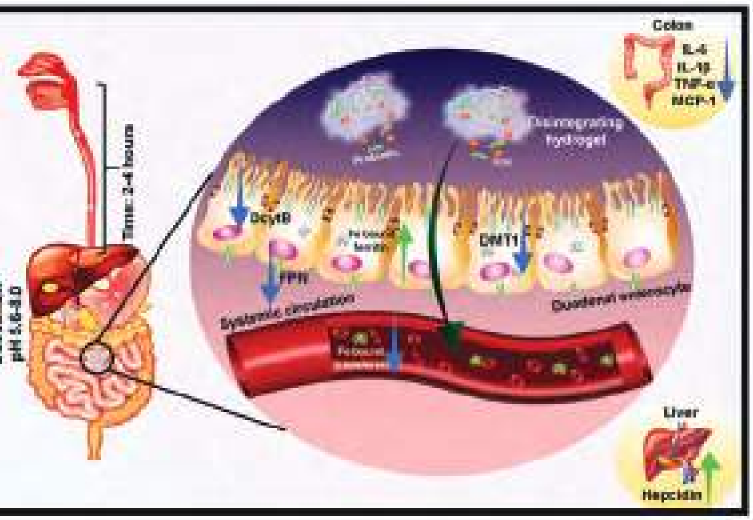
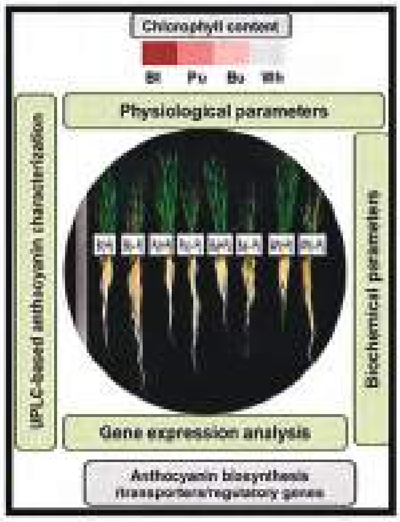
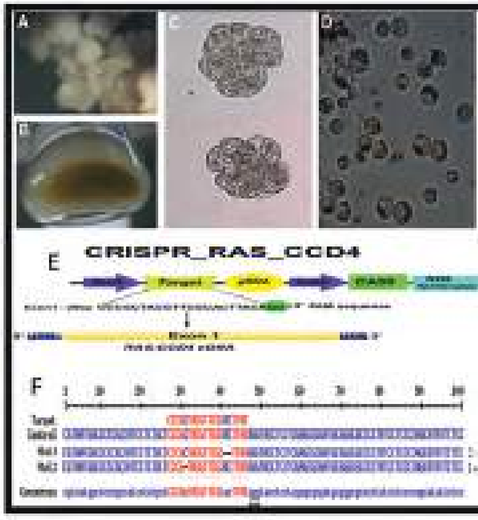
Department of Biotechnology
Government of India

ANNUAL REPORT 2021-22







NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE (NABI)

An Autonomous Institute of the Department of Biotechnology, Government of India

Mohali, Punjab 140306 INDIA

Published by
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Publication Committee
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Dr. Nitin Singhal
Dr. Koushik Mazumder
Sh. Arun Kumar

CONTENTS

From the Desk of the

EXECUTIVE DIRECTOR

I take immense pleasure in presenting the Annual Report of the National Agri-Food Biotechnology Institute (NABI) for the financial year 2021-22. NABI was established with the objectives to promote and coordinate research of high calibre in basic and translational aspects at the interface of Agriculture, Food and Nutrition. The institute has a clear vision to provide food and nutritional security for all through agri-food biotechnology research and innovation. The mission of the institute is to be a centre of excellence and provide leadership in agri-food biotechnology research. To achieve these vision and mission, NABI has designed seven core research areas that deal with the (i) Development of designer crops with high nutrition, increased shelf-life and processing quality; (ii) Genomics and computational biology for marker and gene discovery; (iii) Basic biology for crop improvement; (iv) Functional foods and nutraceuticals for better health; (v) Food and GM crops biosafety; (vi) Nutrigenomics for health and human welfare and (vii) Post-harvest biotechnology for value addition and increasing shelf life.

During the last financial year, several significant achievements have been made in the above-mentioned research areas despite the difficulties faced due to the second wave of Covid-19. Our scientists are actively engaged in applying molecular breeding and biotechnological tools for improving micronutrients, antioxidants, resistant starch, dietary fibers and reducing the anti-nutrient properties in the various identified crop plants.

An extensive collection of wheat germplasm is being maintained at NABI. The collection was multiplied at the research farm in the past Rabi season 2020-21. It comprises about 500 indigenous and exotic wheat genotypes including landraces; 1,200 ethyl methyl sulphonate (EMS)-treated M10 populations; ~250 aneuploid stocks; and several bi-parental segregating populations (F1s, F2s, F6s, F7s). The mutant lines showed variation in amylose content and resistant starch. High amylose mutant lines are being used for the introgression of amylose into high-yielding wheat varieties. Further, the study is also going on to understand the molecular and genetic basis of high amylose deposition in seeds. Biofortified colored wheat with high anthocyanin content has been developed by the breeding approach and would be exploited for different nutraceutical applications. Different food products prepared from colored wheat have shown better storage life, higher retention of antioxidant activity and lower amino acid losses than the traditional white wheat. Efforts are being made to increase consumer awareness about the new product. Institute is also working in the direction to understand the molecular basis of the transcriptional regulation pattern in pigmented (black, blue and purple) wheat. The study has been conducted to understand the physiological and molecular response of coloured wheat seedlings against phosphate deficiency. The study is in progress to decipher the role of molecular components involved in iron (Fe) uptake and mobilization in wheat grains. The research is directed to identify the genes encoding the transporter, transcription factors, and miRNAs and their validation are underway by using functional genomics tools.

The ambitious banana biofortification project has now been extended further with support from BIRAC for the multi-location event selection trials. The most promising banana (cultivar Grand Naine) events have shown near to 50-fold higher accumulation of pro-vitamin A in fruit-pulp of ratoon crops (2nd generation) and has been considered for the regulatory approvals for event selection trials. The expected outcome of the project is to identify the most promising genetic engineered banana line/s by testing them under various agroclimatic conditions for commercial release. For this purpose, the regulatory clearances from IBSC and RCGM have been obtained. The modern approach of genome editing has also been used to develop golden banana at NABI. For the first time, we have developed transgene-free genome-edited cultivar Rasthali lines by the editing of the carotenoid cleavage dioxygenase 4 (CCD4) gene. A non-transgenic approach to CCD4 editing showed that the β-carotene content was increased 1.4 to 2.7 times higher in the leaves and roots of two edited banana cv. Rasthali lines than in the control. Edited plants are presently growing in the net house for maturity and fruit analysis. This work was published in the prestigious Journal of Experimental Botany (Awasthi et al., 2022).

Lathyrus is an important legume crop but is not promoted widely for its cultivation owing to the presence of a neurotoxin L-oxalyl-2,3- diaminopropionic acid (β-ODAP). Mutation breeding and genome-editing approaches are being used to produce Lathyrus cultivars with low or no ODAP. Several putative low ODAP mutant lines have been screened by HPLC and some of these have been identified as the low ODAP mutants in the M3 seeds. Constructs for editing of three ODAP biosynthetic pathway genes have been developed and tested for editing successfully in hairy roots. These constructs are currently being used for whole plant stable transformation experiments. Rice Bran Oil (RBO) is abundantly rich in essential fatty acids, proteins, vitamins, minerals and antioxidants like oryzanol which makes it the healthiest edible oil. However, during milling, the triacyl-glycerol (TAG) stored as oil bodies (OBs) in the rice bran are degraded by the endogenous lipases resulting in the release of free fatty acids and glycerol which ultimately leads to the stale flavor and oxidative rancidity. In this direction, lipases were identified and also characterized for their role in lipid degradation. A CRISPR/cas9 mediated editing of rice-derived-lipase1 (Oslip 1) was performed in rice and mutant lines are being analysed for characteristic features. Various genomics, molecular breeding and computational biology tools are being utilized for the marker and gene discovery. Subsequently, the outcome of these analyses will be utilized for the improvement of the nutritional and processing quality traits of various crop plants.

Similarly, other significant achievements have been made in the research areas of the Food and Nutritional Biotechnology division. Research has been initiated on the use of plant-derived extracellular vesicles (P-EVs) as a therapeutic reagent in mitigating obesity. High-quality exosomes were isolated from ginger juice and their inhibitory effects were demonstrated on the differentiation of preadipocyte 3T3 cell line to oil-filled adipocytes. A subsequent study is planned to perform the transcriptomic and proteomic analysis of the cells treated with plant-derived vesicles to decipher how lipid accumulation can be alleviated through exosome treatment. Previously, the probiotic characterization of the anti-inflammatory potential of indigenous Bifidobacterium strains was demonstrated in the cell lines.

This study was extended further and evaluated the role of Finger millet polyphenols (FM-PRE) and Kodo millet polyphenols (KM-PRE) in preventing lipid accumulation and associated liver inflammation and oxidative stress using human liver cells (HepG2 cells). The study has demonstrated that the bioactive from Finger millet and Kodo millet could counteract obesity and hepatosteatosis development. Previously identified Transient Receptor Potential (TRP) channels were linked with dietary constituents like capsaicin, menthol and cinnamaldehyde and have shown a potential role to prevent weight gain and its related complications like type-2 diabetes and cardiovascular complications in mice model. The current study has demonstrated that the dietary capsaicin can produce an anti-obesity effects in mice. This study will be directed to develop a diet-based strategy for obesity and related complications.

Employing nanotechnology-based approaches, two types of bacterial-specific antibody conjugated gold nanoparticles were synthesized with simple steps for rapid visible detection of food-borne bacteria within 5 minutes. Colourimetric and different microscopic techniques showed specific binding of antibody conjugated gold nanoparticles on the bacterial cell surface. By graphene oxide coating, the sensitivity of the antibody conjugated gold nanoparticles has been increased by10 times. A handy nanodevice based on carbohydrate, antibody and aptamer-based nanoprobes with high sensitivity and selectivity for detection of food-borne bacteria has been developed. In the area of development of ultrasensitive, portable, and rapid biosensors for the detection of heavy metals, pesticides and food contaminants using functional hybrid nanostructure, our scientists have developed polyaniline and gold nanoparticles composites for biosensing metal ions and pesticides using a green chemical route. This spectroscopic and colour-based sensing probe can detect arsenic lower down to 0.002 and 0.004 ppb, respectively in water and food samples. Further, research work has shown that gut bacteria could provide beneficial modulation for mitigating metabolic disorders and have developed a novel anti-inflammatory symbiotic formulation which in pre-clinical studies is useful for preventing ulcerative colitis. In the area of development of novel cobiotic formulations for metabolic disorders, our scientists have shown that Allicin rich garlic extract modulates TRPA1 and rectifies gut hormone dysregulation under diet-induced obesity and associated comorbidities suggesting TRPA1 agonism may be exploited as a nutraceutical/functional food for the prevention of over-nutrition related complications. New research programs towards crop improvement for stress tolerace have also been initiated at the institute.

In the previous year, significant progress has been made in the development of new state-of-the-art research facilities and human resource generation. The establishment of an advanced Supercomputing Facility at NABI is one of the major steps towards R&D excellence. Hon'ble Minister of Science & Technology and Earth Sciences, Dr. Jitendra Singh inaugurated the advanced 650 teraflops supercomputing facility at NABI, Mohali on 2nd November 2021 through online mode. The facility has come up under the National Supercomputing Mission (NSM) in collaboration with C-DAC, Pune. This high-end facility will be a boon for the analysis of BIG DATA accruing from the large-scale genomics, functional genomics, structural genomics and population studies being carried out at various Institutes and universities of national and international repute. Further, NABI has also established Accredited Test Laboratory (ATL) for Tissue Culture Raised Plants. This project for setting up the National Certification System for Tissue Culture Raised Plants (NCS-TCP) at NABI has been sanctioned by DBT with five-year financial support and is being implemented through NIPGR, New Delhi as NCS-TCP management Cell of DBT from October 1, 2021. In our drive for a paper less governance, we have fully implemented the e-office at the Institute. We have also developed and implemented a unique web-based application "NABI-LABIFY" for the routine indenting and procurement of scientific supplies at the institute. Interestingly this application has been codeveloped by our own startup incubate – Samviti and NABI staff. NABI has also established a bio-incubator with the vision of catalysing Agri-Food Biotechnology Innovations and entrepreneurship for sustainable food and nutritional security. The institute currently has 142 students who are working in different capacities such as Ph.D. students, junior research fellows, senior research fellows, project assistants, MK Bhan fellows and post-doctorate research associates. During the year, NABI also trained 27 trainees from various institutes and universities. The selected students were exposed to different research tools to provide their expertise in the specialized niche areas of Agri-biotechnology and Food-Nutritional Biotechnology. In the last financial year, the institute obtained extramural research grants from different national and international funding agencies. On the scientific output, last year the institute published more than 100 high-impact research publications in various peer-reviewed journals. In this year, NABI has filed 03 patents and signed 04 MoUs. In future, the institute will focus more on product and process development in the defined areas of research on which considerable leads have already been achieved. Special efforts will be made to move in a translational mode for product development and the scientisits will be encouraged to file patents as acuring from their research.

I would like to place on record my sincere thanks to Dr. Jitendra Singh ji, Hon'ble Minister of State (Independent Charge) of the Ministry of Science & Technology and Earth Sciences and President of NABI Society for his keen interest, constant support and encouragement. The persistent and whole-hearted support by Dr Renu Swarup and Dr. Rajesh S Gokhale, Secretary, Department of Biotechnology (DBT) has been a critical factor in shaping up the institutional agenda. I profusely thank Dr Vamsi Krishna and Dr. Niloo Srivastava, Scientist - E, DBT for their untiring support and guidance. I am also thankful to Sh. Vishvajit Sahay, Financial Advisor DBT and Sh. Sunil Kumar, Joint Secretary DBT for their constant help in various financial and administrative issues of the institute. My sincere thanks are due to Prof. Ramesh Sonti, Chairman Scientific Advisory Committee (SAC) and all the members of SAC for the scientific advice, suggestions and guidance to improve the ongoing research programs at the Institute.

I acknowledge the unstinting support from the Scientists, Technical, Administrative and Supporting staff of NABI who have stood by me in various institute activities during this difficult pandemic time. Finally, I thank the Annual Report Publication Committee (Dr. Siddharth Tiwari, Dr. Koushik Mazumdar, Dr. Nitin Singhal and Sh. Arun Kumar) for compiling and shaping this document.

-s/d

Prof. Ashwani Pareek
Executive Director, NABI-Mohali

VISION

Food and nutritional security for all through agri-food biotechnology research and innovation.

MISSION

To be a centre of excellence and provide leadership in agri-food biotechnology research.

GOAL

Improving nutritional quality and availability of affordable agri-food and food products through innovations.

Human resource & Scientific Achievements

(From 2010 to 31st March 2022)

Ph.D Awarded: 35	Research Publications: 449
Patents Filed:10	Conference Attended/ Lectures Delivered: 288
Guest Lectures: 66	Conference & Workshop Organized: 10
Trainees: 382	Scientific & Administrative Staff: 42
Research Scholars: 125	MoU Signed: 64

MANAGEMENT OF NABI

MANAGEMENT OF THE INSTITUTE

A. Members of NABI Society

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Hon'ble Minister of State of the Ministry of Science & Technology and Earth Sciences (Independent Charge), New Delhi

Hon'ble Minister of Science, Technology and Environment (Govt. of Punjab),
Member (Ex-officio)
Punjab Civil Secretariat -1, Sector 1, Chandigarh

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Dr. Trilochan Mohapatra
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Indian Council of Agricultural Research (ICAR), Krishi Bhavan, New Delhi

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Mysuru

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Department of Gastroenterology & Human Nutrition,
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Vice Chairperson,
National Innovation Foundation,
Ahmedabad

Dr. Abhay Jere
Nominated Member
Chief Innovation Officer,
Ministry of Education (Govt of India),
New Delhi

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Nominated Member
Director,
Shree Baidyanath Ayurved Bhawan Pvt. Ltd., Kolkata

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Sector – 81 (Knowledge City)
Mohali – 140306

MANAGEMENT OF THE INSTITUTE

B. Governing Body

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Secretary
Department of Biotechnology
Ministry of Science & Technology
New Delhi – 110003
(Chairman – Governing Body)

Sh. Sunil Kumar
Joint Secretary (Administration)
Department of Biotechnology
Ministry of Science & Technology
New Delhi – 110003

Sh. Vishvajit Sahay
Financial Adviser
Department of Biotechnology
Ministry of Science & Technology
New Delhi – 110003

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National Agri-Food Biotechnology Institut
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Mohali – 140306

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Puducherry – 605014

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Shillong - 793022

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Mohali – 140306

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(Member Secretary)

MANAGEMENT OF THE INSTITUTE

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Financial Adviser
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Ministry of Science & Technology
New Delhi – 110003
(Chairman)

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Executive Director
National Agri-Food Biotechnology Institute
Sector – 81 (Knowledge City)
Mohali – 140306

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Government of India
New Delhi

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Small Industries Development Bank of India (SIDBI)
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Director
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Mohali – 140306

Sh. Suneet Verma
Manager Finance
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Mohali – 140306
(Member Secretary)

MANAGEMENT OF THE INSTITUTE

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Indian Institute of Science, Education and Research
Tirupati - 517507
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Dr. Kuldeep Singh
Head, Genebank - Consultant
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)
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CSIR-Institute of Himalayan Bioresource Technology
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Director, State Agricultural Biotechnology Centre
Director, Centre for Crop & Food Innovation
Food Futures Institute, Murdoch University Australia – WA6150

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Plant Physiologist
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Prof. Ashwani Pareek
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National Agri-Food Biotechnology Institute
Sector – 81 (Knowledge City)
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MANAGEMENT OF THE INSTITUTE

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Former Director, NIPGR
New Delhi - 110007
(Chairman)

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Executive Director
National Agri-Food Biotechnology Institute
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Sh. Dinesh Rangra
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National Institute of Fashion Technology
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Mohali – 140306

Assistant Engineer (Civil)
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Mohali – 140306
(Non -Member Secretary)

RESEARCH PROGRESS

PROGRAM - AB01

AGRICULTURE BIOTECHNOLOGY (AB)

DEVELOPMENT OF DESIGNER CROPS WITH HIGH NUTRITION, INCREASED SHELF LIFE AND PROCESSING QUALITY

AGRICULTURE BIOTECHNOLOGY

Ashwani Pareek, PhD
Plant Biology

RESEARCH ASSOCIATE
Jayram Bagri

AB 1.1: Generating high-depth genomics information for the Himalaya rice cultivars for improving nutritional quality and stress tolerance

Iron and zinc play an important role in human as well as plant metabolism. As per WHO estimates, approximately 25% of the world's population suffers from anemia (WHO, 2008), and Fe- deficiency anemia led to the loss of over 46,000 disability adjusted life years (DALYs) in 2010 alone (Murray and Lopez, 2013). Zn is an indispensable micronutrient component of human diet, deficiency of which leads to severe metabolic impairment. It is estimated that 17.3% of people worldwide are at the risk of inadequate Zn intake (Wessells and Brown, 2012). Various genomics resources for enhancing Fe/Zn levels in rice are available in the public domain (Garcia-Oliveira et al., 2009, Lalasa et al., 2012). These resources include the QTLs/ candidate gene markers. Through marker-assisted back crossing, they can be introgressed in the background of our interest. Further understanding abiotic stress tolerance mechanisms in plants is important for improvement of such traits. However, heterogeneity and missing linkage between available genomic data on Fe/ Zn and germplasm causes difficulty in the validation of marker-trait associations in any new rice germplasm set as we have in Himalaya rice germplasm. Therefore, it is important to have the genome re-sequencing of our Himalaya rice germplasm to characterize it and to identify novel QTLs, markers and genes for traits of interest. It would be further accelerated with the available 3K rice genome sequence data.

RESEARCH PROGRESS

We have analyzed 300 unique rice genotypes, which include 150 rice germplasm from Jammu and Kashmir (J&K) and 150 rice genotypes from the North East region (NER) for iron (Fe) and zinc (Zn) estimations at NABI, Mohali (Fig 1).

Estimation of Fe and Zn content in rice grain: The iron (Fe) and zinc (Zn) in these seeds have now been estimated using inductively coupled plasma mass spectrometry (ICP-MS) with three biological replicates as per described by Kumar et al., 2022 with some modifications. The measurement of grain Fe and Zn content

Figure 1. Picture depicts 300 rice genotypes (J&K and NER region) growing in the field at National Agri-Food Biotechnology Institute (NABI), Mohali, Punjab, India.

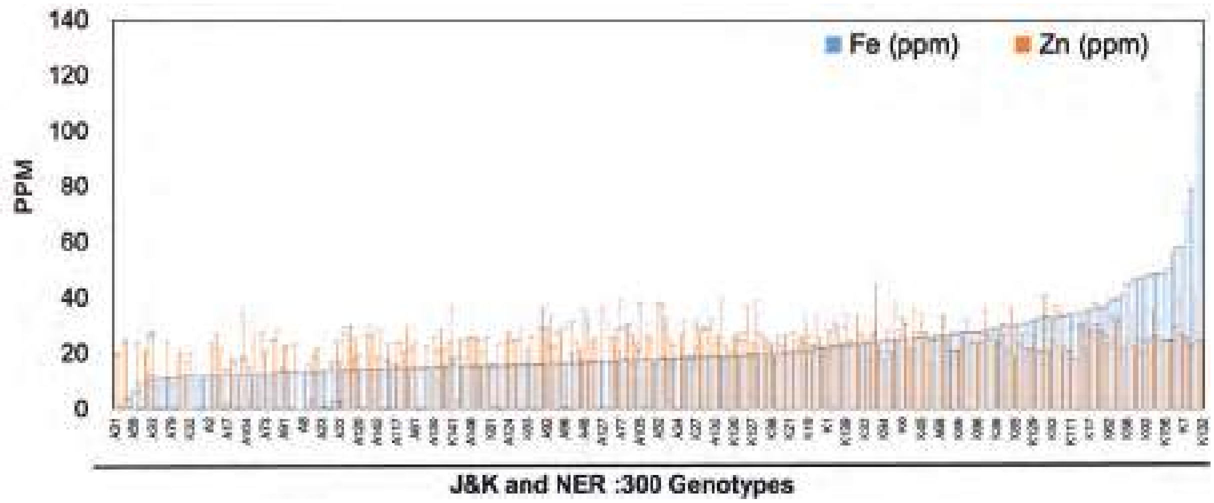


Figure 2. Content of iron (Fe in ppm) and zinc (Zn) in the 300 rice genotypes (J&K and NER region).

in the 300 rice genotypes used in the present study suggested significant variations between the genotypes. The highest mean (130.9±0.49 in ppm) grain Fe content was found in 'K132' to least (0.035±.0042 in ppm) in 'A31', with an average 22.24 ppm. Similarly, the highest mean (40.81±0.51 in ppm) Zn content in grain was quantified in 'K122' as lowest (0.0419±0.011 ppm) in 'A12', with an average 21.72 ppm. Figure 2 depicts a content of iron (Fe in ppm) and zinc (Zn) in the 300 rice genotypes from Jammu and Kashmir (J&K) and north east region (NER) region.

SALIENT ACHIEVEMENTS

- We have successfully analyzed 300 unique rice genotypes, which include 150 rice germplasm from Jammu and Kashmir (J&K) and 150 rice genotypes from the North East region (NER) for iron (Fe) and zinc (Zn) estimations.

FUTURE PERSPECTIVES

- Generation of high quality and high depth whole genome sequences and databases.
- Generation of tissue-specific and development stages specific transcriptome data.

AGRICULTURE BIOTECHNOLOGY

Ashwani Pareek, PhD
Plant Biology

RESEARCH ASSOCIATE
Saurabh Prakash Pandey

AB 1.2: Effect of Brassinosteroids (BRs) on amelioration of salinity stress in the two contrasting rice genotypes

Rice grain is a good source of calories and one of the major staple food crops for half of the world's population, including India. Increasing land salinization has progressive and often profound effects on plant growth and development. Salinity stress (S) hinders growth by disrupting many physio-biochemical and molecular processes at the most critical phase of a plant's life, i.e., the seedling stage. Survival of the seedlings not only exerts an important influence on the size, persistence, and genetic variability of plant populations, but it also ultimately affects the net crop yield. The current study focuses on the potential role of exogenously applied 24-epibrassinolide (BL) for ameliorating the adverse consequences of salt in two contrasting rice genotypes, IR64 (high yielding and stress-sensitive) and Pokkali (low yielding and stress-tolerant). To study the effect of BL on ameliorating salt stress tolerance, hormone concentration was optimized for seedlings of both rice genotypes.

RESEARCH PROGRESS

Effect of exogenously applied 24-epibrassinolide (BL) on the phenotypes of two contrasting rice genotypes (IR64 and Pokkali): In this study, 7-day old rice seedlings of two contrasting rice genotypes, IR64 and Pokkali, were used. Seedlings were treated with BL for hormonal treatment and salt stress of 200 mM NaCl was given for different time durations, i.e., 24 h, 48 h, and 72 h (Fig 3). Salinity-stressed rice seedlings without BL treatment (S) showed severe wilting and yellowish foliage mostly throughout the stem. On contrary, salt-stressed plants treated with BL (BL + S) showed comparatively better growth (healthier green foliage with less wilted leaves), as revealed by phenotypic observations (Fig. 1). Salt stress also induced several detrimental effects on the growth of rice plants; for instance, the shoot and root lengths were reduced when compared to non-stressed control (C) seedlings. The treatment of BL showed larger shoot and root

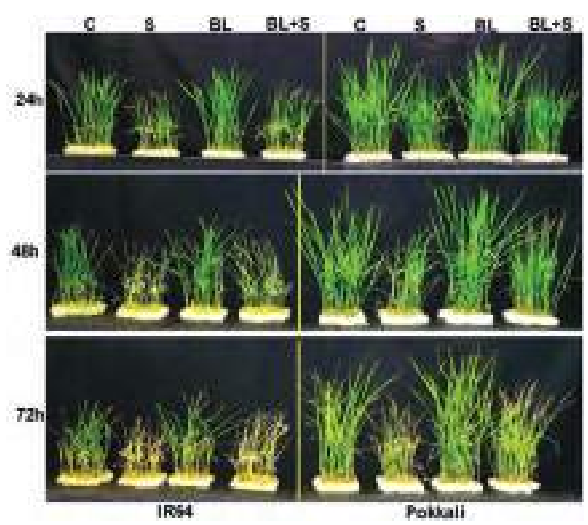


Figure 1. The effect of exogenously applied 24-epibrassinolide (BL) on the phenotypes of two contrasting rice genotypes (IR64 and Pokkali) as affected by progressive salinity stress.

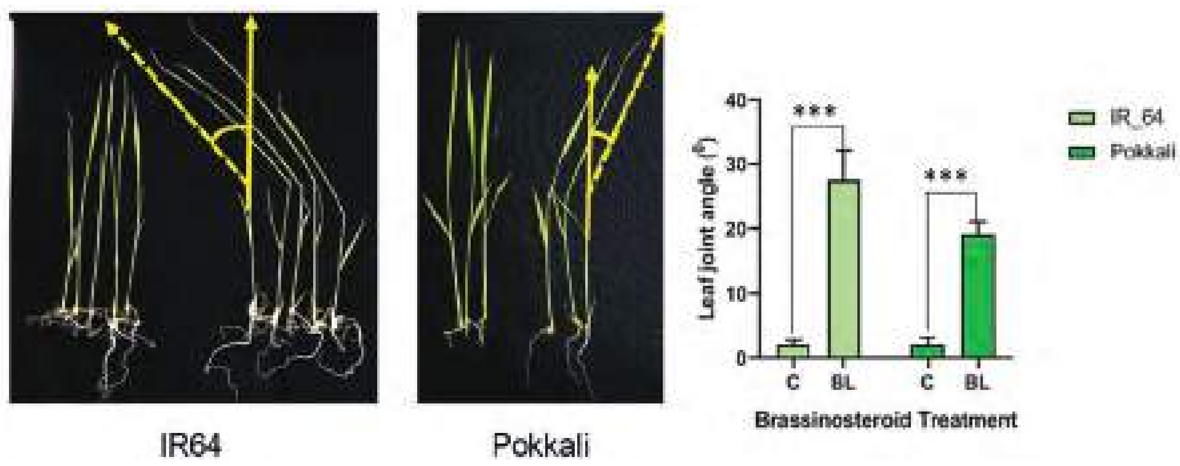


Figure 2. Leaf joint angle bending of rice seedlings of contrasting genotypes (IR64 and Pokkali).

lengths in both IR64 and Pokkali. Additionally, leaf bending was observed highly in IR64 compared to Pokkali under BL treatment (Fig. 2).

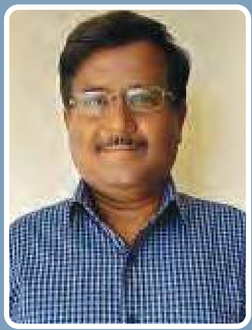
SALIENT ACHIEVEMENTS

- Our study demonstrated the importance of brassinosteroid in ameliorating salt stress effect in two contrasting rice plants. However, physiological parameters (increased shoot, root length, increased chlorophyll content etc.) suggests brassinosteroid hormone have partial effect in mitigating the adverse effect of salt stress.
- Moreover, BL induced amelioration effect has been observed with greater effect in Pokkali compared to IR64.

FUTURE PERSPECTIVES

- Detailed investigation of the role of brassinosteroid in ameliorating salt stress effects in two contrasting rice plants through omics approaches.

AGRICULTURE BIOTECHNOLOGY



Joy K Roy, PhD
Genetics & Genomics

RESEARCH FELLOWS
Ankita Mishra
Saba Rahim
Afsana Parveen
Vinita Sharma
Prashant Kumar
Akansha Madhawan
Vikas Fandade

AB 1.3: Gene discovery for improvement of processing and nutrition quality in wheat

Starch affects processing, cooking and organoleptic qualities as well as nutritional value of food products. The availability of variation in starch quality in wheat germplasm and knowledge of genome-wide distribution of genes/chromosome regions controlling processing and nutritional quality are pre-requisite for starch quality improvement. In this project, variation in amylose content is induced via non-transgenic approach, through chemical treatment, ethyl methane sulphonate (EMS). The mutant lines showing variation in amylose content and resistant starch are identified in the EMS-treated lines. Some of high amylose mutants are being used for introgression of high amylose into high yielding varieties as well as for molecular and genetic basis of high amylose. The progress of the following research activities is given below..

RESEARCH PROGRESS

Wheat germplasm: The wheat germplasm collection was multiplied at NABI's research farm in the last Rabi season 2021-22. It comprises about 500 indigenous and exotic wheat genotypes including landraces, 1200 EMS treated M11 population, and several biparental segregating populations (F1s, F2s, F7s). It also includes a set of 101 mutant lines (M11) showing variation in amylose content.

Low glycemic wheat lines

Two low glycemic wheat lines were multiplied at large scale in the village, Raipur Khurd, Mohali in the last Rabi season, 2021-22.

Identification of quantitative trait locus gene (QTG) for high amylose in RIL population

Development of a recombinant inbred line (RIL) population: In this study, a recombinant inbred line (RIL) mapping population (named NABI-HAS-RIL1) was developed by crossing a high amylose mutant line ('TAC 75') with ~66% amylose with a recipient high yield wheat variety 'WH 1105' with ~22% amylose. The 350 F6 progenies was advanced to F7 generation using spike to row sowing method in the last Rabi season (2021-22) at three different locations (i) NABI, Mohali, (ii) CCSU, Meerut, and (iii) Agarkar Institute, Pune.

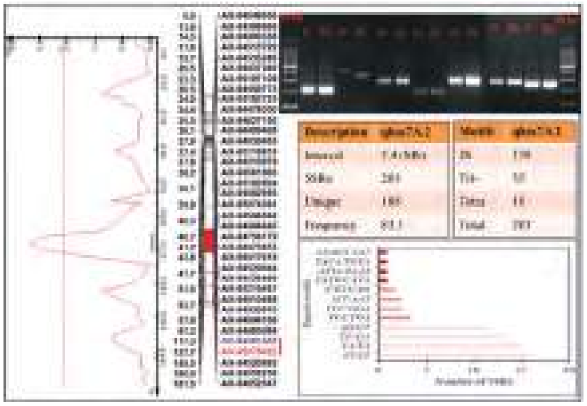


Figure 1. Development of SSR markers within the high amylose QTL region, qhas7A.2.

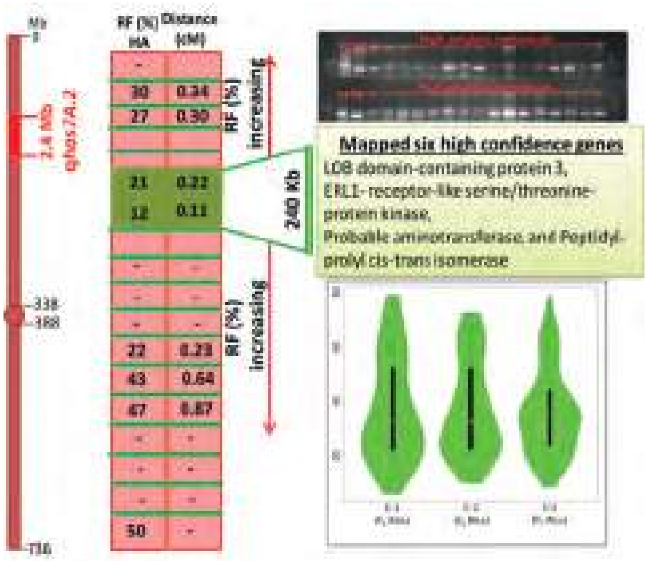


Figure 2. Flow diagram representation of the process for QTL saturation and QTG identification.

QTL mapping of amylose: Genome wide QTL mapping identified a total of 124 QTLs for high amylose starch detected in E-1 (37), E-2 (46), and E-3 (41) environments using composite interval mapping. We identified four major QTLs qhas5B.2, qhas7A.2, qhas7B.1, and qhas7D.2 represented in either of the two environments except for QTL qhas7A.2 which showed its consistent occurrence in all the three environments and multiple generations (F2, F3, and F6). The phenotypic variance explained by qhas7A.2 ranged from 10 to 78%. Therefore, from the above findings we identified qhas7A.2 as the major QTL for high resolution mapping and identification of QTG.

QTG within the major qhas7A.2 QTL region: The major QTL qhas7A.2 spanned a map length of 2.4 Mb on 7A chromosome with low SNP density. We designed 188 genomic SSR markers from the 3.4 Mb genomic scaffold including QTL region, and used to screen polymorphism content between the two parents. Out of the 188 SSR markers 22 (11%) SSRs showed polymorphic between parents (Figure 1).

High resolution mapping of high amylose starch: For high resolution mapping of high amylose starch in NABI-HAS-RIL1 population, the bulk extreme (58 high amylose RILs and two parents) and recessive class approach was used to calculate the recombination frequencies (c) for the polymorphic SSR markers. We identified two markers 'qhas7A-33' and 'qhas7A-178' with a recombination fraction of 0.12 (qhas7A-32) to 0.20 (qhas7A-178) respectively. The genetic distance (Kosambi, cM) for the above mentioned markers in the qhas7A.2 region was 0.11 (qhas7A-33) and 0.22 (qha7A-178) cM. Using this approach, the QTL region was narrowed down to 240 Kb interval on the physical map (Figure 2).

QTL gene identification: The physical position of the SSR markers qhas7A-178 and qhas7A-33 were used to extract the genomic sequences flanking the region. A total of six high confidence genes (LOB domain-containing protein 3, ERL1- receptor-like serine/threonine-protein kinase, Probable aminotransferase, and Peptidyl-prolyl cis-trans isomerase) were identified between the marker interval. Further the genomic sequences will be used for functional analysis and validation of the genes for high amylose biosynthesis.

Other activites involving high amylose lines:

» Protein Targeting to Starch 1, a functional protein of amylose biosynthesis in wheat was functionally validated using in vitro experiments.
» The long non-coding RNA regulating amylose-lipid complexity was characterized in bread wheat (Triticum aestivum L.).

SALIENT ACHIEVEMENTS

- A RIL population in wheat is advanced to F7 comprising of ~350 progenies.
- Candidate QTGs for high amylose biosynthesis were identified using saturation mapping.
- Two low glycemic wheat lines were evaluated in a large field. The expression of interest was floated for commercialization. Two companies have shown interest to buy the technology.

FUTURE PERSPECTIVES

- Registration of two ('TAC 28' and 'TAC 35') high amylose (low glycemic) wheat lines under PPV & FRA, New Delhi.
- Commercialization of high amylose wheat ('TAC 28' and 'TAC 35') through the expression of interest.
- QTL-gene isolation and characterization for high amylose starch.
- Genetic dissection of starch-protein interactions using RIL population.

AGRICULTURE BIOTECHNOLOGY

Monika Garg, PhD
Wheat processing & Nutritional quality

RESEARCH FELLOWS
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AB 1.4: Improvement of nutritional and processing quality in wheat

Plant phytochemicals such as anthocyanins can act as antioxidants and show anti-inflammatory, anti-cancer, anti-aging activities and prevent cardiovascular diseases and type-2 diabetes. In the present proposal, we aim to develop colored wheat lines with high anthocyanin content that could be exploited for nutraceutical applications. It has advantage over anthocyanin rich fruits and vegetables, as later has very short shelf life and cannot be stored for long. Wheat is major farmer crop, with all required machinery in place. Colored wheat can be used as novel ingredient resource for the development of value added products and functional foods. The project revolves around generation of high yielding, localy adapted colored wheat commercial lines with breeding technologies, chemical characterization of different anthocyanins, preclinical and clinical studies to enhance outreach and commercial abilities, development of value added and functional food products for better human health, generation of public awareness about the benefits, large scale multiplication with the involvment and additional income generation of farmers and technology transfer to different milling and baking industries.

RESEARCH PROGRESS

Development and characterization of colored wheat lines

Improvement of colored wheat lines and commercialization: In order to increase the yield and rust resistace of colored wheat lines several new crosses were made, screened and selected. Industrial linkages are being developed for its commercialization and outreach. Efforts are being made to increase consmer awareness about the new product.

Understanding the transcriptional regulation pattern in pigmented (black, blue and purple) wheat: The present study investigated the regulation of colored development in wheat. For studying the pigmentation pattern in wheat seeds, developing color wheat seeds in 28DAA (Day after Anthesis) or Zadok scale of 85 (soft dough) were collected at 30°C day/17°C to 34°C day/18°C (day/night) temperature from NABI fields and subjected for RNA seq analysis. Black wheat had 1092 differentially expressed genes (DEGs), followed by blue (741) and purple (220) (Fig. 2A). We suspected black wheat exhibited blue and purple transcriptional signatures. This theory was erroneous when 464 DEGs were shared by black and blue, 29 by purple and black, and 26 by purple and blue (Fig. 2A). 51 transcripts were common

Figure 1. Seeds of differet colored wheat lines.

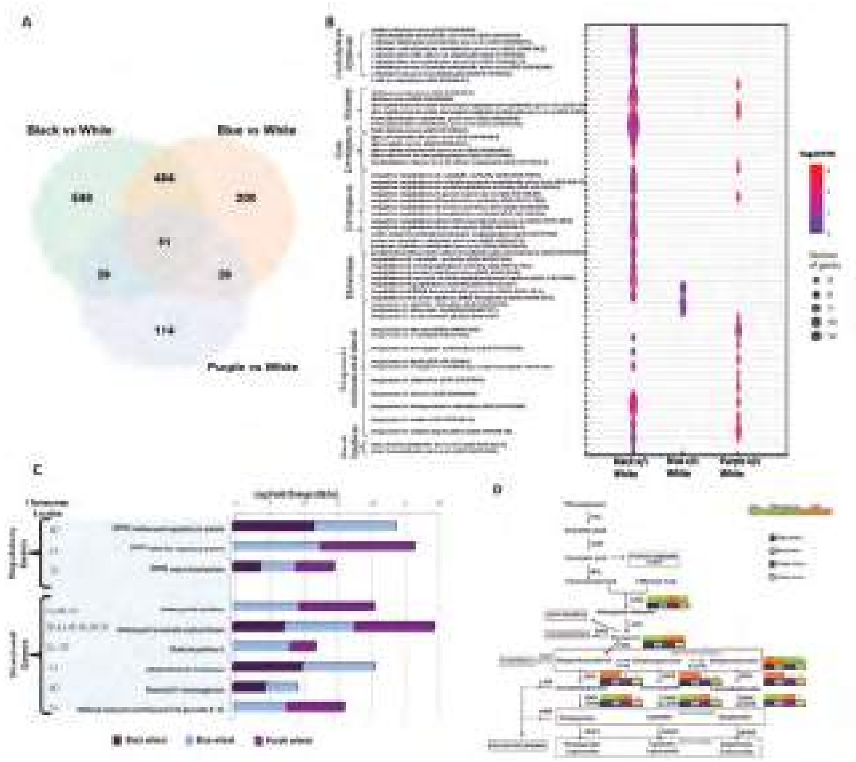

Figure 2. Transcriptional Signatures of three colored wheat seeds. (A) Venn Diagram showing the number of common and unique DEGs among three colored wheat samples compared to white wheat. Black wheat showed the highest number of DEGs and shared more DEGs with blue wheat in comparison to purple wheat. (B) Biological processes in GO enrichment analysis of DEGs. Only significantly enriched values with corrected $P < 0.05$ were included. The color and size of each point represented the -log10 (FDR) values and enrichment scores. A higher -log10 (FDR) value and enrichment score showed a greater enrichment. (C) Highly expressed genes of Anthocyanin regulatory and structural genes, where DEGs with > 3.5 log2 Fold value represented along with their chromosomal position. (D) TPM values of anthocyanin structural genes in phenylpropanoid pathway among three colored wheat lines and white wheat represented by a colored bar, where high TPM values were shown in red color and low TPM in green color.

to all three wheat colors. Black, blue, and purple wheat had 548, 200, and 114 distinct transcripts. The distinctive expression pattern may be related to morphological, physiological, and rheological grain modifications other than pigment synthesis. To determine DEGs' biological and functional importance, Gene Ontology (GO) terms were enriched (Fig. 2B). Black wheat represented more biological processes than purple and blue. Enrichment of biological activities, including metabolic processes and their control, carbohydrate metabolism, germ cell growth, and maturity processes, indicated that the seeds were near to maturity, and the endosperm was enriched in carbohydrates. Several biological processes associated with an environmental stimulation were evident in black and purple wheat, with purple showing a higher false detection rate than black. In contrast, processes relevant to starch synthesis were enriched only in black wheat.

Expression of genes involved in anthocyanin biosynthesis and regulation Regulatory

Regulatory Genes: Our research indicated that three transcription factors (TFs) were activated in colored wheat samples. Two detected TFs belonged to the MYC family and one to the MYB family. The first MYC gene was on chromosome 4D and was significantly expressed in black, blue, but not purple wheat (TraesCS4D02G224600, 4D-MYC; anthocyanin regulatory R-S protein-like) (Aegilops tauschii subsp. strangulata) (Fig. 2C). The second MYC, on chromosome 2A, was strongly expressed in blue and purple wheat (TraesCS2A02G409400, 2A-MYC; anthocyanin regulatory R-S protein-like isoform X1) (Triticum dicoccoides) (Figure 1C). The third MYB gene, on chromosome 7D, was highly expressed in black, blue, and purple wheat (TraesCS7D02G166500, 7D-MYB; anthocyanin regulatory C1 protein) (Aegilops tauschii subsp. strangulata) (Fig. 2C). All colored wheat types expressed both MYC & MYB transcription factors or at least one of the two differentially expressed MYCs. Our findings showed that Purple pericarp is regulated by 2A-MYC and 7D-MYB, and blue by 4D-MYC, 2A-MYC, and 7D-MYB.

