-2009):** Department of Psychiatry, McKnight Brain Institute, University of Florida, Gainesville, Florida, USA.
3. **Assistant Professor (Temporary) (2007-2008):** Department of Pharmacology, University Institute of Pharmaceutical Sciences (UIPS), Panjab University, Chandigarh.
4. **Research Assistant (2004-2005):** Pharmacology Division of Bioresearch Division, Sun Pharma Advanced Research Centre (SPARC), Vadodara, Gujarat.

Major Publications:

1. Cao DS, Zhong L, Hsieh TH, Abooj M, **Bishnoi M**, Hughes L and Premkumar LS (2012). Expression of TRPA1 and its role in insulin release from rat pancreatic beta cells, PLoS ONE. 7(5): e38005.
2. **Bishnoi M**, Bosgraaf CS, Abooj M and Premkumar LS (2011). Involvement of TRPV1 and inflammation in STZ-induced early thermal hyperalgesia is independent of glycemic state of rats. Molecular Pain. 7: 52.
3. **Bishnoi M**, Bosgraaf CS and Premkumar LS (2011). Preservation of acute pain and efferent functions following intrathecal resiniferatoxin-induced analgesia. Journal of Pain. 12: 991-1003.
4. **Bishnoi M**, Chopra K and Kulkarni SK (2009). Co-administration of nitric oxide (NO) donors prevents haloperidol-induced orofacial dyskinesia, oxidative damage and change in striatal dopamine levels. Pharmacol Biochem Behav. 91: 423-429.
5. **Bishnoi M**, Chopra K and Kulkarni SK (2008). Modulatory effect of neurosteroids in haloperidol-induced orofacial dyskinesia: An animal model of Tardive Dyskinesia, Psychopharmacology. 196: 243-254.

Name: Dr. Koushik Mazumder

Date of Joining: 01-02-2012

Designation: Scientist C

Area of Research Interest: Carbohydrate chemistry, glycobiology.

Past Appointment:

1. **Post Doctoral Research Associate (2008-2011):** Complex Carbohydrate Research Centre, University of Georgia, 315 Riverbend Road, Athens, Georgia 30602, USA.



Major Publications:

1. Correia MAS, **Mazumder K**, Brás JLA, Firbank SJ, Zhu Y, Lewis RJ, York WS, Fontes CMGA and Gilbert HJ (2011). The structure and function of an arabinoxylan-specific xylanase. *Journal of Biological Chemistry*. 286: 22510-22520.
2. **Mazumder K** and York WS (2010). Structural analysis of AXs isolated from ball milled switchgrass biomass. *Carbohydrate Research*. 345: 2183-2193.
3. **Mazumder K**, Choudhury BP, Nair GB and Sen AK (2008). Identification of a novel sugar 5, 7-diacetamido-8-amino-3,5,7,8,9-pentadeoxy-D-glycero-D-galacto-non-2-ulosonic acid present in the lipooligosaccharide of *Vibrio parahaemolyticus* O3:K6. *Glycoconjugate Journal*. 25: 345–354.
4. Zhu X, Pattathil S, **Mazumder K**, Brehm A, Hahn MG, Dinesh-Kumar SP and Joshi CP (2010). Virus induced gene silencing offers a functional genomics platform for studying plant cell wall formation. *Molecular Plant*. 3: 818-833.
5. Kulkarni AR, Peña MJ, Avci U, **Mazumder K**, Urbanowicz B, Yin Y, O'Neill MA, Roberts AW, Hahn MG, Xu Y, Darvill AG and York WS (2011). The ability of land plants to synthesize glucuronoxylans predates the evolution of tracheophytes. *Glycobiology*. 22: 439-451.

Name: Dr. Nitin K. Singhal

Date of Joining: 02-03-2012

Designation: Scientist C

Area of Research Interest: Synthetic nano-biochemistry, liposome based gene delivery, fabrication of nano / microstructures, cellular assays, organic synthesis of small molecules, biosensors, biomarker based disease diagnostic.



Past Appointments:

1. **Post Doctoral Fellow (2008-2009):** Pennsylvania State University, State College, USA
2. **Post Doctoral Fellow (2009-2012):** Seoul National University, Seoul, South Korea

Major Publications:

1. Kumar A, Ramanujam B, **Singhal NK**, Mitra A and Chebrolu PR (2010). Interaction of aromatic imino glycoconjugates with jacalin: experimental and computational docking studies Carbohydrate Res. 345: 2491-2498.
2. Amit K, **Singhal NK**, Nagender RR, Siva KN and Chebrolu PR (2009). C1- and C2-Imino-based glycoconjugates: inhibition aspects towards glycosidase activities isolated from soybean and jack bean. Glycoconjugates Journal. 26: 495-510.
3. **Singhal NK**, Mitra A, Ramanujam B and Chebrolu PR (2009). Variation in the sensitivity and selectivity of M^{2+} as a function of the stereochemistry of the carbohydrate: Selectivity differences between galactosyl and ribosyl derivatives towards Cu^{2+} attributed to the structural differences. Dalton Tran. 39: 8432-8442.
4. **Singhal NK**, Ramanujam B, Mariappanadar V and Rao CP (2006). Carbohydrate-based switch-on molecular sensor for cu (ii) in buffer: Absorption and fluorescence study of the selective recognition of cu (ii) ions by galactosyl derivatives in HEPES buffer. Organic Letters. 8: 3525-3528.
5. Ahuja R, **Singhal NK**, Ramanujam B, Ravikumar M and Rao CP (2007). Experimental and computational studies of the recognition of amino acids by galactosyl-imine and -amine derivatives: An attempt to understand the lectin-carbohydrate interactions. Journal of Organic Chemistry. 72: 3430-3442.