Structural Genes: Exploration of structural genes indicated that the TPM values of all of them were higher in colored wheat than in white. High-fold differentially expressed genes were investigated. F3'H, DFR, and ANS were substantially expressed in black and blue wheat. ANS isoforms were also identified. Two isoforms were seen in blue and 3 in purple. Blue and purple wheat express CHS significantly (Fig. 2C & D). Genes involved in anthocyanin acylation were highly expressed (Fig. 2C). 5-aromatic acyltransferase, which transfers hydroxycinnamic moieties to anthocyanin glucosyl groups, was up-regulated in purple, blue, and black wheat (Fig. 2C). All highly expressed structural genes and isoforms are on 2A, 2D, 4A, 4B, 4D, 5A, 5B, 5D, 6A, 6B, 6D. It showed that structural genes were spread throughout chromosomes (Fig. 2C). Based on transcriptional expression differences, black wheat is more like blue than purple. In black and blue wheat, carbohydrate metabolism and core metabolic processes are less expressed than in purple and white wheat, which may contribute to the oblong seed shape in black and blue and plump in purple and white. Patterns common in black and purple wheat represented environment-related responses, including biotic and abiotic stresses indicating their role in plant stress adaptation.

Understanding physiological and molecular response of colored wheat seedlings against phosphate deficiency

The idea was to explore how colored wheat could perform under different nutrient conditions for the maintenance of growth and development. The present study investigated the differential response of phosphorous (Pi) deficiency at the seedling stage using hydroponics. Our results showed that Pi-deficiency triggered typical response in the wheat along with the changes in the plant root morphology, total biomass, micronutrient concentration and distinct anthocyanin accumulation. Our physiological and biochemical data revealed that these parameters were positively altered under stress in the colored wheat and the adaptation followed the trend of white < blue <purple < black (Fig. 3). Our results also confirmed that stress induced accumulation of distinct anthocyanins including derivatives of cyanidin,

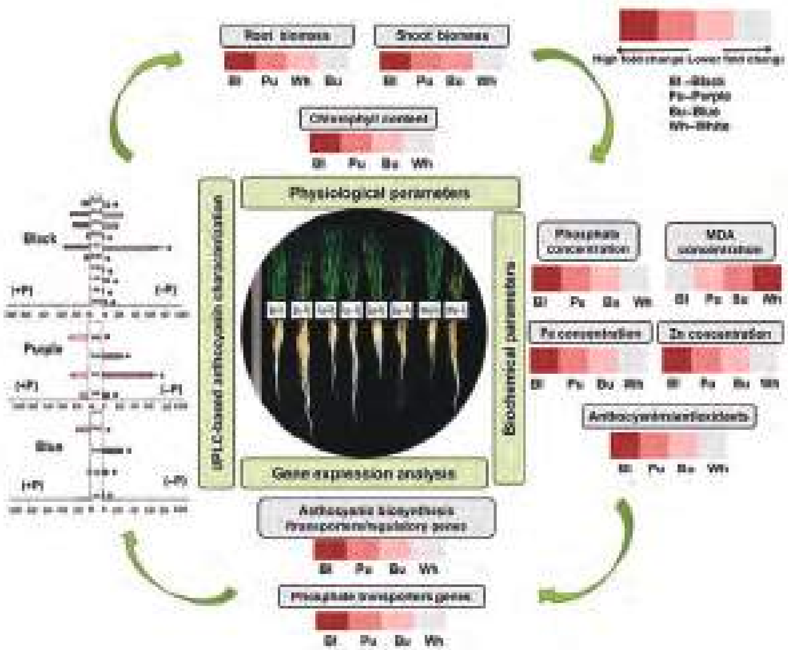

Figure 3. Physiological and biochemical analysis during Pi-deficiency revealed that these parameters were positively altered under stress in the colored wheat and the adaptation followed the trend of white<blue<purple<black. The stress induced differential expression of transcripts encoding phosphate transporters, anthocyanin biosynthesis, putative transporters, regulators and accumulation of distinct anthocyanins. Bl = Black wheat, P = Purple wheat, B = Blue wheat and W =White wheat, (+P) = phosphate sufficient condition, (-P) = phosphate deficient condition.

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delphinidin and peonidin in a genotype dependent manner. Differential expression pattern visualized for the transcripts encoding phosphate transporters, anthocyanin biosynthesis, putative transporters and regulators may be one of the underlying factors. Altogether, our data showed that the black wheat genotype with highest anthocyanin content could be able to adapt better with the P stress. This study will help in identifying suitable colored wheat genotype adapting the stress condition and have potential for influence on the future agricultural cultivation practices.

Elucidating the nutritional profile of colored wheatgrass juice

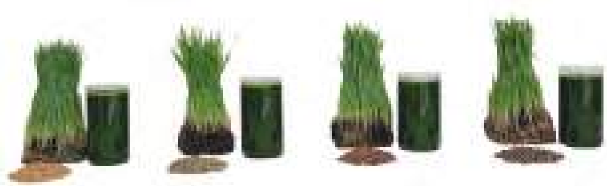


Figure 4. The wheat grains, corresponding wheatgrass and wheatgrass juice (ascending order of color variation was white<blue<purple<black based on anthocyanin and chlorophyll content).

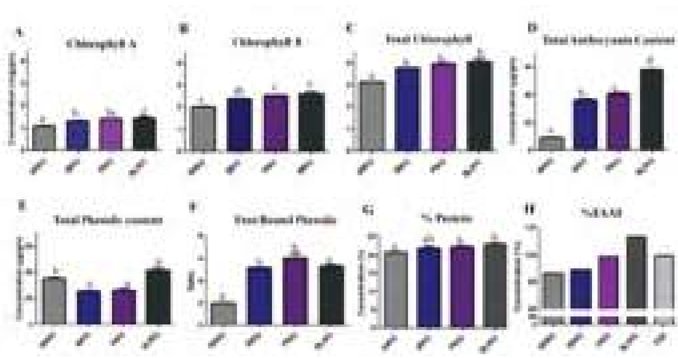


Figure 5. Comparison of phytochemicals from different colored wheat grass lyophilized powders (WJLP). The BLWG exhibited the highest value of chlorophyll A, B, total chlorophyll and total anthocyanin, protein and EAAI followed by PWG, BWG and WWG. The total phenolic content was highest in BLWG and lowest in BWG and PWG. WWG- white wheat grass; BWG- blue wheat grass; PWG- purple wheat grass; BLWG- black wheat grass.

Figure 6. The vermicelli prepared from white, purple and black wheat grains (ascending order of color variation was white<purple<black based on anthocyanin content).

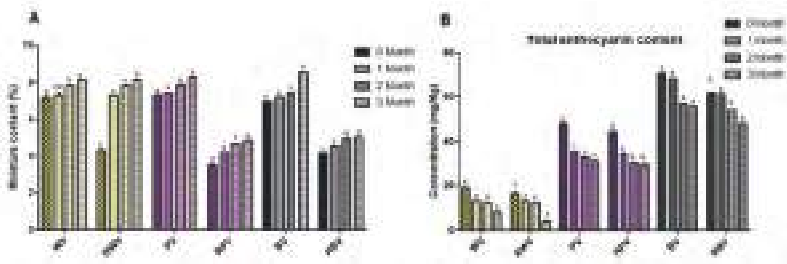


Figure 7. Understading effect of storage on the different colored vermicelli A) Moisture content; B) total anthocyanin content of different color vermicelli's. WV-white wheat vermicelli, RWV-roasted white wheat vermicelli, PV-purple wheat vermicelli, RPV-roasted purple wheat vermicelli, BV-black wheat vermicelli and RBV-roasted black wheat vermicelli.

Wheatgrass juice is a low-acid functional drink extracted from germinated white wheat. It is cherished by consumers due to its potent health benefits. The present study was conducted to develop wheatgrass juice from colored wheat varieties rich in phytochemicals to meet consumers' increasing demand for a novel functional drink (Fig. 4). The conventional wheat-grass juice was found to contain significant chlorophyll content (0.3%), soluble phenolic content (0.6%), protein content (20%), moderate essential amino acid index (EAAI; 86%), high K (RDA; 11.6%) and antioxidant activity. Whereas, black wheatgrass juice had 564.2% higher anthocyanins, 30.5% higher chlorophyll, 87.5% higher soluble phenolic content, 10.2% higher proteins, 31.5% higher EAAI, and 112.5% higher antioxidant activity compared to white wheatgrass juice (Fig. 5). Overall, the biochemical parameters of wheatgrass juice were increased in the order of white<blue<purple<black. The findings of the present study will be beneficial for food industries to launch novel functional drinks.

Food product development of colored wheat and understanding effect of storage on product quality

This study aimed to develop vermicelli from black and purple colored wheat and compared it with non-colored white wheat (Fig. 6). Vermicelli developed from different colored wheat and the effect of processing on the quality characteristics such as cooking quality, shelf life, sensory parameters, macronutrients, anthocyanins, phenolics, flavonoids and antioxidant activity was evaluated in roasted and unroasted forms (Fig. 7). The order of relative content of protein, anthocyanins, phenolics and flavonoids in both the forms was black>purple>white. Higher flour solubility and higher cooking losses in the soup gave black wheat a better texture and taste. Extrusion and roasting reduced anthocyanins, flavonoids, and soluble phenolics but enhanced insoluble phenolics and antioxidant activity. Three-month storage did not affect its quality attributes. Major anthocyanin in black wheat flour and its products was delphinidin-3-o-rutinoside. Several new anthocyanins, including coumaric acid derivatives were identified through MS/MS analysis. Anthocyanin stability followed the trend of peonidin-3-o-glucoside>cyanidin-3-o-glucoside>malvidin-3-o-glucoside>peonidin-3-rutinoside>delphinidin-3-o-galactoside>cyanidin-3-o-rutinoside. Thus, black wheat vermicelli with good shelf life was found to be best in terms of antioxidant activity, protein content, taste, and sensory attributes.

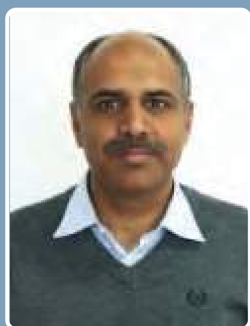
SALIENT ACHIEVEMENTS

- At least one isoform of MYC and MYB transcription factors is sufficient to regulate the anthocyanin synthesis in pericarp or aleurone layers of colored wheat seed.
- Anthocyanins present in the anthocyanin biofortified wheat might be a potential phytochemical that could help it in better adaptation against phosphate deficiency.
- Black wheatgrass juice is better alternative to white wheatgrass juice.
- Nutritional profile and sensory score of black wheat vermicelli was better than white.

FUTURE PERSPECTIVES

- Development of improved colored wheat lines with disease resistance and specific processing quality
- Characterisation of colored wheat for nutrients and anti-nutrients
- Evaluation of colored wheat seed/grass for the reduction/treatment of diet induced iron deficiency anemia, anti-diabetic and anti-cancerous potential
- Clinical trials of colored wheat.

AGRICULTURE BIOTECHNOLOGY



Ajay K Pandey, PhD
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AB 1.5: Functional genomics strategies for improving micronutrient transport and its bioavailability in wheat

Iron (Fe) is one of the critical micronutrients involved in many primary metabolism reactions like photosynthesis, respiration, nitrogen metabolism, chlorophyll biosynthesis and many catabolic and anabolic reactions. Higher plants have a considerable difference in their Fe uptake mechanism to cope with Fe unavailability in the geosphere. In general, plants modulate their Fe responsive genes according to their surrounding environmental changes. Studying the early molecular changes in response to the deficiency responses allowed us to understand and pinpoint the events that help the plants cope with the nutrient limiting condition. Although genes involved in sensing the intracellular Fe are now investigated, work is still required for their response during the changing regimes of Fe. Hexaploid wheat (Triticum aestivum L.) is the third most cultivated crop and staple food for the major part of the world. However, Fe deficiency is becoming one of the most widespread soil constraints affecting wheat productivity, especially with calcareous soils or high pH. Previously, Fe deficiency wheat genes were reported by our group (Kaur et al., 2019; JExB). Though these studies identified similar elements of responsive genes mainly involved in the strategy-II mode of Fe uptake, these remained broadly conserved. Yet, the early molecular events leading to the early adaption of the Fe deficiency was unaddressed. In the present work, our goal was to decipher the early response to the Fe deficiency and resupply for a better understanding of the dynamic expression responses in the wheat roots. To attain this, we performed RNAseq analysis to identify the signaling cascades at the early phase of Fe deficiency. Two-time points, 4-(early) and 8- days (symptomatic) post-Fe deficiency was chosen at the onset of the chlorosis and post-recovery from the Fe stress.

RESEARCH PROGRESS

Identifying the molecular components involved in micronutrient uptake and mobilization in wheat

Morphological and physiological features at an early phase of Fe deficiency:- To examine the early signaling response changes, roots of wheat seedlings were subjected to 4- and 8d of Fe-deficiency (2 μM). After the respective deficiency periods, roots were then exposed to surplus Fe (80 μM) for additional two days.
No significant changes in the chlorosis were observed among the control (+Fe) and the 4d of Fe deficient (–Fe) wheat seedlings. Whereas, the onset of the chlorotic response due to Fe deficiency was observed at 8d compared to its control (Figure 1A and B). Typically, a significant reduction in SPAD values was observed at the 8d post –Fe (Figure 1C). At both, the time points, a significant decrease in the root length was consistently observed in –Fe condition compared to controls (Figure 1 D). Reduction in root size was in accordance with the previously observed phenotype (Kaur et al., 2019).

» Overall, we also observed the impact on the fresh weight of the roots at both the time points (Figure 1E). Although no effect on the fresh biomass of shoots was observed at 4 d, but significant reduction was observed at 8d of Fe deficiency (Figure 1C). Overall, our data indicate that Fe deficiency could impact the phenotype of wheat seedlings at the early phase.
» Genome-wide transcriptional response reveals distinct cluster: We performed a comparative analysis of RNAseq data to explore the distinct response reflected by each of the time points. PCA analysis suggests clustering of the biological replicate with each other.
» Our analysis indicated that 2282 and 1110 genes were significantly up- and down-regulated ($p>0.05$; LFC>1.5) at 4 d of deficiency. Whereas, at the 8 d of Fe-deficiency, 3085 and 2459 genes were significantly up- and down-regu-

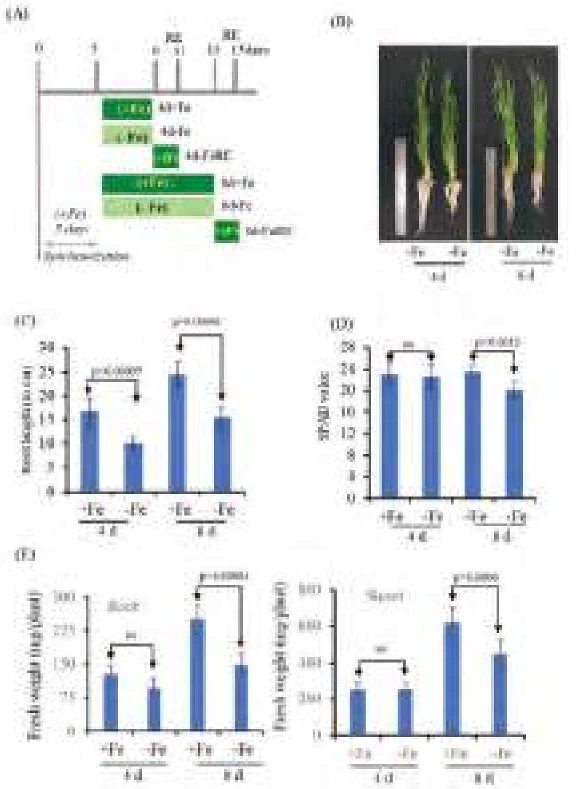

Figure 1. Phenotypic effect of short-term Fe deficiency in hydroponic growth conditions. (A) Five-day-old wheat seedlings grown with sufficient Fe (80 μM, labelled +Fe) were subjected to Fe deficiency (2 μM Fe, labelled -Fe) for 4 d and 8 d. Control seedlings were grown for the same length of time under Fe sufficiency. For later RNAseq analysis, seedlings grown -Fe were transferred to Fe-sufficient medium and grown for another 2 days (re-supply, labelled RE). (B) Phenotypic changes in the wheat seedlings under Fe-deficiency condition. (C) Root length (D) Chlorophyll content by SPAD analysis). (E) Fresh weight of roots and shoots (mg/plant). Values at the indicated days of treatment are given as averages ± SD (n = 12).

lated. This suggests an increasing number of DEGs with the time points. In total, 2006 (59.29%) DEGs at 4 days were commonly regulated between these time points, with 150 genes showing opposite expression patterns across the time-points.

» Multiple membrane transporters such as TaYSL19, nitrate transporters, sugar transporters, ABC transporter and a H+-transporting ATPase were also represented in this analysis. The transcriptomic rearrangement was apparent when these seedlings were subjected to a resupply of Fe for an additional two days. Upon analysis with the respective resupply time points, a total of 1269 (37.41%) and 3840 (69.27%) common DEGs were noted post 4- and 8-days Fe deficiency (Figure 3B and C). These suggest that few genes were commonly regulated to maintain the Fe homeostasis in the roots.

» Fe status affects the genome induction bias in hexaploid wheat roots: Subsequently, we also analysed the relative expression bias within triads that suggested a similar pattern to the induction bias at 4 d of Fe deficiency, with an increase in balanced expression upon Fe resupply.

» Across the conditions, B homoeologs were predominantly suppressed while D and A homoeologs show higher dominance. We also checked if the response to Fe deficiency is limited to certain regions of the genome, and therefore distribution of the DEGs on the hexaploid wheat genome was analysed. Our analysis indicates a higher contribution from chromosomes 2, 3, 5 and 7 while upon resupply, comparatively higher contribution was observed from chromosome 2.

» We further went on to perform the comparative induction bias of TFs (1761 triads) and transporters (94d triads) those falling in the triads. Our analysis suggests that the percentage bias was significantly similar for the TFs. This biasness was not consistent for the category of genes encoding for transporters. We observed an enhanced biasness of the triads with the time period of Fe deficiency stress suggesting the compounding temporal effect of the stress on the genome genome-specific expression. This suggests that differential bias expression of the transporters is an important feature to adapt deficiency tolerance in hexaploid wheat.

» Multiple genes show Fe status dependent expression responses that suggest the strong correlation for their demand-based participation in the nutrient mobilization (Figure 2). In addition, multiple YSL genes were identified those which are showing Fe dependent induction and were reverted to the basal level/downregulated during the Fe resupply condition. The YSL showing such Fe status dependent expression response are been characterized further.

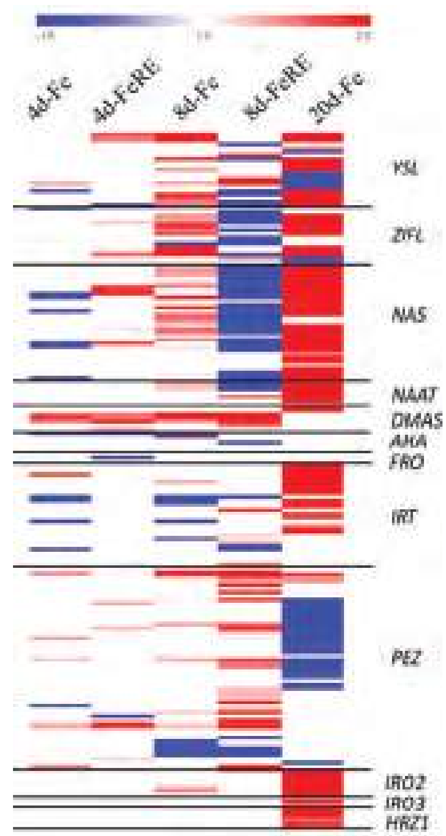

Figure 2. Heatmap analysis the Fe-responsive genes and their abundance in the wheat roots grown under different Fe-status. Changes in the response of the Fe-deficiency were noted with a p-values of <0.05 were selected for analysis Heatmap analysis the Fe-responsive genes and their abundance in the wheat roots grown under different Fe-status. Changes in the response of the Fe-deficiency were noted with a p-values of <0.05 were selected for analysis.

Our study categorizes multiple genes and regulators, genome bias, and induction bias expression, specific to the early and symptomatic stage of Fe deficiency. Overall, this study broadens our understanding of the molecular events in hexaploid wheat in response to Fe deficiency.

Wheat germplasm: An extensive collection of wheat germplasm is being maintained at NABI. The collection was multiplied at NABI's research farm in the last Rabi season 2020-21. It comprises about 500 indigenous and exotic wheat genotypes including landraces; 1,200 EMS treated M10 population; ~250 anueploid stocks; and seve shown to contribute to amylose content, through additive interaction of alleles coming from both parents ('WH1105' and 'TAC75') (Table S6). However, the above identified epistatic QTLs could not explain any significant variation due to environmental interactions, suggesting that environment could not affect the expression of genes with epistatic effects on phenotype.

SALIENT ACHIEVEMENTS

- A RIL population in wheat is advanced to F6 comprising of ~350 progenies and a subset is advanced to F7 progenies.
- Linkage maps was constructed in wheat using 1,293 SNPs, spanned over 3,903.4 cM.
- QTL mapping identified QTLs for amylose starch in wheat.
- QTL x environment interactions and epistatis (QTL x QTL) interactions were identified
- Majority of major QTLs have negative additive effect indicating the contribution of alleles coming from the high amylose mutant line, 'TAC 75'.

FUTURE PERSPECTIVES

- Major QTLs will be validated in back cross population.
- The regions of major QTLs will be augmented to identify the markers (SNPs or SSRs) closer to the QTLs.
- Other mapping populations will be advanced to next generation.

AGRICULTURE BIOTECHNOLOGY

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AB 1.6: Genetic Transformation of Banana for Quality Improvement

With the goal of utilizing the expertise and accomplishments of Queensland University of Technology (QUT), Australia for the development, validation, and transfer of specific traits in two Indian banana varieties, cv. Grand Naine and Rasthali, the BIRAC-supported banana biofortification project was launched in November 2012. For the creation of genetically engineered (GE) Indian banana cultivars, QUT has provided the best pro-vitamin A (PVA) gene constructs in the project's initial phase. These constructs contain the phytoene synthase (APsy2a) gene derived from the Asupina banana and are controlled by the ubiquitin (Ubi) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) promoters. In addition, we have made further attempts to create our own native gene construct, ubiquitin>1-Deoxy-D-xylulose-5-phosphate synthase (NEN-DXS2), for the improvement of PVA in banana cultivars. These three constructs—ACO>APsy2a and Ubi>APsy2a from QUT in Australia and Ubi>NEN-DXS2 created by NABI were used in several rounds of genetic transformation studies. Nearly 400 events from the Grand Naine and Rasthali cultivars were generated and verified by PCR. Following the DBT Biosafety Guidelines, these allegedly GE plants were moved into the net-house for fruit analysis and growth monitoring. To test the stability of the trait, plants with PVA ≥ 20 μg/g dry weight (threshold value) were chosen for additional growth and study in the following generation (ratoon crop). The banana project task force committee has suggested that BIRAC continue to support this project for the second phase of the research activities from February 2021 to March 2024 for three years based on the data produced by the analysis of major as well as ratoon crops. The main goal of the project extension is to identify the most promising GE Grand Naine cultivar event/s (line/s) by testing under various agro-climatic conditions for additional biosafety approvals and commercial release. The progress made during the previous year is summarized below.

RESEARCH PROGRESS

Transfer and Evaluation of Indian Banana with Pro-Vitamin A (PVA) Constructs

Multiplication and hardening of 10 promising selected NABI-PVA events: The sword suckers of promising events and control Grand Naine plants were used for micropropagation (Figure 1). In brief, trimmed suckers were soaked in a solution of 0.5% Bavistin (fungicide) for half an hour and washed with tap water. The trimmed sucker (4 to 5 cm) was surface sterilized with 0.1% HgCl2 for 10 min with constant shaking and washed with autoclaved water 4-5 times inside the laminar airflow. The explants (2.5 to 3 cm) were inoculated in the multiple shoot induction medium containing MS salts, MS vitamin supplemented with BAP (2 mg/l), ADSO4 (35 mg/l), citric acid (10 mg/l) and kanamycin (200 mg/l). The culture was refreshed after three to four weeks on the media containing MS salts, MS vitamin supplemented with BAP (2.5 mg/L), ADSO4 (3 mg/l), L-tyrosine (2.5 mg/l) and kanamycin (200 mg/l) for repetitive organogenesis. Elongated shoots (3-4 cm) were cultured onto the root induction medium containing MS salts, MS vitamin supplemented with IAA (1mg/l) and kanamycin (200 mg/l). Well-rooted plants were shifted in the Soilrite mix soil pots for acclimatization in the growth chamber. Various stages of tissue culture raised promising GE banana lines are displayed in Figure 1 (A-E).

Regulatory approvals for multi-location event selection trials of GE banana: For multi-location event selection trials, various application forms related to GE material transfer (Form B7), receiving (Form B5) and confined field trials (Form D3) were generated by using the IBKP portal. Material Transfer Agreement (MTA) has been signed between NABI-NRCB (15/03/2021), NABI-TNAU (14/06/2021), NABI-NAU (28/09/2021), and NABI-AAU (23/09/2021) for the exchange of GE banana materials

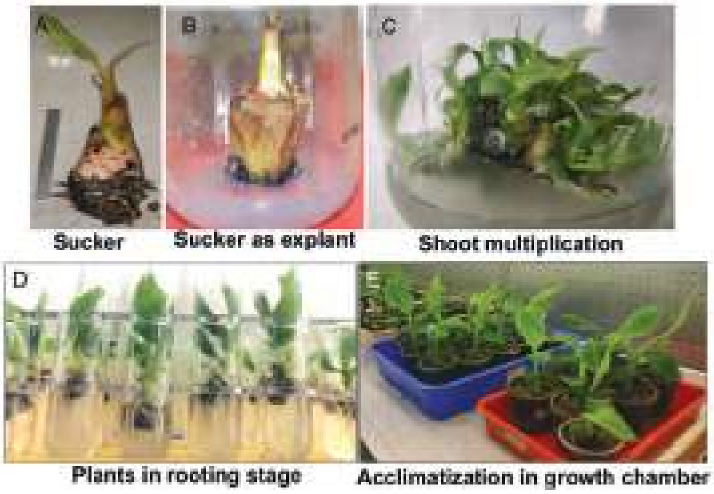


Figure 1. *In-vitro* multiplication and maintenance of tissue culture raised promising GE banana lines. (A) Sucker, (B) Sucker used as explant, (C) Shoot multiplication, (D) Plants at rooting stage, (E) Acclimatized plants in soil-pots.

by following DBT regulatory procedures. NABI applications have also been considered by IBSC and RCGM for the transfer of PVA events in all centers and receiving of NRCB-PVA and Iron events from NRCB. NABI has also submitted Form D3 to RCGM for PVA and Iron events multi-location event selection trials. Various queries have been received and addressed appropriately, then finally Forms D3 have been reviewed and recommended to GEAC by RCGM in its 222nd meeting held on 23/12/2021. GEAC in the letter dated 25th January 2022, recommended for State Government NOC for consideration of proposal related to event selection trials of GE Banana plants. As a national coordinator of project, NABI has sent request letters as well as draft formats to all partners i.e., NRCB, TNAU, NAU, AAU (VC/Director/Director of Research) for getting NOC from respective State Governments. Soft and hard copies of letters along with supporting documents were sent to NRCB, TNAU, NAU, and AAU on 11th Feb. 2022. Meantime, NABI had also submitted a request letter to the Department of Agriculture & Farmer's Welfare, Government of Punjab for NOC on 15th Feb. 2022. Subsequently, NABI had asked for representation from the Punjab Government to defend the proposal and to get NOC for event selection trials of GE Banana lines at NABI. The meeting was held on 5th April 2022 under the Chairmanship of Financial Commissioner (Agriculture) cum Vice-Chancellor, Punjab Agricultural University (PAU) at Punjab Civil Secretariat, Chandigarh. Currently, NABI is looking forward to the decision from Punjab Govt. and then will proceed with our application for GEAC approval to start trials inside the NABI campus.

Molecular characterization of promising banana events

a) Optimization of Southern blotting: The promising GE banana lines showing enhanced PVA content were analyzed for their transgene copy number in their genome by Southern blotting analysis. We have optimized several parameters for Southern blotting such as required genomic DNA content, suitable restriction enzymes, membrane transfer protocol, the concentration of DNA probe, and primary and secondary antibody dilutions. Subsequently, we have performed Southern blotting for one of the promising GE banana line 552 showing the integration of a single gene copy (Figure 2). Genomic DNA was digested with two different restriction enzymes EcoRI and BamHI and their combination, separated on 0.8% agarose gel and transferred onto the nylon membrane by capillary action. Then, the membrane was subjected to probe hybridization followed by biotin-specific primary antibody and HRP labeled secondary antibody probing and ECL development.

b) Optimization of genome walking: The promising GE banana lines need to be analyzed for transgene integration sites in their genome. For that, we have adopted the protocol from Clontech Laboratories, Inc. Universal GenomeWalker™ 2.0 kit. We have optimized the protocol in our laboratory for performing genome walking experiment. This protocol follows PCR-based walking of genomic DNA to identify sequences near the gene of interest. Genomic DNA was digested with four different restriction enzymes and creates libraries after ligation with GenomeWalker

Figure 2. Southern blotting analysis of GE banana line 552 (NABI-DXS) showing integration of single gene copy.

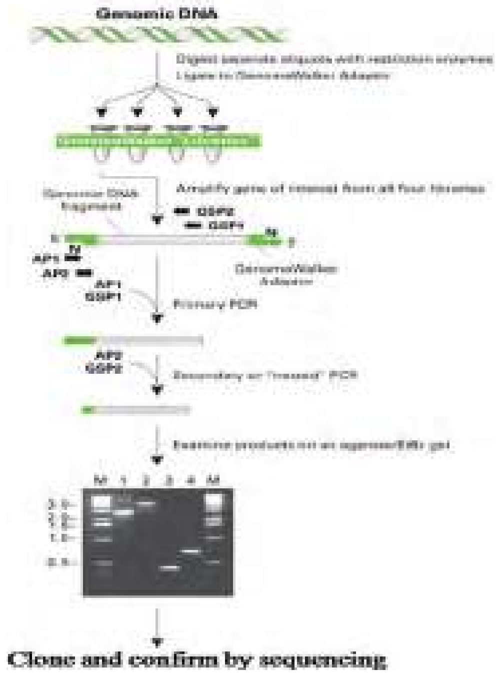


Figure 3. Steps involved in genome walking.

adaptors. Primary PCR amplifies gene of interest with the help of gene-specific and adapter-specific primers. Further, nested PCR enriches the amplified product which will be characterized by cloning and sequencing. Steps involved in genome walking protocol are depicted in Figure 3.

Metabolic engineering for enhanced biosynthesis of pro-vitamin A in Indian banana fruit

The goal of the research is to create and use the CRISPR/Cas genome editing tool to modify specific genes for the biofortification of pro-vitamin A (PVA) in banana fruit. We are dealing with the genes those are responsible for diverting the carotenoid flux (lycopene ε-cyclase; LCYε) and degrading the β-carotene (β-ring hydroxylase; BCH and carotenoid cleavage dioxygenase; CCD) in the carotenoid biosynthesis pathway. As anticipated, the PVA accumulation and stability in banana fruit will be improved by the mutation in these genes. These results will also aid in our understanding of the fundamental regulatory mechanisms governing the buildup and breakdown of β-carotene in banana fruit pulp. Previously, using CRISPR/Cas9, we edited the genes for phytoene desaturase (PDS) and lycopene epsilon-cyclase (LCYε) in the commercial cultivars Grand Naine and Rasthali. When LCYεin the Grand Naine genome was modified, the amount of β-carotene deposited in the fruit pulp was up to six times higher (23.80 ± 1.26 g/g) than it was in the control plants that were not edited. We have carried out bioinformatics and gene expression studies of CCD genes in addition to our genome editing work. The CRISPR/Cas9 construct was created using the CCD4 gene, which was identified for its function in the degradation of β-carotene in banana fruit-pulp.

Transient expression of CRISPR_RAS_CCD4 construct in Rasthali protoplasts by electroporation: A specific vector for RAS-CCD4 was designed for the transformation of cv. Rasthali protoplasts. A 20 bp sequence (gRNA) from the 1st exon of RAS-CCD4 gene was selected using the Breaking-Cas tool. The selected gRNA was incorporated into the pRGE31vector (Addgene plasmid # 50929) under the rice snoRNA U3 promoter using BsaI restriction site. The prepared vector was named as CRISPR_RAS_CCD4 (Figure 4E). The somatic embryos from Rasthali callus were used for preparing embryogenic cell suspension (ECS) culture (Figure 4A, B). The protoplast isolated from ECS culture (Figure 4C, D) were electroporated with CRISPR_RAS_CCD4 construct (Figure 4E). Total DNA was extracted from protoplasts after 48 h of incubation. The target region of RAS-CCD4 was amplified and sequenced. Out of 100 clones, two clones have shown deletion (1/2 nucleotides) at the target region of RAS-CCD4 (Figure 4F). This protoplast based system can be utilized for in-vivo validation of sgRNA cleaving efficiency.

Generation and analysis of stable transgene-free CCD4 edited banana lines: To overcome the barrier of protoplast regeneration, the CRISPR_RAS_CCD4 (Figure 4E) cassette was transferred into embryogenic ECS culture (Figure 5A) of cv. Ras-

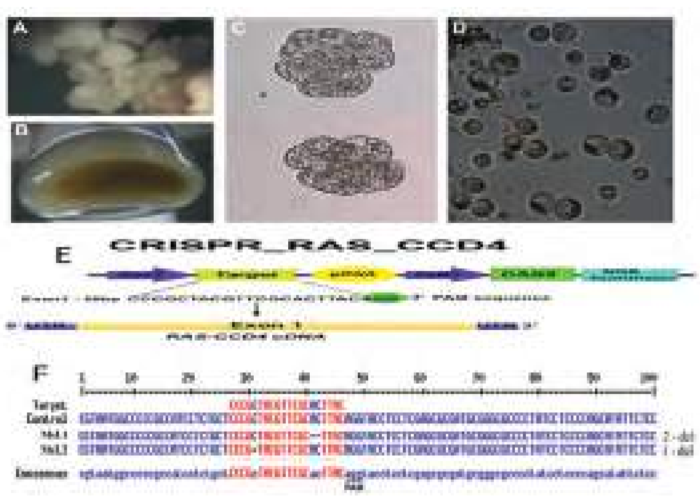


Figure 4. Development and validation of transgene-free CRISPR/Cas9-based genome editing in protoplasts of banana cv. Rasthali.

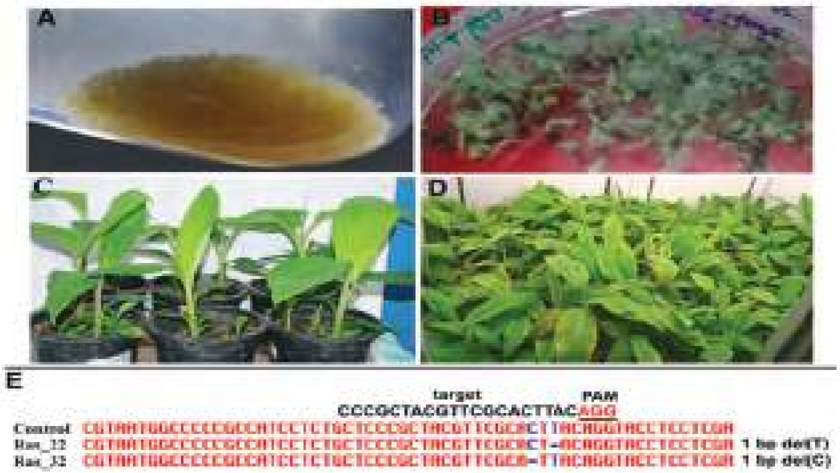


Figure 5. Regeneration and analysis of transgene-free edited lines of banana cv. Rasthali.

thali by particle bombardment for transient expression. The bombarded cells were allowed to germinate on a medium without any antibiotic selection pressure (Figure 5B, C). More than 300 plantlets (Figure 5D) were generated and screened by using the genomic DNA mixed pool method. Total genomic DNA was isolated using the CTAB method. The target region was amplified using CCD4-specific primers. The amplified fragment was analyzed by a 3730x1 DNA analyzer (Applied Biosystems, USA). Sequencing has confirmed the editing of RAS-CCD4 with two different patterns of indels (Figure 5E).

SALIENT ACHIEVEMENTS

- Promising ten PVA enriched events, as well as control Grand Naine plants, are multiplying under in-vitro conditions for distribution to partner institutes in NRCB, Tamil Nadu, TNAU Tamil Nadu, NAU Gujarat and AAU Assam and also plantation in the NABI research field for event selection trails by following DBT biosafety guidelines.
- The regulatory clearance from IBSC and RCGM has been taken. Following the GEAC recommendation, we are processing the application for getting a No Objection Certificate (NOC) from the state government for the event selection trials under confined field conditions.
- Southern blotting and genome walking analysis of promising GE banana lines has been optimized.
- Demonstrated transgene-free genome editing of carotenoid cleavage dioxygenase 4 (CCD4) as a negative regulator of β-carotene in banana cultivar Rasthali and recently published this work in "Journal of Experimental Botany" (2022).

FUTURE PERSPECTIVES

- Multilocation event selection trails of promising GE banana events will be started by following biosafety guidelines.
- Plantation of CRISPR_RAS_CCD4 edited plants in the green-house/net-house for further analysis.
- Identification of putative target of β-carotene hydroxylase (BCH) for unraveling its role in β-carotene degradation.
- Optimisation for western blotting and experiments related to Southern blotting and genome walking of the promising lines to be performed.

AGRICULTURE BIOTECHNOLOGY

Pramod K Kandoth, PhD
Plant Molecular Biology

RESEARCH FELLOWS
Anjali Verma
Lovenpreet Kaur

AB 1.7: Application of genomics approaches for reducing ODAP content in *Lathyrus* spp.

Lathyrus is an important legume crop cultivated mainly in India, Bangladesh, Nepal, Ethiopia, and some parts of Europe. Though this leguminous crop is a rich source of protein, second only to soybean in terms of protein content, it is not promoted widely for cultivation owing to the presence of neurotoxin -L-oxalyl-2,3- diaminopropionic acid (β-ODAP), which if consumed in large quantities lead to a condition called neurolathyrism. However, the plant has important agronomical attributes such as drought tolerance, pathogen resistance, and the ability to withstand waterlogged situations. This crop is also valuable for health-promoting compounds such as l-homoarginine. Global warming is leading to a reduction of cultivable land area worldwide, this crop needs promotion as it can be a hardy crop with guaranteed yield in problem soils.

Breeding efforts in the past have led to the development of several low ODAP cultivars. Still, in these cultivars, the ODAP content is not stable owing to the influence of environmental factors. A biotechnological approach could circumvent this problem. Our efforts are focused on understanding the pathways leading to the production and accumulation of ODAP in the seeds, its relationship with stress tolerance, and thereby use genome editing approaches to produce a Lathyrus cultivar with low or no ODAP production. The activities undertaken during this work deals with: a) Identification of genes and pathways influencing ODAP content in seeds by genomic approaches; b) Isolation of genes involved in the ODAP pathway and; c) silencing of genes that lead to the development of cultivars with no/ reduced ODAP in the seeds.

RESEARCH PROGRESS

Developing an EMS mutant population of Lathyrus: We developed an Ethyl methane sulphonate (EMS) mutant population of Lathyrus. Currently we have a population of more than 550 mutant lines.

Screening of mutants to identify low ODAP mutants: We, so far, screened 102 M2 lines for reduced ODAP content by HPLC method standardized earlier. For each line, wherever possible, 5-6 seeds were germinated and the seedling shoot tissue was collected and ODAP content was estimated. The plants were maintained to collect M3 seeds. The results of this screen are given in Fig 1A. The preliminary analysis identified 23 lines with reduced ODAP concentration compared to the variety used for mutagenesis experiments. The M3 seeds from some of the promising lines were further analyzed to confirm the low ODAP mutants and the homozygosity of the mutants (Fig 1B). Some of the mutants were stable with low ODAP content while we have some segregating lines. We are screening the rest of the mutants. Fig. 1 A) Screening of Lathyrus M2 mutant seedlings for low ODAP content by HPLC. ODAP levels in unmutated Pusa-24 seedlings are shown in blue. Low ODAP content lines are denoted by golden bars. B) Some of the mutant lines which showed low ODAP in the M2 screen is tested again in the seeds (M3) along with controls/ mutants with no difference in ODAP levels.

Genome editing of Lathyrus: We made constructs using single guide RNAs and double guide RNAs and demonstrated editing in transgenic hairy roots. The use of double gRNA construct with multiple gRNAs targeting the same gene improved editing efficiency tremendously. So far, we successfully edited three genes in the ODAP biosynthetic pathway in the hairy roots.

Transformation protocols for transgenic Lathyrus plants: We used a GUS gene construct for optimizing conditions for transformation of Lathyrus. We use multiple shooting with cotyledon node explants, and another method of callus formation with leaf explants, in parallel for transformation experiments. The transformation events are monitored by GUS staining (Fig. 2C). The conditions are being further optimized. The experiments with leaf explants and GUS constructs to derive

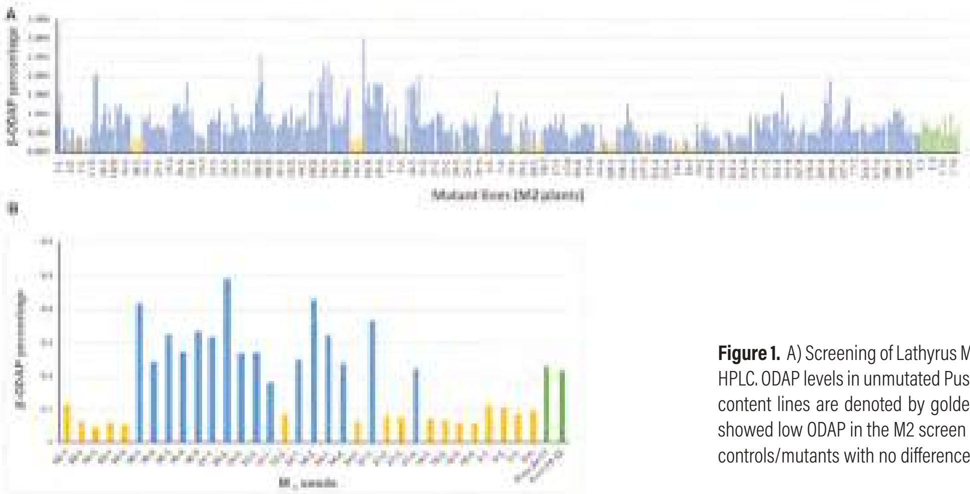


Figure 1. A) Screening of Lathyrus M2 mutant seedlings for low ODAP content by HPLC. ODAP levels in unmutated Pusa-24 seedlings are shown in blue. Low ODAP content lines are denoted by golden bars. B) Some of the mutant lines which showed low ODAP in the M2 screen is tested again in the seeds (M3) along with controls/mutants with no difference in ODAP levels.

transgenic calli is ongoing. We used one of the editing constructs to generate edited transgenic plants using multiple shoot organogenesis method. This method has produced some plants that are tested positive for Kanamycin and Cas9 gene (lane 1, 3 and 6; Fig 3D)

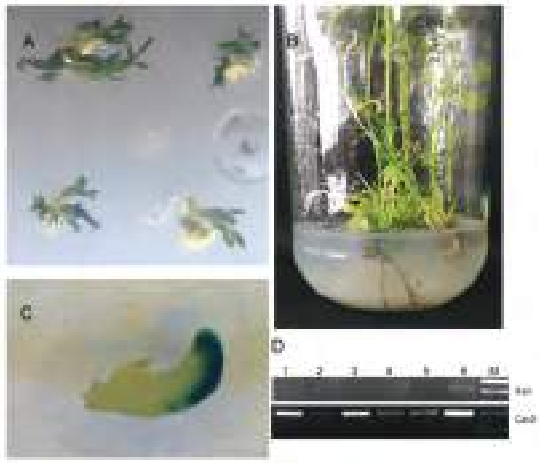


Figure 2. A) Multiple shoot organogenesis with cotyledon explants, B) Rooted plants from multiple shoot organogenesis approach C) GUS staining in the leaves of GUS transformed plants D) PCR of putative transgenic Lathyrus plants using Kanamycin and Cas9-specific primers with one of our editing constructs.

SALIENT ACHIEVEMENTS

- We identified several putative low ODAP mutant lines by HPLC screening of M2 seedlings. Some of these mutant lines are further confirmed as low ODAP mutants in the M3 seeds.
- Constructs for editing of three ODAP biosynthetic pathway genes developed and tested for editing successfully in hairy roots. These constructs are currently used in whole plant transformation experiments.

FUTURE PERSPECTIVES

- Generating transgenic Lathyrus plants with biosynthetic pathway gene mutations.
- Completing the screening of mutant population for low ODAP mutants. Advancing the identified mutants to subsequent generations and phenotypic and genotypic characterization of these mutants.

AGRICULTURE BIOTECHNOLOGY

Prafull Salvi, PhD
Plant Molecular Biology

RESEARCH FELLOWS
Rabishankar Ojha
Nishu Gandass

AB 1.8: Molecular and functional analysis of seed specific Dof transcription factor(s) in rice

Rice is one of the most important cereal crops, as it is consumed by one-third of the global population. Despite an enormous rise in rice cultivation, its productivity is severely compromised by different environmental stresses including extreme temperature, water scarcity, salinity stress, heavy metal stress, etc. Besides, the dwindling agrarian land and continuously growing population have further escalated the gap between its demand and supply. To combat the stressful conditions, diverse molecular responses are instigated at the cellular level which include signalling cascade, phytohormonal regulation, altered gene expression, as well as transcriptional regulation, post-transcriptional and post-translational modification. Transcriptional control involves the regulation mediated via transcription factors (TFs) and transcriptional regulators (TR). TF bind to cis-elements present in the promoter region of the target gene while TR indirectly regulates the gene expression by modulating the DNA-protein interaction through chromatin remodelling. In rice, about 2,048 gene are predicted to encode for TFs while 328 as transcriptional regulators. We have studied an uncharacterized DOF-TF, which is highly disordered in nature and appeared to play an important role in thermal stress response in rice. Intrinsically disordered proteins are characterized as protein with enriched disordered region and devoid of an ordered three-dimension structure, yet they are capable to execute diverse cellular functions. As compared to prokaryotes, eukaryotic proteomes are known to possess higher disordered protein. This aspect of abundant IDRs in eukaryotes also indicated the correlation of IDRs with the complexity at functional and organism level. These IDRs emerged as a new field in structural biology which are largely studied in animal system. However, due to their central role in regulation of metabolic and cell signalling process they are attaining much attention in plant science as well. Especially, TF are profuse with IDR, which offer the functional plasticity and versatility to the TFs. Overall, we used diverse molecular, In-silico and functional approaches to explore the role of DOF27 in rice with emphasis to heat stress.

RESEARCH PROGRESS

- We have investigated the role of *OsDOF27* and characterised it as an intrinsically disordered protein. Further, the molecular role of *OsDOF27* in thermal stress tolerance has been elucidated.
- The qRT-PCR analysis revealed that *OsDOF27* significantly upregulated under different abiotic stress treatments in rice, particularly under heat stress.
- The stress-responsive transcript induction of *OsDOF27* was further corelated with enriched abiotic stress related cis-regulatory elements present in its promoter region.
- The invivo functional analysis of the potential role of *OsDOF27* in thermotolerance was further studied in yeast and in planta.
- Ectopic expression of *OsDOF27* in yeast implicates thermotolerance response.
- Further, the rice transgenic lines with overexpressing *OsDOF27* revealed its positive role in mitigating heat stress tolerance.
- Collectively, we represent the intrinsically disorderedness in DOF-TF and its role in thermal stress response in rice.

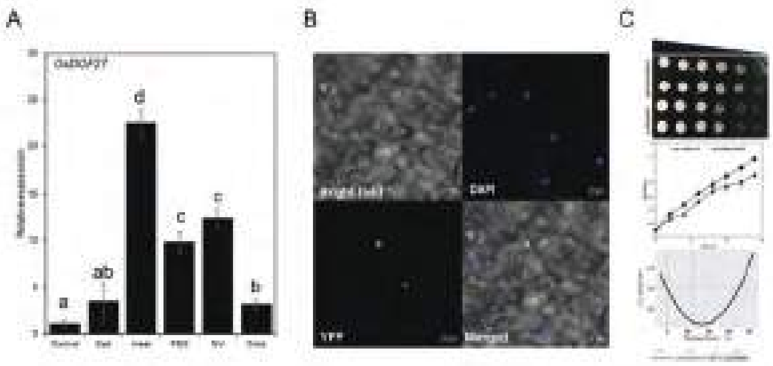


Figure 1. A) qRT-PCR for expression analysis of OsDOF27 under different abiotic stress conditions. B) Subcellular localization of OsDOF27 protein. C) Thermotolerance potential of yeast is shown by spot assay and growth curve analysis, and protein stability curve.

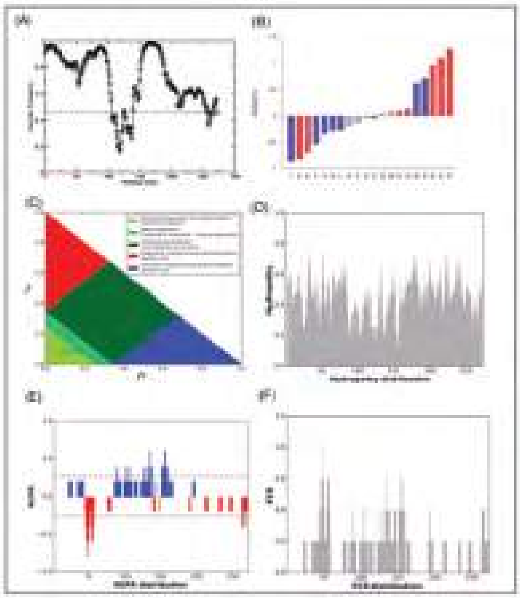


Figure 2. Analysis of disorder regions present in the protein sequence of OsDOF27 based on the amino acid composition.

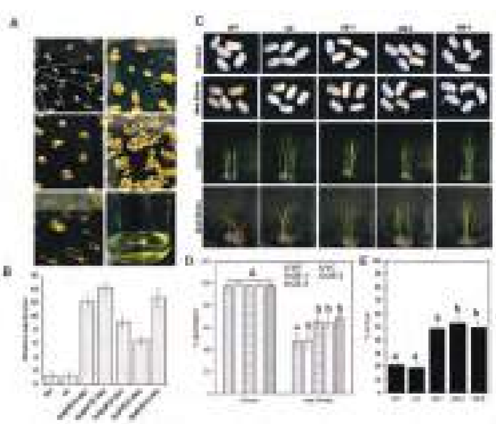


Figure 3. A) Schematic representation of the Agrobacterium-mediated rice transformation of OsDOF27. B) qPCR analysis of positive transformants. Comparative stress tolerance potential of transgenic lines overexpressing OsDOF27 and its control counterpart (WT, wildtype plants and VC, vector control plants) at C) germination stage and (D) seedling stages and E) percent survival of transgenic lines in comparison to WT and VC under heat stress treatment.

SALIENT ACHIEVEMENTS

- We have studied an uncharacterized DOF-TF, i.e., OsDOF27, from rice and identified it as an intrinsically disorder protein.
- We have established the molecular role of OsDOF27 in mediating thermal stress tolerance in rice.

FUTURE PERSPECTIVES

- Functional analysis of DOF-TF (RPBF) transgenic lines with emphasis to seed physiology.
- Generation of promoter-gus reporter lines using agrobacterium mediated genetic transformation of rice.
- Protein expression and purification for RPBF protein.
- Identification and validation of DNA-Protein interaction for RPBF-TF.

AGRICULTURE BIOTECHNOLOGY

Rupam K Bhunia, PhD
Plant Lipid Biochemistry

RESEARCH FELLOW
Kshitija Sinha

AB 1.9: Metabolic engineering of triacylglycerol (TAG) biosynthetic pathway to trigger TAG content and stability in plants

Rice Bran, the outer brownish layer of a dehusked rice grain is the source of rice bran oil (RBO). Being rich in essential fatty acids, proteins, vitamins, minerals and antioxidants like oryzanol makes it one of the healthiest oil which has also been recommended by WHO. However, during milling i.e. separation of the husk from the grain, the triacylglycerol (TAG) stored as oil bodies (OBs) in the rice bran are degraded by the endogenous lipases resulting in the release of free fatty acids and glycerol which ultimately leads to stale flavour and oxidative rancidity. Many conventional physical, chemical and biological methods are being used by the rice bran industries to reduce bran lipid spoilage but all these conventional methods come with their drawbacks which ultimately compromise the quality of rice bran lipids and therefore, there is a need to understand the mechanism of this process at the molecular level. The main players that we identified, play an important role in rice bran oil deterioration are FAD2, TAG lipases and lipoxygenases. Amongst these factors, the main contributors to the degradation of bran lipids are lipases and lipoxygenases as they participate extensively in hydrolytic and oxidative rancidity of RBO. In our previous work, we identified the putative lipase genes and the three out of 18 shortlisted lipases i.e. Oslip1, Oslip12 and Oslip13 were chosen for functional characterisation. We also developed CRISPR/ Cas9 mediated edited rice lines (Taipei-309) of one of the selected lipases i.e. Oslip1.

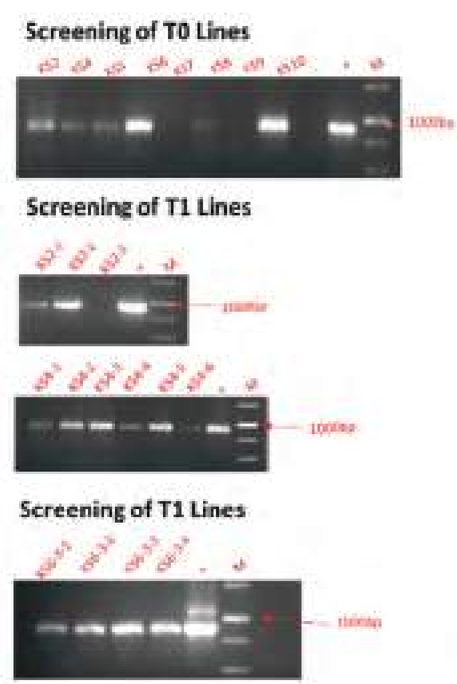


Figure 1. Screening of positive transformants by PCR amplification using Cas9 internal primers.

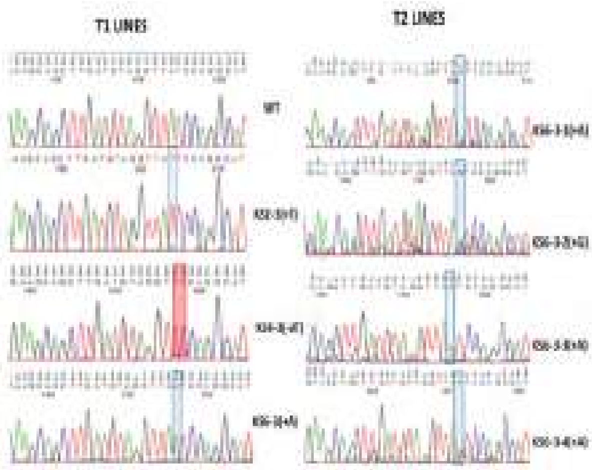


Figure 2. Chromatogram analysis of positive CRISPR/Cas9 Oslip1 edited plants.

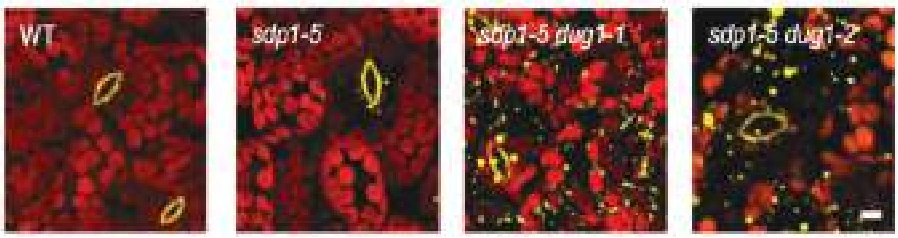


Figure 3. Effect of promoter fusion on lipid metabolism in rosette leaves of 7 weeks old plants. LSCM images of lipid droplets accumulating in leaves. Leaf sections were harvested ~6 h after dawn. Lipid droplets and guard cell cuticular ledges yellow (Nile red stained) and chloroplasts red (chlorophyll florescence).

RESEARCH PROGRESS

» **CRISPR/Cas9 mediated editing of Oslip1 in rice and generating Cas9-free edited lines for analysis:** We synthesised CRISPR constructs of the aforementioned genes, transformed them in Agrobacterium tumefaciens (LBA4404) in order to study the role of the genes in rice, we used Taipei-309. T1 lines of KS2 and KS4 and T2 lines of KS6 have till now been obtained and the process of screening for Cas9-free edited plants is on-going (Fig.1 and Fig.2).

» **Achieving gain-of-function phenotypes using CRISPR/Cas9:** Gain-of- function phenotypes were developed without inserting a foreign DNA by fusing the promoter of a non-essential photosynthesis-related gene to

Figure 4. T0 RB1 plants transferred from hydroponics for Acclimatization.

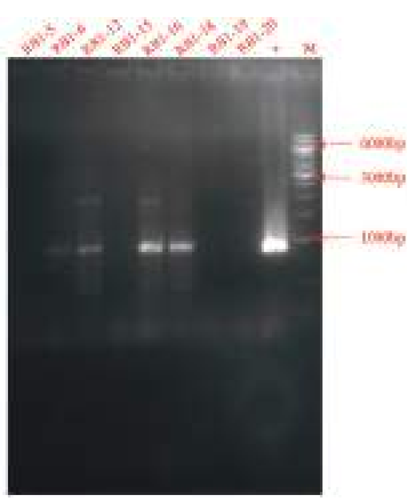


Figure 5. Cas9 Screening of T0 RB1 plants using Cas9 specific primers.

DGAT 2 (DIACYLGLYCEROL ACYLTRANSFERASE 2) which plays a pivotal role in TAG accumulation in plants (Fig. 3). By deleting the intervening genomic sequence using dual-guide CRISPR/Cas9, DGAT2 expression enhanced more than 24 fold and the TAG content increases by 34 folds in the lipase-deficient sugar-dependent1 mutant of Arabidopsis thaliana. This strategy provides a transgene-free mode of genetic engineering that relies on transcriptional gain-of-function.

» **CRISPR/Cas9 mediated editing of multiple genes in rice and checking its effects on bran lipid stability:** In order to increase the effects of editing on rice bran stability, we designed CRISPR constructs with multiple guide RNAs to target multiple genes. Genes which were found to be highly expressing in rice bran of germinating grains i.e. Oslip1, Oslip12, Oslip13, LOX2, LOX3 and LOX4 were targeted in different combinations. Two constructs i.e. RB1 and RB2 were made and transformed in the rice calli (Taipei-309) via Agrobacterium (Fig.4).

» Regenerating shoots were transferred for rooting and fully grown shoots have been transferred in hydroponics for acclimatization and hardening. Genomic DNA of the plants obtained have been isolated and the process of screening for Cas9 positive edited plants is on-going (Fig.5). Till now 8 lines have been screened using Cas9 specific primers out of which 4 lines showed positive bands (~1000bp).

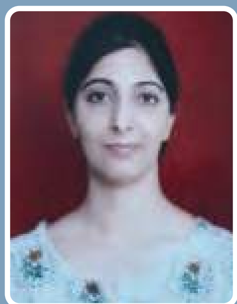
SALIENT ACHIEVEMENTS

- The genomic deletion strategy offers a transgene free route to engineering traits that rely on transcriptional gain of function, such as producing high lipid forage to increase the productivity and sustainability of ruminant farming.
- Designed and assembled CRISPR/Cas9 DNA constructs for knocking out lipase and lipoxygenase genes identified by transcriptomics and bioinformatics analysis.
- Rice lipase genome edited lines were created, which could play a role in rice bran rancidity.

FUTURE PERSPECTIVES

- Generate low rancidity rice lines by targeted mutagenesis of lipase and lipoxygenase using CRISPR/Cas9.

AGRICULTURE BIOTECHNOLOGY



Humira Sonah, PhD
Molecular Biology

AB 1.10: Improvement of seed oil, protein content and nutritional quality in soybean *(Glycine max (L.) Merr.)*

Soybean (Glycine max (L.) Merr.), one of the most important leguminous crops is known for its high oil and protein content in seeds. Soybean oil is widely used as edible oil and as an industrial commodity. A typical oil concentration in soybean is 20% of dry seed weight and its quality is determined by the fatty acid composition. Soybean possesses five principal fatty acids, palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3). Depending upon the application of soybean oil, different concentrations of particular fatty acids are desirable. Fatty acid concentration can be manipulated in a breeding program if the genetics of the trait are well known and desired sources of germplasm are available. Several effort has been made for dissection of these traits but precise location of trait and knowledge about molecular mechanism involved in the trait development are one of the major limitations. To overcome these limitations, high-precision mapping and identification of novel regulatory loci, subsequent candidate genes evaluation and genetic gain through genomic selection approaches looks promising steps.

Recent advancement in sequencing technology provides several options to obtain over a million informative markers in a time and cost effective manner. One of the most efficient and cheap option is genotyping-by-sequencing (GBS) which can provide million markers genotyping for large mapping population. The improved GBS has been utilised for genome-wide association study (GWAS), genetic linkage analysis, genomic selection, genomic diversity study, markers discovery and many other marker assisted application in crop improvement programs. Several GWAS efforts have been made towards understanding the genetic regulation of seed oil and protein in soybean. Little progress has been made for the identification of candidate gene involved in seed composition traits besides having information of many QTLs. The genomic selection (GS) based on

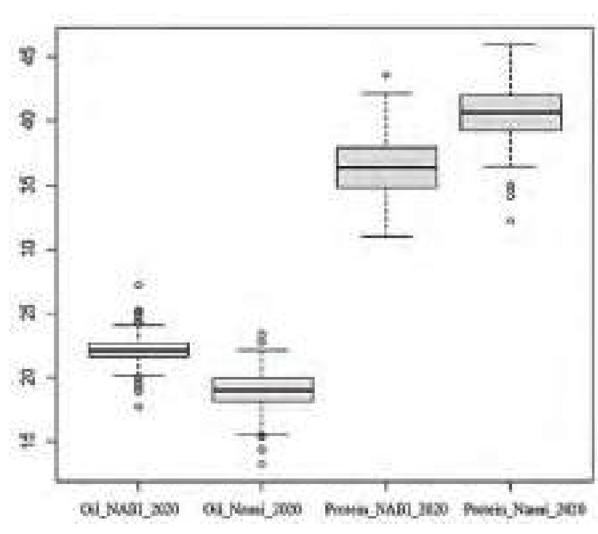


Figure 1. The distribution of seed oil and protein content at two different locations. The graph depicts the phenotypic variation for seed oil and protein content in soybean.

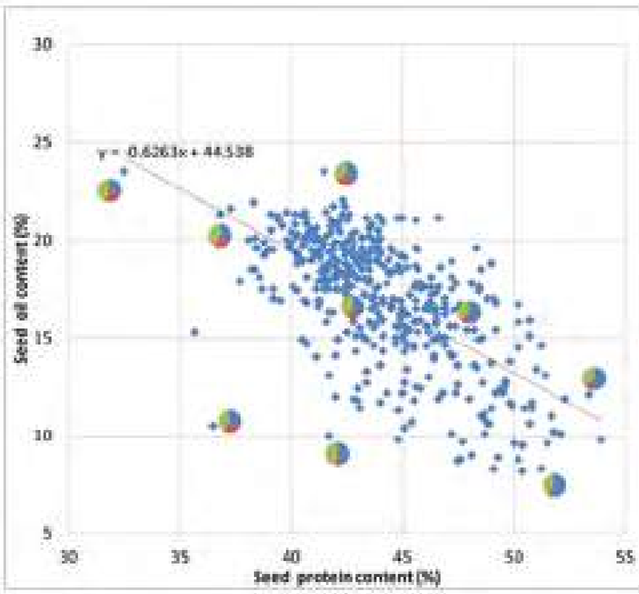


Figure 2. Scatterplot for correlation analysis between seed oil and protein content in soybean. The scatter plot shows significant negative correlation between seed oil and protein content in soybean.

statistical model, utilised the phenotypic and genotypic data of training population, to predict phenotype of genotyped individual. The GS approach is being considered as most efficient to accelerate the trait improvement in soybean and other crop breeding programs. In this regards, GS model for different traits will be developed and evaluated for the prediction efficiency. This will help to achieve required analytical expertise and confidence to implement the technology. The meta-QTL analysis will be performed for oil and protein content and other seed composition trait to identify any such loci which are specific to particular trait and then develop informative markers for the breeding application. GWAS, along with GS, will be performed to identify loci governing seed quality-related traits by keeping the Indian climatic conditions under consideration. The general goal of this project is to integrate genomics and breeding research leading to the development of superior cultivars and food-grade soybean and to expand the utilization of soybean crop in India.

RESEARCH PROGRESS

- Phenotypic evaluation of seed oil and protein content at different locations was performed. The soybean seed oil content varies from 15% to 25% and protein content varies from 25% to 50% (Figure 1). As expected, significant variation in oil and protein content was observed at two different locations.
- A negative correlation was observed between the seed oil and protein content in soybean (Figure 2). The genotyping by sequencing (GBS) method was used for genotyping of soybean germplasm which was further used for GWAS. Population Structure analysis was performed by using FastStructure. The phylogenetic tree was constructed for using filtered SNPs from soybean germplasm using the neighbor joining clustering method and the resulting Newick tree was visualized with the 'Archaeopteryx tree' tool.
- The kinship matrix and principal component analysis was constructed by

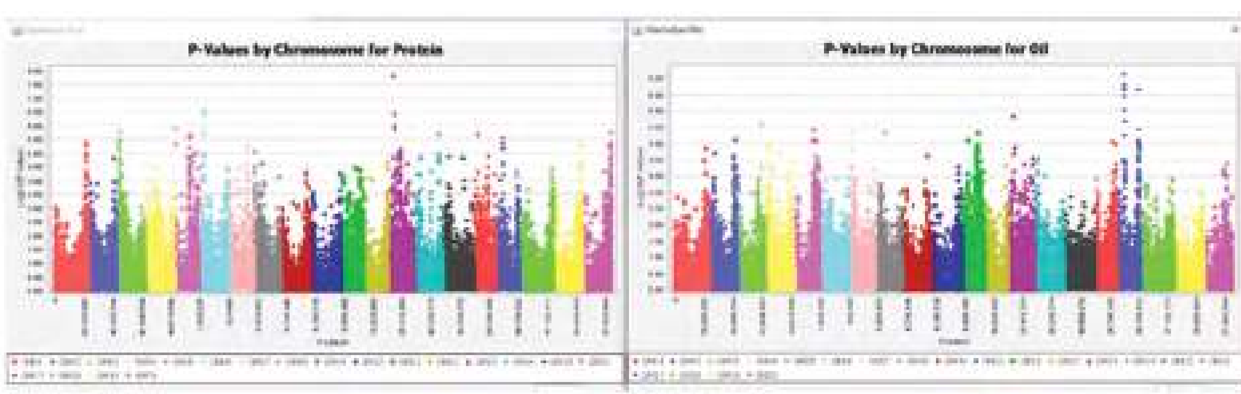


Figure 3. Manhattan plot showing significant loci governing seed oil and protein content in soybean.

using Van Raden method within the Genome Association and Prediction Integrated Tool.

» The GWAS was performed using the Trait Analysis by aSSociation, Evolution and Linkage (TASSEL v5.2.67) statistical software. Multidimensional scaling (MDS) output was used as a covariate for running Mixed Linear Model (MLM) to account for the population structure. The kinship matrix was constructed by using the Centered IBS kinship method. Several models were used to confirm the significant associations. Significant loci for seed oil and protein content was observed respectively on chromosome 13 and 17 (Figure 3) which is further being evaluated and will be followed by candidate gene identification.

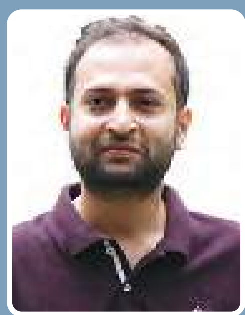
SALIENT ACHIEVEMENTS

- Phenotypic characterization for selected traits was done.
- Significant loci governing seed oil and protein content were identified
- Potantial donar lines for high oil and high protein content were identified.

FUTURE PERSPECTIVES

- Multi-enviroment phenotypic evaluation of seed quality-related traits will be performed
- Genome-wide association study for the seed quality-related traits will be performed.
- Candidate gene identification within the significant loci governing seed oil and protein content will be performed.

AGRICULTURE BIOTECHNOLOGY



Abhishek Bhandawat, PhD
Molecular Biology

RESEARCH FELLOWS
Manisha Godara

AB 1.11: Unraveling the role of plastidial transporters in regulation of starch biosynthesis in bread wheat (*Triticum aestivum L.*)

Triticum aestivum L. commonly known as bread wheat has broadest adaptation, and is among the most consumed staple crops across the globe. Its seeds majorly comprise of starch which is a vital source of calories in our diet. The major portion of starch (about 75%) comprises of highly branched D-glucopyranosyl chain known as amylopectin and less abundant (about 25%) proportion of linear D-glucopyranosyl chain known as amylose. The ratio of amylose: amylopectin determines the pasting properties and nutritional value, and hence the desirability of the bread wheat varieties for various applications. Amylopectin has high glycemic index, digest rapidly in gut and increases the blood sugar level which is a leading cause of obesity, diabetes Type-2 and other cardiovascular diseases, if consumed in excess. Amylose is a form of resistant starch which digests slowly in upper gut, and undergoes fermentation by beneficial microbes in the lower gut to produce short chain fatty acids, imparting health benefits. Thus, wheat varieties with enhanced amylose proportion are of high nutritional and industrial significance. ADP-glucose is known to be the primary precursor for synthesis of starch in seed endosperm. Classically, the biosynthesis of storage starch in cereal endosperm involves a series of cytosolic conversions of sucrose to form ADP-glucose, which is acted upon by granule-bound starch synthases (GBSS I) to form amylose or non-bound starch synthases and various starch branching (SBE) and debranching (DBE) enzymes to form amylopectin. Overexpression of GBSSI and downregulation of starch branching enzymes either lead to minor increase in amylose content or at the cost of loss of starch yield. Enhancing the substrate availability can enhance the expression and biosynthesis of downstream pathway genes. Transporters play a key role in unloading of substrates and energy molecules to the plastid. For instance, ADP-glucose/ADP translocator (Brittle 1 or BT1) is known to transport active glucose from cytosol to stroma. Other transporters such as glucose-6-phosphate transporter, ATP/ADP transporter have been reported in starch biosynthesis in various cereal and non-cereal plants. However, the role of these substrate transporters in bread wheat largely remains unexplored. The aim of the current study is to identify and validate key plastidial transporters regulating amylose and starch biosynthesis in bread wheat (Figure 1a). This will not only expand our understanding about amylose biosynthesis but provide a suitable target for developing varieties with better nutritional properties for food and industrial applications.

RESEARCH PROGRESS

Identification and expressional analysis of transporters

The cDNA sequence data of *Triticum aestivum* was mined from ENSEMBL plants database (https://plants.ensembl.org/info/data/ftp/). To identify the genes encoding plastidial transporters, blastx homology search against various protein databases, including Swissprot and rice. Rice and arabidopsis was performed to predict their functional role. The homeologs of these transporters were retrieved for designing homeolog specific primers using PrimerQuestTM tool (https://www.idtdna.com/pages/tools/primerquest) for qPCR studies. Seeds of three different developmental stages: 4, 14 and 21 days after anthesis were targeted to examine the relative expression of transporters (Figure 1b). A significant variation in expression was evident in different homeologs across different seed developmental stages.

Optimization of agrobacterium-mediated transformation

A novel plasmid comprising of RUBY construct encoding for three individual polypeptides that utilizes tyrosine and produce red colour pigment (betalain) has recently been reported (He et al. 2020). A hypervirulent strain of *Agrobacterium tumefaciens* (AGL1) containing the RUBY plasmid was utilized for standardizing the transformation protocol. Initially the construct was tested for visible RUBY signal in 3-4 weeks old *Nicotiana benthamiana*. Overnight grown culture of AGL1 contain-

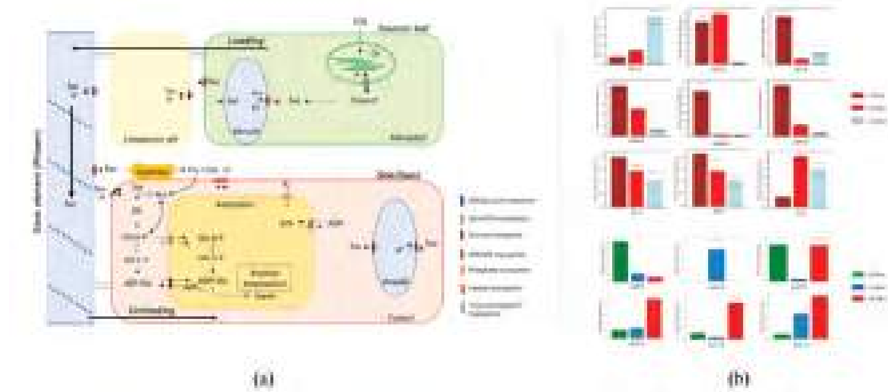


Figure 1. (a) An overview of transporters and their role in starch biosynthesis in sink organ (b) Quantitative real-time PCR profiling of candidate bread wheat transporter homeologs across various seed developmental stages of bread wheat variety C 306.

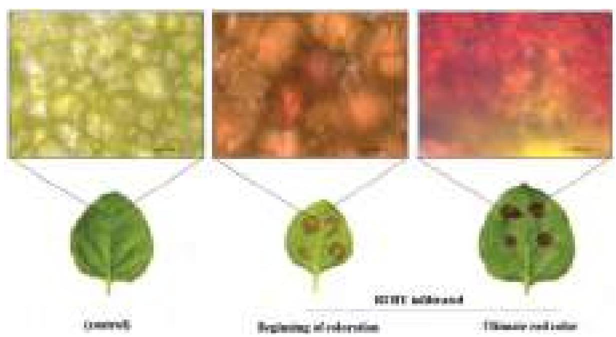


Figure 2. Visible red phenotype 3-4 days after agrobacterium-mediated transient transformation of N. benthamiana leaves with 35S: RUBY.

ing RUBY plasmid (under suitable antibiotic selection) was resuspended in a MES buffer containing acetosyringone and used for infilteration. Visible red signal was observed within 3-4 days of infiltration (Figure 2). Currently, the transformation protocol using modified RUBY construct is being standardized in different bread wheat varieties using various types of explant source such as mature embryo, shoot tip, leaves and callus (Figure 3).

Figure 3. Callus induction from (a) growing shoot tip and (b) mature embryos of bread wheat variety PBW 343 on MS agar plates supplemented with 2,4-D.

SALIENT ACHIEVEMENTS

- Candidate transporters and homeologs involved in starch biosynthesis were identified and their relative qPCR expression was studied in bread wheat.
- Protocol for callus induction and regeneration for subsequent agrobacterium-mediated transformation of bread wheat is being standardized.

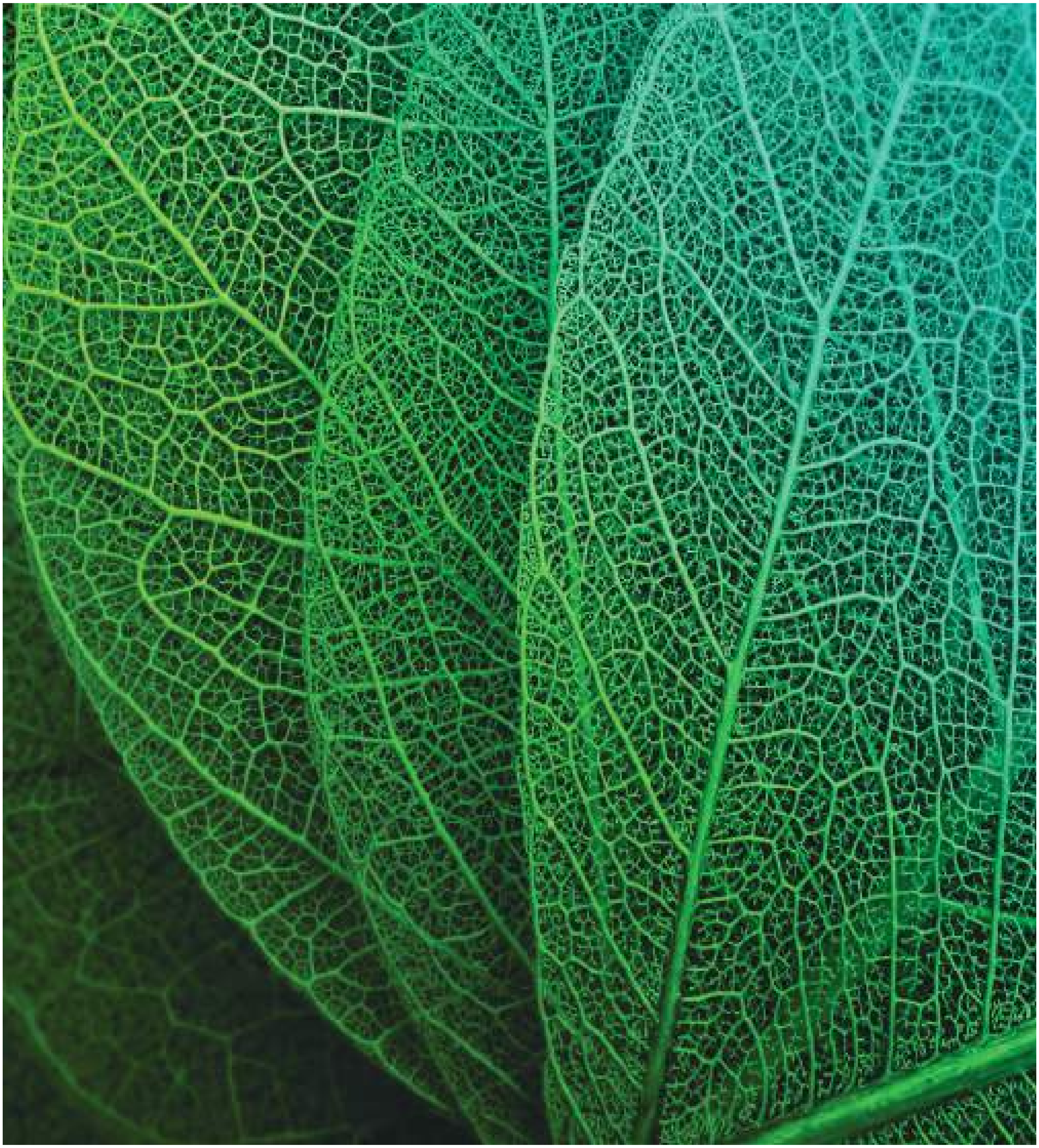
FUTURE PERSPECTIVES

- To develop robust protocol for agrobacterium-mediated transformation in bread wheat
- Investigate the effect of candidate transporter gene overexpression and/or silencing on altered starch and amylose.

PROGRAM - AB02

AGRICULTURE BIOTECHNOLOGY (AB)

GENOMICS AND COMPUTATIONAL BIOLOGY FOR MARKERS AND GENE DISCOVERY



AGRICULTURE BIOTECHNOLOGY

Shrikant S Mantri, PhD
Computational Biology

RESEARCH ASSOCIATE
Dalwinder Singh, PhD

RESEARCH TRAINEE
Hemant Singh

AB 2.1: Microbiome data mining to discover novel patterns

Gut microbiome is the totality of trillions of microorganisms and their genetic material present inside the intestinal tract. Gut microbiome comprises bacteria, virus, fungi, protozoa with maximum composition covered by bacteria (over 90%). These microbes play a crucial role in determining the health of a host by providing it with metabolic enhancements, immune system education, xenobiotic degradation capabilities etc. Data mining is revolutionising the field of metagenomics and proteomics as it is fast and accurate and less laborious. What work would have been done in years of time now can be done within days with the help of high-performance computing (HPC). With the help of HPC we tried to mine metagenome data of individuals of extreme longevity (up to 109 years of age) to understand the modulations of metagenome functions in accordance with age and understand the biosynthetic potential of gut microbiome and then co-relate it with the trait of extreme longevity. With the help of computational gene cluster annotation tools, you can annotate novel Biosynthetic gene clusters and annotate secondary metabolites related to them. And also with the help of profiling tools you can taxonomically profile and visualise metagenomes thus can establish further patterns according to various study requirements.

RESEARCH PROGRESS

- Development of a pipeline for fast profiling and visualisation of metagenome (using kraken2 and pavian) and for rapid bacterial metagenome assembly (metaspades), Biosynthetic gene cluster annotation (antismash and BiG-SCAPE) as shown in figure 1.
- Kraken 2 profiling results have suggested that there is significant difference between gut microbiome composition of males and females of extreme longevity which can be further correlated with global metadata of females comprising over 70% in the centenarian and super centenarian population.
- Cross referencing to identify novel BGCs by matching with MiBig databases suggest that out of 2110 identified BGCs most of them are novel.
- Again cross referencing with a larger database called BIGFAM to truly identify how many of identified clusters are novel.
- Family clustering of the novel Biosynthetic gene clusters and annotating family of project interest i.e family with BGCs particular to trait of extreme longevity suggests that 9 particular cluster families have possible role in trait of extreme longevity.
- Modulation of working scripts. For example, multithreading to decrease wall-time used to finish a particular job and addition of various flags in command for enhanced, accurate and more clear result.

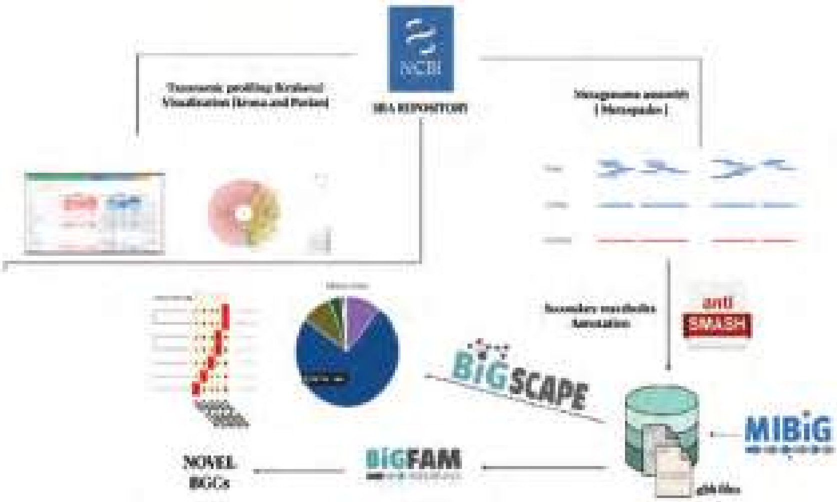


Figure 1. Workflow of taxonomic profiling and BGCs annotation.

SALIENT ACHIEVEMENTS

- Pipeline for fast profiling and visualization of metagenome, rapid bacterial metagenome assembly and Biosynthetic gene cluster annotation is developed.
- Through family clustering and annotating of the novel BGCs it is suggested that 9 particular cluster families have a possible role in the trait of extreme longevity.

FUTURE PERSPECTIVES

- Identification of bacterial taxa to which our cluster family of interest and related BGCs belong.
- After identification of taxa, study of BGCs present in most abundant gut bacterial species of people of extreme age and continuation with synthesis or expression of these BGCs.
- Then using High Performance computing and high throughput metabolomics identification of novel secondary metabolites that could be playing a significant role in the trait of extreme longevity.
- We are also developing a docker container containing all the tools and applications required for this pipeline and writing a combined script so that this process can be automated and large metagenome sample sets can be processed within a shortspan span of time which will help us to discover Biosynthetic potential of various ecosystems and speed up the discovery of novel secondary metabolites which can be used as replacement of antibiotics , can serve as probiotics and can have several implications in pharma industry.

AGRICULTURE BIOTECHNOLOGY

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Plant Genetics

Shrikant S Mantri, PhD
Computational Biology

RESEARCH ASSOCIATE
Dalwinder Singh, PhD

RESEARCH FELLOW
Raminder Kaur

AB 2.2: Predicting Protein-Protein Interaction with deep learning

The coiled-coil is a fundamental element of molecular engineering. It consists of two or more alpha helices curved in supercoiled form and encoded in a seven amino acid repeat designated as heptad (abcdefg). Coiled coils have been extensively used in protein oligomerization, resulting in protein-protein interactions and possess various applications such as in hydrogel material construction and to recruit the ligands to nanoparticles. Protein-protein interactions are essential for almost cellular functions. The notion to know about two interacting proteins is important for understanding protein function as well as for being able to disrupt their interactions. The basic leucine-zipper transcription factors (bZIPs) are a class of eukaryotic coiled coils transcription factors that form either homodimers or heterodimers that bind to dsDNA in a site-specific manner. bZIPs are similar in sequence and structure. The protein-protein interactions of bZIPs are specific, and this specificity is crucial for determining which DNA sites are being bound there. The simple structure makes them experimentally amenable and well suited for developing models of interaction specificity. While current models perform well at distinguishing interactions from non-interaction, they are not fully accurate or able to predict interactions.

RESEARCH PROGRESS

Following work has been completed so far towards the completion of this project:

» **Data Acquisition:** Data has been collected from the experimental studies, and it includes homo-dimer, heterodimer and non-binding instances of bZIPs proteins. The bZIPs proteins in the acquired data belong to Homo sapiens, Drosophila melanogaster, Caenorhabditis elegans, Saccharomyces cerevisiae, Ciona intestinalis, and Danio rerio species. In the current state of dataset, more than 800 instances represent interaction pairs while over 2000 instances are non-interacting pairs. An effort to collect more data is being undertaken.