Name: Dr. Shailesh Sharma

Date of Joining: 02-01-2012

Designation: Project Scientist

Area of Research Interest: Detecting single feature polymorphisms in *Triticum aestivum* using affymetrix arrays. Transcript annotation of *Triticum aestivum* obtained from affymetrix.



Past Appointment:

1. **Post Doctoral Scientist (2010 – 2011):** USA - India joint research training program. This program was hosted by the Seattle Biomedical Research Institute at the University of Washington, USA and at The International Centre for Genetic engineering and Biotechnology, New Delhi. Research program was funded by the Fogarty International Center of the National Institutes of Health, USA.

Major Publications:

1. **Sharma S, Cavallaro G and Rosato A (2010).** A systematic investigation of multiheme c-type cytochromes in prokaryotes. *J. Biol. Inorg. Chem.* 15: 559-571.
2. **Sharma S and Rosato A (2009).** Role of the N-terminal tail of metal-transporting P (1B)-type ATPases from genome-wide analysis and molecular dynamics simulations. *J. Chem. Inf. Model.* 49: 76-83.

Name: Dr. Nishima

Date of Joining: 04-01-2012

Designation: Project Scientist

Areas of Research Interest: Nanobiotechnology and its applications, microcantilever based detection of biomolecules, polypeptides based microelectronics, chemical modification of haptens and physicochemical characterization, natural product chemistry



Past Appointment:

1. **Post Doctoral Research Fellow (2010-2011):** Nanyang Technological University, Singapore

Major Publications:

1. **Wangoo N**, Shekhawat G, Jin-Song Wu, Suri CR, Bhasin KK and Dravid V (2012). Green synthesis and characterization of size tunable silica capped gold core-shell nanoparticles. *Journal of Nanoparticle Research* (In Press).
2. **Wangoo N**, Kaushal J, Bhasin KK, Mehta SK and Suri C (2010). Zeta potential based colorimetric immunoassay for the direct detection of diabetic marker HbA1c using gold nanoprobe. *Chemical Communications*. 46: 5755-5757.
3. **Wangoo N**, Bhasin KK, Mehta SK and Suri CR (2008). Synthesis and capping of water-dispersed gold nanoparticles by an amino acid: Bioconjugation and binding studies. *Journal of Colloid and Interface Science*. 323: 247-254.
4. **Wangoo N**, Bhasin KK, Boro R and Suri CR (2008). Facile synthesis and functionalization of water-soluble gold nanoparticles for a bioprobe. *Analytica Chimica Acta*. 610: 142-148.

Name: Dr. Sudhir P. Singh

Date of Joining: 16-01-2012

Designation: Project Scientist

Area of Research Interest: Molecular biology of seed development in wheat with the objective to identify bottlenecks by preventing iron translocation from outer bran layers to endosperm in bioavailable form. To identify the candidate genes for inducing seedlessness in fruit crops.



Past Appointment:

1. **Research Associate (2010-2011):** National Agri-Food Biotechnology Institute, Mohali, Punjab

Major Publications:

1. Kumar J, **Singh SP**, Kumar J and Tuli R (2012). A novel mastrevirus infecting wheat in India. Archives of Virology. 157(10): 2031-2034. ISSN: 0304-8608.
2. Kumar J, Kumar A, **Singh SP**, Roy JK, Lalit A, Parmar D, Sharma NC and Tuli R (2012). First report of leaf curl virus infecting okra in India. New Disease Reports. 25: 9. ISSN No. 2044-0588.
3. **Singh SP**, Pandey T, Srivastava R, Verma PC, Singh PK, Tuli R and Sawant SV (2010). BECLIN 1 from Arabidopsis thaliana, under the generic control of regulated expression systems, a strategy for developing male sterile plants. Plant Biotechnology Journal, 8(9): 1005–1022. ISSN: 1467-7652.
International Patent (Published): Sawant SV, Tuli R and **Singh SP**. Method for producing male sterile plants. PCT WO/2010/061276; A gene for inducing male sterility. India: 2697/DEL/2008; USA: US2011289631; European Union: EP2350290; China: CN102257143; Australia: AU2009321261.
4. Lodhi N, Ranjan A, Singh M, Srivastava R, **Singh SP**, Chaturvedi CP, Ansari SA, Sawant SV and Tuli R (2008). Interactions between upstream and core promoter sequences determine gene expression and nucleosome positioning in tobacco PR-1a promoter. Biochimica et Biophysica Acta (BBA). 1779 (10): 634-644. ISSN: 1874-9399.
5. Singh RK, **Singh SP** and Singh SB (2005). Correlation and path analysis in sugarcane ratoon. Sugar Tech. 7(4): 176-178. ISSN: 0972-1525.

Extramural Grants Received: Title: A novel strategy for developing scion plants of desired phenotype, by using RNAi delivering rootstock. Sponsoring Agency: Science and Engineering Research Board, Department of Science and Technology (DST).

Name: Ms. Vandana Mishra

Date of Joining: 23-01-2012

Designation: Project Scientist

Area of Research Interest: Bioinformatics: Development of genomics resources for grain crops.