» **Data Pre-processing:** We have removed all the sequences as well as labels containing any character other than 20 known amino acid symbols. Further, the subregions of the bZIPs proteins have been extracted to obtain the binding regions as well as to generate the negative instances replicating the real-world scenario. Further, the non-interacting pairs are made by selecting the sub-regions randomly from the non-bzip proteins to strengthen the data quality.

» **Data Encoding:** It is an important step to convert the symbol data into numeric one to provide the input to machine learning algorithms. Several data encoding schemes are available in the literature which will be tested for input data for learning. So far, one-hot and integer encoding with embedding have been applied. We aim to compare these schemes to select the best one. Moreover, multimodal learning scheme is also under consideration to improve recognition by extracting the secondary and tertiary structures of proteins.

» **Model Construction:** The advance deep learning approaches, such as transformers, are being explored to build the prediction model due to their better problem solving as well as generalization capabilities. So far, experiments have been performed with the transformers, and bi-directional Long short-term memory (LSTM) approaches.

» **Model Validation:** The datasets are split with a holdout approach and train-

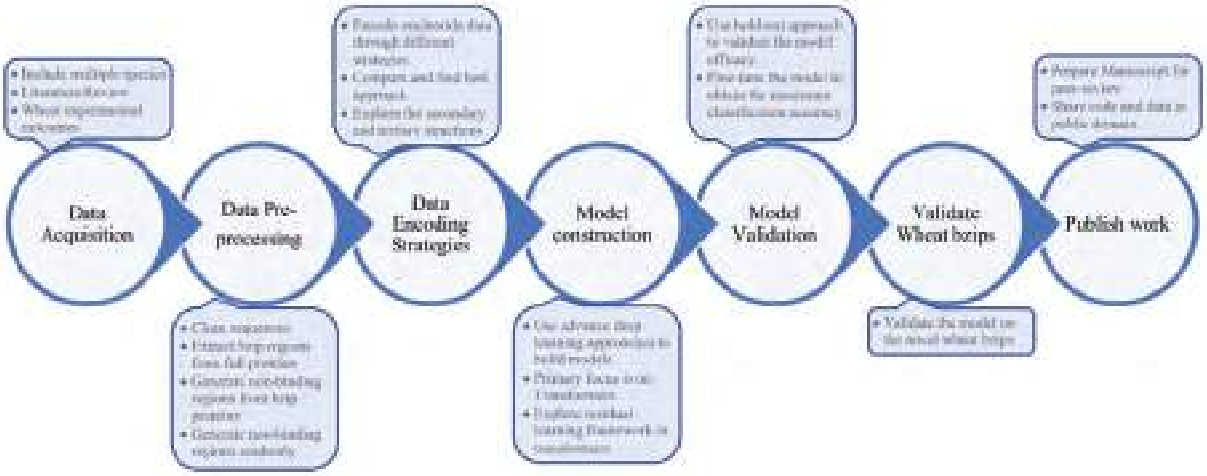


Figure 1. Workflow of predicting the bZIPs PPI interaction with deep learning approaches.

ing sets are used for building the model. The test set is used to validate and analyse the model capabilities in terms of under and over-fitting. Further, the proposed model will be tested on wheat bzips that were obtained through experiments by Dr. Vikas Rishi Lab to validate its efficacy.

» **Publish work:** Lastly, the proposed model will be published in a peer-review journal. Both code and datasets will be shared with the public to replicate results or find new bzips interaction pairs and advance research in this domain.

SALIENT ACHIEVEMENTS

- Transformers have been successfully applied to solve the BZIP binding problem.
- An automated tool is developed that takes an input of two protein sequences to estimate their interaction. There is no need to manually search for the zipper regions of proteins and their register for prediction.
- Protein interactions are predicted without considering the specific binding rules. Therefore, a generalized tool is developed to search for more interactions across kingdoms.
- A large number of BZIP proteins have been collected from the literature review which opens up possibilities to advance the work in this domain. For instance, a public database can be built from this collection of proteins.

FUTURE PERSPECTIVES

- The experiments will be performed to validate the model predictions on the novel BZIPs detected from the crop plants
- More BZIP data will be collected from the literature review to build a more powerful classification model
- The latest transformers such as residual learning mechanism, will be explored to improve the model performance.
- The tertiary structure of proteins predicted with the AlphaFold will be utilized to improve the model performance.

AGRICULTURE BIOTECHNOLOGY

Sivasubramanian, PhD
Plant Genomics

RESEARCH FELLOWS
Deepak Duhan
Shivani Gajbhiye
Damini

AB 2.3: Genomics assisted breeding for disease resistance against necrotrophic pathogens of Brassica crops

Oilseeds of the Brassica genus are significant contributors to the total edible oil consumed worldwide, second only to soybean oil. *Brassica juncea* is an important oilseed crop in Asia, while *Brassica napus* is mostly grown in Europe and Canada. Fungal diseases are major destabilizers with respect to growth and yield of mustard. Our work focuses on Alternaria blight or black spot, a widespread and destructive disease caused mostly by *A. brassicae*, infecting all aboveground parts of the plant. The high incidences of *A. brassicae* infection is accompanied by infections on the seed pods thereby affecting oil quality and quantity. *Alternaria* spp. are also one of the major factors contributing to post-harvest losses in the vegetable Brassicas. In India, the yield losses are particularly high in the hilly regions and foothills of the mountain ranges where the climate is more conducive (cool and foggy) for infection and growth of *A. brassicae*. The high incidence of *A. brassicae* has practically restricted the cultivation of oilseed Brassicas in these regions. No source of resistance is available in any of the cultivated Brassica species. The activities undertaken during this project deals with 1) prospecting for resistance genes in the landraces and wild germplasm of Brassicas and related species for resistance 2) characterisation of the pathogenic effectors and identification of their interacting partners in the host. Subsequently, strategies will be determined to utilize the resistance/susceptibility genes in breeding programmes through the use of marker-assisted breeding/gene-editing technologies.

RESEARCH PROGRESS

Prospecting for resistance genes in the landraces and wild germplasm of Brassica species

- Genome-wide association mapping was carried out to identify the genes/loci responsible for secreted peroxidase (sPOX) activity in response to culture filtrate of *A. brassicae*. We found 28 SNPs in six genomic loci to be significantly associated with the trait. Using the information of linkage disequilibrium in the six genomic loci we could map the 28 top SNPs to 134 putative candidate genes.
- In order to prioritize the number of candidate genes to be taken up for functional validation, we undertook a transcriptomic study to evaluate the genes that are differentially expressed in *B. juncea* upon infection with *A. brassicae*. We used two different time points of infection (2- and 4 -days post infection) to define early and late-responsive genes to the invading pathogen. We used a stringent criterion of Log2FC > |1.5| and padj < 0.05 to define significantly differentially expressed genes. Using this criterion, we found 8022 genes to be differentially expressed at 2 dpi and 7484 genes to be differentially expressed at 4 dpi. Out of the 8022 genes, 4959 genes were significantly upregulated, whereas 3063 genes were significantly downregulated. Similarly, 4424 genes were upregulated and 3060 genes were downregulated at 4 dpi.
- Further, we overlapped the two datasets viz. the GWAS candidate genes and the differentially expressed gene sets to prioritize putative candidates for further functional studies. Using this approach, we could narrow down the list of putative candidates to 18 genes. These 18 genes are now being validated using reverse genetics approaches.

Characterisation of the pathogenic effectors and their interacting partners in the host

- We identified an effector (ABRSC13.48) that was highly upregulated during infection of the host (B. juncea). ABRSC13.48 was upregulated ~400-fold at 2 dpi and ~800-fold at 4 dpi with respect to plate grown mycelia. ABRSC13.48 had no sequence similarity to any known domain or protein in the NCBI or Uniprot

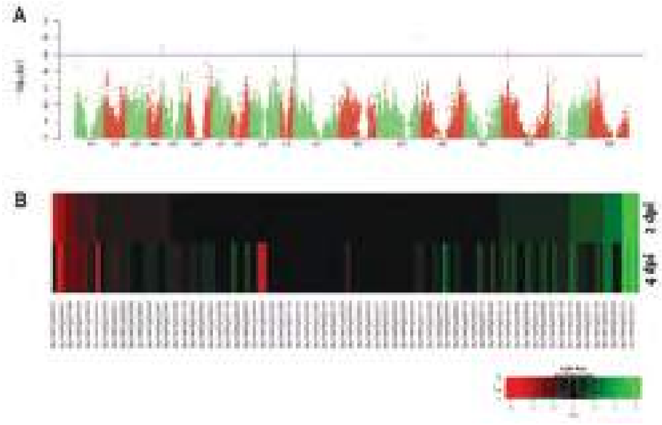


Figure 1. GWAS and prioritization of candidate genes. A) GWAS of sPOX activity in 261 accessions of B. juncea; B) Heatmap of expression values of 134 candidate genes from GWA analysis.

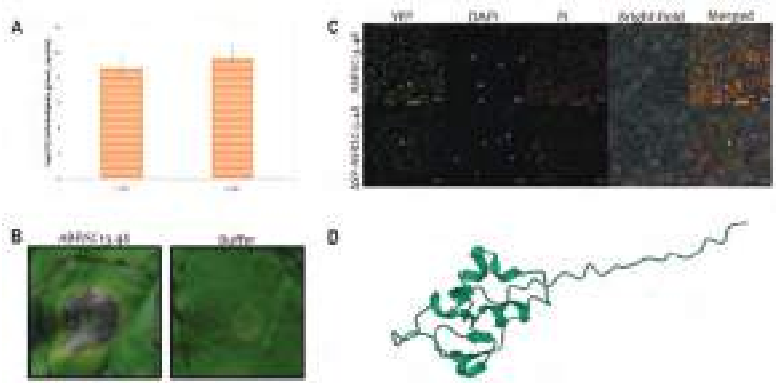


Figure 2. Functional characterisation of ABRSC13.48. A) Relative expresson of ABRSC13.48 during infection; B) Induction of necrotic cell death in host (B. juncea) and nonhost species (N. benthamiana); C) Subcellular localization of ABRSC13.48 in N. benthamiana; D) 3-D structure of ABRSC13.48 as modeled by AlphaFold2 (AI system developed by DeepMind).

databases. Agrobacterium-mediated expression of ABRSC13.48 in N. benthamiana led to induction of cell death in the plant. We found that ABRSC13.48 localized to the apoplastic space but did not induce any pattern-triggered immunity in nonhost as well as host plants. Experiments are underway to determine the host-interacting partner of this effector.

SALIENT ACHIEVEMENTS

- GWA mapping of PTI responses of B. juncea in response to A. brassicae CF.
- Transcriptome analysis of B. juncea leaves infected with A. brassicae at two different time points of infection.
- Identification of an effector of unknown function that is highly expressed during infection of the host – B. juncea.

FUTURE PERSPECTIVES

- Functional validation of 18 candidate genes identified in GWA mapping through reverse genetics approaches.
- Identification of interacting partners of the unknown effector in the host as well as nonhost plant.

PROGRAM - AB03

AGRICULTURE BIOTECHNOLOGY (AB)

BASIC BIOLOGY FOR CROP IMPROVEMENT

AGRICULTURE BIOTECHNOLOGY

Vikas Rishi, PhD
Plant Genetics

RESEARCH FELLOW
Harshita Pandey

AB 3.1: Improved specificity of CRISPR/Cas genome-editing system in the presence of divalent metal ions

The clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9) system consists of an array of identical repeats separated by spacers of viral origin, flanked by diverse cas genes. In nature, CRISPR plays a role in adaptive bacterial immunity by fending off viruses and other foreign genetic elements. The system has been repurposed as a powerful RNA-guided DNA targeting platform for genome editing, transcriptional perturbation, epigenetic modulation, genome imaging etc. Immunity by CRISPR-Cas involves three different stages, namely, a) adaptation, b) expression and c) interference; In the adaptive phase, bacteria and archaea capture snippets of sequences called protospacers from the foreign viral or plasmid genome. In the second phase, called the expression phase, the transcription of crRNA occurs. The third phase is interference, in which crRNA forms a ribonucleoprotein complex along with tracrRNA and Cas9 to mark the silencing of complementary foreign sequences. Significant developments in CRISPR-Cas have led to its wide use in many translational science and medicine applications. Although a versatile and expeditious method for genome editing, CRISPR's limitations lie in the tool's specificity. Sometimes Cas nuclease cleaves dissimilar but homologous sequences bearing some sequence similarity to the intended target, cleavage of which could lead to loss of function mutations in a functional gene. Knowing the versatility, vast and widespread use of this technology, it's evident that the specificity of this tool is highly desirable for its better use in basic sciences and therapeutics. Our efforts are focused on understanding the basic mechanism that underlies off-targeting, suggesting ways to increase the target specificity of this technology. We are interested in understanding the role that divalent ions, namely magnesium and manganese, play in

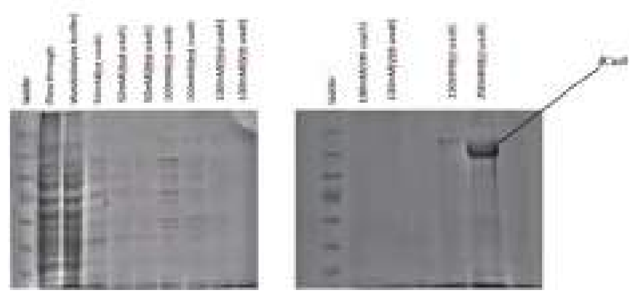


Figure 1. 8% SDS-PAGE after hydroxyapatite purification, elution was done in 250mM elution buffer.

Figure 2. 2% agarose gel showing sgRNA (101 bp); lane 1, 50 bp ladder, lane 2, sgRNA.

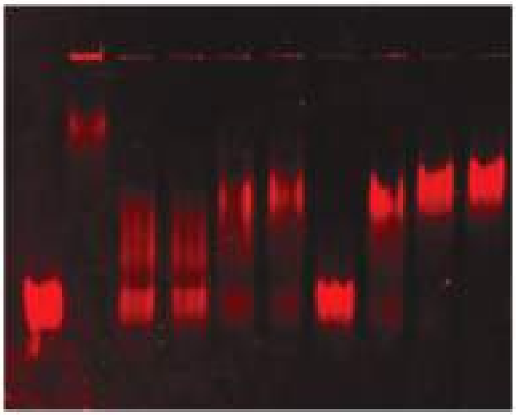


Figure 3. 5% Native-PAGE showing dCas9-sgRNA binding with the DNA duplex (lane 1, IR700 labeled DNA only, lane 2, DNA with dCas9, lane 3-6 increasing concentrations of complex with duplex, lane 7, binding in presence of poly di-dc (protein with OE tag), lane 8-10, increasing concentrations of complex with duplex (protein without OE tag).

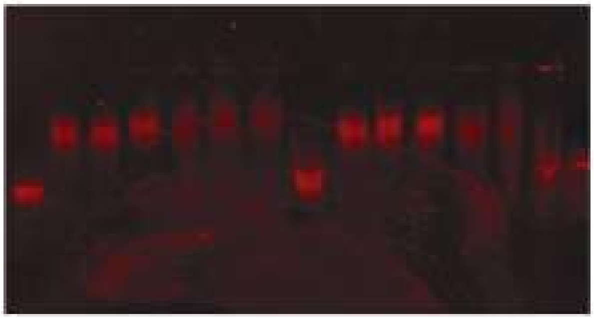


Figure 4. 5% Native PAGE showing binding of dCas9-sgRNA complex to both on- and off target site (lane1-7 on target), lane 8-14 off-target); lane 1, control, lanes 2-4 (300-800 nM dCas9-sgRNA; in absence of magnesium chloride), lanes 5-7; 300-800nM dCas9-sgRNA in presence of magnesium chloride, lane 8 control, lanes 9-11 (300-800nM dCas9-sgRNA in absence of magnesium chloride), lanes 12-14, 300-800nM dCas9-sgRNA in presence of magnesium chloride.

target specificity. These two ions are present in the enzyme's catalytic domains. Another part of this study focuses on analysing the off-target mutations by changing the composition and structure of sgRNA. Changes in guide RNA will be made to study the mismatch tolerance range of the guide RNAs. Gains from such a study will help us design and optimise the CRISPR/Cas system for efficiently editing a eukaryotic genome.

RESEARCH PROGRESS

Cloning, expression, characterisation and purification of dCas9 protein: We cloned dCas9 in the pet5 vector. The clone was confirmed by restriction digestion and Sanger sequencing. The protein was expressed in BL21 DE3 and further purified using hydroxyapatite purification (Fig 1). The identification of Cas9 protein was confirmed using Nano LC-MS/MS.

Assessment of activity of dCas9: Oligos were designed to bind the defined target site. sgRNA was synthesised with the help of an in-vitro transcription kit (Fig 2). The protein's activity (with and without an overexpression (OE) tag) was checked using gel mobility shift assays (Fig 3).

Electrophoretic Mobility Shift Assay (EMSA) in presence of magnesium ion: Binding assays were performed in the presence of 10 mM magnesium chloride (in reaction) to observe its effect on binding. Stretch of GFP & YFP was chosen as on- and off-target sites, respectively. dCas9-sgRNA complex bind to both the DNA, but the presence of 10mM Magnesium chloride causes a 3-fold decrease in binding to off-target sites (Fig 4).

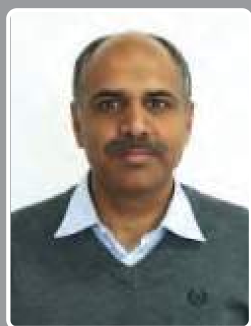
SALIENT ACHIEVEMENTS

- Cloning, expression, purification and assessment of the activity of dCas9 have been completed.
- Binding assays in the presence of divalent ions showed that magnesium causes a decrease in binding to off-targets.

FUTURE PERSPECTIVES

- Binding assays in the presence of another divalent metal ion, i.e., manganese and 150 mM KCl, will be performed. Also, binding assays with sgRNA targeting oligos with mismatches at various positions at the distal end will be performed.
- In vitro cleavage assays will be performed in the presence and absence of metal ions.
- Interaction studies to decipher the binding, stability and thermodynamics of the RNP complex formation.

AGRICULTURE BIOTECHNOLOGY



Ajay K Pandey, PhD
Plant Molecular Biology

RESEARCH FELLOWS
Anuj Shukla

AB 3.2: Functional characterization of wheat inositol pyrophosphate kinase

Inositol pyrophosphates (PP-InsPs) are high-energy derivatives of inositol, involved in different signaling and regulatory responses of eukaryotic cells. Distinct PP-InsPs species are characterized by the presence of phosphate at a variable number of the 6-carbon inositol ring backbone, and two distinct classes of inositol phosphate kinases responsible for their synthesis have been identified in Arabidopsis, namely ITPKinase (inositol 1,3,4 trisphosphate 5/6 kinase) and PP-IP-5Kinase (diphosphoinositol pentakisphosphate kinases). Plant PP-IP5Ks are capable of synthesizing InsP8 and were previously shown to control defense against pathogens and phosphate response signals. However, other potential roles of plant PP-IP5Ks, especially towards abiotic stress, remain poorly understood. Earlier we show that, PP-IP5K like protein referred as TaVIH2 when overexpressed in Arabidopsis resulted in drought tolerance or water deficit stress. In this report we show the protein-protein interaction of wheat VIH2-3B protein.

RESEARCH PROGRESS

In plants, VIH proteins have been reported in multiple plants, including wheat. We plan to check if the wheat VIH2 could posses PP-IP5K like activity that is similar to yeast and also perform protein-protein interactions studies to obtain clues for its new functions.

» Herein we attempted to check if wheat VIH2 could possess PPIP5K like protein kinase activity. We generated IP7 mouse IP6K1 enzyme and IP6 as a substrate. The purified IP7 was then used as a substrate for further studies. The TaVIH proteins were incubated with InsP7 as a substrate for two-time points (1 and 2 hr), and the products were resolved by PAGE. Our experiments suggest that InsP8 was synthesized only by TaVIH2-KD when InsP7 was provided as a substrate (Figure 1). During this period of incubation, no detectable levels of

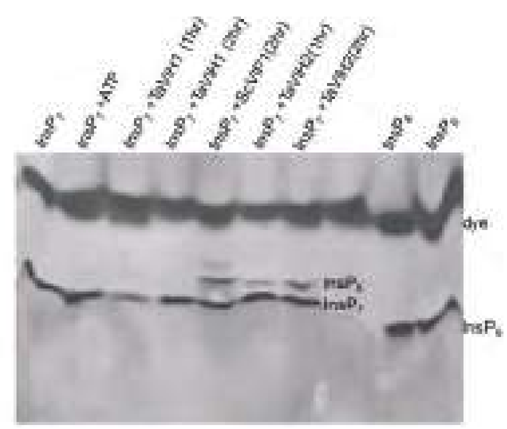


Figure 1. Visualization of PP-InsP products on the PAGE-GEL (33%). The In-vitro kinase reactions were performed using 30 ng of ScVIP1-KD (KD-kinase domain), TaVIH1-KD and TaVIH2-KD purified proteins for 1 and 2 hr at 28o C. The reactions were then resolved on the gels (TBE-PAGE). The photo was taken after staining by Toluidine Blue.

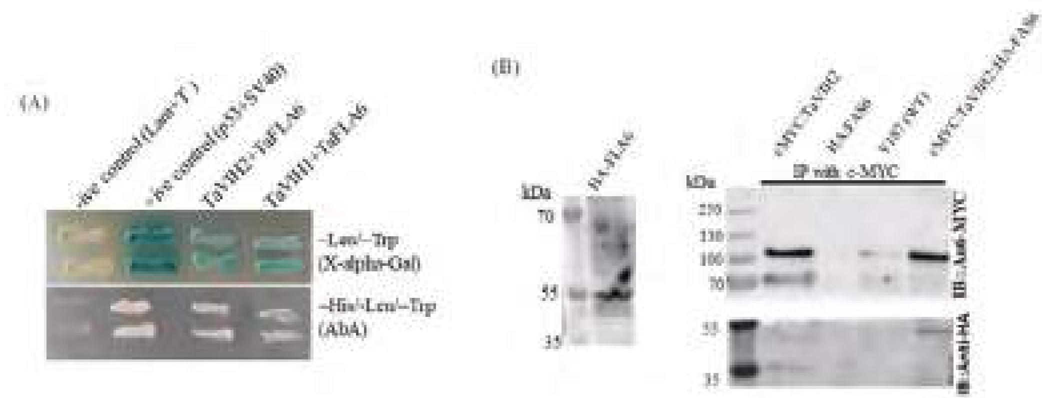


Figure 2. Interaction of wheat VIH2 with FLA6. (A) Confirmation of the yeast two hybrid screen of TaVIH2 with TaFLA6 on the SD-LHT plates supplemented with X-alpha-Gal. (B) Co-immunoprecipitation of TaFLA6 and Western analysis of c-MYC fused TaVIH1 or TaVIH2 proteins in the yeast strain to confirm the in-vivo interactions in the yeast.

the product were seen for the TaVIH1-KD reactions. In contrast, upon a longer incubation with substrates (~9 hrs), we observed that InsP8 was generated by both VIH1 and VIH2 proteins, suggesting that TaVIH1 may have a lower enzyme activity compared with TaVIH2. We have further confirmation the formation of the phosphorylated inositol molecules by using MALDI-ToF- MS analysis.

» The analysis of Human PPIP5K and yeast VIP1, resulted in the study of protein-protein network analysis with nodes pointing towards the PHO mediated interactors for the VIP and involvement of HsPPIP5K in other biochemical activities (Figure 1). A similar analysis for wheat VIH shows multifunctional globular proteins such as Actin as a probable interactor.

» Yeast two hybrid interaction was done using the TaVIH2-3B as a bait resulted in identification of many protein interacting clones and majority of them encoded for the cell-wall plasticity related transcripts. Further Co-immunoprecipitation with one such clone referred as FLA6 encoding for Fasciclin like protein was shown to be plasma-membrane localized (Figure 2). Further studies are ongoing to evaluate the localization of the membrane protein/s that may be dependent of the IP7/IP8 levels in the cells.

SALIENT ACHIEVEMENTS

- Role of functional PPIP5K like activity of the wheat VIH proteins was confirmed by using the enzymatic activity.
- Yeast two hybrid screen provides insight into the interaction proteins of VIH2 with multiple proteins involved in cell-wall related plasticity.

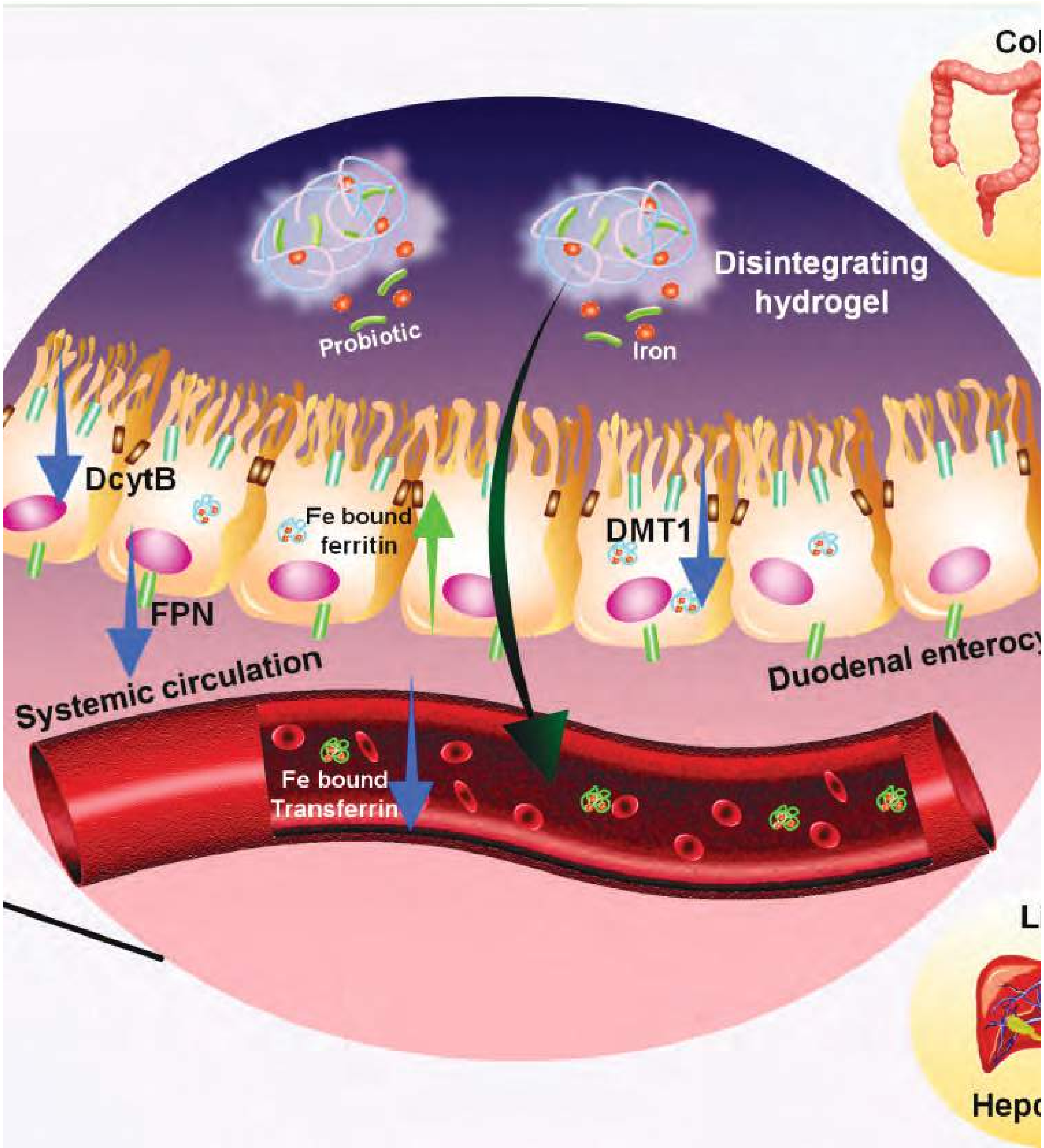
FUTURE PERSPECTIVES

- Biochemical characterization of the Arabidopsis lines overexpressing the TaVIH2.
- Functional assays for the interacting partners of TaVIH2 with multiple proteins and their localization dependency on the presence of IP7/IP8.

PROGRAM - FNB 01

FOOD & NUTRITIONAL BIOTECHNOLOGY (FNB)

FUNCTIONAL FOODS AND NUTRACEUTICAL FOR BETTER HEALTH



FOOD & NUTRITION

FNB 1.1: Effects of plant-derived microvesicles on adipogenesis

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Eukaryotic cells secret membrane-derived, heterogeneous extracellular vesicles (EV) of different sizes (30-2000 nm). Plant-originated vesicles known as Plant-derived EVs (P-EVs) contain proteins, RNAs, lipids, and other metabolites that can be isolated from the juice and tissues of the plants. Purified from several edible species, these EVs are biocompatible, biodegradable, and their cargo- bioavailable, making them useful for cell-free therapy. Emerging evidence from clinical and preclinical studies suggests that P-EVs have many beneficial effects on addressing human morbidities. Among the population of EVs, microvesicles (100-1000 nm) have the potential to be used as therapeutics due to ease of isolation. Mu J et al. (2014) demonstrated interspecies communication between plant and mouse gut host cells through edible plant-derived exosome-like nanoparticles derived from carrot, ginger, grape etc. These edible nanoparticles lead to the activation of anti-inflammatory cytokines, Wnt signalling and antioxidants, which help in maintaining intestinal homeostasis. Ginger rhizomes-derived exosomes alleviate inflammation-related complications in mice models, including cancer. No such studies are carried out where ginger-derived microvesicles have been used to address lifestyle diseases like obesity. This study attempts to analyse the effects of ginger microvesicles on the differentiation of preadipocytes to lipid-laden adipocytes that directly affect human obesity..

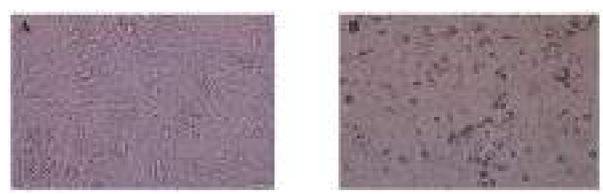

Figure 1. 3T3-L1 differentiation. (A) cells before differentiation, (B) cells after differentiation.

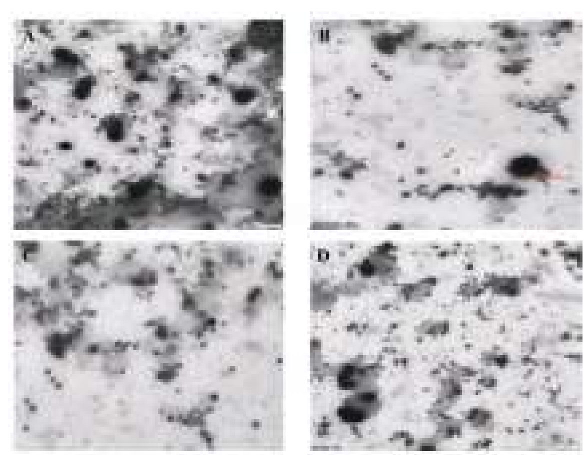

Figure 2. Morphology of microvesicles derived from ginger under transmission electron microscope (TEM).

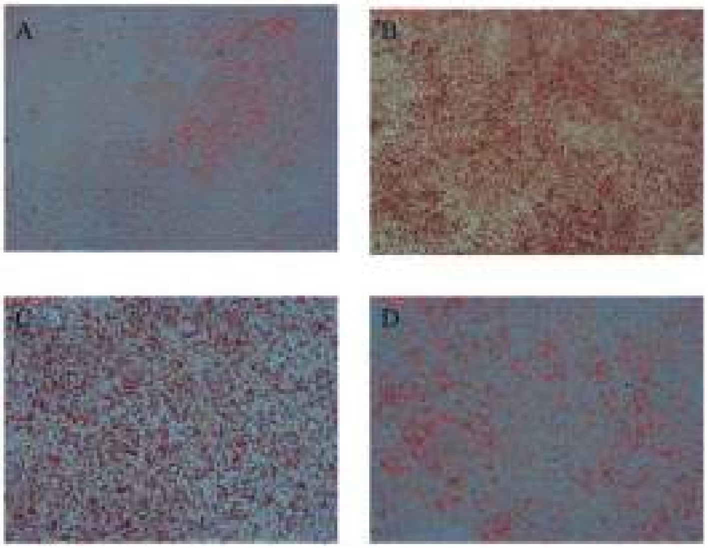

Figure 3. Inhibitory effect of ginger juice-derived microvesicles on lipid accumulation in 3T3-L1 adipocytes. 3T3-L1 cells were incubated with 10, 20 micrograms of ginger-derived microvesicles for 8 days Cells were stained with Oil Red O. (A) undifferentiated cells, (B) control without treatment, (C&D) treated with 10 micrograms ginger microvesicles (D) treated with 20 micrograms ginger microvesicles.

RESEARCH PROGRESS

Cell line: 3T3-L1 cells (NCCS, Pune), a preadipose cell line developed from murine Swiss 3T3 cells, are used for the ex-vivo analysis of ginger-derived microvesicles' effects on cell differentiation leading to adipogenesis. Cell lines were grown in tissue culture plates with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100U/ml penicillin, and 100 µg/ml streptomycin at 37°C in a 5% CO2 atmosphere. Microvesicles from ginger were isolated from the rhizome juice. Unlike exosomes, Microvesicles are isolated at lower centrifugation (15000 rpm), circumventing the need for ultracentrifugation. 3T3-L1 cells were differentiated in the presence and absence of ginger-derived microvesicles (10 and 20 micro grams) for 8 days. It was found that there is a significant decrease in lipid accumulation at 10 and 20 µg (Fig. 9C and fig. 9D).

SALIENT ACHIEVEMENTS

- High-quality exosomes were isolated from ginger juice using a moderate centrifugation protocol. Inhibitory effects of microvesicles were demonstrated on the differentiation of preadipocyte 3T3 cells to oil-filled adipocytes. Treatment with 10 & 20 micrograms of vesicles inhibits oil accumulation by 3T3 cells as demonstrated by oil o red staining.

FUTURE PERSPECTIVES

- We plan to undertake the transcriptomic and proteomic analysis of the cells treated with plant-derived vesicles to decipher how lipid accumulation can be alleviated through exosome treatment.

FOOD & NUTRITION

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FNB 1.2: Beneficial manipulation of gut bacteria as a strategy for the management of metabolic disorders

Human gut comprises various microorganisms which get affected due to the diet they consume. Diet rich in beneficial nutrients and antioxidants help in enriching the gut microbiota with beneficial microbes. One such component are polyphenols that are antioxidant and anti-inflammatory in nature. Besides these properties, they are recently recognised to stimulate the healthy gut microbiota and may provide beneficial effects to the host health. Our previous studies suggested that (i) finger millet and kodo millet whole grain and bran supplementation prevented high fat diet induced obesity by reducing chronic low grade inflammation; (ii) finger millet arabinoxylan also could prevent high fat diet induced obesity through beneficial modulation of the gut bacteria and reducing the metabolic endotoxemia and (iii) finger millet polyphenol rich extracts (FM-PRE) and Kodo millet polyphenol rich extracts (KM-PRE) could counteract the high fat diet induced obesity through preventing the adipose tissue hypertrophy and reducing the inflammation. However, there are no studies on the ability of millet polyphenols in counteracting the high fat high sucrose diet induced metabolic aberrations and gut dysbiosis. Hence, the objectives of the study are to evaluate the role of Finger millet polyphenols (FM-PRE) and Kodo millet polyphenols (KM-PRE) in preventing lipid accumulation and associated liver inflammation and oxidative stress using human liver cells (HepG2 cells) and to evaluate their beneficial effect in counteracting the high fat and high sucrose diet induced obesity and associated metabolic complications.

RESEARCH PROGRESS

***In vitro* lipid accumulation in HepG2 cells and its amelioration using FM-PRE and KM-PRE:** The human hepatoma HepG2 cancer cells were cultured in Dulbecco Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), and 1% Penicillin-Streptomycin (P/S). The cells were incubated under 37°C with 5%CO2. Effect of various doses of palmitic acid (50, 100, 150, 250, 400 and 500 µg/ml), FM-PRE and KM-PRE treatments on cell viability was determined using the MTT assay method.

Lipid accumulation by the HepG2 cells: HepG2 cells were seeded on culture plates and incubated with FM-PRE and KM-PRE at a dose of 1mg/ml respectively along with 250 µM/ml of palmitic acid for 24 h. Then the intra cellular lipids accumulated were visualised by Oil Red O staining and quantified after DMSO extraction of the dye and measuring the optical density at 490 nm using a microplate reader. Furthermore, the cells were disrupted and intracellular ROS and oxidative stress parameters were determined as per the established protocols. In the cell culture supernatants, levels of various inflammatory markers were determined using commercially available ELISA kits as per the manufacturer's instructions.

Protective effects of millet polyphenols on the high fat high sucrose diet induced hepatic steatosis in mice: The entire animal study protocol was approved by the institutes animal ethics committee of NABI, Mohali (approval number: NABI/2039/CPCSEA/IAEC/2019). Male C57BL/6 mice (8-10 weeks of age and weight of 18-20 g) were acclimatized under the standard experimental conditions (temperature 22°C; humidity 55 ± 5 %; 12 h light and dark cycles) for one week at the Animal Experimentation Facility of NABI, Mohali. Water and food were provided ad libitum. Weight normalized mice were randomized into the following 6 groups (n=8): Control; High fat high sucrose diet (HFHSD); FM-PRE per se; FM-PRE+HFHSD; KM-PRE per se and KM-PRE+HFHSD groups. The polyphenol intervention groups were orally fed with FM-PRE and KM-PRE respectively in PBS by gastric gavage on every day for 12 weeks during the light cycle whereas the HFHSD group was supplemented with PBS as vehicle. The HFHS diet contained 45% calories

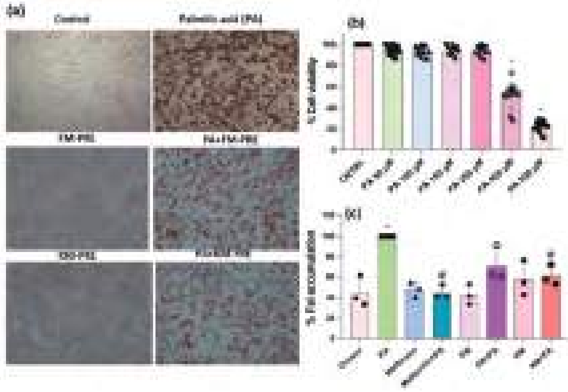

Figure 1. Effect of Palmitic acid, FM-PRE and KM-PRE co-treatment on HepG2 cells (a) Lipid accumulation by ORO staining; (b) Cell viability; (c) Lipid accumulation (%). * vs control; # vs significant to PA treated cells.

derived from lard and 35% from sucrose. Morphometric parameters, food and water intake were recorded every alternate week. At the end of the 12th week, an oral glucose tolerance test (OGTT) was determined. On the day of sacrifice, blood samples were collected by retro-orbital plexing. Blood was allowed to clot and centrifuged at 3000 g for 10 min at 4oC. The animals were sacrificed by cervical dislocation and various tissues, faeces and cecum contents were collected. Serum, tissues, faeces and cecum contents were stored at -80 °C until further use.

Serum biochemistry: Lipid profiling, IL-1β, IL-6, and TNF-α levels in serum were determined using the ELISA method as per the manufacturer's instructions.

Liver biochemical analysis: Approximately 10-20 mg of liver tissues were homogenized in ice-cold PBS containing a mixture of protease inhibitors followed by sonication and centrifugation (10,000 g; 10 min; 4°C). TNF-α, IL-6 and IL-1β levels were determined in the supernatant using ELISA kits as per the manufacturer's instructions. Furthermore, total cholesterol, triglycerides, HDL, LDL were estimated using Accurex kits. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined using Accurex kits as per the manufacturer's protocol.

***Ex vivo* gut permeability assay:** Gut permeability assay was performed ex vivo using 2 cm colon tissue after thorough washing and flushing with ice cold saline. Further, 70μL of FITC based nanoparticles was filled in the saline flushed colon and the ends were tied using silk thread which is further placed in a 150 mL prefilled PBS buffer having pH of 7.4. The solution kept on continuous mild stirring. Then, 1 mL of PBS was taken from the respective PBS unit at 15 min time intervals (0, 15, 30, 45, 60, 75, and 90 min) and replaced with the same amount of fresh PBS buffer to maintain the exact condition. Fluorescent intensity of FITC loaded nanoparticles released from the colon into the buffer was measured at different time intervals. Images of the tubes were captured using IVIS Lumina Series-III in ILS mode (Perkin Elmer, USA) and analysed using image 5.0 software (Perkin Elmer, USA) at 480 nm excitation and 520 nm emission.

Oxidative stress biochemical analysis: A 50 mg of the tissue from liver, colon, ileum, brain, WAT and BAT were homogenized in ice cold PBS containing triton x-100 and protease inhibitor cocktail. Homogenates were centrifuged at 12000 g for 15 min at 4°C and the supernatants were collected and stored at -20 °C until further experiments. Protein content in the homogenate was determined by the Bradford method. The tissue levels of Malondialdehyde (MDA), Superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were determined as per the established protocols.

Relative abundance of selected gut bacteria in ceacal contents: Genomic DNA was extracted from 100 mg of ceacal contents from each mouse and the purified DNA was quantified using Infinite M200 PRO Nanoquant. Furthermore, the integrity of the DNA was checked on 0.8% agarose gel. SYBR green based RT-PCR was performed to determine the abundances of selected gut bacteria. The data was normalized using the total bacteria and the results are expressed as relative fold change of bacterial DNA abundance compared with the control group.

RNA extraction and qPCR: Total RNA was extracted from colon and liver using the trizol based RNA extraction method, integrity was confirmed on the 1.2% agarose gel and the amount of RNA was quantified using Nanodrop at 260/280 nm. Genomic DNA was removed by DNase digestion and 1 μg RNA was reverse transcribed to cDNA and then the changes in the gene expression were determined by SYBR green based qPCR. The results were expressed as fold change relative to the control group.

Statistical analysis: GraphPad Prism 8 software (GraphPad prism 8.0 software Inc., La Jolla, CA, USA) was used to analyse the data. Values were expressed as

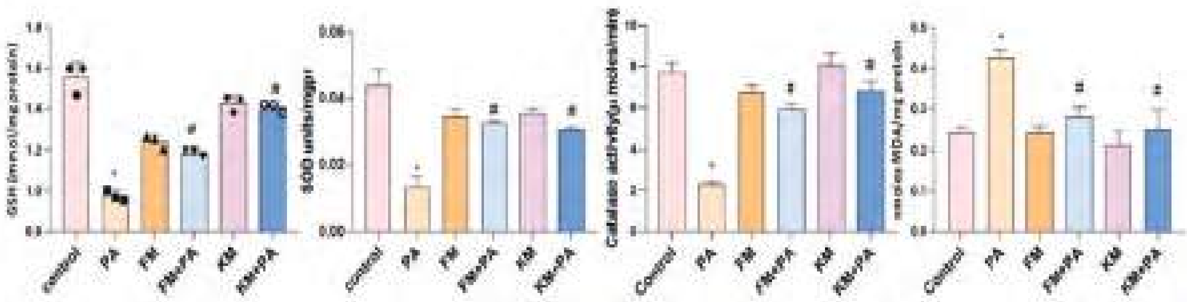

Figure 2. Effect of Palmitic acid, FM-PRE and KM-PRE co-treatment on oxidative stress parameters in HepG2 cells. * vs control; # vs significant to PA treated cells.