Past Appointments:

1. **Junior Research Fellow (Project- Next generation sequencing) (2011-2012):** Plant Immunity Laboratory, National Institute of Plant Genome Research, JNU campus, New Delhi.
2. **Application Specialist (Biotechnology instruments/bioinformatics software) (2010-2011):** NSW Biotech Pvt. Ltd, New Delhi.
3. **Junior Research Fellow (Project- Structural bioinformatics) (2009-2010):** Department of Biochemistry, Delhi University, South campus, New Delhi.
4. **Bioinformatician (2008-2009):** Infovalley Biosystems (India) Pvt. Ltd, Bangalore.

Major Publications: Nil



RESEARCH PUBLICATIONS

I. Publication based on research initiated at NABI:

1. Kumar J, Singh SP, Kumar J and Tuli R (2012). A novel mastrevirus infecting wheat in India. **Archives of Virology**. 157(10): 2031-2034.

II. Publications based on work done by faculties or initiated at their earlier institutes:

2012

1. **Bishnoi M** and Boparai RK (2012). An animal model to study the molecular basis of tardive dyskinesia. **Methods Mol Biol**. 829: 193-201.
2. Bruijnzeel AW, Ford J, Rogers JA, Scheick S, Ji Y, **Bishnoi M** and Alexander JC (2012). Blockade of CRF1 receptors in the central nucleus of the amygdala attenuates the dysphoria associated with nicotine withdrawal in rats. **Pharmacol Biochem Behav**. 101(1): 62-8.
3. Chitale VS, Tripathi P, Behera MD, Behera SK and **Tuli R** (2012). On the relationships among diversity, productivity and climate from an Indian tropical ecosystem: a preliminary investigation. **Biodiversity and Conservation**. 21: 1177-1197.
4. Jena SN, Srivastava A, Rai KM, Ranjan A, Singh SK, Tarannum N, Srivastava M, Bag SK, **Mantri S**, Asif MH, Yadav HK, **Tuli R** and Sawant SV (2012). Development and characterization of genomic and expressed SSRs for levant cotton (*Gossypium herbaceum L.*). **Theor Appl Genet**. 124: 565-576.
5. **Kumar J**, Kumar A, **Singh SP**, **Roy JK**, Lalit A, Parmar D, Sharma NC and **Tuli R** (2012). First report of leaf curl virus infecting Okra in India. **New Disease Report** (earlier BSPP Plant Pathology), 25: 9, ISSN No 2044-0588.
6. Madnala R, Gupta V, Singh PK and **Tuli R** (2012). Development of chloroplast transformation vectors, and a new target region in the tobacco plastid genome. **Plant Biotechnol Reports**. 6: 77-87.
7. Ranjan A, Nigam D, Asif MH, Singh R, Ranjan S, **Mantri S**, Pandey N, Trivedi I, Jena SN, **Tuli R**, Pathre U and Sawant S (2012). Genome wide expression profiling of two accessions of *Gossypium herbaceum L.* in response to drought. **BMC Genomics**. 13: 94-99.
8. Soumit K, Behera , Mishra AK , Sahu N , Kumar A, Singh N, Kumar A, Bajpai O, Chaudha L B, Khare PB and **Tuli R** (2012). The study of microclimate in response to different plant community association in tropical moist deciduous forest from northern India. **Biodiversity and Conservation**. 21: 1159-1176.
9. **Singh SP**, Singh Z and Swinny EE (2012). Climacteric level during fruit ripening influences lipid peroxidation and enzymatic and non-enzymatic antioxidative systems in Japanese plums (*Prunus salicina Lindell*). **Postharvest Biology and Technology**. 65: 22-32.
10. **Tiwari S** and **Tuli R** (2012). Optimization of factors for efficient recovery of transgenic peanut (*Arachis hypogaea L.*) **Plant Cell Tissue and Organ Culture**. 109: 111–121.
11. Upadhyay SK, Singh S, Chandrashekar K, **Tuli R** and Singh PK (2012). Compatibility of garlic (*Allium sativum L.*) leaf agglutinin and CryAc -endotoxin for gene pyramiding. **Applied Microbiol & Biotechnol**. 93: 2365-2375.
12. Warren CL, Zhao J, Glass K, **Rishi V**, Ansari AZ and Vinson C (2012). Fabrication of duplex DNA microarrays incorporating methyl-5-cytosine. **Lab Chip**. 12 (2): 376-80.

2011

1. **Bishnoi M**, Bosgraaf CA, Abooj M and Premkumar LS (2011). Involvement of TRPV1 and inflammation in STZ-induced early thermal hyperalgesia is independent of glycemic state of rats. **Molecular Pain**. 7: 52.
2. **Bishnoi M**, Bosgraaf CS and Premkumar LS (2011). Preservation of acute pain and Efferent functions following intrathecal resiniferatoxin-induced analgesia. **Journal of Pain**. 12: 991-1003.
3. Correia MAS, **Mazumder K**, Brás JLA, Firkbank SJ, Zhu Y, Lewis RJ, York WS, Fontes CMGA and Gilbert HJ (2011). The structure and function of an arabinoxylan-specific xylanase. **Journal of Biological Chemistry**. 286: 22510-22520.
4. Jena SN, Srivastava A, Singh UM, Roy S, Banerjee N, Rai KM, Singh SK, Kumar V, Chaudhary LB, **Roy JK**, **Tuli R** and Sawant SV (2011). Analysis of genetic diversity, population structure and linkage disequilibrium in elite cotton (*Gossypium* L.) germplasm in India. **Crop and Pasture Science**. 62: 859-875.
5. Premkumar LS and **Bishnoi M** (2011). Disease-related changes in TRPV1 expression and its implications for drug development. **Curr Top Med Chem**. 11(17): 2192-209.
6. Raju M, Gupta V, Deebea F, Upadhyay SK, Pandey V, Singh PK and **Tuli R** (2011). A highly stable Cu/Zn superoxide dismutase from *Withania somnifera* plant: gene cloning, expression and characterization of the recombinant protein. **Biotechnology Letters**. 33: 2057-2063.
7. Sidhu OP, Annarao S, Chatterjee S, **Tuli R**, Roy R and Khaterpal CL (2011). Metabolic alterations of *Withania somnifera* (L.) Dunal fruits at different developmental stages by NMR spectroscopy. **Phytochemical Analysis**. DOI 10.1002/pca.1307.
8. **Tiwari S**, Mishra DK, Chandrashekar K, Singh PK and **Tuli R** (2011). Expression of d-endotoxin CryIEC from an inducible promoter confers insect protection in peanut (*Arachis hypogaea* L.) plants. **Pest Management Science**. 67: 137-145.
9. Upadhyay SK, Sharad S, Singh R, Rai P, Dubey NK, Chandashekar K, Negi KS, **Tuli R** and Singh PK (2011). Purification and characterization of a lectin with high hemagglutination property isolated from *Allium altaicum*. **Protein Journal**. 30: 374-83.

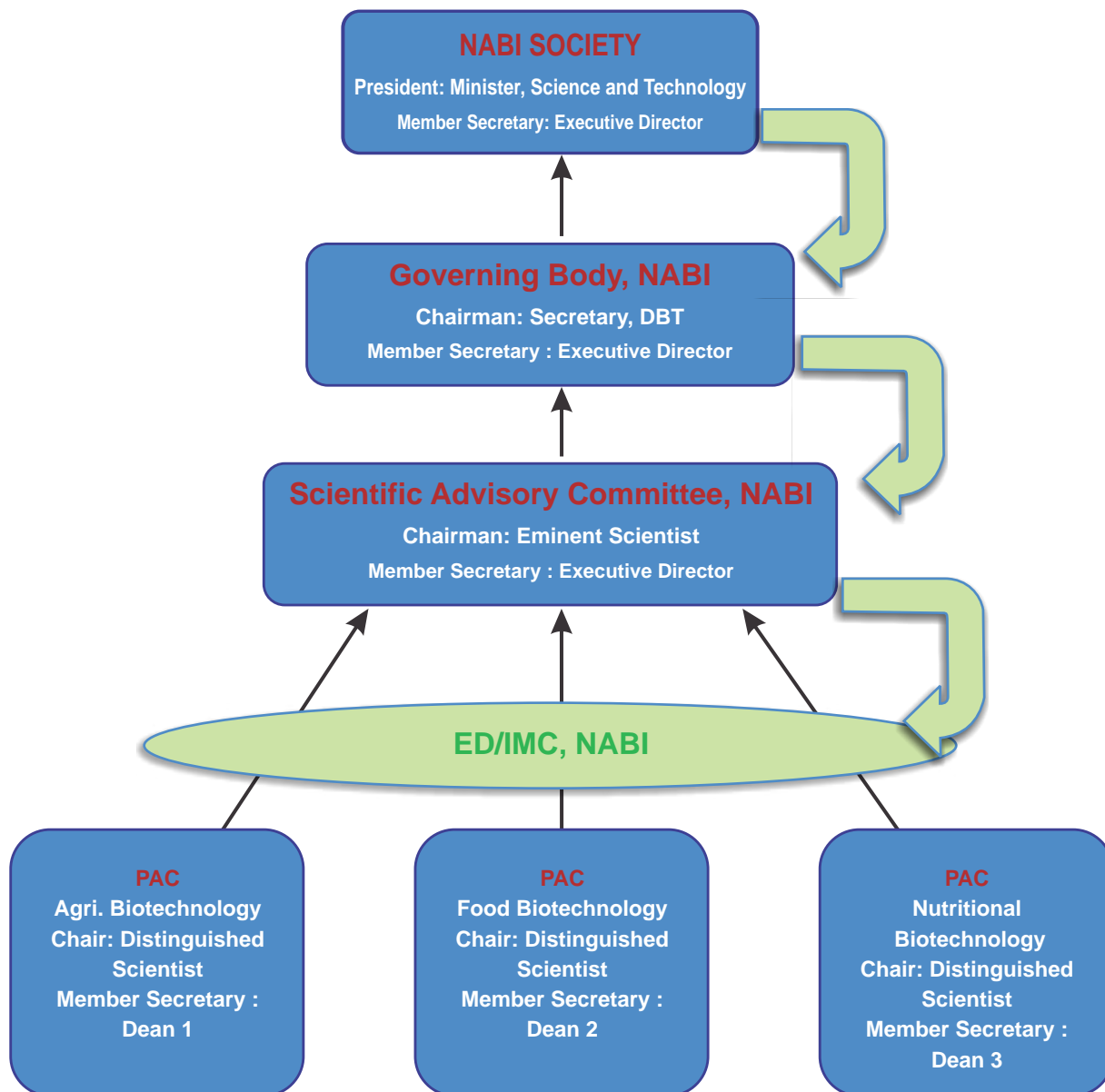


EXTRAMURAL GRANTS AND FUNDING

S. No.	Investigator	Title of the Project	Funded by
1	Dr. Rakesh Tuli	J. C. Bose Fellowship	DST, Govt. of India
2	Dr. Sudhir P. Singh	A novel strategy for developing scion plants of desired phenotype by using RNAi delivering rootstock	SERB, DST, Govt. of India