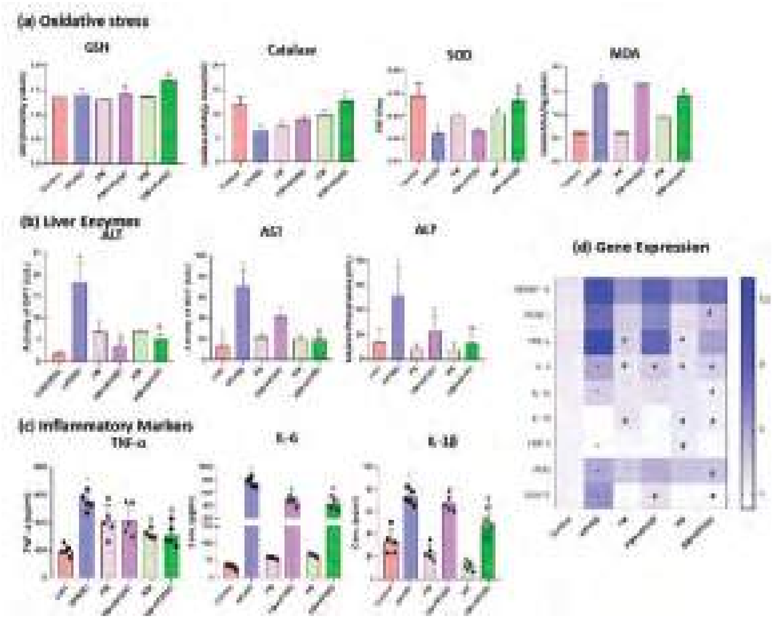

Figure 3. Effect of FM-PRE and KM-PRE supplementation on HFHS diet induced liver abnormalities. (a) Oxidative stress parameters; (b) Hepatic Enzymes; (c) Inflammatory markers and (d) Gene expression changes.

mean ± SEM. One-way ANOVA followed by Tukey's post-hoc test was used to compare differences among the experimental groups. $P \leq 0.05$ was considered as statistically significant.

***In-vitro* lipid accumulation in hepatic HepG2 cells and its amelioration using FM and KM-PRE:** Palmitic acid was more potent in inducing lipid accumulation in liver cells as compared to oleic acid at 250 μM concentration. In Oil Red-O staining, we could observe prominent lipid accumulation in the PA group which was prevented upon FM-PRE and KM-PRE treatments (Fig.1). In the PA group, increased oxidative stress was observed where there were higher MDA levels and decreased levels of GSH, SOD and catalase were observed while FM-PRE and KM-PRE co-treatment with PA prevented these harmful changes (Fig.2). It signifies that FM-PRE and KM-PRE were potent to ameliorate the palmitic acid-induced lipid accumulation suggesting their use in ameliorating MAFLD progression.

Prevention of high fat high sucrose diet induced metabolic changes: HFHS diet disrupted the glucose homeostasis as there was higher fasting blood glucose levels in the serum and impaired glucose tolerance relative to the control animals. FM-PRE and KM-PRE co-supplementation along with HFHS diet prevented these harmful changes. HFHS diet feeding caused hepatic steatosis as elevated levels of total lipids, triglycerides, total cholesterol, LDL and decreased levels of HDL was observed. Besides this, HFHS diet also caused elevation in oxidative stress, levels of ALT, AST and ALP along with elevated and inflammatory cytokines in the liver homogenates (Figure 3a-3c). Gene expression of some of the marker genes in lipid biosynthesis pathway were significantly elevated (Figure 3d). FM-PRE and KM-PRE supplementation along with HFHS diet prevented these lipid derangements, steatosis, lowered the oxidative stress, levels of ALT, AST and ALP and decreased the levels of inflammatory cytokines. HFHS diet caused dysbiosis and significant loss in barrier integrity as high levels of total radiance in HFHSD as compared to control group was observed in the ex vivo studies on the colon tissue. FM-PRE and KM-PRE supplementation along with HFHS diet improved the commensal bacterial abundances and prevented the loss in barrier function. Even though FM-PRE and KM-PRE offered protection, KM-PRE was more potent in preventing these harmful changes.

SALIENT ACHIEVEMENTS

- Chronic low grade inflammation, a hallmark of obesity, mainly contributes for multiple comorbidities such as insulin resistance, type 2 diabetes and hepatosteatosis. Bioactives from finger millet and kodo millet could counteract obesity and hepatosteatosis development.

FUTURE PERSPECTIVES

- Understanding the molecular events in the protective ability of millet polyphenols against hepatic lipid accumulation caused by Palmitic acid.

FOOD & NUTRITION

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FNB 1.3: Development of novel cobiotic formulations for metabolic disorders

Over the years, many anti-obesity medications have been developed for the management of obesity, but most have been withdrawn. These medications reduce or control weight by affecting one or more of the fundamental physiological processes of hunger and satiety by controlling neuronal and hormonal signals. Because these processes are essential for growth and development, these medications contain the potential for adverse effects, including cardiovascular and neurological complications. The experience with anti-obesity drugs has produced well-founded cynicism about new drugs. Given the rapid development and major risks of the current obesity epidemic, how long can we wait for effective and safe treatments? Life style change (diet and exercise) has been seen that best and most effective way to counterweight gain. It is important to have dietary regulations or physical activity to prevent weight gain associated life style problems rather than to search for the treatment. Available literature suggests that sensory ion channel receptor system, Transient Receptor Potential (TRP) channels, are possible candidates to regulate energy metabolism and thermogenesis, via different mechanisms. In our previous reports we have shown that TRPV1, TRPM8

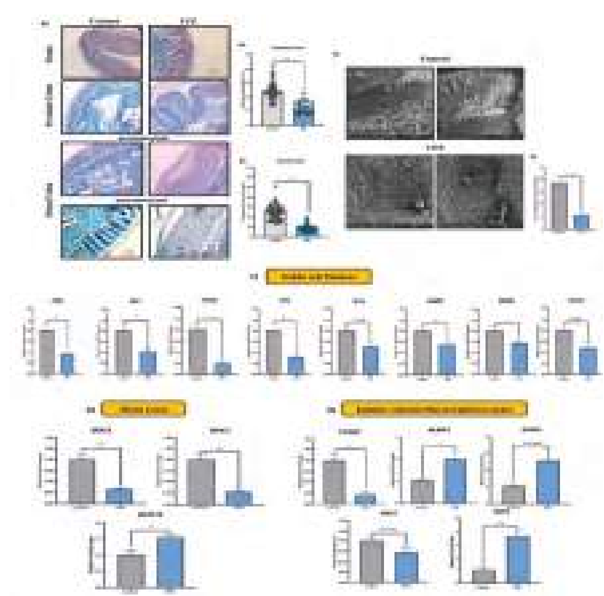


Figure 1. Effect of CPZ rectal administration on colonic mucus production: Histology representative images of A) Hematoxylin & Eosin – Alcian blue staining in the ileum, proximal and distal colon (n=3), High iron diamine – Alcian blue staining in distal colon (n=3), B) Thickness of mucus layer in the proximal colon, C) Thickness of mucus layer in distal colon. D) Representative images for SEM of colon tissues and E) Number of colonic mucus extrusion (n=3). Gene expression analysis for F) Goblet cell markers, G) Mucin genes and H) Immune response/mucus regulation in the colon (n=6). Mice were divided into two groups – Control (administered vehicle rectally) and CPZ (administered 531 μM CPZ rectally). Treatment was given for 2 weeks. After sacrifice, colon tissues were fixed in Carnoy's solution and subjected to serial dehydration with gradient ethanol (25%, 50%, 75%, 90%, and twice in 100%) for 2 h each followed by clearing in xylene (1 h twice). The colon tissues were embedded in paraffin and 5 μm sections were cut. For staining, the sections were deparaffinized in xylene (5 min, twice) followed by rehydration in gradient ethanol (100% twice, 90%, 70%, 50%, 25%, and PBS, 2min each). The samples were treated with respective stains (H&E: 2min each, AB: 15min, HID: 18h, AB: 30min) followed by washing (5min, thrice after each stain) in water. Sections were dehydrated, treated with xylene, and mounted with DPX mounting medium. Microscopy was performed for imaging and mucus thickness was measured using Imagescope. For H&E-AB staining, images were captured at 10X and

for HID-AB staining, images were captured at 20X. The mucus layer in H&E-AB stain tissues is presented with red arrows in the figure. For SEM, colon sections were cut open (lumen was exposed) and fixed in 2.5% glutaraldehyde. Following, the tissues were washed with PBS (15 min, thrice at 4 °C). The tissues were post-fixed in 1%osmium tetroxide for 2 h and again washed with PBS (15 min, thrice at 4 °C). The tissues were then serial dehydrated in acetone (30%, 50%, 70%, 90%, 95%, 100%, 30 min at 4 °C). The samples were air-dried and spur-coated with gold (10 nm) and images were taken on a scanning electron microscope at 1000X. In SEM image analysis, (M in red) depicts colonic mucus extrusions, (GC in green) depicts goblet cells, and (C in yellow) depicts crypts of the colon. The mucus extrusions were counted using Imagescope. For gene expression studies, RNA was extracted from colon tissues and gene expression was performed for goblet cell markers, mucin genes, and genes involved in immune response and mucus regulation using Nanostring nCounter multiplex gene expression assay. All data is represented as mean ± SEM. Intergroup variations were assessed using Student unpaired t-test. *p<0.05, **p<0.01, *** p<0.001 versus Control.

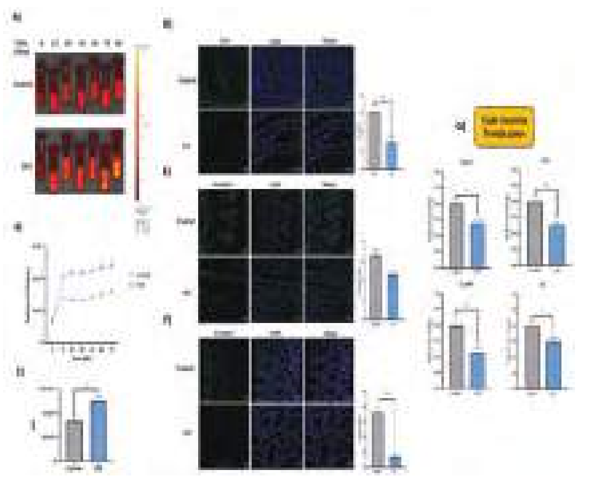


Figure 2. Effect of CPZ rectal administration on intestinal permeability and tight junction proteins. A) In-vitro intestinal permeability – representative images for the release of FITC-loaded nanoparticles from the colon at different time points (n=3), B) Time-based plot for radiance efficiency and C) AUC of the radiance efficiency. Representative images for immunofluorescence in colon tissues for expression of - D) Zona occludens-1 (ZO-1), E) Occludin (OCC) and F) Claudin-1 (CLDN-1) (n=3). Fluorescence intensity was measured using ImageJ and data is presented after normalization with DAPI. G) Gene expression analysis for tight junction proteins. Mice were divided into two groups – Control (administered vehicle rectally) and CPZ (administered 531 µM CPZ rectally). Treatment was given for 2 weeks. After sacrifice, colon tissues (n=3) were fixed in Carnoy fixative, paraffin-embedded molds were prepared and 5 µm sections were obtained. The sections were subjected to IHC with antigen retrieval using citrate buffer pH 6.0 (90°C, 60 min) followed by blocking in 5% goat serum in PBS-Tween20, overnight incubation in primary antibodies, and secondary antibody treatment for 2 h and DAPI for 30-60s. High-resolution images at 40X magnification were captured using confocal microscope and fluorescence intensity was measured using ImageJ. For gene expression studies, RNA was extracted from colon tissues and gene expression was performed for tight junction proteins using Nanostring nCounter multiplex gene expression assay. All data is represented as mean ± SEM. Intergroup variations were assessed using Student unpaired t-test. *p<0.05, **p<0.01, *** p<0.001 versus Control.

and TRPA1 activating dietary constituents like capsaicin, menthol and cinnamaldehyde have potential to prevent weight gain and its related complications like type-2 diabetes and cardiovascular complications by different mechanisms (Baboota et al., 2014 a,b; 2018; Khare et al., 2016; 2018; 2019, 2021, Kumar et al., 2020; 2022). TRPV1-positive neurons profusely innervate the gut, including intestines, and mediate responses to stimuli via local release of neurotransmitters and/or by communicating to the central nervous system. In a novel finding from our previous study, resiniferatoxin-induced TRPV1 ablation in rats led to drastic decrease in mucus production and dysbiosis in colon (Kumar et al., 2020). Further, we used a diet-induced obesity model in mice, to study alterations in gut barrier function, and explore what role CAP, TRPV1, gut microbiota and particularly mucus itself, might play in ameliorating high fat diet-induced impairments. Dietary capsaicin produced anti-obesity effects similar to the exogenous mucin feeding in mice. Anti-obesity action of capsaicin is not exclusive to TRPV1 agonism and colonic mucin secretagogue action of capsaicin plays a crucial part (Kumar et al., 2022). TRPV1 sensory neurons co-express with TRPA1 in gastrointestinal tract. It is important to

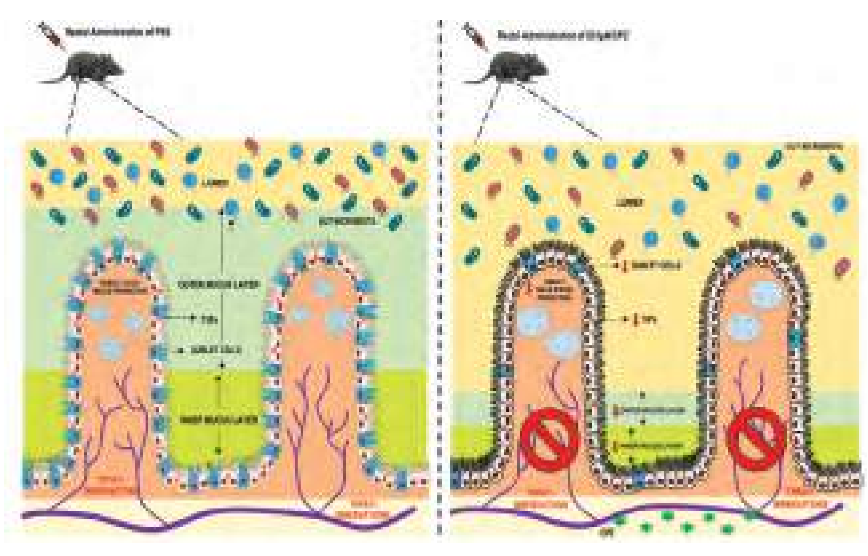


Figure 3. Representative image for overall effects of CPZ-induced desensitization of peptidergic nociceptive TRPA1 neurons on colonic mucus production and gut health.

understand the role of TRPA1 in mucin homeostasis. The work presented for this year is to understand the significance of TRPA1 (high expression in colonic sensory neurons and co-expression with TRPV1) in gut mucosal health using rectal capsazepine-induced systemic TRPA1 desensitization model in mice.

RESEARCH PROGRESS

Studies on Transient Receptor Potential (TRP) channel mediated modulation of adipogenesis & obesity (browning) by dietary molecules: Histological analysis of the colon on day 14 revealed that CPZ administration markedly reduced the thickness of the mucus layer in lumen of the proximal and distal colon (Fig. 2A, B, C) as compared to control animals. In addition to this, in distal colon, a significant decrease in goblet cell numbers, as well as alcian blue (AB) and high iron diamine (HID) stain intensity (as a measure of diminished sialomucins and sulfomucins), was observed after CPZ treatment (Fig. 2A). Ileum did not show any morphological changes. SEM analysis showed a major decrease in the number of mucus extrusions (M) from goblet cells (GC) and crypts (C) of the colon indicating reduced mucus release after CPZ treatment. Also, the mucosal architecture with a smooth velvet appearance in the control group was lost upon CPZ treatment (Fig. 2D, E). Substantiating these findings, expression levels of genes involved in goblet cell functioning, mucin production and immune response were altered (Fig. 2F, G, H).

In-vitro intestinal permeability was evaluated by time-dependent fluorescent imaging. In this study, the diffused FITC-loaded nanoparticle samples withdrawn at different time points were imaged and analyzed for membrane barrier function. We have observed intense fluorescent intensity in the CPZ group as compared with the control group (Fig. 3A, 3B). Furthermore, the area under curve (AUC) in CPZ treated animals indicated a higher diffusion rate of FITC-loaded nanoparticles from the colon and suggested a compromised intestinal permeability (Fig. 3C). It has also been observed that the expression of Tight Junction Proteins (TJPs; Zona occludens-1 (ZO-1), Occludin (OCC), and Claudin-1 (CLDN-1)) in colon sections were downregulated in the CPZ group as compared to the control group as indicated by immunohistochemical evaluation (Fig. 3D, E, F). Gene expression studies also indicated a similar trend of reduction in expression of *Cdh, Cldn-1, Zo-1,* and *Occ* in the colon of CPZ-treated animals as compared to the control group (Fig.3G).

SALIENT ACHIEVEMENTS

- CPZ-induced acute desensitization of TRPA1 nociceptive neurons impaired the colonic mucus production and intestinal barrier integrity.
- The findings from this study serves the basis for further investigations on the role of TRPA1 nociceptors in mucin health and host: microbe interactions for a better understanding of side effect profiles of these approaches.

FUTURE PERSPECTIVES

- To study the involvement of TRPA1 nociceptive neurons in the beneficial effects for short chain fatty acids in colon.
- To establish chronic model of TRPA1 desensitization using CPZ and studying the effects of HFD feeding on colonic mucosal health in the functional absence of TRPA1 nociceptive neurons.
- To explore the explore preventive effects of the prebiotic co-treatment on the colonic alterations owing to HFD feeding in functional absence of TRPA1 nociceptive neurons.

FOOD & NUTRITION

FNB 1.4: Development of therapeutic formulations for nutritional and lifestyle disorders

Nitin K Singhal, PhD
Nanobioscience

RESEARCH FELLOWS
Poonam Sagar
Geetika Bajaj

Two major health issues governing India's major concerns are Iron deficiency Anemia and Type 2 Diabetes Mellitus. These are long term metabolic/lifestyle disorders and affect a large fraction of the Indian population. We are working towards identification and execution of different therapeutic approaches for these major disorders.

- Iron deficiency Anemia is caused by two major reasons, one is poor availability of iron in diet and the second one is poor absorption of iron in the diet. Generally, the poor absorption of iron in the gastrointestinal tract is the limiting factor that is associated with several adverse effects that could be local or systematic. These adverse effects are the main reason behind the non-acceptability of conformist iron supplements. Our group has developed synbiotic hydrogel beads co-encapsulating iron and probiotic bacteria for enhanced oral iron bioavailability and reduced side effects. These hydrogels have demonstrated sustained release along with enhanced iron absorption in diet induced mice model. Further, lower inflammation signals were observed in the group treated with hydrogels as compared to ferrous sulfate or non-encapsulated iron. To further progress toward clinical validation of these hydrogels, we compared the relative iron bioavailability of these hydrogel with that of commercially available supplements.
- Diabetes mellitus or T2DM is characterized by a group of metabolic disorders such as hyperglycemia over a long period, impaired carbohydrate, lipid, and protein metabolism caused by the pancreas's failure to produce enough insulin, and insufficient cells responding properly to the produced insulin. Presently, India has 61.3 million people affected by diabetes. The numbers are estimated to rise to 101.2 million by 2030. The current strategies to alleviate diabetes mellitus 2 includes anti-diabetic drugs, like Metformin, Sulfonylureas, Thiazolidinediones, Dipeptidyl peptidase-4 inhibitors, SGLT2 inhibitors, and Glucagon-like peptide-1 analog and painful insulin Injections. These strategies are costly and require regular adequate dosage to prevent the increased glucose level in the body. Hence, new treatment interventions are required to treat T2DM. The emergence of nanotechnology has enabled new prospects for drug therapies in T2DM, including controlled drug release or targeted drug

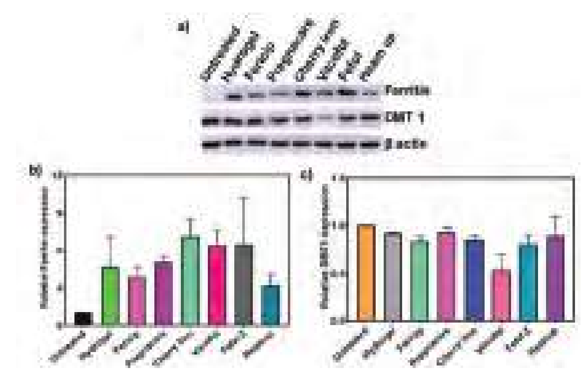


Figure 1. Analysis of protein expression through western blotting: a) Western blotting of DMT 1 and Ferritin, b) Densitometric analysis of Ferritin expression, c) Densitometric analysis of DMT 1 expression. Abbreviations: DMT 1 (Divalent metal transporter).

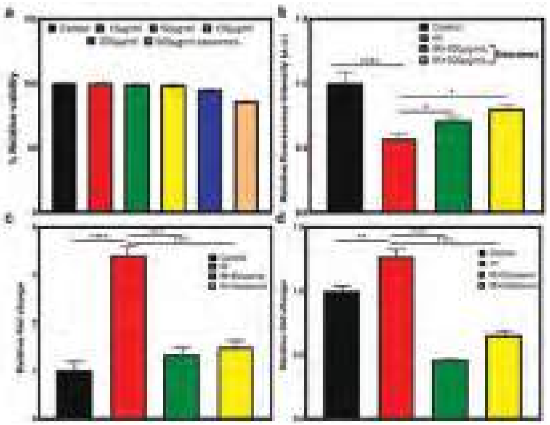


Figure 2. (a) Percentage relative viability of cells as assessed by MTT assay (b) Percentage relative 2-NBDG fluorescence intensity determined by fluorescence spectroscopy. (c) Relative mRNA expression levels quantified by quantitative RT-PCR of PCK-1, G6PC genes respectively in terms of fold change under a different set of conditions

delivery. Exosomes like vesicles are the smallest class of extracellular vesicles consisting of proteins, lipids, nucleic acids and various microRNAs. They are ideal drug delivery vehicles with various advantages over the existing synthetic delivery systems due to their low immunogenicity, high tolerance, biodegradability, and nontoxic capacity. Hence, in this work, we are exploiting the therapeutic effect of exosomes derived from various medicinal plants as effective therapeutics against T2DM. Furthermore, as insulin resistance is the hallmark of T2DM condition in which the cells stop responding to the insulin that is released by the pancreas which increases glucose levels in the blood leading to hyperglycemia. When this condition arises, liver starts its endogenous production of glucose via the process of gluconeogenesis. This further increases the blood glucose levels.

RESEARCH PROGRESS

Iron Deficiency Anemia: In this study, the bioavailability of iron from hydrogel was compared with that of commercial iron supplements in Caco2 cell lines. Major protein that are affected during iron deficiency are ferritin and DMT 1. Ferritin is a marker of iron stories in the body, whereas DMT 1 is involved in absorption of iron from duodenal enterocytes. During the course of iron deficiency, the expression of ferritin reduces and that of DMT 1 increases. Major increase in the ferritin expression in the groups treated with Cherry iron, Vitcofol, Fefol Z, and hydrogel (Figure 1 a, b) was observed. The group treated with HeamUp shows the lowest change in the expression level of ferritin. However, insignificant changes were observed in the DMT 1 expression except the group supplemented with Vitcofol (Figure 1 a, c). However, the formulation did reduce the expression of DMT1 at mRNA level. These results support the equivalent potential of the synbiotic hydrogels in enhancing iron absorption. The synbiotic hydrogels are able to provide enhanced iron bioavailability even at lower dosages. The sustained and targeted release of iron from hydrogels enable the intestinal enterocytes to absorb iron more efficiently. With the help of this advantage these hydrogel beads can reduce the side effects that occur due to overdose of micronutrients. Presence of probiotic within the hydrogels also prevent intestinal inflammation associated with malabsorption of micronutrients. The efficacy of these hydrogels can be more accurately estimated in randomized clinical trials in human volunteers threatened by the risk of iron deficiency.

Type II Diabetes Mellitus: The aim of present study is to explore the therapeutic effect of exosomes in insulin resistance condition via evaluating its effect on insulin dependent glucose uptake (2-NBDG) in insulin resistant cells. Further, evaluation of the effect of exosomes on expression of key gluconeogenic genes (phosphoenolpyruvate carboxykinase-1 (PCK-1) and G6PC (Glucose 6 phosphatase) was also studied. As shown in figure 2, (a) Cell viability was assessed after treatment with exosomes at different concentrations. It was observed that treatment with exosomes, even at the concentration of 500 µg/ml was not showing any significant loss in cell viability. Hence, it was concluded that exosomes could be a safer, non-toxic and cost effective alternate for T2DM therapeutics. (b) In order to study the therapeutic effect of exosomes in insulin resistance condition, in-vitro model (using HepG2 cells) of insulin resistance was generated followed by exosome treatment for 24 hours. These cells were then incubated with 2-NBDG and 100nM insulin to evaluate the effect of exosomes on insulin dependent glucose uptake. It was observed that exosomes at the concentration of 500 µg/ml were significantly able to increase the glucose uptake and ameliorate insulin resistance condition in HepG2 cells. (c) Liver is one of the major organs affected by insulin resistance which results in unchecked gluconeogenesis and impaired glucose uptake from blood in an insulin dependent manner. Hence, the expression of gluconeogenic enzymes is increased during insulin resistant condition. It was further observed that treatment with exosomes at the concentration of 10 µg/ml was able to significantly improve the expression of this enzyme, eventually improving insulin resistance.

SALIENT ACHIEVEMENTS

- The sustained and targeted release of iron from hydrogels enable the intestinal enterocytes to absorb iron more efficiently.
- The iron bioavailability provided by synbiotic hydrogels is equivalent to commercially available iron formulations.
- Dosage of exosome at various concentrations was observed to cause no toxicity.
- Treatment with exosomes derived from ginger were observed to improve insulin resistance and increased glucose uptake in insulin resistant HepG2 cells.
- Exosome treatment was also observed to improve liver gluconeogenesis via downregulating key enzymes of gluconeogenesis..

FUTURE PERSPECTIVES

- Fasting blood glucose estimation of diabetic mice after exosome treatment at different concentrations.
- Metabolic tolerance tests (OGTT, ITT) to evaluate effect of oral dose of exosomes in diabetic mice.
- RT-PCR analysis of gluconeogenic genes in T2DM mice after exosome treatment.

FOOD & NUTRITION

Koushik Mazumder, PhD
Carbohydrate Chemistry

RESEARCH FELLOW
Rohit Maurya

FNB 1.5: Production, characterization and biological significance of health beneficial of prebiotics

Diabetes and obesity are the most common lifestyle-related disease of today time. Several studies have clearly indicated the consumption of more sugar in the diet could be linked to the rise in diabetes and obesity among the population. Isomalto-oligosaccharides (IMOs) are a low-calorie sweetener which have prebiotic and immunomodulatory activity. IMOs are also approved by the European Food Safety Authority (EFSA) and FDA food safety as a novel food ingredient. Therefore, IMOs have huge marketing potential as functional food ingredient. However, there are only few commercial producers of IMOs worldwide and has not been commercialized in India. Our invention is focused on cost-effective production of IMOs from food processing by-product (potato peel starch) which involves in-house production of enzyme and magnetic nanoparticle immobilization of the enzyme for repetitive usability. The aim of the invention is to demonstrate upscaling production (gram/ kg level) of IMOs. Further, initiatives will be made to prepare health beneficial functional food products based on IMOs.

RESEARCH PROGRESS

For the IMOs production potato peel starch used as the raw material. Starch was isolated from wet potato peel with a yield of 6.5% (±1.1). IMOs production was produced by three enzymatic pathways such as liquefaction, saccharifiction, and transglycosylation. In the first step the optimum condition for nearly complete liquefaction of potato starch was determined as: time: 1h, temperature: 95 °C, pH: 6.9 and enzyme to substrate ratio: 0.7 U/g. The completion of liquefaction was identified by dispersion of color of starch iodine complex during reaction. Further in the second step, Fungamyle was used for the saccharification of liquefied starch. The optimum condition for complete saccharification of liquefied starch was determined as time: 4h, temperature: 50 °C, pH: 6 and enzyme to substrate ratio: 1.2 U/g. During saccharification reaction, large oligosaccharides were converted into malto-oligosaccharides which were identified by TLC and HPAEC. The analysis of the saccharified product revealed the presence of maltose (92 g/l), maltotriose

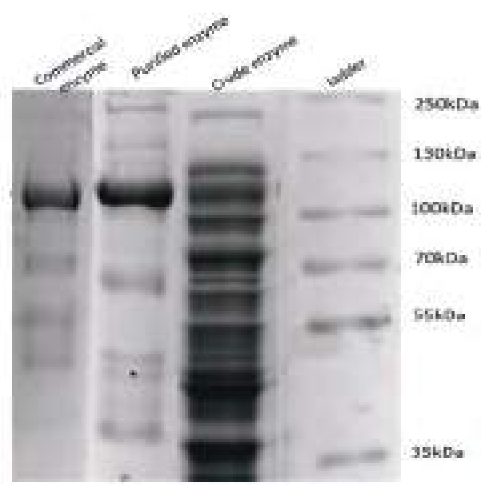


Figure 1. SDS PAGE analysis of purified α-transglucosidase.

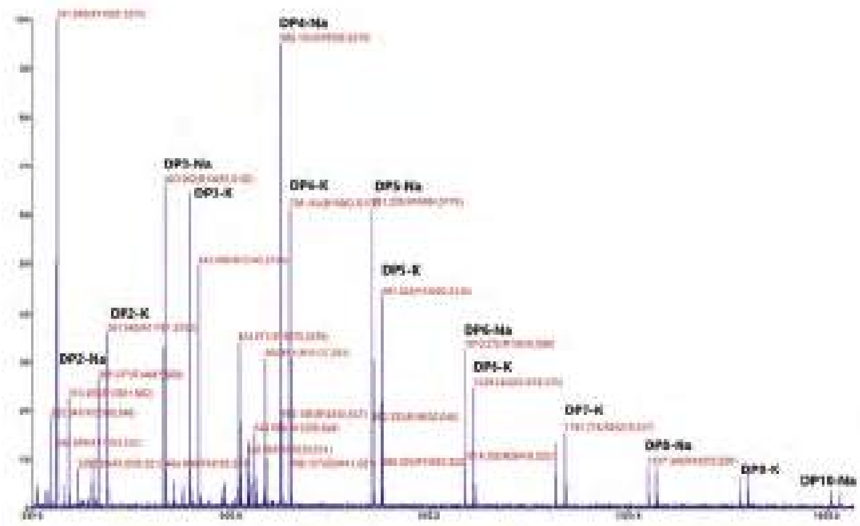


Figure 2. MALDI-TOF spectrum of IMOs.

(46.5 g/l) as major constituents along with small amount of glucose (11 g/l). Further, the gene (α-transglucosidase) from A. niger was cloned in pET-28a vector and further overexpressed in *E.coli*. The purified α-transglucosidase was analyzed by SDS PAGE, indicated the molecular weight of ~110 kDa. The molecular weight of the purified α-transglucosidase was found be comparable to the commercial available enzyme.

In order to determine the optimum transglycosylation reaction conditions for the production of IMOs, the effect of four variables temperature, pH, time and enzyme to substrate ratio was investigated. The response surface methodology (RSM) experimental data revealed that the highest yield (70 g/l) of IMOs with small amount glucose and maltose obtain at optimum condition such as: temperature: 45°C, pH: 5.5, time: 9 h and enzyme to substrate ratio: 6.9 U/g. The progress of transglycosylation was identified by TLC and HPAEC. The product obtain after transglycosylation of saccharified starch was separated by size exclusive chromatography. Further, the purified IMOs rich fraction was analyzed by HPEAC which indicated the fraction devoid of glucose. MALDI-TOF-MS analysis (Fig. 2) revealed the quasimolecular ions at m/z 365 Da, 527 Da, 683 Da, 851 Da, 1013 Da, 1175 Da, 1337 Da which suggested the presence of IMOs oligosaccharide with degree of polymerization (DP) ranging from 2-10. However accordingly to the use m/z peak analysis of sodium molecule ions, the amount of oligosaccharide were DP>10 were very low and undetectable. After purification of the IMOs by size exclusion chromatography, the glucose concentration in the purified IMOs was reduced to 4 g/l along with iso-maltose (37.7 g/l), iso-maltotriose (21.6 g/l), panose (17g/l), maltose (3 g/l), malto-triose (10 g/l) and other isomalto-oligosaccharides (10 g/l, DP> 2) where the purity of the IMOs was estimated as ~84%.

SALIENT ACHIEVEMENTS

- In our study, the in-house production of α-transglucosidase of high purity was achieved. Further, the process for lab scale enzymatic production of IMOs was optimized by response surface methodology (RSM) and the oligosaccharides was quantified by HPAEC. Further, the detailed structural characterization of IMOs suggested the DP of oligosaccharides in the range of 2-7. The detailed study for upscaling production is under progress.

FUTURE PERSPECTIVES

- Cost-effective and up-scaling production of IMOs.
- Detailed structural characterization of IMOs using advanced analytical techniques (MS and NMR).
- Developed of food products based on IMOs and evaluation of health promoting effects of IMOs.

FOOD & NUTRITION

Mohit Kumar, PhD
Nutritional & Neuroscience

RESEARCH FELLOW
Nitin Rawat

FNB 1.6: A ketogenic diet approach to reverse palatable food addiction: Implications for metabolic and neurological disorders

Overconsumption of ultra-processed food leads to major health complications including gut dysbiosis, metabolic diseases (type 2 diabetes, obesity) and co-morbid neurological disorders (anxiety, depression and cognitive deficits) affecting an individual/a family emotionally, socially and economically. Ultra-processed foods with high amount of refined sugar, fat and salt are highly addictive in nature hence consumption of ultra-processed food is drastically increasing in India and becoming the integral part of daily food habits especially in young and urban population. India's fast-food industry is expanding at the rate of 40% every year and ranks 10th in the fast food per capita spending figures with 2.1% of expenditure in annual total spending. Refined sugars are the major constituent of processed foods and are the primary source to induce palatability due to their sweet taste. Pre-clinical studies in lab conditions have shown that high sugar containing foods show their pleasurable effects and induce positive reinforcement by releasing excessive amount of dopamine (the main neurotransmitter associated with rewards and pleasure) in the brain, stimulating endogenous opioid signalling and activating mesocorticolimbic reward neurocircuitry overriding the eating behaviour from a homeostatic eating to hedonic overeating. These neuroadaptations developed during excessive sugar intake leads to sever withdrawal (WD) symptoms and cravings when an individual try to cut short the daily intake of refined sugar. Moreover, literature has also suggested that excessive sugar intake causes blunted sweet taste sensation leading to overconsumption of food and obesity phenotype through dysregulated neuronal circuits controlling satiety and hunger. Since sugar has the potential to activate brain reward neurocircuit, people on early stage of sugar WD may further increase the chances to relapse either on sugar or on other illicit drugs (nicotine, opioid, alcohol) if exposed to these drugs earlier. Considering these facts, understanding of neural correlates and underlying molecular mechanisms of sugar dependence especially early WD symptoms and development of novel effective therapies are urgently warranted for the management of sugar associated stress driven comorbid disorders including obesity, diabetes, major depressive disorder, substance use disorder, depression and anxiety.

Brain utilizes ~20% of total energy expenditure of the body to maintain its homeostatic functions and unavailability of primary energy source during early sugar WD develops an energy deficit environment and metabolic stress in the brain which could be one of the primary reasons for observed physical and mental symptoms during early sugar WD. Fluctuations in brain glucose concentration have been shown to activate brain resident immune cells "microglia" and suggested to be the central player in glycaemic variability-induced cognitive deficits. Release of pro-inflammatory cytokines from activated microglia are strongly associated with neuronal dysfunctions, compromised neurovascular functions, increased blood brain barrier permeability, hampered neuronal recovery and behavioural deficits such as anhedonia and anxiety, which are also the endophenotypes of sugar WD. Therefore, interventions reversing activation of microglia and neuroinflammation could be promising future therapies to help people to quit use of addictive foods and other illicit drugs. A ketogenic diet is well known for its health benefits including anti-inflammatory effects by re-establishing healthy gut microbiota, reducing blood glucose levels, inducing nutritional ketosis and increasing the plasma levels of ketone bodies especially 3-beta-hydroxybutyrate (BHB), here we are hypothesising that shifting to a ketogenic diet during the early sugar WD may normalises the blood glucose levels, prevents the development of sugar WD symptoms, cravings for sugar, chances to relapse and development of long term obesity possibly by alleviating neuroinflammation and improving neurovascular functions. Leading this goal, in the present study we used human derived microglia cells (CHME3) as an in vitro model to decipher the underlying molecular mechanisms driving activation of microglia, compromised microglial phagocytic activity and increased pro-inflammatory cytokines during early glucose WD. The study's long-term implications will be to develop personalised diet regime and

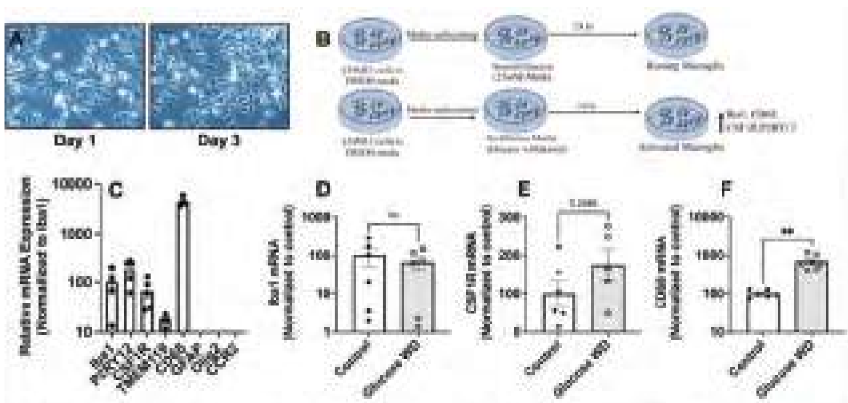


Figure 1: Representative images of the CHME3 cells at Day 1 and Day 3 after passaging (A); Treatment paradigm followed for the study (B); Bar graph showing relative mRNA expression of pan-microglia (Iba1, P2RY12, CSF1R, TMEM119, CD68), astrocyte (GFAP), oligodendrocyte (Olig2) and monocyte (CCR2) markers in CHME3 cells assessed by real time RT-PCR (C); Bar graph showing relative mRNA expression of microglia activation markers (Iba1, CSF1R, CD68) in control and glucose withdrawal treated CHME3 cells (D-F).

ketogenic dietary supplements for the use of clinical purpose in the patients with palatable food and drug dependence.

RESEARCH PROGRESS

Establishment of human microglia cell line: CHME3 is a human microglia cell line and is an excellent in vitro model to study the role of microglia in the pathogenesis of neurological disorder, development of novel therapeutics and unrevealing the underlying molecular mechanisms regulating microglia phenotype under pathological conditions. The results obtained from this cell line can be directly implicated to the clinical purpose due to their human origin. We have successfully established the CHME3 cell line in our cell culture facility here at NABI. Authenticity of the cell line was confirmed by analysing the mRNA expression of pan-microglia markers (Iba1, P2RY12, CD68, CSF1R and TMEM119) using real time RT-PCR assay (Figure 1). Observed mRNA expression of microglia marker genes confirmed the authenticity of CHME3 cell line. Absence of other glial cell type markers such as astrocyte (GFAP), oligodendrocyte (Olig2) and monocyte (CCR2) further confirmed the non-availability of these cell types and purity of microglial cell culture.

Effects of acute glucose withdrawal on microglia activation and phenotype: In this study we aimed to evaluate the effects of acute glucose WD on the genetic markers of microglia activation (Iba1, CD68, CSF1R, P2RY12), inflammatory cytokines/factors (TNF-α, IL1b, IL-6, TGF-β, ARG1), phagocytosis (Trem2) and reactive oxygen species (Nox2) in CHME3 cells mimicking the clinical conditions of acute sugar WD. For the purpose, CHME3 cells after getting 80-90% of confluency

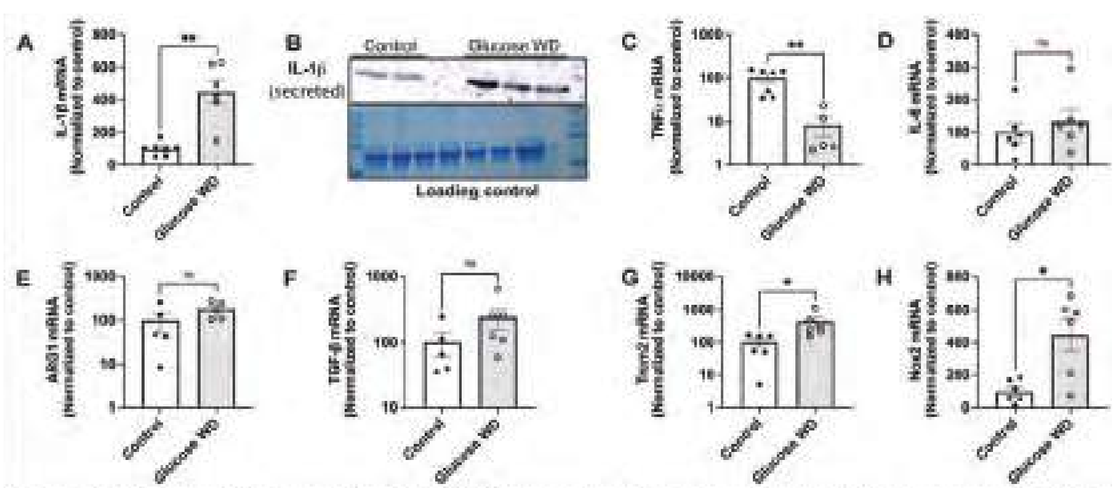


Figure 2: Relative mRNA expression of IL-1β (A); Representative images of western blot for secreted IL-1β (B); Relative mRNA expression of Tnf-α (C); IL-6 (D); ARG1 (E); TGF-β (F); Trem2 (G); Nox2 (H) in control and glucose withdrawal treated CHME3 cells.

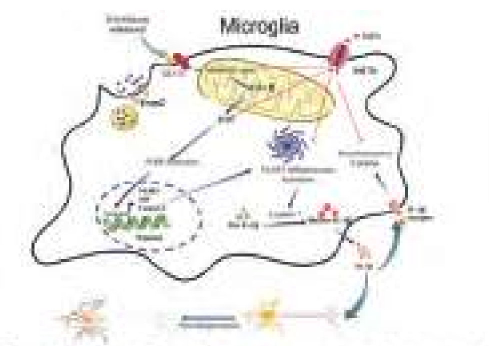


Figure 3: Summary figure of the present research work and proposed hypothesis for future research

were divided into two groups: Control (25mM glucose) and Glucose WD (0mM glucose) by replacing the culture media containing respective glucose concentrations. After 24 hr, cells were harvested, total RNA was isolated and reverse transcribed into cDNA. Real-time RT-PCR was performed to assess the relative mRNA expression of desired genes. A significant increase in the gene expression of pan-microglia activation marker (CD68) was observed in microglia treated with glucose WD as compared to control cells (Figure 1) suggesting that glucose deprivation during sugar WD leads to activation of microglia. These findings lead us to our second objective to determine if activation of microglia is also associated with increased levels of pro-inflammatory cytokines. We found a significant increase in the mRNA expression of pro-inflammatory cytokines especially IL1β in the cells exposed to glucose WD as compared to controls. Western blot analysis of secreted IL1β in media further confirmed that acute glucose WD enhances the secretion of IL1β in surroundings as compared to control cells (Figure 2). Moreover, the mRNA expression of Trem2 was also increased in the cells exposed to glucose WD suggesting compromised phagocytosis capacity of microglia during acute sugar WD.