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(Upto July 11, 2011)

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National Agri-Food Biotechnology Institute,
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Mysore

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Centre for Cellular & Molecular Biology
(CCMB),
Hyderabad

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Deptt. of Genetics,
University of Delhi,
South Campus, Dhaura Kuan,
New Delhi

Dr. Anura V. Kurpad

Professor of Physiology,
St John's Medical College,
Bangalore

Dr. H. P. S. Sachdev

Senior Consultant (Paediatrics),
Sitaram Bhartia Institute of Science & Research,
B-16, Kutub Institutional Area,
New Delhi

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Former Director,
Central Food Technological Research Institute,
Mysore

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Bhabha Atomic Research Centre,
Mumbai

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Executive Director,
National Agri-Food Biotechnology Institute,
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Advisor, DBT
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Lodi Road,
New Delhi

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

Dr. C. R. Bhatia

Former Secretary,
Department of Biotechnology,
New Delhi
(*Chairman*)

Dr. Kailash Chander Bansal

Director,
National Bureau of Plant Genetic Resources
(NBPGR),
New Delhi

Dr. Akhilesh Kumar Tyagi

Director,
National Institute of Plant Genome Research,
New Delhi

Dr. G. K. Garg

Director, ITR
Krishidhan Research Foundation Pvt. Ltd.,
Aurangabad Road,
Jalna
(*Chairman*)

Dr. K. Madhavan Nair

Deputy Director,
National Institute of Nutrition,
Hyderabad

Dr. (Prof.) Sunil Kumar Mukharjee

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

Dr. Kiran K. Sharma

International Crops Research Institute for
the Semi-Arid Tropics (ICRISAT),
Hyderabad

Dr. Ramesh Sonti

Deputy Director,
Centre for Cellular & Molecular Biology
(CCMB),
Hyderabad

Dr. Rajesh Kapur

Advisor, DBT
CGO Complex,
Lodi Road,
New Delhi

Dr. Ashok K. Singh

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

Dr. T. Mohapatra

Principal Scientist,
NRC Plant Biotechnology,
Indian Agricultural Research Institute,
New Delhi

Dr. Rakesh Tuli

Executive Director,
National Agri-Food Biotechnology Institute,
Mohali

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

Dr. V. Prakash

(Former Director, CFTRI)
Distinguished Scientist,
Council of Scientific and Industrial Research,
Mysore
(*Chairman-Food Biotechnology*)

Dr. B. Siva Kumar

Former Director,
National Institute of Nutrition,
Secunderabad
(*Chairman-Nutrition Biotechnology*)

Dr. Appu Rao

Scientist,
Central Food Technological Research Institute,
Mysore

Dr. V. K. Batish

Emeritus Scientist,
Molecular Biology Unit,
National Dairy Research Institute (NDRI),
Karnal

Dr. K. Madhavan Nair

Deputy Director,
National Institute of Nutrition,
Hyderabad

Dr. S. K. Roy

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

Dr. H. P. S. Sachdev

Sitaram Bhartia Institute of Science & Research,
B-16, Kutub Institutional Area,
New Delhi

Dr. H. N. Mishra

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

Dr. Bhupendar Khatkar

Chairman, Department of Food Technology,
Guru Jambheshwar University of Science &
Technology,
Hissar

Dr. M. C. Varadraj

Scientist,
Central Food Technological Research Institute,
Mysore

Dr. Rajesh Kapur

Advisor, DBT
CGO Complex,
Lodi Road,
New Delhi

Dr. Rakesh Tuli

Executive Director,
National Agri-Food Biotechnology Institute,
Mohali

H. Building Committee

Dr. G. P. Talwar

Director Research,
Talwar Research Foundation,
New Delhi

Dr. N. Sathyamurthy

Director,
Indian Institute of Science & Education
Research,
Mohali

Dr. P. K. Seth

Chief Executive Officer,
Biotech Park,
Lucknow

Sh. Sanjay Goel

Director (Finance),
Department of Biotechnology,
New Delhi

Dr. Rajesh Kapur

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

Er. N. S. Bhatti

Former Chief Engineer,
Punjab Administration,
Chandigarh

Dr. K. K. Kaul

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

Er. S. K. Jaitley

Former Chief Engineer,
UT Sctrariat,
Chandigarh

Er. S. L. Kaushal

Former Chief Architect Punjab,
Chandigarh

Ms. Balwinder Saini

Chief Architect,
Govt. of Punjab,
Chandigarh

Er. Kuldeep Singh

Former Chief Engineer
Chandigarh Adminstration,
Chandigarh

Dr. S. B. Katti

Scientist G,
Central Drug Research Institute,
Lucknow

Dr. U. V. Pathre

Scientist,
National Botanical Research Institute,
Lucknow

Dr. Jagdeep Singh

Coordinator,
Indian Institute of Science Education and
Research,
Mohali

Er. A. A. Malik

Supretendent Engineer,
National Botanical Research Institute,
Lucknow

Dr. Rakesh Tuli

Executive Director,
National Agri-Food Biotechnology Institute,
Mohali
(Chairman)



HUMAN RESOURCE

I. Research Faculty

i. Regular:

S. No	Name	Designation	Date of Joining
1	Dr. Rakesh Tuli	Executive Director	08-02-2010
2	Dr. Vikas Rishi	Scientist E	01-03-2012
3	Dr. Joy K. Roy	Scientist D	09-08-2010
4	Dr. Ajay K. Pandey	Scientist D	14-11-2011
5	Dr. Siddharth Tiwari	Scientist C	28-07-2010
6	Sh. Shrikant Subhash Mantri	Scientist C	18-08-2010
7	Dr. (Ms.) Monika Garg	Scientist C	30-11-2010
8	Dr. Sukhvinder P. Singh	Scientist C	06-12-2010
9	Dr. Kanthi K. Kiran	Scientist C	02-09-2011
10	Dr. Mahendra Bishnoi	Scientist C	16-12-2011
11	Dr. Koushik Mazumder	Scientist C	01-02-2012
12	Dr. Nitin K. Singhal	Scientist C	02-03-2012

ii. On Contract:

S. No	Name	Designation	Date of Joining
1	Dr. Shailesh Sharma	Project Scientist	02-01-2012
2	Dr. Nishima	Project Scientist	04-01-2012
3	Dr. Sudhir P. Singh	Project Scientist	16-01-2012
4	Ms. Vandana Mishra	Project Scientist	23-01-2012

II. Technical and Engineering Support

S. No	Name	Designation	Date of Joining
1	Sh. E. Subramanian	Computer Operator	27-02-2010
2	Er. Jatin Singla	Assistant Engineer (Civil)	21-01-2011
3	Ms. Aakriti Gupta	Senior Tech Assistant	22-02-2011
4	Sh. Jagdeep Singh	Senior Tech Assistant	01-03-2011
5	Sh. Sukhjinder Singh	Computer Operator	23-02-2012
6	Sh. Jaspreet Singh	Assistant Engineer (Civil)	19-03-2012

III. Administration

S. No	Name	Designation	Date of Joining
1	Sh. Rattan Lal Sharma	Associate Director (Accounts and Finance)	25-5-2011
2	Sh. S. Krishnan	Store & Purchase Officer	10-03-2010
3	Sh. Vikram Singh	Administrative Officer	01-04-2011
4	Sh. Suneet Verma	Finance Officer	15-09-2011
5	Sh. Sabir Ali	Executive Assistant (Administration)	21-01-2011
6	Ms. Hema Rawat	Executive Assistance (Accounts)	01-04-2011
7	Sh. Vishal Kumar	Management Assistant (Accounts)	08-09-2011

IV. Human Resource Development

i. Research Scholars:

S.No	Name	Designation	Date of Joining
1	Sh. Jitendra Kumar	Senior Research Fellow	04-06-2010
2	Sh. Yogesh Gupta	Junior Research Fellow	09-08-2010
3	Ms. Anuradha Singh	Junior Research Fellow	11-09-2010
4	Sh. Rohit Kumar	Junior Research Fellow	06-06-2011
5	Ms. Anita Kumari	Junior Research Fellow	29-08-2011
6	Sh. Anshu Alok	Junior Research Fellow	09-09-2011
7	Ms. Shaweta Arora	Junior Research Fellow	09-09-2011
8	Ms. Manpreet Kaur Saini	Junior Research Fellow	09-09-2011
9	Sh. Jitesh Kumar	Junior Research Fellow	09-09-2011
10	Sh. Gaurab Das	Junior Research Fellow	10-10-2011
11	Sh. Kaushal Kumar Bhati	Junior Research Fellow	14-11-2011
12	Ms. Anuradha Swami	Junior Research Fellow	23-02-2012
13	Ms. Roohi Bansal	Junior Research Fellow	24-02-2012
14	Ms. Monica Sharma	Junior Research Fellow	01-03-2012
15	Sh. Raja Jeet	Junior Research Fellow	12-03-2012

ii. Trainees:

S.No	Name	Designation	Date of Joining
1	Sh. Vikas Bindal	Trainee	02-01-2012
2	Ms. Arushi Sharma	Trainee	02-01-2012
3	Ms. Natasha Paul	Trainee	02-01-2012
4	Ms. Manila Kashyap	Trainee	02-01-2012
5	Sh. Ravindra Dhakad	Trainee	02-01-2012
6	Sh. Anuj Kumar Pandey	Trainee	03-01-2012
7	Sh. Rakesh Kumar	Trainee	03-01-2012
8	Ms. Iram Preet Kaur	Trainee	04-01-2012
9	Ms. Arti Katoch	Trainee	04-01-2012
10	Ms. Guneet Kaur	Trainee	04-01-2012
11	Ms. Lipika Sahota	Trainee	09-01-2012
12	Ms. Ramandeep Kaur	Trainee	09-01-2012
13	Sh. Deepak Soni	Trainee	13-01-2012
14	Sh. Nishant Garg	Trainee	03-02-2012
15	Sh. Shashank Maheshwari	Trainee	03-02-2012



PROGRESS OF INFRASTRUCTURE AT MAIN CAMPUS

Proposed Master Plan of the main campus,
Sector-81, Ajitgarh (Mohali)





Main entrance gate of the campus at sector-81, Mohali.



Low lying area at the main campus and a view of IISER campus on the other side of boundary wall.



PROGRESS OF INFRASTRUCTURE AT INTERIM FACILITY

Development of NABI interim facility



Inauguration of construction work at 1st floor at interim facility: July 4th, 2011



Upper and lower left: Analytical instruments rooms at 1st floor.

Upper Right: New laboratories at 1st floor.