Glucose deprivation during acute sugar WD induces a metabolic stress in microglia encouraging cellular mitochondria (energy house of the cell) to generate more ATP to fulfil the required energy demand of the cell. We further argued since mitochondria are the primary source of reactive oxygen species in the cell produced during oxidative phosphorylation, overproduction of ATP during glucose WD may leads to oxidative stress. To confirm the hypothesis, we analysed the mRNA expression of NADPH oxidase 2 (Nox2) encoded gene, which is the primary enzyme catalysing the reaction producing free radicals at outer mitochondrial membrane and is specifically expressed in microglia. Interestingly, a 4-fold increase in the mRNA expression of Nox2 was observed in microglia treated with glucose WD suggesting Nox2 mediated mechanism as a key driver to activate microglia during acute sugar WD. Therefore, interventions providing an alternative source of energy relieving metabolic stress during acute sugar WD might be helpful to reverse pro-inflammatory phenotype of microglia during sugar WD and may prevent development of sugar dependence. Here we are proposing to use BHB (a ketone body) as an alternate energy source to microglia either though direct supplementation or though promoting nutritional ketosis using a ketogenic diet during acute sugar WD to prevent exacerbated neuroinflammation and subsequent behavioural deficits (Figure 3).

SALIENT ACHIEVEMENTS

- Human microglia CHME3 cell line has been successfully established in NABI cell culture facility.
- Novel neuroinflammatory drivers of sugar dependence (IL1β and Nox2) have been successfully identified which can be used as potential therapeutic targets for the management of sugar dependence and comorbid metabolic and neurological disorders.
- Identifying novel molecular mechanisms will pave a way to invent new nutraceutical strategies to purposefully manipulate the microglia phenotype to improve health.

FUTURE PERSPECTIVES

- To explore the use of BHB as an alternate energy source and potential nutritional therapy to reverse microglia phenotype during glucose withdrawal in the in vitro model system.
- Implementation of preclinical animal models to understand the physiological, neurochemical and behavioural pattern of palatable food addiction and withdrawal phenotype.
- To evaluate the underpinning molecular mechanisms driving the beneficial effects of a ketogenic diet and nutritional ketosis in palatable food addiction and comorbid metabolic (obesity, type 2 diabetes) and neurological (depression, anxiety, cognitive deficits) disorders.

FOOD & NUTRITION

FNB 1.7: Unraveling epigenetic mechanisms triggering cognitive dysfunction in hepatic encephalopathy- Role of neutraceuticals

Aanchal Aggarwal, PhD
Epigenetics

RESEARCH FELLOW
Shiwangi Gupta

Hepatic encephalopathy (HE) is a debilitating, neuropsychiatric and metabolic syndrome, complicating the course of liver disease. Cirrhotic patients suffering from HE have profound impact on the quality of life of patients and their caregivers adding the socio-economic burden faced by them. HE is defined as "brain dysfunction resulting from liver insufficiency and/or portal systemic shunting that exhibits a wide spectrum of neurological abnormalities". Despite the fact that HE is a well-known risky disorder, the exact pathogenesis remains unclear. Among the broad spectrum of causative factors associated with HE, ammonia is the key neurotoxin involved in the development of HE that may sensitize the brain to various precipitating factors. Metabolism of ammonia in the liver is severely compromised in liver dysfunction resulting in the elevation of ammonia levels in blood known as "Hyperammonemia". Under these conditions, astrocytes in the brain utilize the amino acid glutamate to detoxify ammonia by converting it to glutamine. Therefore, this intracellular rise of glutamine (an osmolyte) owing to liver failure leads to accumulation of water inside astrocytes causing them to swell and thereby causing cerebral edema and increased intracranial hypertension. Ammonia being one of the central neurotoxic molecules is linked to multiple complex signal cascades like oxidative stress, mitochondrial dysfunction, increased BBB permeability, inflammation responsible for cognitive dysfunction in HE. All these pathologies corroborate to metabolic imbalance of central nervous system (CNS) including depletion of acetyl CoA (low glucose uptake), reduced S-adenosylmethionine (SAM) and aberrant signaling cascades (MAPK/ERK) that may trigger diverse epigenetic mechanisms contributing to HE etiology.

Epigenetic mechanisms regulate the transcriptional access to gene playing significant role in the etiology of various neurodegenerative disorders. Behavioral functions have been found to depend on histone modifications (e.g., acetylation, phosphorylation, methylation) and DNA methylation. Dnmt1 and Dnmt3a exhibited abnormalities in long-term potentiation (LTP) and long-term depression after stimulations in CA1 synapses, and these dysfunctions are associated with impaired learning and memory. Histone methylation and histone acetylation in the hippocampal formation play an important role in modulating synaptic strength after neuronal activation. It is hypothesized that these epigenetic mechanisms may play major role in induction of excitotoxicity, calcium overload, oxidative stress, inflammation and apoptosis that are the major debilitating mechanisms responsible for HE pathology. This suggested the possibility of a link between HE phenotype and the epigenetics. Role of histone modifiers (HATs (histone acetyl transferases), HMTs (histone methyltransferases), HDMs (histone demethylases)) as well as histone modifications and their correlation with differential expression of memory encoding genes in HE will open a new direction towards therapeutics in this field. Notably in recent years, it has been demonstrated that dietary phytochemicals like Curcumin, Resveraterol, Querecetin, Sulforaphane, Epigalocatechin gallate, Genistein etc. as well as their metabolites has been determined to induce epigenetic changes by affecting the regulation of histone deacetylases, histone acetyltransferases, DNA methyltransferase I, and miRNAs controlling various biological processes. They are commonly present in fruit, vegetables, beverages and spices and have potential therapeutic potential to treat various diseases like cancer, neurodegeneration, diabetes, cardiovascular problems. Interestingly, HDAC inhibitors like Trichostatin-A, Valproic acid etc. have been seen as treatment options for many neurodegenerative disorders like Alzheimer's, Huntington's, Parkinson's disease and Hdac-2 knockout model has been found to increase memory and learning in Alzheimer's condition. Therefore, it would be interesting to analyse these naturally occurring epigenetic modifiers as treatment option for HE. In view of the above facts, this study aimed to decipher epigenetic mechanisms related to cognitive dysfunction in Hepatic encephalopathy in vitro and in vivo model as well to study the role of various neutraceuticals in overcoming the problem.

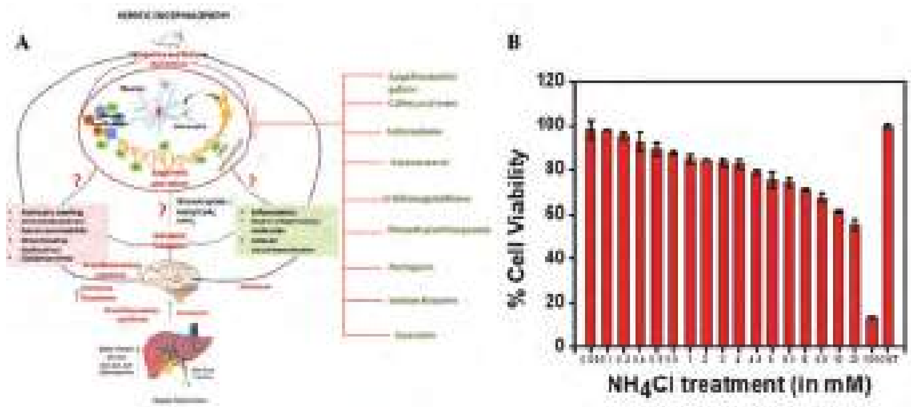


Figure 2. A) Hypothetical model proposing the role of ammonia in altered epigenetic mechanism leading to the development of Hepatic Encephalopathy. B) Percent cell viability of SH-SY5Y cells after NH4Cl treatment.

RESEARCH PROGRESS

Optimization of Sub-lethal dose of NH_4Cl to induce in vitro Hepatic encephalopathy model: Neuronal cell lines SH-SY5Y and U87MG were curated from NCCS Pune to analyze the dose and time-dependent effect of NH_4Cl on neuronal and glial lineage human cell lines. Three days post-treatment at various conc. of NH4Cl (1 µM-100mM) showed 70% and 60% cell viability at 5mM and 10mM respectively. Cells treated with these NH4Cl conc. (i.e 5 mM and 10 mM) were further used to extract RNA, Cytoplasmic, and Nuclear Proteins.

Optimization of bile duct ligation model mimicking chronic Hepatic encephalopathy: Hepatic encephalopathy was induced in Rat by bile duct ligation surgery. Animals were prepared for surgery using the pre-operative and anesthetic procedures. Laparotomy was performed under general anesthesia. The lobes of the liver were gently retracted to expose the bile duct. The bile duct was carefully isolated, doubly ligated, and cut between the two ligatures. The abdominal incision was closed with absorbable suture (2-0) and the skin incision was closed with normal suture.

Evaluating the effect of NH_4Cl on different cell lines: To analyze the effect of NH_4Cl on the Histone Acetylation profile, cells were treated with 5mM and 10mM doses of NH_4Cl, the obtained proteins were analyzed using HDAC fluorometric assay. Further, RNA was extracted to obtain cDNA and to quantify the effect of NH4Cl on the expression of different HDACs and memory-associated genes. A shift in histone acetylation profile was obtained after NH_4Cl treatment. We will further explore the effect of NH_4Cl on different mRNA and protein accumulation of HAT and HDACs to understand the significance of NH_4Cl in regulating the transcription and translation profile of the genes.

Figure 2. Surgical procedure for BDL A) Rat anesthetized using IsoFlurane, B) Abdominal midline skin and musculature incision made, C) Small intestine exposed, D)Bile duct doubly ligated, E - F) Bile duct cut between two ligatures, G) Abdominal suture being closed with absorbable suture and H) Skin incision closed with normal suture.

SALIENT ACHIEVEMENTS

- Ideal doses for inducing Hepatic encephalopathy in cells were identified and their relative protein and mRNA estimation was done.
- Protocol for bile duct ligation was established to mimic the chronic Hepatic encephalopathy model.

FUTURE PERSPECTIVES

- To investigate changes in cognitive function associated with the Hepatic Encephalopathy model.
- To establish various epigenetic modifications in the different brain regions of bile duct ligated rats.
- To correlate these epigenetic changes with cognitive function in rats.
- *In vitro* and *In vivo* testing of nutraceuticals.

POST HARVEST BIOTECHNOLOGY

Maninder Meenu, PhD
Food Science

RESEARCH FELLOWS
Swati Kanwar
Sakshi Gumber

FNB 1.8: Encapsulation of anthocyanin from colored wheat for food and nutraceutical applications

Recently, the concerns regarding the natural colorant in foods are increasing as the synthetic colorants are reported to present adverse impacts on human health. To address this issue, researchers are actively exploring the application of anthocyanins as a natural colorant. Anthocyanins are the largest group of water-soluble pigments responsible for the different color of flowers and fruits of plants. The addition of anthocyanins in food not only contributes to the aesthetic value of food but also enhances the potential health benefits of food. The colored wheat varieties developed at the National Agri-Food Biotechnology Institute, Mohali, Punjab was reported to contain a significant amount of anthocyanins. Thus, these wheat varieties can also be explored as a rich source of natural food colorant that also exhibits therapeutic potential. From a technological point of view, black carrots are an excellent source of anthocyanins due to their good taste, brilliant shade and considerable stability. However, it is a seasonal fruit and when compared with black wheat and its bran which costs Rs 35-40/kg and 15-20/Kg, respectively black carrots are expensive generally ranging from Rs 50 to 65/Kg. Wheat bran, regarded as a waste product, is a rich source of anthocyanins and is commonly used as an animal feed. Thus, it can be used as a promising low-cost raw material for the extraction of anthocyanins. However, the application of anthocyanins in foods is challenging due to their low stability during food processing and storage.

Thus, we are working with research objectives:

» To optimize the extraction of anthocyanins from colored wheat to develop a rapid, cost-effective and efficient method for the extraction of anthocyanins from wheat and wheat bran.
» To encapsulate wheat anthocyanins to enhance their stability and bioavailability followed by determining their physicochemical properties.
» fortification of various food formulations with free anthocyanins and encapsulated anthocyanins to improve the nutritional, sensory and shelf life.

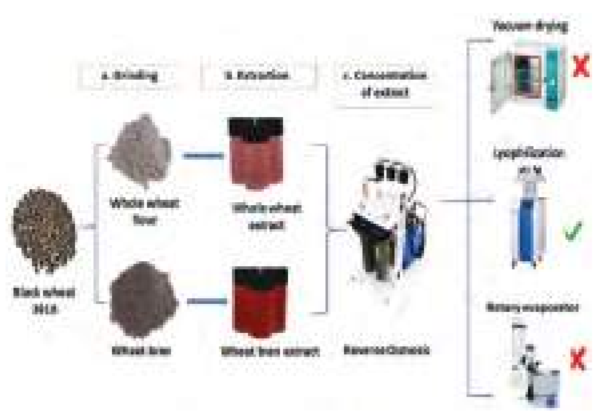


Figure 1. Schematic view of work progress.

RESEARCH PROGRESS

Grinding of whole wheat flour and separated bran using Milcent flour mill with B1 sieve to achieve same particle size; also tried FOSS grinding will with 0.5 mm sieve that was rejected due to insignifant difference in total anthocyanin content of resultant samples and grind only small amount of sample, (b) anthocyanins were extracted by shaking (120 RPM) at room temperature with 1% HCl in water for 6+5 h with 10+10 mL solvent; tried 140 different solvents, 11 different time intervals and 3 different solvent volume (10, 20 and 10+10 mL). Final extraction conditions were selected based on the less use of harmful chemicals, reduced cost, less time and energy consumption. (c) Furthermore, the large volume of extract obtained was reduced by about 85% with the reverse osmosis technique, remaining extract was tried to further dry by using rotary evaporator, vacuum dryer and lyophilizer. Rotary evaporator took 7-8 hr to completely dry the extract. However, the desired final product is anthocyanin in powder form which was not obtained by rotary evaporator. Whereas, vacuum dryer took around 7 days to dry the same amount of extract and resulted in visible degradation of compounds. Lyophilization of the extract seems to be satisfactory method for drying compared to rotary evaporator and vacuum dryer. In addition, the results of lyophilization will also be compared with the spray drying method. The method which will fast and result in the least degradation of anthocyanins which selected for further study.

SALIENT ACHIEVEMENTS

- Optimized solvent for anthocyanin extraction from whole black wheat and its bran.
- Optimized time for anthocyanin extraction from whole black wheat and its bran.
- Selected flour mill and sieve size for optimized anthocyanin extraction.
- Selected methods for concentrating anthocyanin extracts.

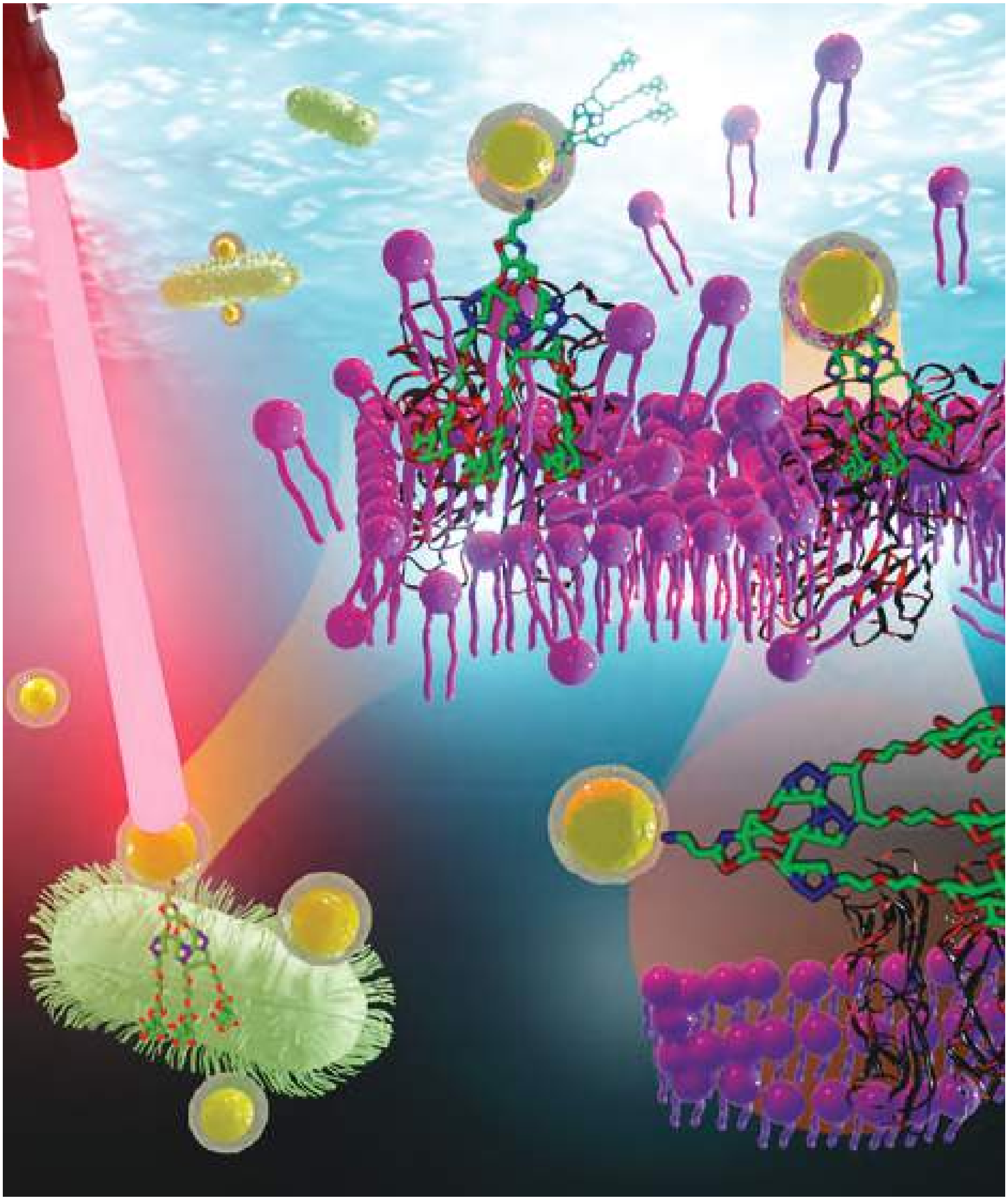
FUTURE PERSPECTIVES

- Encapsulation of anthocyanins will be carried out using either top-down or bottom-up techniques based on the compatibility with material, availability, and cost.
- Determination of physicochemical properties of encapsulated anthocyanins using various tools and techniques.

PROGRAM - FNB 02

FOOD & NUTRITIONAL BIOTECHNOLOGY (FNB)

FOOD AND GM CROPS BIOSAFETY



FOOD & NUTRITION

Nitin K Singhal, PhD
Nanobioscience

RESEARCH FELLOWS
Ritika Gupta
Nitesh Priyadarshi
Priyanka Garg
Sunaina Kaul

FNB 2.1: Development of nanobiosensor for the detection and ablation of food borne bacteria

Several fold increase in bacterial strains and associated diseases have become a cause of concern for the civilization. Amongst all ailments, food-borne diseases have been referred to be a major reason behind morbidity and mortality. These conditions add a tremendous financial burden to our health care systems. Infection usually occurs through ingestion of contaminated food or water and is highest in developing countries, particularly in areas with poor sanitation. Currently available techniques like Enrichment media-based detection, polymerase chain reaction and ELISA are time consuming and tedious. Although considered as rapid, many techniques for detection involve pre-enrichment, which takes upto 12-24 hrs depending upon the strain. Polymerase chain reaction (PCR) is a sensitive and rapid technique. However, it can be inhibited by several factors present in food and other complex environmental samples. PCR based techniques also require pre-enrichment of the pathogens. ELISA although sensitive, is a time-consuming procedure. Progression in nanotechnology has empowered to incite new diagnostic tools for sensitive and fast discovery of pathogens and toxin. Specific identification and killing of bacterial species in food is a proximate commitment to ensure food safety. Utilizing nanomaterials we have utilized following approaches to ensure contribution in the development of new biosensors against food/water borne pathogens.

- Aptamer (ssDNA molecule) based detection has gained interest due to its simple cost-effective synthesis, high specificity, and sensitivity. Aptamers against the Salmonella typhimurium and *E. coli* by cell-SELEX (Systematic evolution of ligands by exponential enrichment) method were synthesized. FRET mechanism was utilized that involved quenching the fluorophore tagged selected aptamers if present within 10 nm range. In the presence of target bacteria, aptamer binds with it and increases the distance between fluorophore and Graphene Oxide thus regaining the fluorescence intensity.
- A probe of fluorescent gold nanoparticles with TCPP as a linker has been utilized as a detection and a killing platform for bacteria. TCPP a unique photosensitizer, when excited at a certain wavelength, results in production of ROS that is able to ultimately ablate the target cell. Galactose provides specificity towards *Pseudomonas aeruginosa* (*P. aeruginosa*) as the lectins present on its surface have great affinity with galactose. With the developed biosensor detection of *P. aeruginosa* upto 10^6 CFU/mL, was possible.
- Enzymatic substrates, sensitive bacterial diagnostic tools have made specie-specific bacterial detection possible. Microbial cell lysis provides an analytical methodology for biomolecules based bacterial identification. Existing lysis methods involve thermal, chemical, electrical and mechanical microfluidic platform for bacterial identification. The concept of nanostructures for mechanical microbial lysis have been recently gaining pace. Their penetration is sufficient enough to tear apart the bacterial membrane releasing the cytosolic components and induce the associated cell apoptosis. Gold nanostars (AuNst) are a different class of nanostructures that possess spiky topology. These spikes as a cell rupturing medium and thus detection has been studied in the following work.

To increase sensitivity and selectivity, we developed carbohydrate and aptamer functionalized gold nanoparticles and mechanobactericidal gold nanostars based biosensors against different food borne pathogens. These approaches will contribute in the development of new food borne pathogen biosensor.

RESEARCH PROGRESS

Aptamer functionalized gold nanoparticles based biosensors: For multiplexed detection of bacteria, *S. typhimurium, E. coli,* and *P. aeruginosa*, three differ-

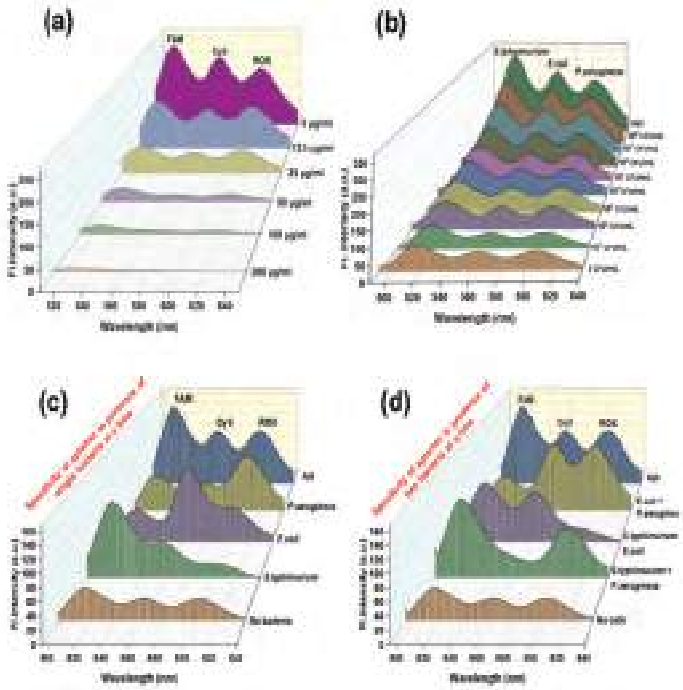


Figure 1. Multiplexed bacteria detection using graphene oxide and differently fluorophore labeled high binding affinity aptamer: (a) Quenching of fluorescence with the increase in Graphene oxide concentration; (b) Increase in fluorescence intensity with the increase in cell concentration; (c) Specificity analysis using single bacteria at a time, and (d) Specificity analysis using two target bacteria at a time.

ent aptamers were labeled with FAM, Cy3, and ROX dye respectively. To develop a detection assay, Graphene Oxide (GO) sheets were used which have a quenching property. Based on the FRET mechanism, fluorescence of fluorophore tagged aptamers quenched when present within 10 nm range of GO. The labeled aptamers adsorbed on the surface of GO by ϖ-ϖ interaction and lose their fluorescence. In the presence of target bacteria, aptamer binds with it and increases the distance between fluorophore and Graphene Oxide thus regaining the fluorescence intensity. Fluorescence regained of only that aptamer whose target bacteria was present. The limit of detection can be concluded as 100 CFU/mL for *S. typhimurium and E. coli* while 10 CFU/mL for *P. aeruginosa* as shown in Figure 1.

To design a multiplexed detection device, the assay was performed in a microfluidic chip using GO as a fluorescence quencher. Here, three aptamers Sal5, ECo, and PAe have been labelled with FAM fluorophore at the 5'end and incubated with 50 μg/mL of GO for the quenching of fluorescence. On adding the respective target, fluorescence was restored. This experiment was performed on microfluidic chips. Microfluidic chips were fabricated using PMMA polymer. The microfluidic chips consisted of four small wells and one big well. Round printer paper sheets coated with the detection probe were dried and placed in small wells. The chip was then sealed from the bottom. Round paper cutting was done using a paper punch. The four small wells consist of FAM-labeled Sal5, ECo, and PAe aptamer. The centre bigger well was used for adding the sample (Bacteria). In single paper cut, single type of aptamer targeting single respective bacteria were adsorbed on GO, and dried. Similar procedure was done for other aptamers as well. In control well, any one of the aptamers at same concentration was placed. After that mixture of bacteria (*S. typhimurium, E. coli* and *P. aeruginosa*) were diluted from concentration 10^7 CFU/mL to 10 CFU/mL. 80 μl of the bacteria was then added in the middle well. The bacteria move to small wells through microchannels. In figure 2, it can be observed that the intensity of fluorophores was increased with increasing concentration of bacterial cell number.

TCPP functionalized gold nanoparticles based biosensor: Carbohydrates are useful building blocks for supramolecular assemblies offering the possibility of multiple hydrogen bonding. Chemical synthesis of linear and branched carbohydrates helps to get insights into multivalent carbohydrate-protein interaction. Multivalency plays a pivotal role in biological processes, particularly in the adhesion of bacteria/viruses or antibodies/macrophages to the cell surface. In contrast to weak monovalent binding, multivalent interactions result in high specificity and in thermodynamic and kinetic stability. Foodborne and water borne infections are among the significant reason for cost, illness, and mortality over around the world. The presence of microorganisms, such as *Escherichia coli Salmonella, Listeria,* & *Vibrio* in food and Pseudomonas aeruginosa (*P. aeruginosa*) in water possess risk to health, especially for infants, the elderly & immune deficient patients. Therefore, there is an urgent need to develop bacterial biosensors for rapid detection of bacteria in contaminated environment. Moreover, lectin-carbohydrate interactions have been employed by various research groups to synthesize specific nanomate-

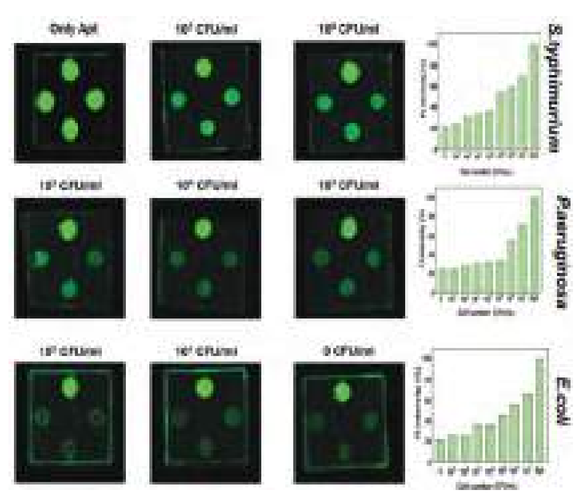


Figure 2. Multiplexed detection in microfluidic chips. (a) Microfluidic chip design and the fluorescence signal in the presence and absence of GO; (b) Increase in fluorescence with the increase in bacterial concentration.

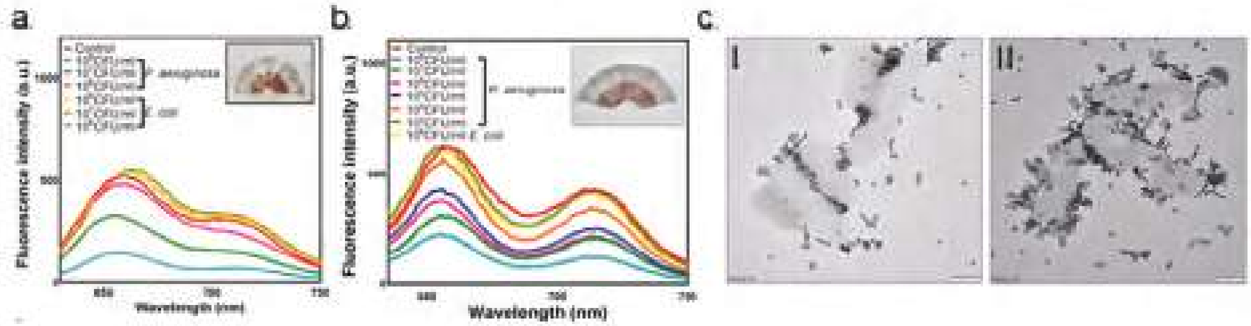


Figure 3. Bacterial aggregation and fluorescent spectra of P. aeruginosa and E. coli with a. galactose monopod probe b. Galactose tripod probe. c. TEM images of *P. aeruginosa* with I. Galactose monopod probe II. Galactose tripod probe.

rials to detect bacteria. Glycan based colorimetric biosensors are example of such fabricated nanomaterials which have been proved to be useful for bio-sensing applications. The present study aims towards developing specific glycoconjugate based fluorescence biosensor and photoablation platform for which tetra carboxy phenyl porphyrin (TCPP), a photosensitizer was incorporated. Galactose monopod and tripod has been used as monovalent and multivalent carbohydrate to detect P. aeruginosa in suspension. Characterization of synthesized nanomaterial and its efficacy in detecting P. aeruginosa were analysed using various spectroscopy and microscopic techniques. Moreover, photoablation property of nanoprobe was analysed by plating assay and ROS production. The results clearly indicate that the synthesized multivalent carbohydrate nanoprobe is very fast, effective, sensitive and specific in detecting upto 10^4 CFU/mL P. aeruginosa in 10 minutes (Figure 3).

Gold nanostar based biosensor: In this study, Gold nanostars (AuNst) have been used as a lytic agent against bacterial species. With the characteristic chemically tuned spike length of AuNst of ~120 nm, piercing of the Gram negative bacterial membrane (10nm thickness) was made possible. The release of cytosolic enzymes from the bacterial strain *Escherichia coli* (*E. coli*), *Salmonella typhimurium* (*S. typhimurium*) was quantified by chromogenic substrates Para-Nitrophenyl-ß-D Glucuronide and magenta caprylate respectively. The addition of non-ionic detergent Triton X-100, enhanced the release of bacterial lysate that improved the generation of coloured product due to the availability of released cytosolic enzymes.

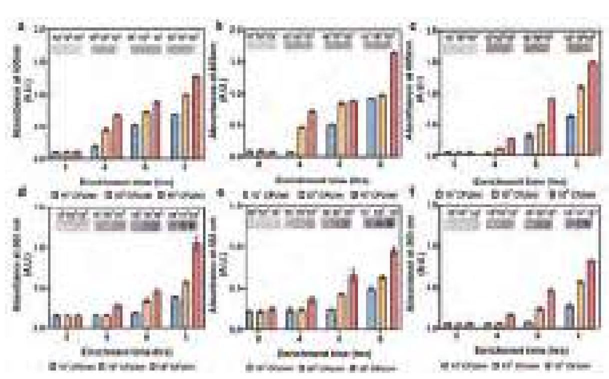


Figure 4. Bacterial lysis estimation. a) Real time food sample bacterial contamination detection after enrichment. a) ß-Glucuronidase detection in *E. coli* spiked milk samples, b) ß-Glucuronidase detection in *E. coli* spiked bread homogenate samples, c) ß-Glucuronidase detection in *E. coli* spiked meat homogenate samples, d) esterase detection in *S. typhimurium* spiked milk samples, e) esterase detection in *S. typhimurium* spiked milk samples, f) esterase detection in *S. typhimurium* spiked bread homogenate samples, g) esterase detection in *S. typhimurium* spiked meat homogenate samples.

Utilizing AuNst as a cell rupturing agent, *S. typhimurium* was lysed by AuNst and various other lysing agents like probe sonication, only Triton X-100 and a combination of AuNst with Triton X-100. Each colorimetric enzyme substrate assay was first optimized using pure enzyme, ß-Glucuronidase for *E. coli* and esterases for *S. typhimurium*. Optimal concentrations of each colorimetric substrate were first established by determining the saturation of the colorimetric intensities at respective absorbance maximum, i.e. 2 mM for PNPG and 10mM for magenta caprylate. Further, to estimate the minimum load of bacterial cells that can be detected, bacterial cells were serially diluted from 10^9-10^4 CFU/ml. Addition of Triton X-100 with mechanical lysis of AuNst improved the lysate generation making bacterial detection possible upto 10^5 CFU/ml for *E. coli* and 106CFU/ml for *S. typhimurium*. In order to detect food contamination, 10^1, 10^2, 10^3 CFU/ml of *E. coli*/ *S. typhimurium* were spiked with ten times diluted samples of milk, and homogenate of bread and meat pieces. The food samples then underwent the process of enrichment making 10^3 CFU/ml of visual detection possible after 4 hours. While after 8 hours, 10^1 CFU/ml could also be faintly visualized as shown in Figure 4.

SALIENT ACHIEVEMENTS

- The limit of detection can be concluded as 100 CFU/mL for *S. typhimuirum* and *E. coli* and 10 CFU/mL for P. aeruginosa.
- Microfluidic-chip based detection has been made possible from a mixture of bacterial strains (*S. typhimurium, E. coli* and *P. aeruginosa*)
- The results with synthesized multivalent carbohydrate nanoprobe are sensitive and specific in detecting upto 10^4 CFU/mL of *P. aeruginosa* in 10 minutes.
- Using AuNst and Triton X bacterial detection was possible upto 10^5 CFU/ml for *E. coli* and 10^6 CFU/ml for *S. typhimurium*.
- Detection of food based bacterial contamination was successful upto 10^1 CFU/ml for both *E. coli* and *S. typhimurium* after an enrichment period of 8 hours.

FUTURE PERSPECTIVES

- To prepare smartphone based device for multiplexed bacterial detection with microfluidic assay.
- To probe the photoablation efficiency of synthesized carbohydrate conjugated nanoprobe.
- To develop AuNst based fast colorimetric bacterial detection assay.

PROGRAM - FNB 03

FOOD & NUTRITIONAL BIOTECHNOLOGY (FNB)

NUTRAGENOMICS FOR HEALTH AND HUMAN WELFARE

FOOD & NUTRITION

Vikas Rishi, PhD
Proteomics

Shrikant S Mantri, PhD
Computational Biology

RESEARCH ASSOCIATE
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RESEARCH FELLOW
Binduma Yadav

FNB 3.1: Mining dynamic DNA methylation pattern during differentiation of Preadipocytes to adipocytes

Obesity is a complex disease involving abnormal/excessive fat accumulation that may impair health. It is the result of a combination of both genetic and epigenetic factors. However, the epigenetic basis of obesity is not very well studied. Recently many groups have found correspondences of methylation with obesity. Specifically, DNA methylation contributes vastly to major hereditary epigenetic disorders. Our study focuses on how alterations in DNA methylation patterns occur under the influence of nutrition during adipocyte differentiation, leading to remodelling or changes in chromatin. In the initial studies, we found that the DNA methylation pattern is dynamic and changes as the differentiation progresses. This variation is not just global but varies prominently at specific locations described as hotspots. Preliminary studies show how the hotspots involved in preadipocyte differentiation are affected. We have used 3T3 cell lines to study the dynamic DNA methylation as cell differentiates from preadipocytes to oil-filled adipocytes. The 3T3 L1 NIH Mus musculus cells are cultured to high confluency, followed by the DNA isolation at different growth phases of cells treated with a hormonal differentiation cocktail. Later, methylation status was determined by

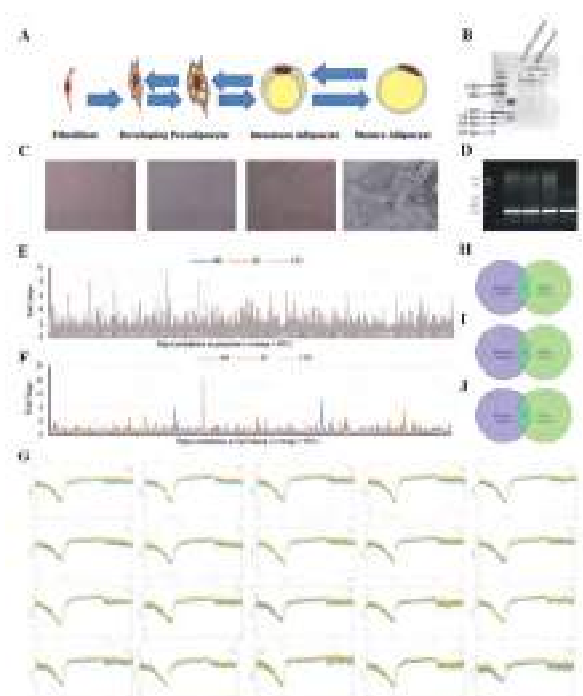

Figure 1. Dynamic DNA methylation pattern during differentiation of preadipocytes. (A) In vitro protocol of differentiation of preadipocytes to adipocytes, (B) McrBc enzyme digested 3T3-L1 genomic DNA, (C) Bright-field images of adipocytes at various stages of differentiation in undifferentiated and differentiated cells at 4 hrs, 2 days, 15 days, (D) PCR amplification of hotspots at chromosome 8, (E) Hypermethylation at promoters, (F) Hypermethylation at CpG Islands, (G) Chromosome-wise gene body plots and overlapping of DMRs and hotspots at 4 hrs (H), 2 days (I) and 15 days (J).

digesting the isolated DNA sample with the McrBc restriction enzyme, followed by short-chain fatty acid treatment to analyse their effect on epigenome during pre-adipocyte differentiation. Also, the whole genome bisulphite sequencing of undifferentiated and differentiated samples has been done, from which we have derived methylome information. The finding of this study will be a milestone in relating methylation with obesity. Further, it will also help in identifying epigenetic therapeutic targets.

RESEARCH PROGRESS

» **Experimental workflow:** Genomic DNA was extracted from NIH 3T3L1 undifferentiated and differentiated cells, which Was further sequenced by whole genome bisulphite sequencing.

» **Bioinformatic Analysis:** Raw sequences were Trimmed using Trimmomatic and Quality control using FastQC. Further, the reads were Deduplicated using bismark and then methyl extraction was done. Methylpy was used to call methylation sites for all Cs all over the genome. Visualisation of Bigwig files was done using IGV. Differentially methylated region analysis done. A comparison of Hotspot Vs DMR is being made.

» **Novel Findings:** Mature adipocytes had the highest number of Differentially methylated regions. Also, the DNA methylation pattern is dynamic as the differentiation of preadipocytes progresses.

SALIENT ACHIEVEMENTS

- Analysis of whole genome bisulphite sequencing and downstream visualisation of the results.
- Differentially methylated regions were explored in Promoter, Hotspot and DMRs.

FUTURE PERSPECTIVES

- To find out how chromatin remodelling is associated with changes in DNA methylation patterns.
- The pattern of differentially methylated regions (DMRs): specifically at promoters and CpG island.
- Whether Hotspots are part of Differentially methylated regions or not.
- Comparison of Hi-C data to further determine how methylation played an essential role in chromatin looping during differentiation of NIH 3T3L1.

PROGRAM - FNB 04

FOOD & NUTRITIONAL BIOTECHNOLOGY (FNB)

POST HARVEST BIOTECHNOLOGY FOR VALUE ADDITION AND INCREASING SHELF LIFE

POST HARVEST BIOTECHNOLOGY

Koushik Mazumder, PhD
Carbohydrate Chemistry

RESEARCH FELLOWS
Swati Kanwar
Sakshi Gumber

FNB 4.1: Development of edible coating and biodegradable packaging materials for the post-harvest shelf life improvement of fresh fruits

Absence of postharvest treatment, traditional storage on farms, infestation of microorganism and pests, non-availability of processing methods are the responsible factors for the highest rate of postharvest losses in fruit and vegetable in India. Due to limited availability of cold chain facilities especially during storage and transportation, development of coating materials to prolong the shelf life of fruits and vegetables is the high priority in this research area. Biodegradable and edible polysaccharides provide a thickening effect and have film forming ability which can be used to prepare coating materials to extend the shelf life of fruits maintaining the sensory and safety qualities. In majority of cases, the coating technology is simple and can be applied even in the farm level; therefore development of coating materials to prolong the shelf life of fruits and vegetables is the high priority, so that spoilage during transportation and marketing is reduced. The present study relates to extraction of polysaccharides from several agricultural and food processing by-products (e.g. wheat straw and potato peel). Further, novel strategies were adopted to structurally modify the polysaccharides via esterification (fatty acid esterification and acetylation) to prepare hydrophobic derivatives to improve their functional properties. These hydrophobic polysaccharide esters were further blended with hydrophilic polysaccharide to prepare composite coating formulations and biodegradable packaging materials for the shelf life improvement of the fresh fruits during transportation and storage.

RESEARCH PROGRESS

Study 1: The aqueous emulsion of arabinoxylan (AX)-stearate esters of cellulose (SAC) (60:40, w/w) and arabinoxylan (AX)-stearate esters of starch (SAS) (60:40, w/w) in the concentration range of 1-4% were prepared. Further, antimicrobial component (Cinnamon bark oil, 0.1%, v/v) was incorporated in the film forming emulsion and applied on the surface of commercially important fruit (guavas) by using dipping method. In the present study efficacy of AX-SC and AX-SAS composite coatings in the concentration ranges of 1-4% was further evaluated for the postharvest quality improvement of fruit (guavas) under recommended ambient storage conditions. During the study, the efficacy of the antimicrobial AX-SAC and

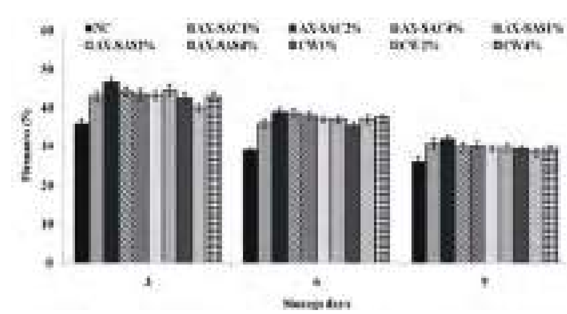

Figure 1. Effect of coating material on the firmness of guavas stored at room temperature (NC: uncoated; AX: arabinoxylan, SAC: stearic acid cellulose ester; SAS: stearic acid starch ester; CW: carnauba wax) during 9 days of storage.