Lower right: Plant tissue culture and animal cell culture facility developed at 1st floor.



Plant Tissue Culture facility at 1st floor.



Scientists working at Animal Cell Culture facility, 1st floor.



IMPORTANT EVENTS

Important events:

1. Independence Day celebration: August 15th, 2011. Dr. Rakesh Tuli, hoisted the National flag at interim facility.
2. Tree plantation programe at the main campus: August 18th 2011. Dr. Rakesh Tuli and staff participated in the tree plantation programe. Pollution tolerant species were planted along with the boundary wall of NABI campus with the objective of making the campus green and colourful.
3. Republic Day celebration: January 26th, 2012. Dr. Rakesh Tuli, hoisted the national flag at interim facility.
4. The 2nd Foundation Day of NABI was celebrated at interim facility: Feburary 18th, 2012. Padma Bhushnan Dr. R. S. Paroda, former DG, ICAR & Secretary, Department of Argicultural Research and Education (DARE) delivered the Foundation Day lecture “*Harnessing biotechnology in Indian agriculture: Challenges and Opportunities*”. Padma Bushna Dr. N K Ganguly, former DG, ICMR gave Presidential Address on “*Food Security and Nutritional Challenges for Marginalised Populations*”. They also released the Foundation Year Annual Report 2010-2011 of the institute.
5. promote Indo-Canada partnership development activities: August 14th, 2011.
6. Visit of Nutrition Science and Biotechnology Faculty Selection Committee experts: October 29th, 2011.
7. Visit of Food Science and Biotechnology Faculty Selection Committee experts : November 27th – 28th, 2011.
8. Visit of National Dairy Research Institute delegates: December 27th, 2011.
9. Second Scientific Advisory Committee (SAC) and Programe Advisory Committee (PAC) meeting : February 18th - 19th, 2012.
10. Visit of University of Agricultural Sciences delegates: February 27th, 2012.

National/International visits of NABI staff:

1. Dr. Joy K. Roy was invited to participate in the symposium organized by the Indian National Science Academy (INSA) under its Bilateral Exchange Programe with the German National Academy of Sciences, Leopoldina on “Plant Biology” which was held during October 18th-20th, 2011 at New Delhi. He also presented his research work entitled “Towards understanding of molecular basis of processing quality in wheat”.
2. Dr. Sudhir P. Singh visited, Canadian Light Source, Saskatoon, Canada from September 26th to October 2nd, 2011 to perform experiments on mineral localization in the seed tissues of wheat. The Beam time was awarded by the Canadian Light Source to perform X-Ray Fluorescence and XANES experiments on VESPERS beam line.
3. Sh. Shrikant Mantri, attended a seminar on Genomeet (A Decade of Genomics) held at IGIB, New Delhi on January 1st, 2012.
4. Dr. Sudhir P. Singh attended the first ICC International Grains Conference held at

Meetings

1. Visit of Agri-Biotechnology Faculty Selection Committee experts : July 12th, 2011.
2. First Programe Advisory Committee (PAC) meeting : July 26th, 2011.
3. First Scientific Advisory Committee (SAC) meeting : August 2nd, 2011.
4. Third Governing Body (GB) meeting : August 2nd, 2011.
5. Visit of Canadian Delegates from Saskatoon-cluster, Saskatchewan, to

New Delhi during January 16th -18th, 2012. He presented his work entitled “New insights into iron transport from maternal tissues to endosperm in mature wheat seed using synchrotron radiation”.

5. Dr. Joy K. Roy attended the first “ICC International Grains Conference”, which was held during January 16th - 18th, 2012 at New Delhi. He presented his work entitled “Gene discovery for improvement of processing quality in bread wheat”.
6. Dr. Nishima, participated in a seminar on “Green Chemistry” held at Ludhiana on February 24th, 2012.
7. Dr. Rakesh Tuli, attended Biotechnology-Industry Research Assistance Council meeting at Australia during March 6th-10th, 2012 and visited to Queensland University Technology, Brisbane, Australia to discuss about the project on banana improvement.

International visitors to NABI

1. Canadian delegates visited NABI campus and interacted with NABI Scientists: August 14th, 2011.
2. Dr. Bikram Singh Gill from Kansas State University, Kansas, USA visited NABI on January 23rd, 2012. He discussed research progress with NABI scientists. He gave his presentation on recent approaches in wheat genomics.
3. Dr. Harbans Bariana from University of Sydney, Sydney, Australia, Plant Breeding Institute, Australia visited NABI on February 9th, 2012 to share his thoughts and interacted with NABI faculty in the area of plant breeding and genetic resources.
4. Prof. Leon A. Terry from Cranfield University, Bedfordshire, England visited NABI on February 15th, 2012 and discussed about his research work.



PHOTO-GALLERY

Agri-Biotechnology Faculty Selection Committee: July 12th, 2011



Visit of experts to interim facility. Dr. Joy K. Roy explaining his work on Affymetrix microarray to (from left) Dr. A. K. Tyagi, Dr. C. R. Bhatia, Dr. J. P. Khurana, Dr. P. K. Gupta, Dr. Imran Siddiqi and Dr. Rakesh Tuli.



Visit of experts to the main campus, Sector-81, Mohali.

First PAC Meeting: July 26th, 2011



PAC meeting being chaired by Dr.V. Prakash.



Distinguished experts from Agri, Food and Nutrition Biotechnology during the PAC meeting.

First SAC Meeting: August 2nd, 2011



First SAC meeting being addressed by Dr. M.K. Bhan and chaired by Dr. C.R. Bhatia.



Dr. Rakesh Tuli, presenting the research plans of NABI to PAC and SAC members.

Third Governing Body Meeting: August 2nd, 2011



The third GB meeting being chaired by Dr. M. K. Bhan, Secretary, DBT, Dr. (Mrs.) Manju Sharma, Former Secretary, DBT and member GB is seen on the right.



Dr. Rakesh Tuli, discussing the progress, opportunities and challenges in Agri-Food researches with the distinguished GB members.

Independence Day Celebrations at NABI: August 15th, 2011



Dr. Rakesh Tuli, hoisted the national flag at NABI interim facility and addressed the staff.



Independence Day celebrations at the main campus, Sector 81, Mohali.

Tree Plantation Programme at the Main Campus: August 18th, 2011



Tree plantation programme was organised at the main campus, Sector 81, Mohali. Dr. Rakesh Tuli and staff participated in the programme. Pollution tolerant species were planted along the boundary wall to make campus look green and colourful, under the guidance of Dr. S. C. Sharma, former Dy. Director, NBRI, Lucknow.

Visits of Delegates



Visit of Canadian delegates from Saskatoon-cluster, Saskatchewan, to promote Indo-Canada partnership: August 14th, 2011. **From Left:** Sh. G. V. Shankar, Dr. Venkatesh Meda, Dr. Rakesh Tuli, Dr. Sudhir P. Singh and Dr. Robert (Bob) Tyler.



Visit of Scientific delegation from National Dairy Research Institute: December 27th, 2011.

Republic Day Celebrations at NABI: January 26th, 2012



Dr. Rakesh Tuli, hoisted the national flag.



Republic day celebrations at NABI interim facility.

Second PAC Meeting: February 18th, 2012



First row from left: PAC meetings being chaired by Dr. V. Prakash (PAC – Food Biotechnology) , Dr. B. Siva Kumar (PAC – Nutrition Biotechnology) and Dr. R. S. Paroda (PAC – Agricultural Biotechnology).

Second row from left: Discussion during the combined PAC meeting of Agri, Food and Nutrition Biotechnology.

Third row: Visit of the experts to the main campus at Sector 81, Mohali.

Second SAC Meeting: February 19th, 2012



SAC meeting being chaired by Dr. R.S. Paroda, Chairman SAC and former Director General, ICAR.



Experts in Agri, Food and Nutrition Biotechnology having discussion with faculty, during the SAC meeting.

Second Foundation Day: February 18th, 2012



First row from left: Dr. R.S. Paroda, Dr. N.K. Ganguly, Dr. B. Siva Kumar and other distinguished guests invited on the occasion. Dr. R.S. Paroda was the Chief Guest and Dr. N.K. Ganguly presided over the function.

Second row from left: Dr. Rakesh Tuli, reading Annual Report of NABI. Signing of MoU between NIPER and NABI.

Third row from left: Dr. R. S. Paroda delivering a talk on “*Harnessing Biotechnology in Indian Agriculture: Challenges and Opportunities*”. Dr N.K. Ganguly gave Presidential address “*Food Security and Nutritional Challenges for Marginalised Populations*” on the occasion. Dr. Ajay K. Pandey giving the vote of thanks.

FINANCIALS

Annual Accounts for the year 2011-12:

- The financial resource of the institute is the core grant provided by the Department of Biotechnology, Govt. of India under non-recurring & recurring components.
- The institute received the core grant of Rs. 2,358.00 lac in the year 2011-12.
- Annual accounts for the year 2011-12 are

prepared by the institute on the basis of accrual system of accounting using standard format of accounts prescribed by the Government of India for Central Autonomous Bodies.

- M/s Raj Gupta & Co., (Chartered Accountant) Chandigarh, the statutory auditors of the institute have audited the accounts.

Financial Position:-

(Figure in Rupees)

S.No.	Particulars	As on 31-03-2011	As on 31-03-2012
A.	Capital Fund and Liabilities		
1.	Capital Fund	22,52,20,686	36,08,87,705
2.	Current Liability and Provisions	11,16,888	69,72,351
	Total	22,63,37,574	36,78,60,056
B.	Assets		
1.	Fixed Assets	11,84,15,380	18,07,26,363
2.	Capital Work -in-Progress	76,20,000	3,69,11,630
3.	Current Assets ,Loans & Advances etc.	10,03,02,194	15,02,22,063
	Total	22,63,37,574	36,78,60,056
C.	Receipts of the Institute		
1.	Grants from DBT	22,22,24,014	23,58,00,000
2.	Internal Receipts /Income	23,01,994	91,79,908
	Total	22,45,26,008	24,49,79,908
D.	Utilization		
1.	Equipment	12,08,76,581	7,42,44,835
2.	Furniture and other Fixed Assets	44,67,606	62,47,815
3.	Manpower	77,35,259	2,12,64,257
4.	R&D Expenses	1,07,13,216	1,54,34,510
5.	Administrative Expenses	2,89,08,896	5,44,32,454
E.	Payables/Outstandings	11,16,888	69,72,351
F.	Advances / Receivables	1,63,49,345	1,65,25,554
G.	Margin Money for LCs	5,50,00,000	8,27,47,849
H.	Closing Bank Balance (Excluding Margin Money)	2,89,52,849	5,09,48,660



C-127, Industrial Area, Phase 8, Ajitgarh (Mohali), Punjab, India - 160071

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