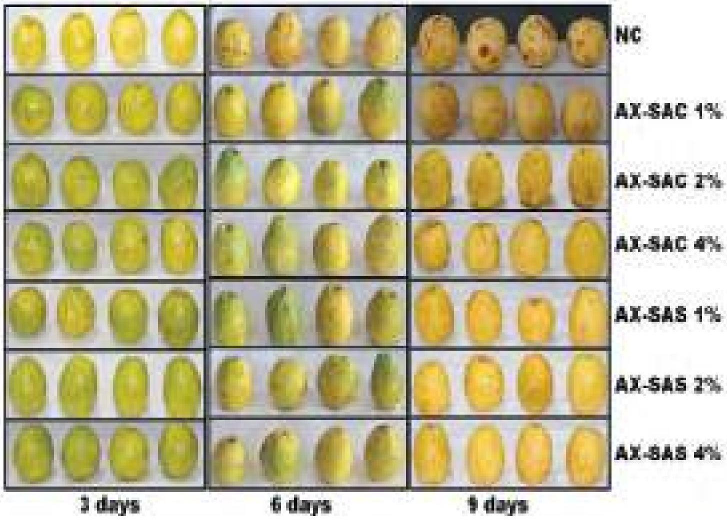

Figure 2. Visual appearance of coated and uncoated guavas (NC: uncoated; AX: arabinoxylan; SAC: stearic acid cellulose ester; SAS: stearic acid starch ester; CW: carnauba wax) during 3-9 days of storage.

AX-SAS coating materials were further compared with commercially used wax (Carnauba). During 3 days storage at 25°C (±2) and 85% RH, all the categories (uncoated and coated guavas) had nearly similar weight loss of less than 3%. However significant differences were observed during the storage period 6-9 days and 85% relative humidity (RH). During 6 days storage, weight loss of 6.96 % was observed in uncoated guavas whereas 5.4-6.3 % for guavas coated with AX-SAC and AX-SAS (1-4%). Weight losses were continuous throughout the storage period, the uncoated guavas experienced weight loss of 10.7% during 9 days storage which was higher than the coated guavas. The result suggested AX-SAC and AX-SAS emulsions had beneficial effect on reducing weight loss whereas similar efficacy of carnauba wax coatings (1-4%) in reducing weight loss was also observed. Endogenous ethylene production is a primary characteristic of ripening in climacteric fruit. The study revealed that during 3 days of storage at 25°C and 85% RH, ethylene production rate of the uncoated and coated guavas are nearly similar up to 0.01 mL/Kg.h. However during 6-9 days storage, ethylene rate of uncoated guavas rapidly raised to 0.36 mL/Kg.h whereas, AX-SAC and AX-SAS (1-4%) coated guavas had much lower ethylene production rate in the range of 0.23-0.29 mL/Kg.h. Carnauba wax coatings had also moderate effect in reducing ethylene production rate (0.16 mL/Kg.h) of guavas compared to uncoated guavas.

The fruit firmness is an important physical parameter which are used for assess the quality of fruits during ripening, storage and distribution. During the storage period of 3-9 days, the firmness of guavas decreased gradually as the storage period progressed. However, the guavas coated with AX-SAC (1-4%) and AX-SAS (1-4%) retained maximum fruit firmness with the textural values in the range of 29.8-38.9 N and 29.4-44.5 N respectively during 6-9 days which might be due to reduced or delayed respiratory activity during storage. Similar efficacy of canauba wax coating (1-4%) was observed in retaining the firmness of guavas (28.2-42.7 N). The least firmness (26.2-28.9 N) (Fig. 1) was recorded for the uncoated guavas which might be due to the higher moisture loss and increased metabolic activities. Surface color is an important index to evaluate the quality and ripening process in guava fruit. The change in the peel color of coated and uncoated guavas during storage of 3-9 days was investigated and presented in Fig.2. In the case of coated guavas (AX-SAC and AX-SAS, 1-4%), the peel color was retained as mostly greenish up to 6 days storage whereas the peel color of un-coated guavas turned to complete yellow during earlier storage of 3 days. At the end of 9 days storage, uncoated fruits developed brownish/black spots of the surface of peel due to deterioration in quality which was not evident in coated fruits. The detailed microbiological analysis further revealed that in uncoated guavas, the molds and yeast increased to 5.29 log cfu/g during 9 days of storage. However, antimicrobial AX-SAC (1-4%) coated guavas exhibited significantly lower molds and yeast count in the range of 4.2-4.7 log cfu/g whereas 3.2-4.0 log cfu/g counts were observed for antimicrobial AX-SAS (1-4%) coated guavas during 9 day of storage.

Study 2: AX and cellulose were extracted from wheat straw with 18% and 45% yield respectively. The compositional analysis of AX as alditol acetate derivatives indicated the presence of arabinose and xylose as major sugar whereas glucose was the major sugar found in cellulose. AX also contained uronic acid (9.6%) and phenolic compounds (23.8 mg GAE/g sample). Further, cellulose was converted

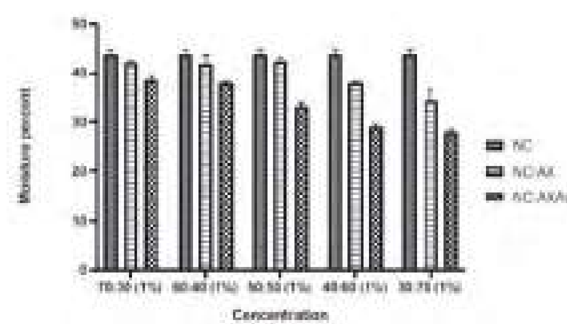

Figure 3. Moisture barrier data of native and nanocomposite films.

into nano-cellulose (NC) by high-pressure homogenization and qualitatively analysed by Dynamic Light Scattering (DLS). DLS data of nano-cellulose clearly indicated the mean particle in the range of 340-520 nm with negative zeta potential value (-34.5mV) which suggested higher stability of NC emulsion. Further, TEM images of NC indicate the long thread like interconnected fibers in the range of 15-20 nm length. AX was esterified by pyridine and acetic anhydride to prepare acetyl esters of AX (AXAc). FTIR spectra were obtained to record the conjugation of acetate group to AX. The absorption band in the 1700-1750 cm-1 range was associated with the stretching vibrations of –C=O bond side groups of acetate. The absorption band at 1034-1219 cm^{-1} was linked with -C-OH stretching in C-O-C band vibrations of polysaccharide. A sharp stretching peak in the region of 1743 cm^{-1} suggested the presence of C=O group of carboxyl ester moiety of esterified AX. Additionally, DS value of 1.85 was observed for AXAc at reaction temperature of 80°C for 1h. Further increase in the reaction interval from 1h to 4h caused no significant changes in DS value which varied in the range of 1.85-1.89.

In our study, native NC (1%) was blended with AX and AXAc for the preparation of nanocomposite films (NC-AX and NC-AXAc) in various proportions (w/w%) such as 70:30 (1%), 60:40 (1%), 50:50 (1%), 40:60 (1%) and 30:70 (1%) in presence of 15% (w/v) glycerol as plasticizer. Water absorption capacity of nanocomposite films was measured under various relative humidity conditions. Under high relative humidity (85%) incorporation of AXAc into NC films caused significant decrease in water content value as compared to the NC and NC-AX films (Fig. 3). Further, it was observed that with the increase in the proportion of AXAc into NC films from 30% to 70%, moisture percentage value was remarkably decreased by nearly in the range of 1.2-1.5 fold. These results suggested incorporation of AXAc to NC had beneficial effect in reducing moisture absorption capacity of the NC-AXAc composite packaging material which could further prevent moisture loss of perishable commodities (fruits/vegetables) during transportation and storage.

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SALIENT ACHIEVEMENTS

- In our study polysaccharides from agricultural and food processing by-products (e.g. wheat straw and potato peel) were extracted to produce edible coating and biodegradable packaging materials for the shelf-life improvement of fruit crops. The studies on lie post-harvest qualities of guavas suggested that AX-SAC and AX-SAS coating formulations (1-4%) significantly improved the quality of the coated fruits during 6 days storage.
- Further, novel stagey was adopted to derivatize AX to produce hydrophobic acetylated arabinoxylan (AXAc) for the improvement in the functional properties for packaging applications. Further, the hydrophobic acetylated arabinoxylan (AXAc) was blended with nano-cellulose (NC) to prepare composite food packaging materials for shelf life extension of perishable commodities.

FUTURE PERSPECTIVES

- Detailed studies on efficacy evaluation of AX-SAC and AX-SAS coating materials for the post-harvest quality improvement of various perishable fruit crops.
- Upscaling and cost-effective production of AX-SAC and AX-SAS coating materials.
- Detailed safety studies (*in vivo* toxicological) on AX-SAC and AX-SAS coating materials.
- Efficacy evaluation of NC-AXAc composite packaging materials for the quality improvement of packed perishable commodities during transportation and storage.

Human RESOURCES

RESEARCH FACULTY

S. No	Name	Designation	Date of Joining
Regular Faculty			
1.	Prof. Ashwani Pareek	Executive Director	01-01-2021
2.	Dr. Vikas Rishi	Scientist F	01-03-2012
3.	Dr. Joy K. Roy	Scientist F	09-08-2010
4.	Dr. Ajay K. Pandey	Scientist F	14-11-2011
5.	Dr. Siddharth Tiwari	Scientist E	28-07-2010
6.	Sh. Shrikant S. Mantri	Scientist E	18-08-2010
7.	Dr. (Mrs.) Monika Garg	Scientist E	30-11-2010
8.	Dr. Kanthi Kiran Kondepudi	Scientist E	02-09-2011
9.	Dr. Mahendra Bishnoi	Scientist E	16-12-2011
10.	Dr. Koushik Mazumder	Scientist E	01-02-2012
11.	Dr. Nitin K. Singhal	Scientist E	02-03-2012
Other Faculty			
12.	Dr. Hena Dhar	Inspire Faculty	25-04-2017
13.	Dr. Gulshan Kumar	Inspire Faculty	11-05-2017
14.	Dr. Pramod Kaitheri Kandoth	Ramalingaswami Fellow	17-08-2017
15.	Dr. Rupam Kumar Bhunia	Inspire Faculty	01-01-2018
16.	Dr. Rupesh Deshmukh	Ramalingaswami Fellow	23-04-2018
17.	Dr. Vanish Kumar	Inspire Faculty	24-04-2018
18.	Dr. Humira Sonah	Ramalingaswami Fellow	07-05-2018
19.	Dr. Sivasubramanian	Inspire Faculty	03-10-2018
20.	Dr. Ravindra Pal Singh	Ramalingaswami Fellow	01-06-2019
21.	Dr. Prafull Salvi	Inspire Faculty	01-04-2019
22.	Dr. Karambir Kaur	Project Scientist	13-08-2019
23.	Dr. Anjil Kumar Srivastava	Ramalingaswami Fellow	08-03-2021
24.	Dr. Vadthya Lokya	Project Scientist	27-03-2021

TECHNICAL & ENGG. SUPPORT

S. No	Name	Designation	Date of Joining
1.	Sh. Jagdeep Singh	Senior Technical Assistant	01-03-2011
2.	Sh. Jaspreet Singh	Assistant Engineer	19-03-2012
3.	Sh. Sushant Vats	Assistant Engineer	02-04-2012
4.	Dr. Mainpal Singh	Senior Technical Assistant	24-12-2012
5.	Sh. Atul Kumar Kesarwani	Senior Technical Assistant	21-01-2013
6.	Sh. Kamalendra	Senior Technical Assistant	18-03-2013
7.	Sh. Baldev Krishan	Computer Operator	11-12-2019
8.	Sh. Sandeep	Senior Technical Assistant	20-12-2019
9.	Sh. Sandeep Kumar	Senior Technical Assistant	26.10.2021
10.	Sh. Jayhind Nishad	Senior Technical Assistant	11.11.2021

ADMINISTRATION

S. No	Name	Designation	Date of Joining
1.	Sh. S. Krishnan	Manager Administration	10-03-2010
2.	Sh. Suneet Verma	Manager Finance	15-09-2011
3.	Dr. Munish Singla	Finance Officer	01-01-2020
4.	Sh. Sabir Ali	Management Assistant (Admin.)	21-01-2011
5.	Mrs. Hema Pharswan	Management Assistant (Accounts)	01-04-2011
6.	Sh. Ashish Arora	Management Assistant (Admin.)	15-06-2012
7.	Sh. Arun Kumar	Management Assistant (Public Relation)	21-06-2012
8.	Mrs. Anukiran Bagga	Library Assistant	19-12-2012

HUMAN RESOURCE DEVELOPMENT

S. No	Name	Designation	Date of Joining
National Post-Doctoral Fellows			
1.	Dr. Pradeep Singh	Agri-Biotechnology	03-03-2022
M K Bhan Fellows			
1.	Dr. Abishek Bhandawat	Agri-Biotechnology	09-04-2018
2.	Dr. Mohit Kumar	Nutrition Science	01-10-2021
3.	Dr. Aanchal Aggarwal	Nutrition Science	01-10-2021
4.	Dr. Maninder Meenu	Agri-Biotechnology	01-10-2021
5.	Dr. Ajit Pal Singh	Agri-Biotechnology	01-10-2021

Research Scholars

S. No	Name	Area of Research	Awarding University-Institute
Student awarded Ph.D. degree			
1.	Sh. Anil Kumar	Expression and functional characterization of yellow stripe like transporters in hexaploid wheat.	Panjab University, Chandigarh
2.	Ms. Shivani Sharma	Molecular characterization and expression profiles of membrane transporters involved in Fe and Zn homeostasis in hexaploid wheat.	Panjab University, Chandigarh
3.	Ms. Neha Thakur	Identification, cloning and functional characterization of Glucuronokinase gene from wheat (Triticum aestivum L.)	Panjab University, Chandigarh
4.	Sh. Mohammad Saba Rahim	Genetic Dissection of starch quality traits in wheat and evaluation of its health benefits.	Central University of Punjab
5.	Sh. Nitesh Priyadarshi	Multivalent Carbohydrate functionalized gold nanorod based nano biosensor for detection and ablation of food borne bacteria.	Panjab University, Chandigarh
6.	Sh. Rajdeep Jaiswal	Identification, Molecular cloning and characterization of secretory protein wheat leaf rust fungus (Puccinia triticina)	Panjab University, Chandigarh
7.	Ms. Ruchi Bansal	Identification and characterization of genes for hypersensitive response in rice magnaporthe oryzae interaction.	Panjab University, Chandigarh
8.	Ms. Shimayali Kaushal	Gold nanorod based Nano biosensor for sensitive detection, isolation and photoablation of food borne bacteria.	Panjab University, Chandigarh
9.	Sh. Vishal Singh	Development of siRNA funtionalized graphene oxide based therapeutic Nano formulation to ameliorate type-2 diabetes mellitus (T2DM)	Panjab University, Chandigarh
10.	Ms. Gazaldeep	Understanding the molecular basis of iron and phosphate homeostasis in hexaploid wheat.	Panjab University, Chandigarh
11.	Sh. Venkatesh Chunduri	Dynamics of heat stress response in wheat.	Panjab University, UIET

HUMAN RESOURCE DEVELOPMENT

S. No	Name of the Student	Position at Present	Date of Joining
1.	Ms. Shimayali Kaushal	Senior Research Fellow	21-01-2016
2.	Ms. Raminder Kaur	Senior Research Fellow	01-09-2016
3.	Ms. Poonam Sagar	Senior Research Fellow	21-06-2017
4.	Ms. Vinita Sharma	Senior Research Fellow	24-07-2017
5.	Sh. Siddhanth Chaturvedi	Senior Research Fellow	11-09-2017
6.	Ms. Swati Kanwar	Senior Research Fellow	13-09-2017
7.	Ms. Sunaina Kaul	Senior Research Fellow	22-09-2017
8.	Ms. Ritika Gupta	Senior Research Fellow	20-02-2018
9.	Sh. Vibhu	Senior Research Fellow	10-10-2018
10.	Ms. Jasleen	Senior Research Fellow	10-10-2018
11.	Mrs. Ruchi Bansal	Senior Research Fellow	11-12-2018
12.	Ms. Surbhi Kumawat	Senior Research Fellow	15-01-2019
13.	Ms. Ruchika Bhatia	Senior Research Fellow	24-08-2019
14.	Ms. Nitika Rana	Senior Research Fellow	13-11-2020
15.	Sh. Mohammad Saba Rahim	Senior Research Fellow	01-01-2021
16.	Mrs. Shika Sharma	Senior Research Fellow	11-03-2021
17.	Mrs. Anita Kumari	Junior Research Fellow	02-05-2018
18.	Ms. Varnika Rana	Project Fellow-I	18-06-2018
19.	Sh. Himanshu Sharma	Research Associate-III	05-10-2018
20.	Sh. Mithlesh Kumar Sardar	Project Field Assistant	29-03-2019
21.	Ms. Satveer Kaur	Senior Research Fellow	01-05-2019
22.	Sh. Gurvinder Singh	Field Assistant	02-05-2019
23.	Sh. Hemraj	Field Assistant	02-05-2019
24.	Ms. Shivani Sharma	Junior Research Fellow	03-05-2019
25.	Sh. Jitendra Kumar	Research Associate-I	06-05-2019
26.	Mrs. Lakhani Hiralben L.	Senior Research Fellow	20-05-2019
27.	Sh. Pargat Singh	Field Assistant	21-05-2019
28.	Mrs. Aanchal Chaudhary	Senior Research Fellow	31-05-2019
29.	Ms. Lovenpreet Kaur	Senior Research Fellow	07-06-2019
30.	Sh. Kalyan Vaid	Junior Research Fellow	24-06-2019

S. No	Name of the Student	Position at Present	Date of Joining
31.	Sh. Koushik Shah	Research Associate-I	22-07-2019
32.	Sh. Gaurav Raturi	Senior Research Fellow	30-08-2019
33.	Ms. Sunaina	Project fellow-I	02-09-2019
34.	Sh. Deepak Duhan	Project fellow-I	02-09-2019
35.	Mrs. Shivani Sharma	Project Fellow-I	21-10-2019
36.	Ms. Jasmeen Dhiman	Project Fellow-I	21-10-2019
37.	Sh. Himanshu Yadav	Project Fellow-I	01-11-2019
38.	Sh. Dalwinder Singh	Research Associate-II	18-11-2019
39.	Ms. Gunashri Ganesh Padalkar	Senior Research Fellow	10-12-2019
40.	Ms. Navpreet Kaur	Junior Research Fellow	16-12-2019
41.	Ms. Nitika	Junior Research Fellow	01-01-2020
42.	Ms. Nikita	Project Fellow-I	24-12-2019
43.	Ms. Vandana Thakral	Senior Research Fellow	14-01-2020
44.	Sh. Raja Bhaiyya	Junior Research Fellow	21-01-2020
45.	Ms. Sreeja S S	Senior Research Fellow	14-02-2020
46.	Ms. Pallavi	Senior Research Fellow	17-02-2020
47.	Ms. Anjali	Senior Research Fellow	28-02-2020
48.	Ms. Kirti Devi	Senior Research Fellow	05-03-2020
49.	Ms. Kshitija Sinha	Senior Research Fellow	09-03-2020
50.	Ms. Shivani Gajbhiye	Senior Research Fellow	17-03-2020
51.	Ms. Nikita Saini	Project Fellow-I	08-07-2020
52.	Ms. Shivani Jaiswal	Junior Research Fellow	01-09-2020
53.	Ms. Raksha	Project Fellow-I	07-09-2020
54.	Ms. Geetika Bajaj	Junior Research Fellow	14-09-2020
55.	Sh. Tushar Matta	Junior Research Fellow	17-09-2020
56.	Ms. Ankita Mishra	Research Associate-II	18-11-2020
57.	Ms. Nishu Gandass	Project Fellow-I	24-11-2020
58.	Ms. Shikha Sharma	Project Associate-II	01-12-2020
59.	Ms. Priyanka Garg	Junior Research Fellow	13-01-2021
60.	Sh. Rajdeep Jaiswal	Research Associate-I	18-01-2021

HUMAN RESOURCE DEVELOPMENT

S. No	Name of the Student	Position at Present	Date of Joining
61.	Sh. Mandlik Rushil Ramesh	Junior Research Fellow	19-01-2021
62.	Sh. Rishav Sharma	Project Fellow-I	22-01-2021
63.	Sh. Naveen Kumar	Junior Research Fellow	25-01-2021
64.	Ms. Simranjit Kaur	Project Fellow-I	12-02-2021
65.	Ms. Jayshree Niharika Panda	Junior Research Fellow	13-02-2021
66.	Ms. Riya Joon	Junior Research Fellow	26-02-2021
67.	Sh. Dharavath Ravinder Naik	Research Associate-II	09-03-2021
68.	Sh. Yatin	Project Assistant	27-03-2021
69.	Sh. Hardeep Singh	Project Associate-I	27-03-2021
70.	Ms. Lata Pawar	Junior Research Fellow	30-03-2021
71.	Ms Nandni	Junior Research Fellow	03-04-2021
72.	Ms. Nikita	Junior Research Fellow	06-04-2021
73.	Sh. Himanshu Sharma	Research Associate-I	06-04-2021
74.	Sh. Rajdeep Jaswal	Research Associate-I	16-07-2021
75.	Ms. Saloni	Research Associate-I	19-07-2021
76.	Ms. Damini	Junior Research Fellow	23-07-2021
77.	Mrs. Ritu Kapoor	Research Associate-I	30-07-2021
78.	Mrs. Gazaldeep Kaur	Research Associate-I	03-08-2021
79.	Sh. Lakshay Mahajan	laboratory Animal Vet	27-08-2021
80.	Ms. Ankita Negi	Project Fellow-I	11-11-2021
81.	Ms. Afsana Parveen	Senior Research Fellow	11-11-2021
82.	Sh. Raman Kumar	Scientific Assistant	08-12-2021
83.	Ms. Shahirina Khan	Senior Project Associate	09-12-2021
84.	Sh. Robin Sharma	Field Worker	13-12-2021
85.	Ms. Priyanka Kaundal	Research Associate-III	20-12-2021
86.	Ms. Shristi Sharma	Senior Research Fellow	20-12-2021
87.	Sh. Gaurav Augustine	Project Fellow-I	27-12-2021
88.	Sh. Vishal Sharma	Senior Project Associate	06-01-2022
89.	Sh. Saurabh Prakash Pandey	Research Associate-I	07-01-2022
90.	Sh. Harish Jadhav	Project Fellow-I	24-01-2022
91.	Sh. Pradeep Singh	SERB NPDF	03-03-2022
92.	Sh. Rabi Shankar Ojha	Junior Research Fellow	04-03-2022
93.	Ms. Neha Thakur	Research Associate-I	05-03-2022
94.	Sh. Apporv Tiwari	Project Scientist-I	07-03-2022
95.	Sh. Usman Ali	Project Scientist-I	08-03-2022
96.	Ms. Manisha	Junior Research Fellow	31-03-2022
97.	Ms. Promila	Junior Research Fellow	25-03-2022
98.	Ms. Anu Walia	Junior Research Fellow	21-03-2022
99.	Ms. Vandita Tiwari	Project Assistant	31-03-2022

HUMAN RESOURCE DEVELOPMENT

Research Scholars pursuing Ph.D in NABI from Regional Centre of Biotechnology

S. No	Name of the Student	Position at Present	Date of Joining
1.	Ms. Harshita Pandey	PhD Research Scholar	27-07-2018
2.	Ms. Sanskriti	PhD Research Scholar	26-07-2018
3.	Ms. Neha	PhD Research Scholar	23-07-2018
4.	Sh. Prashant Kumar	PhD Research Scholar	24-07-2018
5.	Ms. Anjali Verma	PhD Research Scholar	26-07-2018
6.	Sh. Palvinder Singh	PhD Research Scholar	31-07-2018
7.	Ms. Binduma Yadav	PhD Research Scholar	25-07-2018
8.	Ms. Ruchika Maurya	PhD Research Scholar	31-07-2018
9.	Sh. Rohit Maurya	PhD Research Scholar	02-08-2018
10.	Sh. Virender Kumar	PhD Research Scholar	31-07-2018
11.	Ms. Akansha Madhawan	PhD Research Scholar	25-07-2018
12.	Ms. Varsha Meena	PhD Research Scholar	26-07-2018
13.	Sh. Anuj Shukla	PhD Research Scholar	26-07-2018
14.	Ms. Sakshi Gumber	PhD Research Scholar	23-08-2018
15.	Sh. Surender Singh	PhD Research Scholar	24-07-2019
16.	Ms. Roni Chaudhary	PhD Research Scholar	26-07-2019
17.	Sh. Vikas Fandade	PhD Research Scholar	26-07-2019
18.	Ms. Diksha Choudhary	PhD Research Scholar	22-07-2019
19.	Sh. Yogesh Sharma	PhD Research Scholar	25-07-2019
20.	Ms. Anita Rawat	PhD Research Scholar	25-07-2019
21.	Ms. Ankita Rana	PhD Research Scholar	29-07-2019
22.	Ms. Bhawna	PhD Research Scholar	28-09-2020
23.	Ms. Deepshika Tyagi	PhD Research Scholar	28-09-2020
24.	Ms. Mona	PhD Research Scholar	28-09-2020
25.	Ms. Shruti Saini	PhD Research Scholar	30.09.2021
26.	Ms. Era Chaudhary	PhD Research Scholar	30.09.2021

HUMAN RESOURCE DEVELOPMENT

Trainees

S. No	Name of the Student	Designation	Date of Joining
1.	Sh. Mohd Noman	Trainee	03-01-2022
2.	Sh. Keshav Jamwal	Trainee	03-01-2022
3.	Sh. Veerendra Maraiya	Trainee	06-01-2022
4.	Ms. Shreya Setia	Trainee	03-01-2022
5.	Ms. Manisha	Trainee	01-02-2022
6.	Ms. Muskan Chopra	Trainee	03-01-2022
7.	Ms. Nisha Dahiya	Trainee	01-02-2022
8.	Ms. Humara Mehshar	Trainee	03-01-2022
9.	Ms. Chopada Rinkal Nareshbhai	Trainee	06-01-2022
10.	Ms. Pragati Gupta	Trainee	04-01-2022
11.	Ms. Ridhima	Trainee	01-02-2022
12.	Ms. Simran	Trainee	17-08-2022
13.	Ms. Nikita Sood	Trainee	17-08-2022
14.	Ms. Hrishika	Trainee	20-08-2022
15.	Ms. Gifty	Trainee	17-08-2022
16.	Ms. Shivangi	Trainee	20-08-2022
17.	Ms. Manika	Trainee	20-08-2022
18.	Sh. Pulkit	Trainee	20-08-2022
19.	Sh. Hemant	Trainee	20-08-2022
20.	Ms. Dimpy	Trainee	20-08-2022
21.	Ms. Nimisha	Trainee	20-08-2022
22.	Ms. Magnolia	Trainee	20-08-2022
23.	Sh. Divyanshu	Trainee	20-08-2022
24.	Ms. Sonia Bharti	Trainee	20-08-2022
25.	Ms. Sakshi	Trainee	20-08-2022
26.	Sh. Manjeet	Trainee	20-08-2022
27.	Ms. Aayushee	Trainee	20-08-2022

Research PUBLICATIONS

PEER-REVIEWED ARTICLES

1. Agrawal A, Pareek A, and Dkhar J (2021). Genetic Basis of Carnivorous Leaf Development. Frontiers in Plant Science 12. doi: https://doi.org/10.3389/fpls.2021.825289
2. Anwar K, Joshi R, Dhankher OP, Singla-Pareek SL and Pareek A (2021). Elucidating the response of crop plants towards individual, combined and sequentially occurring abiotic stresses. International Journal of Molecular Sciences 22, 6119. doi: https://doi.org/10.3390/ijms22116119
3. Anwar K, Joshi R, Morales A, Das G, Yin X, Anten NP, Raghuvanshi S, Bahuguna RN, Singh MP, and Singh RK (2022). Genetic diversity reveals synergistic interaction between yield components could improve the sink size and yield in rice. Food and Energy Security, e334. doi: https://doi.org/10.1002/fes3.334
4. Chen T, Shabala S, Niu Y, Chen ZH, Shabala L, Meinke H, Venkataraman G, Pareek A, Xu J and Zhou M (2021). Molecular mechanisms of salinity tolerance in rice. The Crop Journal 9, 506-520. doi: https://doi.org/10.1016/j.cj.2021.03.005
5. Gupta BK, Sahoo KK, Anwar K, Nongpiur RC, Deshmukh R, Pareek A and Singla-Pareek SL (2021). Silicon nutrition stimulates Salt-Overly Sensitive (SOS) pathway to enhance salinity stress tolerance and yield in rice. Plant Physiology and Biochemistry 166, 593-604. doi: 10.1016/j.plaphy.2021.06.010
6. Joshi S, Nath J, Singh AK, Pareek A and Joshi R (2022). Ion transporters and their regulatory signal transduction mechanisms for salinity tolerance in plants. Physiologia Plantarum, e13702. doi: 10.1111/ppl.13702
7. Kumar G, Basu S, Singla-Pareek SL and Pareek A (2022). Unraveling the contribution of OsSOS2 in conferring salinity and drought tolerance in a high-rice. Physiologia Plantarum 174, e13638. doi: 10.1111/ppl.13638
8. Kumar J, Mishra A, Kumar A, Kaur G, Sharma H, Kaur S, Sharma S, Devi K, Garg M and Pandey A K (2022). Whole genome re-sequencing of indian wheat genotypes for identification of genomic variants for grain iron and zinc content. Molecular Biology Reports 49, 7123-7133. doi: 10.1007/s11033-022-07593-w
9. Mishra M, Rathore RS, Singla-SL, and Pareek A (2022). High lysine and high protein-containing salinity-tolerant rice grains (Oryza sativa cv IR64). Food and Energy Security 11, e343. doi: https://doi.org/10.1002/fes3.343
10. Bhatia R, Sharma S, Bhadada SK, Bishnoi M and Kondepudi KK (2022). Lactic acid bacterial supplementation ameliorated the LPS-Induced gut inflammation and dysbiosis in mice. Frontiers in Microbiology. doi: 10.3389/fmicb.2022.930928
11. Bansal Y, Singh R, Sodhi RK, Khare P, Dhingra R, Dhingra N, Bishnoi M, Kondepudi KK and Kuhad A (2022). Kynurenine monooxygenase inhibition and associated reduced quinolinic acid reverses depression-like behavior by upregulating Nrf2/ARE pathway in mouse model of depression: In-vivo and In-silico studies. Neuropharmacology. doi: 10.1016/j.neuropharm.2022.109169
12. Dineshbhai CK, Basaiawmoit B, Sakure AA, Maurya R, Bishnoi M, Kondepudi KK, Patil GB, Mankad M and Liu Z, Hati S (2022). Exploring the potential of Lactobacillus and Saccharomyces for biofunctionalities and the release of bioactive peptides from whey protein fermentate. Food Bioscience. doi: https://doi.org/10.1016/j.fbio.2022.101758
13. Chaturvedi S, Khan S, Bhunia RK, Kaur K and Tiwari S (2022). Metabolic engineering in food crops to enhance ascorbic acid production: crop biofortification perspectives for human health. Physiology Molecular Biology of Plants. doi: https://doi.org/10.1007/s12298-022-01172-w
14. Ashokbhai JK, Basaiawmoit B, Das S, Sakure A, Maurya R, Bishnoi M, Kondepudi KK, Padhi S, Rai AK, Liu Z and Hati S (2022). Antioxidative, antimicrobial and anti-inflammatory activities and release of ultra-filtered antioxidative and antimicrobial peptides during fermentation of sheep milk: In-vitro, in-silico and molecular interaction studies. Food Bioscience. doi: https://doi.org/10.1016/j.fbio.2022.101666
15. Dineshbhai CK, Basaiawmoit B, Sakure AA, Maurya R, Bishnoi M, Kondepudi KK, Patil GB, Mankad M, Liu Z and Hati S (2022). Exploring the potential of Lactobacillus and Saccharomyces for biofunctionalities and the release of bioactive peptides from whey protein fermentate. Food Bioscience. doi: https://doi.org/10.1016/j.fbio.2022.101758
16. Mansinhbhai CH, Sakure A, Liu Z, Maurya R, Das S, Basaiawmoit B, Bishnoi M, Kondepudi KK, Padhi S, Rai AK, Mishra BK and Hati S (2022). Anti-Inflammatory, ACE Inhibitory, Antioxidative Activities and Release of Novel Antihypertensive and Antioxidative Peptides from Whey Protein Hydrolysate with Molecular Interactions. Journal of the American Nutrition Association. doi: 10.1080/07315724.2022.2052201
17. Sharma S, Singh S, Chaudhary V, Mantri S, Chander A, Maurya R, Rajarammohan S, Singh RP, Rishi P, Bishnoi M, Bhadada SK, Kondepudi KK (2022). Isomaltooligosaccharides utilization and genomic characterization of human infant anti-inflammatory Bifidobacterium longum and Bifidobacterium breve strains. 3Biotech. doi: 10.1007/s13205-022-03141-2
18. Singh RP, Niharika J, Kondepudi KK, Bishnoi M and Tingirikari JMR (2022). Recent understanding of human milk oligosaccharides in establishing infant gut microbiome and roles in immune system. Food Research International. doi: https://doi.org/10.1016/j.foodres.2021.110884
19. Kumar J, Mishra A, Kumar A, Kaur G, Sharma H, Kaur S, Sharma S, Devi K, Garg M, Pandey AK, Bishnoi M, Pareek A and Roy JK (2022). Whole genome re-sequencing of indian wheat genotypes for identification of genomic variants for grain iron and zinc content, Molecular Biology Reports. doi: 10.1007/s11033-022-07593-w
20. Kumar V, Kumar V, Mahajan N, Kaur J, Devi K, Dharavath RN, Singh RP, Kondepudi KK, Bishnoi M. (2022). Mucin secretory action of capsaicin prevents high fat diet-induced gut barrier dysfunction in C57BL/6 mice colon. Biomedicine Pharmacotherapy. doi: 10.1016/j.biopha.2021.112452.
21. Kaur G, Kaur D, Kansal SK, Garg M, Krishania M (2022). Potential Cocoa Butter Substitute Derived from Mango Seed Kernel. Food Chemistry. https://doi.org/10.1016/j.foodchem.2021.131244.
22. Mishra A. Singh PK, Bhandawat A, Sharma V, Sharma V, Singh P, Roy J and Sharma H. (2022). Analysis of SSR and SNP markers, bioinformatics, methods and application. In Dev Bukhsh Singh and Rajesh Kumar Pathak (eds) (pp. 131-144). Academic. https://doi.org/10.1016/B978-0-323-89775-4.00017-1.
23. Singh RP, Niharika J, Kondepudi KK, Bishnoi M, Tingirikari JMR (2022). Human milk oligosaccharides in establishing infant gut microbiome and roles in immune system and defence: Current understanding and future trends. Food Research International. doi: 10.1016/j.jff.2020.104074.
24. Sharma V, Mishra A, Sharma H, Kumar P and Roy (2022). Unraveling novel and rare mutations for alpha amylase and key transcription factors in EMS induced wheat mutants for amylose by TILLING. Molecular Biology Reports. https://doi.org/10.1007/s11033-022-07155-0.
25. Verma A, Nidhi N, Kaur G, Mantri S, SharmaTR, Pandey AK, Kandoth PK (2022). Contrasting β-ODAP content correlates with stress gene expression in Lathyrus cultivars. Physiol Plant. 174-1 https://doi.org/10.1111/ppl.13616.
26. Guillaume NM, Mollie L, Bhunia R, Shankhapal AR, Noleto-Dias C, Lomax C, Ward JL, Kurup S, Eastmond PJ (2022). Diverting phenylpropanoid pathway flux from sinapine to produce industrially useful 4-vinyl derivatives of hydroxycinnamic acids in Brassicaceous oilseeds. Metabolic Engineering. https://doi.org/10.1016/j.ymben.2022.01.016.
27. Awasthi P, Khan S, Lakhani H, Chaturvedi S, Shivani, Kaur N, Singh J, Kesarwani AK, and Tiwari S (2022). Transgene-free genome editing supports CCD4 role as a negative regulator of ß-carotene in banana. Journal of Experimental Botany. erac042, https://doi.org/10.1093/jxb/erac042.
28. Kumar J, Kumar A, Mishra A, Mishra VK, and Roy J (2022). Genetic variation, heritability, genetic advance, micronutrients, and grain morphology trait associations in EMS induced mutant lines of wheat (Triticum aestivum L.). Genetic Resources and Crop Evolution, https://doi.org/10.1007/s10722-022-01363-0.
29. Kaur G, Kaur D, Kansal SK, Garg M, Krishania M (2022). Potential cocoa butter substitute derived from mango seed kernel. Food chemistry 372: 131244. doi.

org/10.1016/j.foodchem.2021.131244.
30. Bhutada G, Menard G, Bhunia RK, Hapeta PP, Ledesma-Amaro L, Eastmond PJ (2022). Production of human milk fat substitute by engineered strains of Yarrowia lipolytica. Metabolic Engineering Communications: e00192. https://doi.org/10.1016/j.mec.2022.e00192.
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PATENTS

1. A process for the development of composite films/coating materials based on arabinoxylan-cellulose fatty acid esters and arabinoxylan-starch fatty acid esters from wheat straw and potato peel (Full patent Application No: 202111009769).
2. Mechano-bactericidal action of gold nanostars embedded hydrogel bandage for biosafety Applications (Application No. 202111049490).
3. Mechanistic killing of Bacteria by Gold Nanostar colloid solution for food safety applications (Application No. 202111049491).

INVITED TALKS

1. Dr. Siddharth Tiwari delivered an invited talk on the topic "Experimental design and characterization of genome-edited plants: A practical application in banana" in a national workshop on "CRISPR/Cas9 based Genome Editing Technology" organized by the ICAR-Indian Institute of Agricultural Biotechnology (ICAR-IIAB), Ranchi on 8th April 2021.
2. Dr. Kanthi Kiran delivered a webinar talk on "Pro-, Pre- and synbiotics for healthy living" at the Department of Biomedicine, Medical Faculty, University of Porto, Porto, Portugal on 1st June 2021.
3. Dr. Kanthi Kiran delivered an invited talk on the topic "Beneficial Modulation of the Gut Microbiota as a Strategy for Mitigating Metabolic Diseases" at the Department of Microbiology and Cell Science Genetics Institute, Institute of Food and Agricultural Sciences, University of Florida, United States, 26th June 2021.
4. Dr Siddharth Tiwari delivered an invited talk on the topic "Crop improvement strategies using biotechnological tools (CRISPR and Transgenic)" in the national seminar on "Challenges in Food Production under Resilient Environment" organized by the Department of Botany, Maharshi Dayanand University, Rohtak on 20th July 2021.
5. Prof. Ashwani Pareek delivered an invited talk on the topic "Waste Management Technologies: Biomass and Agro-residue" at IIT Delhi on 31st July 2021.
6. Prof. Ashwani Pareek delivered a talk on the topic "Genetics and Genomics Technologies for Crop Improvement" at Hans Raj College, Delhi University on 01st August 2021.
7. Dr. Kanthi Kiran and Dr. Mahendra Bishnoi attended a workshop on the topic "Gut Microbiota and the Gut-Brain Connection: There is Science in Gut Feeling After All" held at Universiti Teknologi MARA Selangor Branch, Puncak Alam Campus, Malaysia during 2nd - 6th August 2021.
8. Prof. Ashwani Pareek delivered a talk on the topic "Title Excitement in Plant Sciences" at JIIT, NOIDA on 02nd August 2021.
9. Dr. Koushik Mazumder delivered an invited talk on the topic "Post-harvest Management of Horticultural Commodities" at Faculty Development Programme on 3rd August 2021 at JIIT, Noida.
10. Dr. Joy K Roy delivered an invited lecture on the topic "Advancement in next-gen DNA/RNA sequencing technologies" at a Faculty Development Program held on 6th August 2021 at Department of Biotechnology, Jaypee Institute of Information Technology, Noida.
11. Prof. Ashwani Pareek delivered an invited talk on the topic "Ensuring Food Security to Feed Nine Billion" at IISER, Tirupati on 09th August 2021.
12. Prof. Ashwani Pareek delivered an invited talk on the topic "Biodiversity and conservation of Mangroves" at Doon Public School on 17th August 2021.
13. Dr. Mahendra Bishnoi presented an invited lecture at Interdisciplinary Center for Clinical Research (IZKF) workshop for postgraduates, Erlangen, September 2021.
14. Dr. Siddharth Tiwari was invited by International Centre for Genetic Engineering and Biotechnology (ICGEB) to deliver a lecture in the DBT's Science Setu Program held on 28th September 2021. He delivered a lecture on the topic "Biofortification of Crops Through Genetic Engineering"
15. Dr. Siddharth Tiwari was invited to deliver a lecture in the training program on "Biosecurity and Biosafety: Policies, Diagnostics, Phytosanitary Treatments and Issues" organized by ICAR- National Bureau of Plant Genetic Resources (NBPGR), New Delhi from 15th - 24th September 2021. He delivered a lecture on the topic "Gene Editing: A Case Study of Banana".
16. Prof. Ashwani Pareek delivered an invited talk on the topic "Agri-Food Biotechnology for Transforming Food Processing Sector" at NIFTEM, Kundli-India on 15th October 2021.
17. Dr Siddharth Tiwari delivered an invited lecture on the topic "Banana genome engineering towards reducing vitamin A deficiency" on 21st October 2021 at an international conference on "AgOmics'2021" organized by the Tamil Nadu Agricultural University (TNAU), Coimbatore (Tamil Nadu) during 21st – 23rd October 2021.
18. Ms. Payal Kapoor presented a poster on the topic "Anthocyanin biofortified wheat on gut micobiota and associated implications in mice" at the 7th Whole Grain Summit 2021, held online from 25th – 27th October 2021, CREA, Italy.
19. Dr. Mahendra Bishnoi presented an invited lecture at 14th Congress of Nutrition, Belgrade, 8th – 10th November 2021.
20. Dr Siddharth Tiwari was invited to participate in the panel discussion on "Gene-editing technologies and applications" at Bengaluru Technology Summit (BTS) 2021 organized during 17th -19th November 2021.
21. Dr Siddharth Tiwari was invited for the lecture at the National Conference on "CRISPR/Cas: From Biology to Technology" jointly organized by SRM University, Amaravati, Andhra Pradesh and the Institute of Bioinformatics and Applied Biotechnology (IBAB), Bengaluru, Karnataka from 25th -27th November 2021. Dr. Tiwari delivered a talk on the topic "CRISPR-based genome editing of agriculture crops: special reference to banana".
22. Dr. Shrikant Mantri delivered an invited online lecture on the topic "Metagenome mining to discover novel anti-infectives" at "ATAL FACULTY DEVELOPMENT PROGRAM- 2021" held on 7th December 2021 at Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research Pimpri, Pune.
23. Prof. Ashwani Pareek delivered a talk on topic "Planting Excitements" at HRDC JNU in 26th Refresher Course in Life Sciences & Biotechnology on 14th December 2021.
24. Dr Siddharth Tiwari was invited as a keynote speaker and for the panel discussion in conclave on "Global MP Scientists & Technocrats Meet" organized jointly by IIT, Indore, Vigyan Bharati, Malwa Prant and Madhya Pradesh Council of Science & Technology (MPCST), Govt. of Madhya Pradesh on 23rd December 2021.
25. Prof. Ashwani Pareek delivered a talk on the topic "Secondary Agriculture" at College of Agricultural Engineering, University of Agricultural Sciences, Bangalore on 10th January 2022.
26. Dr Siddharth Tiwari was invited to participate in the "Stakeholders Consultation on Genetically Modified (GM) Food and Feed Import and Detection of Unauthorized GM Food Events" organized by the National Academy of Agricultural Sciences (NAAS) on 10th January 2022.
27. Prof. Ashwani Pareek delivered a talk at Goa University for the Refresher Course in Life Sciences (Inter/Multi-disciplinary) on 12th January 2022.
28. Dr. Siddharth Tiwari was invited as a resource person to deliver a lecture (virtual mode) in the AICTE-ISTE sponsored induction/refresher program on the topic "Technology Driven Health Care system" organized by Jawaharlal College of Engineering and Technology, Lakkidi, Palakkad, Kerala from 18th January to 24th January 2022. He delivered a lecture on the topic "Plant Biotechnology in Health Care" on 19th January 2022.
29. Ms. Anjali sharma presented a poster on the topic "Nutritional value of colored wheatgrass varieties (black, blue and purple)" in the First International symposium on "Cereals for Food Security and Climate Resilience held on 20th January 2022, ICAR, Karnal, India.
30. Ms. Anita Kumari presented a poster on the topic "Biochemical characterization and quantification of anthocyanins enriched black and purple vermicelli" in the First International symposium on "Cereals for Food Security and Climate Resilience held on 20th January 2022, ICAR, Karnal, India.
31. Ms. Satveer Kaur presented a poster on the topic "Physiological and molecular response of colored wheat seedlings against phosphate deficiency is linked to accumulation of distinct anthocyanins" in the Ist International Symposium on"Cereals for Food Security and Climate Resilience" held 20th January 2022, ICAR, Karnal, India.
32. Prof. Ashwani Pareek delivered a talk on the topic "Sustaining Food Production under Environmental Stress" at Newton-Bhabha Fund Researcher Link Workshop held from 18th -21st January 2022 through hybrid mode.
33. Dr. Monika Garg delivered a keynote lecture on the topic "Advances in Colored wheat" in the international conference on "Recent trends in Smart and Sustainable Agriculture for Food Security" (SSAFS-2022) held on 21st – 22nd

January 2022, Punjab, India.

34. **Dr. Koushik Mazumder** delivered an invited talk on the topic "Application of Bio-polymers in Post-Harvest Management" at College of Horticulture and Forestry, Hamirpur, Himachal Pradesh on 28th January 2022.
35. **Dr Siddharth Tiwari** was invited for a lecture in the Faculty Development Programme (FDP) on an emerging field of Biotechnology - "Applications of CRISPR-Cas Gene Editing Tool" at Maulana Abul Kalam Azad University of Technology, Kolkata, West Bengal sponsored by AICTE Training and Learning Academy (ATAL) from 7th -11th February 2022. He delivered a talk on "Methods and Applications of CRISPR/Cas9 Mediated Gene Editing in Plants for Improvement of Important Traits" on 10th February 2022.
36. **Prof. Ashwani Pareek** delivered a talk at national webinar on "New Dimensions in Crop Improvement: From Allele Mining to Genome Editing" held at Rani Lakshmi Bai Central Agricultural University, Jhansi (UP) on 26th February 2022.
37. **Prof. Ashwani Pareek** delivered at talk at national webinar on "Biofortified Crops for Balanced Nutrition and Immunity Boosting" organized by ICAR-National Institute for Plant Biotechnology (NIPB) on 28th February 2022.
38. **Prof. Ashwani Pareek** delivered a talk on the topic "From multi-OMICS to Muta-OMICS: Raising Salinity and Drought Tolerant Rice" at Madurai Kamaraj University on 2nd March 2022.
39. **Ms. Payal Kapoor** delivered an oral talk on the topic "Anthocyanin biofortified wheat on gut micobiota and associated implications in mice" at the Agri Vision-2022, Ravenshaw University, held from 6th – 8th March 2022, Cuttack, Odisha, India.
40. **Dr. Monika Garg** delivered an oral presentation on the topic "Anthocyanin biofortified, antioxidants rich colored wheat is a new research trend" in the 6th Edition of Global Congress on Plant Biology and Biotechnology (GPB-2022) held on 24th -26th March 2022, Singapore.
41. **Dr. Joy K Roy** delivered an invited talk on the topic "Improvement of starch quality in wheat" under the scheme "Strengthening & Development of higher Agricultural Education in India" ICAR-1 subhead 9.2.1 (subcomponent: SC-SP) held on 28th March 2022 at School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana.

Extramural GRANTS

S. No.	Name of the PI	Budget Grant	Funding Agency	Project Title	From	To
1.	Dr. Siddharth Tiwari	₹ 301.11	Biotechnology Industry Research Assistance Council (BIRAC) and Department of Biotechnology, Govt. of India	Transfer and Evaluation of Indian Banana with Pro-Vitamin A (PVA) Constructs. This project is a part of the Multi-Institutional Core Project entitled Development and Transfer of Technology from Queensland University of Technology (QUT), Australia to India for Biofortification and Disease Resistance in Banana.	15.11.2012	28.02.2023
2.	Dr. Siddharth Tiwari (PI) Dr. Ajay K Pandey (CO-PI)	₹ 55.92	Department of Biotechnology, Govt. of India	CRISPR/Cas mediated genome editing of genes for high pro-vitamin A accumulation and its stability in banana	26.03.2018	25.03.2022
3.	Dr. Kanthi Kiran	₹ 62.75	Department of Biotechnology, Govt. of India	Development of amaranth core collection using SSR and SNP markers and evaluation of core set for nutritional, yield traits and abiotic stress tolerance	05.03.2019	04.03.2024
4.	Dr. Joy K Roy	₹ 337.80	Department of Biotechnology, Govt. of India	High resolution QTL mapping for iron (Fe), zinc (Zn), grain protein, and phytate content and their introgression in high yielding wheat cultivars	18.03.2019	17.09.2022
5.	Dr. Hena Dhar, INSPIRE Faculty	₹ 35.68	SERB, DST, Govt. of India	Genomics-guided exploration of polyketide synthases for sustaniable production of long chain omega-3 fatty acid via metabolic engineering	30.03.2019	29.03.2022
6.	Dr. Vanish Kumar, INSPIRE Faculty	₹ 41.20	SERB, DST, Govt. of India	Development of bionanoplatform-based ultrasensitive sensor for the detection of food contaminants with enhaced portability and regenerability.	30.03.2019	29.03.2022
7.	Dr. T.R. Sharma Dr. Humaira Sonah Dr. Rupesh Deshmukh Dr. Sivasubramanian	₹ 59.04	Department of Biotechnology, Govt. of India	Imparting sheath blight disease tolerance in rice	27.06.2019	26.06.2022
8.	Dr. Gulshan Kumar	₹ 33.72	SERB, DST, Govt. of India	Development of microalgae based feed supplement for enhanced bioavailability of essential micronutrients for monogastric animals	30.10.2019	29.10.2022
9.	Dr. Rupam Kumar Bhunia	₹ 25.13	SERB, DST, Govt. of India	Metabolic engineering of triacylglycerol (TAG) biosynthetic pathway to enhance the nutritional quality of forage sorghum and rumen protection of dietary lipids	06.03.2020	05.03.2023
10.	Dr. Pramod Kandoth Dr. Ajay K. Panday Dr. Siddharth Tiwari	₹ 32.69	SERB, DST, Govt. of India	Genomic approaches to reduce B-ODABP levels in Lathyurs spp	21.01.2020	20.01.2023
11.	Dr. Rupesh Deshmukh Dr. Humira Sonah	₹ 37.92	SERB, DST, Govt. of India	Understanding the molecular basis of selective transport of silicon and arsenic by modulating Nodulin 26-like Intrinsic protein 2-1 (NIP2-1) in rice (oryza sativa)	05.02.2020	04.02.2023
12.	Dr. Nitin K Singhal	₹ 30.96	SERB, DST, Govt. of India	Development of Glycoconjugates based site directed fluorescent sensor for the detection of bacteria	26.02.2020	25.02.2023
13.	Dr. Rupesh Deshmukh Dr. Humira Sonah	₹ 71.98	Department of Biotechnology, Govt. of India	Developement of superior haplotype based near isogenic Lines (Haplo-NILs) for enhanced genetic gain in rice	02.03.2020	01.03.2023

14.	Dr. Monika Garg Dr. Koushik Majumdar	₹ 78.05	Department of Biotechnology, Govt. of India	Characterisation of Genetic Resources: Germplasm Characterization and Trait Discovery in wheat using Genomics Approches and its Integration for Improving Climate Resilience, Productivity and Nutritional Quality	29.02.2020	28.02.2025
15.	Dr. Ajay K. Pandey	₹ 33.12	SERB, DST, Govt. of India	Elucidating the role of wheat ferroporatins during cellular iron hemeostasis	30.12.2020	29.12.2023
16.	Dr. Ravinder Pal Singh (PI) Dr. Mahendra Bishnoi & Dr. Kanthi Kiran Kondepudi (Co-PI)	₹ 43.07	Department of Biotechnology, Govt. of India	Novel enzymatic strategy for synthesizing human milk oligosaccharids for promoting health of infants	30.12.2020	29.12.2023
17.	Dr. kanti Kiran Kondepudi	₹ 45.92	Indian Council of Medical Research	A Nutrigenomics Driven symbiotic formulation development for the prevention of metabolic syndrome	01.02.2021	31.01.2024
18.	Dr. Sivadubramanian R	₹ 33.12	SERB, DST, Govt. of India	Mining non-host resistance (NHR) genes for enginerring Alteraria Blight resistance in Indian Mustard (Brassica juncea)	18.03.2021	17.03.2024
19.	Dr. Siddharth Tiwari (PI) Dr. Ajay Kumar Pandey (Co-PI)	₹ 156.43	DBT through NIPGR	National Certification System for Tissue Culture Raised Plants	01.10.2021	30.09.2026
20.	Prof. Joy K Roy Prof. Asish Pal	₹ 10.00	Biotechnology Industry Research Assistance Council (BIRAC),	Biomineralized core-shell polymeric hydrogel beads for controlled fertilizer release and moisture retention under adverse weather conditions	06.10.2021	5.4.2023
21.	Dr. Nitin Kumar Singhal Dr. Vikas Rishi	₹ 23.57	Department of Biotechnology, Govt. of India	Development of aptamer-based multiple biosensors for sensing of pesticids in real time	01.12.2021	30.11.2024
22.	Dr. Prafull Salvi Dr. Ajay K. Pandey (Co-PI)	₹ 35.75	SERB, Govt. of India	Investigating the role and regulatory aspect of intrinsically disordered region(s) in DOF-transcription factor(s) with emphasis to rice grain quality	21.12.2021	20.12.2024
23.	Dr.Nitin K Singhal Dr. Mahehndra Bishnoi	₹ 64.22	Department of Biotechnology, Govt. of India	Probiotic-Millet Based Synbiotic Oral Hydrogels for the Amelioration of Iron Deficiency Anemia and Associated Inflamation	05.01.2022	04.01.2025
24.	Dr. Ajay K Pandey	₹ 26.10	Department of Biotechnology, Govt. of India	Elucdating the Genetic Architechture of tillering in wheat through CRISPR/CAS based editing of Ideal Plant Architecture gene (IPA1) and Teosinte Branched 1 (TB1)	12.01.2022	11.01.2025
25.	Dr. Vikas Rishi (PI) Dr. Pramod K Kandoth (Co-PI)	₹ 42.37	Department of Biotechnology, Govt. of India	Efficient genome editing of seed-specific bZIP transcription factors in tomato for small seeded/ seedless phenotype using CRISPR/Cas system	14.01.2022	13.01.2026
26.	Dr. Ravinder Pal Singh (PI) Dr. Mahendra Bishnoi & Dr. Kanthi Kiran Kondepudi (Co-PI)	₹ 32.07	Department of Biotechnology, Govt. of India	Exploring prophylactic activities of diverse structural peetin oligosaccharides: s step towards nutrigenomics	24.01.2022	23.01.2025
27.	Dr. Humira Sonah Dr. Rupesh Deshmukh (Co-PI)	₹ 31.47	Department of Biotechnology, Govt. of India	Developing food-grade soybean using CRISPR/ Cas9 mediated multiplex genome editing	28.01.2022	27.01.2025
28.	Prof. Ashwani Pareek	₹ 133.98	Department of Biotechnology, Govt. of India	Generating high-depth genomics information for the Himalaya rice cultivars for improving nutritional quality and stress tolerance	24.03.2022	23.03.2025

29.	Prof. Ashwani Pareek	₹ 18.30	SERB, DST, Govt. of India	Teachers Associateship for Research Exellence (TARE) (Biophysics,Biochemistry,Molecular Biology and Microbiology)	20.12.2021	19.12.2024
30.	Dr. Joy K Roy	€ 50 000	International Atomic Energy Agency (IAEA)	Decoding the complex molecular machinery associated with drought and salinity tolerance in rice using advanced molecular tools.	11.02.2022	30.04.2027
31.	Training Cordinator - Dr. Joy k. Roy	₹ 49.50	Department of Bio-technology, Govt. of India through PSCST	Skill Vigyan Program	31.05.2019	30.05.2022
32.	Dr. Siddharth Tiwari (PI) Dr. Ajay K. Pandey & Dr. Sandeep (Co-PI)	₹ 156.43	Department of Bio-technology, Govt. of India through NIPGR	Accredited Test Laboratory (ATL) under National Certification System for Tissue Culture Raised Plants (NCS-TCP) at NABI	01.10.2021	30-09-2026
33.	Dr. Siddharth Tiwari (PI) Dr. Ajay K. Pandey (Co-PI)	₹ 8.12	SERB, DST, Govt. of India	Development of CRISPR/Cas9 mediated genome editing in pea (Pisum sativum L.) for characterization of oligosaccharide pathway genes	01-07-2021	30-06-2024

Event
GALLERY

INDEPENDENCE DAY - 15 AUG 2021

A group picture of NABI and CIAB staff during celebration of Independence Day. **Prof. Ashwani Pareek**, Executive Director hoisted the National Flag.

HINDI DIWAS - 14 SEP 2021

Hindi diwas celebration at NABI and CIAB, Mohali

SUPERCOMPUTING FACILITY 02 NOV 2021

Hon'ble Minister of State (Independent Charge) of the Ministry of Science & Technology and Earth Sciences **Dr. Jitendra Singh** inaugurating the advanced 'PARAM SMRITI - Supercomputing Facility' at NABI.

NEWTON BHABHA FUND RESEARCHER LINK WORKSHOP 18 JAN 2022

NABI and CIAB jointly organized the Newton Bhabha Fund Researcher Link Workshop on "Sustaining Food Production under Environmental Stress". Hon'ble Minister of State (Independent Charge) of the Ministry of Science and technology and Earth Sciences **Dr. Jitendra Singh** addressing the Inaugural Session of the workshop.

REPUBLIC DAY - 26 JAN 2022

FOUNDATION DAY - 22 FEB 2022

NABI celebrated 12th Foundation Day on 22-02-2022. **Padma Shri Prof. Sudhir K. Sopory**, Former Vice Chancellor, JNU, New Delhi graced the foundation day celebrations as Chief Guest **Prof. Yunde Zhao**, Director of Plant Biology Food and Fuels for 21st Century, Professor, Cell and Developmental Biology, University of California, San Diego and Editor-in-Chief of Plant Physiology, was invited as the distinguished guest & keynote speaker.

NATIONAL SCIENCE DAY-28 FEB 2022

NABI & CIAB jointly organized 'National Science Day-2022' on the theme **"Integrated Approach in Science and Technology for Sustainable Future",** to promote the Science, Technology, Engineering, and Mathematics (STEM).

NABI in NEWS

चंडीगढ़ भास्कर 19-01-2022

मात्रा नहीं, न्यूट्रिशियन है सबसे बड़ा चैलेंज

Hindustan Times

NABI workshop starts in hybrid mode

Press Information Bureau

अमर उजाला

कोरोना काल में वैज्ञानिकों ने किए अद्भुत काम : जितेंद्र सिंह

Outlook

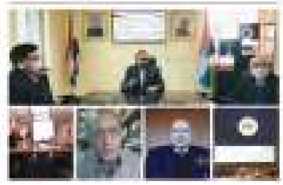
Jitendra Singh Calls For Collaboration Between India, UK On Food Security, Other Issues

United News of India

India's Multi Lingual News Agency

TIMES OF UP

Jitendra Singh addresses India-UK meet on "Sustaining Food Production under Environmental Stress"



चंडीगढ़ भास्कर 23-02-2022

बढ़ती आबादी, कम होती खेती लायक जमीन का ट्रांसजेनिक फसलें ही हैं उपाय

Hindustan Times

NABI celebrates Foundation Day

CHANDIGARH : National Agri-Food Biotechnology Institute (NABI) celebrated its 12th Foundation Day on Tuesday. Executive director Ashwani Pareek highlighted the institute's work, while former JNU vice-chancellor Sudhir K Sopory congratulated the institute on completing 12 years. HTC

एक अरब लोग भूख से पीड़ित : डा. स्नेहलता

अमर उजाला

साइंस डे : उद्योगों को अकादमियों के साथ करने चाहिए नए प्रयोग

Hindustan Times

NABI marks Science Day

MOHALI : With an objective to promote science outreach among school children and the general public, National Agri-Food Biotechnology Institute (NABI) and Center of Innovative and Applied Bioprocessing (CIAB), celebrated the National Science Day (NSD) on Monday. HTC

जग मार्ग

राष्ट्रीय विज्ञान दिवस | विश्व स्तर पर एक अरब लोग सूक्ष्म से पीड़ित और कृषि भूमि सिकुड़ रही: डॉ. स्नेहलता सिंगला-पारीक

नाबी मोहाली में आयोजित कार्यक्रम में स्कूली विद्यार्थियों और माहिरों ने लिया हिस्सा

अनुसंधान और विकास का किया प्रदर्शन

दैनिक भास्कर

चंडीगढ़ भास्कर 23-02-2022

नाबी फाउंडेशन डे •

पानी में आर्सेनिक होगा तो 5 मिनट में जामुनी से नीला हो जाएगा स्ट्रिप का रंग

दैनिक भास्कर

चंडीगढ़ भास्कर 26-02-2022

की बात • मोहाली के नाबी में फसलों की न्यूट्रीशनल वैल्यू बढ़ाने पर होगा काम

जीन एडिटिंग पर देश का पहला ट्रेनिंग सेंटर शुरू होगा नाबी में

• डिपार्टमेंट ऑफ बायोटेक्नोलॉजी ने 5.70 करोड़ रुपये की ग्रांट अप्रूव कर दी है

NABI's ACCOUNT STATEMENT

Goyal Parul& Co.
Chartered Accountants

H.O.: 54, Meedo Complex, Near, Saharanpur Chowk, Dehradun-248001 (UKH)
B.O.: 1180, New Light Co-op House Building Society, Sector-51B, Chandigarh – 160047. Ph.9592888878

AUDITORS' REPORT

TO

THE MEMBERS,

NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE

1. We have audited the attached Balance sheet of NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE as at March31, 2022, the Income and Expenditure account and Receipt &Payment Account for the year ended on that date annexed thereto. These Financial statements are the responsibility of the institution's Management. Our responsibility is to express an opinion on these financial statements based on our audit.

2. We conducted our audit in accordance with auditing standards generally accepted in India. Those standards require that we plan and perform that audit to obtain reasonable assurance about whether the financial statements are free of material misstatements. An audit includes, examining, on tests basis evidence supporting the amount & disclosures in the financial statements. An audit also includes assessing the accounting principles used and significant estimates made as well as evaluating the overall financial statement presentation. We believe that our audit provides a reasonable basis for our opinion.

3. We have obtained all the information and explanation, which, to the best of our knowledge and belief, were necessary for the purpose of audit. In our opinion proper books of accounts as are necessary have been kept so far as it appears from our examination of those books.

4. In our opinion and to the best of our information and according to the explanations given to us, **subject to our observation below and notes on accounts (Schedule 25)**, the financial statements give a true and fair view, in conformity with the accounting principles generally accepted in India:

a) In the case of Balance sheet of the state of affairs of the Institution as at March 31, 2022 and
b) In the case of Income and Expenditure Account, of the surplus/(deficit) of the Institution for the year ended on that date

-Nil -

For Goyal Parul & Co.
Chartered Accountants

(CA Vijay Kumar)
Partner
Membership No.506042
UDIN:22506042AOIPPI7619

Place: Mohali
Date:05.08.2022

FORM OF FINANCIAL STATEMENTS (NON PROFIT ORGANIZATION)

NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE

NABI Campus, Knowledge City, Sector 81, PO Manauli, SAS Nagar, Mohali.

BALANCE SHEET AS ON 31st MARCH 2022

(Amount in Rs.)

CORPUS/ CAPITAL FUND AND LIABILITIES	Schedule	Current Year	Previous Year
Corpus/Capital Fund	1	1,38,95,31,230	1,52,47,93,753
Reserves and Surplus	2	2	1
Earmarked / Endowment / Project Grants	3	5,05,87,800	6,15,87,012
Secured Loans and Borrowings	4	-	-
Unsecured Loans and Borrowings	5		-
Deferred Credit Liabilities	6	-	-
Current Liabilities and Provisions	7	4,76,57,876	6,39,23,103
TOTAL		**1,48,77,76,908**	**1,65,03,03,869**
ASSETS			
Fixed Assets	8	1,33,64,16,672	1,44,11,28,489
Investments- from Earmarked/Endowment funds	9	-	-
Investments - Others	10	-	-
Current Assets, Loans & Advances etc.	11	15,13,60,236	20,91,75,380
TOTAL		**1,48,77,76,908**	**1,65,03,03,869**
Significant Accounting Policies	24		
Contingent Liabilities and Notes on accounts	25		

As per our separate report of even date attached

For National Agri-Food Biotechnology Institute

M/s Goyal Parul & Co.
Chartered Accountants

(DR. MUNISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

(CA VIJAY KUMAR)
PARTNER
Membership No.506042

Dated: 05/08/2022
Place: Mohali

Prof. Ashwani Pareek
Executive Director
National Agri-Food Biotechnology Institute
Dept. of Biotechnology, Govt. of India
Mohali-140306, Punjab, India

Page 1 of 21

FORM OF FINANCIAL STATEMENTS (NON-PROFIT ORGANISTIONS)
NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE
NABI Campus, Knowledge City, Sector 81, PO Manauli, SAS Nagar, Mohali.

INCOME AND EXPENDITURE ACCOUNT
FOR THE YEAR ENDED 31st MARCH 2022

(Amount in Rs.)

INCOME	Schedule	Current Year	Previous Year
Income from Sales/Services	12	-	-
Grants in aid /subsidies	13	16,43,00,000	14,25,00,000
Fees/subscriptions	14	-	-
Income from Investments (Income on investment from earmarked/endowment funds transferred to funds)	15	-	-
Income from Royalty, Publication etc.	16	-	-
Interest Earned	17	21,95,338	98,41,089
Other Income	18	72,91,086	1,04,05,245
Increase/decrease in stock of finished goods & work-in -progress	19	-	-
TOTAL(A)		**17,37,86,424**	**16,27,46,334**
EXPENDITURE			
Establishment Expenses	20	4,79,57,247	3,79,91,206
Other Administrative Expenses	21	9,09,17,353	7,80,65,152
Research & Development Expenditure (Incl. Grants, Subsidies etc)	22	4,09,65,420	3,83,99,852
Interest	23	-	-
Depreciation (net total at the year end-corresponding to schedule 8)		17,93,56,813	18,54,75,249
TOTAL(B)		**35,91,96,833**	**33,99,31,459**
Balance being surplus/(deficit) carried to Capital Fund (A-B)		-18,54,10,409	-17,71,85,126
Significant Accounting Policies	24		
Contingent liabilities and Notes on accounts	25		

As per our separate report of even date attached

For National Agri-Food Biotechnology Institute

M/s Goyal Parul & Co.
Chartered Accountants

(DR. MUNISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

(CA VIJAY KUMAR)
PARTNER
Membership No.506042

Dated: 05/08/2022
Place: Mohali

Prof. Ashwani Pareek
Executive Director
National Agri-Food Biotechnology Institute
Mohali-140306, Punjab, India

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

NATIONAL AGRI FOOD BIOTECHNOLOGY INSTITUTE

NABI Campus, Knowledge City, Sector 81, PO Manauli, SAS Nagar, Mohali.

RECEIPTS AND PAYMENTS ACCOUNT FOR THE PERIOD/YEAR ENDED ON 31.03.2022

(Amounts in Rs.)

RECEIPT	Current Year	Previous Year	PAYMENT	Current Year	Previous Year
(A) Opening Balance			**(A) Establishment Expenses**		
a) Cash in Hand			1. Manpower Salaries and Fellowships	3,91,81,366	3,16,09,665
b) Bank Balances			2. Expenses on Employees Retirement & terminal benefits	1,70,73,715	1,65,69,285
i) In Current Accounts	-	-			
ii) In Deposit Accounts	17,62,75,125	29,72,70,444	**(B) Other Administrative Expenses**		
iii) In Savings Accounts	73,42,600	1,76,64,321	1. Cartage & Carriage inward	35,600	45,590
			2. Honorarium /Sitting Fee	4,24,300	2,40,000
			3. Electricity, power and Water charges	3,17,80,150	2,40,21,687
(B) Grant-in-Aid			4. AMCs/ CMCs	1,47,67,378	94,08,574
(a) Grant from DBT	19,18,00,000	15,75,00,000	5. Vehicles Running & maintenance	5,05,564	4,06,904
			6. Postage,Telephone & communication charges	8,07,859	5,14,871
			7. Printing & stationery	7,17,142	7,49,694
(C) Interest Income			8. Travelling & conveyance expenses	4,40,049	2,22,104
(a) Interest Income	28,14,254	1,00,76,468	9. Outsourcing Manpower Exp	2,19,70,851	2,19,86,986
			10. Legal & Professional charges	9,87,108	3,64,266
			11. Adv. & publicity	3,12,725	18,02,083
(D) Other Incomes			12. Repair & Maintenance Building	43,66,072	30,08,272
1. Tender Fees	10,130	40,000	13. Office & Admn Expenses	14,81,793	9,28,663
2. Sample Analysis	10,81,907	7,853	14. Guest House Expenditure	12,99,679	10,11,949
3. Guest House Income	3,64,170	3,74,250	15. Watch & Ward Expenses	16,28,549	24,53,793
4. RTI Fee	250	20	16. Hostel Expenses	24,17,597	25,77,483
5. Project Income	16,65,685	35,10,845			
6. Training fee	4,62,975	3,45,000	**(C) Research & Development Expenditure**		
7. Staff Quarter License fee	7,57,287	7,15,620	1. Chemicals & Consumables	2,16,46,570	1,87,85,082
8. Hostel License Fee	9,68,947	7,06,915	2. Fellowships	1,11,69,920	1,62,55,052
9. Application fee	26,492	41,908	3. Computer Software & Accessories	9,71,067	6,53,435
10. Rental Income	3,28,917	18,51,514	4. Research Work Expenses	80,048	82,921
11. Technology Transfer	5,67,657	1,54,591	5. Field Expenses	61,91,323	40,10,987
12. Misc. Income	6,81,684	20,95,867	6. Patent Filing Expenses	1,66,794	51,598
13. Campus facilities	30,985	52,287	7. Workshops and seminars	1,84,540	9,286
14. Ph. D Fee	3,30,340	4,84,000	8. Research Publication Expenses	5,22,234	10,15,877
15. Income from Consultancy Services	-	2,16,608	9. Sequencing Expenses	5,97,998	17,38,338
16. I.D Charges	-	6,458	10. Recognition & Membership fee	6,36,452	6,07,836
17. Sale of Garments	1,913	63,969			
18. Staff Car Usage	19,208	700	**(D) Non-Recurring Expenditures**		
(E) Other Projects Receipt (Including interest earned)	8,07,08,616	8,90,09,209	1. Development of Main Campus	25,72,668	1,18,22,948
(F) Other Receipt			2. Scientific Equipments	4,65,83,048	9,16,00,806
1. Biotech Lab	13,283	16,217	3. Computers & Books	12,84,104	99,77,028
2. Caution Money	5,000	20,000	4. Furniture & Fixture	7,09,273	14,62,673
3. M/s Perkin Elmer		1,880	5. Office Equipment	4,77,045	12,06,387
4. Staff welfare fund	2,20,847	54,361	6. Library Books & Periodicals	-	14,990
5. Earnest Money Deposit		10,81,000			
6. Pyramid Builders	13,882	-	**(E) Other Payments**		
7. Security Deposits	1,91,756	-	1. External Project Expenses	9,17,07,829	11,41,43,859
8. Recoupment received from CIAB	12,19,752		2. Nobel Prize Series	1,10,21,769	1,55,21,188
9. M/s RITES Ltd.	10,00,000	1,42,49,278	3. NABI Biotech Club expenses		29,500
10. M/s Media solutions		2,700	4. Security Deposits paid		3,99,811
11. M/s Orient Overseas Container Line Limited		26,223	5. Interest refunded to CFI	93,09,282	1,00,03,376
12. TDS Refund received		4,075	6. Earnest Money Deposits refunded	7,58,782	
13. Adhar ITPO		34,970	7. Academic Administrative Fee	90,000	
14. GST received	1,16,255	38,175			
			(F) Loans & Advances		
			1. Advances to Employees/students	771	4,730
			2. Advances to M/s Pyramid Builders		13,882
			3. Amount recoverable from CIAB		18,44,644
			4. Deposit with PSPCL	3,15,300	
			5. TDS/TCS Receivable	1,02,298	
			6. NICSI	10,97,155	
			(G) Closing Balance		
			a) Cash in Hand	-	-
			b) Bank Balances		
			i) In Deposit Accounts	9,97,10,563	17,62,75,125
			ii) In Savings Accounts	1,88,42,921	73,42,600
Grand Total	46,89,86,955	59,85,37,704	**Grand Total**	46,89,86,955	59,85,37,704

In terms of separate report of even date attached

For National Agri Food Biotechnology Institute

(DR. MANISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

M/s Goyal Paul & Co
Chartered Accountants

(CA VIJAY KUMAR)
PARTNER
Membership No.506042

FORM OF FINANCIAL STATEMENTS (NON-PROFIT ORGANISTIONS)

NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE

NABI Campus, Knowledge City, Sector 81, PO Manauli, SAS Nagar, Mohali.

SCHEDULES FORMING PART OF BALANCE SHEET AS AT 31.03.2022

SCHEDULE-1

CORPUS/CAPITAL FUND

(Amount In Rs.)

Particulars	Current Year	Previous Year
Balance as at the beginning of the year	1,52,47,93,753	1,63,68,86,499
Add : Contributions towards corpus/capital fund	2,75,00,000	1,50,00,000
Add : Fixed Assets Created out of Project Grants	2,26,47,886	4,40,97,380
Add : Gratuity & LE transferred to LIC		59,95,000
Less/(Deduct) : Expenditure over Income transferred from the income & expenditure A/c	-18,54,10,409	-17,71,85,126
BALANCE AS AT THE YEAR -END	**1,38,95,31,230**	**1,52,47,93,753**

SCHEDULE-2

RESERVES AND SURPLUS

Particulars	Current Year	Previous Year
1.Capital Reserves: Land provided by Punjab Govt.	1	1
Fixed Asset transferred by other organizations	1	-
2.Revaluation Reserve	-	-
3.Special Reserve	-	-
4.General Reserve		
TOTAL	**2**	**1**

For National Agri-Food Biotechnology Institute

(DR. MUNISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

M/s Goyal Parul & Co.
Chartered Accountants

(CA VIJAY KUMAR)
PARTNER
Membership No.506042

Dated: 05/08/2022
Place: Mohali

Page 4 of 21

Sr. No.	Project Name	Additions: a) Opening balance as on 01-04-2021	b) Additions during the Year	c) Accrued Interest / Interest Recd. on Investment	TOTAL (a+b+c)	Utilization Expenditure: i) Capital Expenditure	ii) Revenue Expenditure: Fellowships	Chemical & Consumables	Contingency Exp/Travel etc	Overhead Exp	TOTAL	Total Expenses	Refund/Transfer	Net Balance as on 31-03-2022
1	Development and Transfer of Technology from Queensland University of Technology, Australia to India for Bio-fortification and Disease Resistance in Banana (GAP 02)	1,09,34,752		80,452	1,10,15,204		[illegible]	1,79,141	2,80,237	-	33,51,886	33,51,886	58,79,200	17,84,118
2	Genome and Transcriptome Sequencing of Aromatic rices from North Eastern Region (GAP 17)	491			491					491	491	491		-
3	Enhanced rice milling and maximised valorisation of rice milling by product (GAP 21)	-4,88,309	4,88,309		-						-	-		-
4	Pharmacological mimicking of cold via cold thermo-receptors (GAP 22)	9,29,608			9,29,608						-	-		9,29,608
5	Setting up of Secondary Agricultural Food Processing Entrepreneurial Network in Punjab-Phase-I (GAP 23)	10,96,312			10,96,312		72,097	16,970			89,067	89,067	10,07,245	-
6	CRISPR/Cas mediated genome editing of genes for high pro-vitamin A accumulation and its stability in banana (GAP 24)	47,054			47,054						-	-	12,943	34,111
7	Genome-Wide mapping of QTLs controlling healthy amylose starch variation in wheat (GAP 25)	11,08,208			11,08,208		3,20,184	1,80,348	58,616		5,81,252	5,81,252	5,26,956	-
8	Development and validation of technology for production of high energy density biocoal from rice straw and other agri-biomasses (GAP 26)	40,69,567			40,69,567	16,32,721		19,186			19,186	16,51,907		24,17,660
9	Utilizing genome editing tools for nutritional improvement in wheat (GAP-27)	4,84,037	14,07,935	5,302	18,97,274		8,77,440		22,764	30,000	9,30,204	9,30,204	11,932	9,55,138
10	Bio fortified antioxidant rich colored wheat cultivation under organic and agronomic supplementation (Fe, Zn, Protein) strategies to increase farm income (GAP 28)	6,25,036			6,25,036		1,84,000	1,80,348	2,60,688		6,25,036	6,25,036		-
11	High resolution QTL mapping for iron (Fe), zinc (Zn), grain protein, and phytate content and their introgression in high yielding wheat cultivars (GAP 29)	81,21,873	27,63,169	60,505	1,09,45,547		30,07,540	61,16,829	1,55,495		1,02,79,864	1,02,79,864	78,580	5,87,103
12	Genomics-guided exploration of polyketide synthases for sustainable production of long chain omega-3 fatty acid via metabolic engineering (GAP 30)	7,86,206	9,50,000	13,264	17,89,600	5,93,530	4,75,451	3,18,673	25,759	88,121	9,08,005	15,00,565		2,89,035
13	Development of biomacroplatform-based ultrasensitive sensor for the detection of food contaminants with enhanced portability and regenerability (GAP 31)	2,84,409	4,75,064	6,987	7,66,460	18,454	4,71,433	1,43,509	18,781	48,403	6,82,125	7,00,579		67,887
14	Imparting sheath blight disease tolerance in rice (GAP 32)	8,52,555		9,491	8,62,046		6,81,447	2,02,972	11,000		8,95,420	8,95,420	19,187	27,599
15	Development of microalgae based feed supplement for enhanced bioavailability of essential micronutrients for monogastric animals (GAP 33)	80,824	5,50,000	6,518	6,43,342		3,54,000	55,427	22,370	50,000	4,81,797	5,80,797		92,546
16	Metabolic engineering of triacylglycerol (TAG) biosynthetic pathway to enhance the nutritional quality of forage sorghum and rumen protection of dietary lipids (GAP 34)	28,348	8,00,000	12,430	8,40,540		3,72,000	2,00,624	37,130	1,21,329	8,38,583	8,38,583		21,877
17	Genomic approaches to reduce B-ODAHP levels in Lathyrus spp (GAP 35)	80,389	17,00,000	10,432	18,00,821		3,72,000	5,60,659	48,881	1,60,529	11,42,329	11,42,329		6,60,692
18	Understanding the molecular basis of selective transport of silicon and arsenic by modulating Nodulin 26-like Intrinsic protein 2-1 (NIP2-1) in rice (oryza sativa) (GAP 36)	2,94,813	15,60,000	83,703	18,34,722		4,45,600	7,55,585		97,622	13,00,205	13,00,205		5,14,517

Page 6 of 21

Sr. No.	Project Name	Additions: a) Opening Balance as on 01.04.2021	b) Additions during the Year	c) Accrued Interest / Interest Earned on Investments	TOTAL (a+b+c)	Utilization/Expenditure: i) Capital Expenditure	ii) Revenue Expenditure: Fellowships	Chemical & Consumable	Contingency Exp/Travel etc	Overhead Exp	TOTAL	Total Expense	Refund/Transfer	Net Balance as on 31.03.2022
19	Development of Glycoconjugates based site directed fluorescent sensor for the detection of bacteria (GAP 37)	1,28,896	9,00,000	6,760	10,35,656		3,25,800	4,44,977	18,062	72,560	8,61,132	8,84,132		1,51,524
20	Development of superior haplotype based near isogenic Lines (Haplo-NILs) for enhanced genetic gain in rice (GAP 38)	13,35,189		13,045	13,48,234	2,64,405	5,55,677	16,155	3,16,411		8,88,243	11,49,764	75,126	1,46,325
21	Characterization of Genetic Resources: Germplasm Characterization and Trait Discovery in wheat using Genomics Approaches and its Integration for Improving Climate Resilience, Productivity and Nutritional Quality (GAP 39)		33,22,300	19,119	33,41,209		85,937	9,75,699	35,366	50,000	11,76,002	11,76,002		21,64,797
22	Elucidating the role of wheat ferroportins during cellular iron homeostasis (GAP 40)	16,56,182		14,221	16,70,403		3,72,000	4,75,482	10,924		9,18,338	9,21,338		7,59,065
23	Novel enzymatic strategy for synthesizing human milk oligosaccharides for promoting health of infants (GAP 41)	2,41,300	6,75,800		9,21,100		3,72,000	5,07,533	38,428		9,17,961	9,27,963	3,217	-
24	A nutrigenomics driven formulation development for the prevention of metabolic syndrome (GAP-42)	4,77,189	3,87,958	14,209	8,79,356		4,79,309	3,82,685		14,316	8,76,310	8,50,243		1,29,113
25	Mining non-host resistance (NHR) genes for engineering Alternaria Blight resistance in Indian Mustard (GAP-43)	11,11,979		15,537	11,27,516	14,099	2,98,150	4,94,017	40,002	81,760	9,13,929	9,28,027		1,99,489
26	National Certification System for Tissue Culture Raised Plants (GAP-44)		47,70,528	46,980	48,17,508		3,00,450	2,71,455	1,58,912	6,65,000	16,19,823	16,29,823		31,73,677
27	Biomineralized core-shell polymeric hydrogel beads for controlled fertilizer release and moisture retention under adverse weather conditions (GAP-45)		3,80,000	2,885	3,82,885			1,08,843			1,08,843	1,08,843		2,74,042
28	Development of aptamer-based multiple biosensors for sensing of pesticide in real time (GAP-46)		8,81,759	6,683	8,88,442			2,03,132	540		2,03,672	2,03,672		6,84,781
29	Investigating the role and regulatory aspect of intrinsically disordered region(s) in DOF-transcription factor(s) with emphasis to rice grain quality (GAP-47)		11,90,353	9,774	12,00,127		28,000	1,48,577		88,271	2,64,848	2,64,848		9,45,277
30	Probiotic-Millet Based Synbiotic Oral Hydrogels for the Amelioration of Iron Deficiency Anemia and Associated Inflammation (GAP-48)		11,13,408	7,887	11,21,092			9,897	914		10,811	10,811		11,10,281
31	Elucidating the Genetic Architecture of tillering in wheat through CRISPR/CAS based editing of Ideal Plant Architecture gene (IPA1) and Teosinte Branched 1 (TB1) (GAP-49)		8,51,729	5,596	8,57,876			84,233	913		85,146	85,146		7,72,730
32	Efficient genome editing of seed-specific bZIP transcription factors in tomato for small seeded/seedless phenotype using CRISPR/Cas system (GAP-50)		12,47,280	8,417	12,55,697				913		913	913		12,54,784
33	Exploring prophylactic activities of diverse structural pectin oligosaccharides: a step towards nutrigenomics (GAP-51)		13,82,640	5,599	13,88,239	10,831		1,78,678	914		1,79,592	1,90,323		11,97,888
34	Developing food-grade soybean using CRISPR/Cas9 mediated multiplex genome editing (GAP-52)		12,22,640	5,407	12,28,047				12,000	50,000	62,000	62,000		11,66,047
35	Generating high-depth genomics information for the Himalayan rice cultivars for improving nutritional quality and stress tolerance (GAP-53)		34,40,248	500	34,40,748						-	-		34,40,748

Sr. No.	Project Name	Additions: a) Opening Balance as on 01.04.2021	b) Addition during the Year	c) Accrued Interest / Interest Back on Investment	TOTAL (a+b+c)	Utilization expenditure: i) Capital Expenditure	ii) Revenue Expenditure: Fellowships	Chemical & Consumable	Contingency Exp/Travel etc.	Overhead Exp	TOTAL	Total Expenses	Refund/Transfer	Net Balance as on 31.03.2022
36	Teachers Associateship for Research Excellence (TARE) (Biophysics,Biochemistry,Molecular Biology and Microbiology) (GAP-54)		3,35,000		3,35,000						-	-		3,35,000
37	National Super Computer Mission (CDAC)	1,87,00,000			1,87,00,000	1,87,00,000					-	1,87,00,000		-
38	Skill Vigyan Program	16,20,000	50,000	51,029	17,01,029			1,27,950	6,050		1,34,000	1,34,000	18,304	15,48,725
39	Department of Biotechnology (DBT) JRF/SRF Fellowships	2,28,843	29,63,425	-	31,92,268		28,14,044	85,239			28,99,283	28,99,283		2,92,985
40	Council of Scientific & Industrial Research (CSIR) JRF/SRF Fellowships	3,26,592	67,596		3,94,188				2,94,078		2,94,078	2,94,078		1,00,110
41	Indian Council of Medical Research (ICMR) JRF/SRF Fellowships	4,03,499	17,28,096		21,31,595		18,48,571		61,513		19,10,084	19,10,084	1,01,219	1,20,292
42	JC Bose Fellowship	-40,809	20,00,000	11,173	19,80,084		3,25,000	1,07,805		2,00,000	6,28,805	6,34,805		12,81,490
43	DST Inspire Fellowship	1,15,809	3,21,163		4,36,653		4,16,613		2,718		4,19,331	4,19,331		17,282
44	National Post Doc Fellowship	-	10,12,800		10,12,800		55,568			1,00,000	1,55,568	1,55,568		8,57,232
45	Ramalingaswamy Fellowship	13,40,835	1,01,27,686	1,20,447	1,15,87,968		60,71,757	18,80,078		69,881	80,21,114	80,21,114	72,664	34,94,190
46	DST Inspire Faculty Fellowship	40,70,768	1,35,45,879		1,76,16,647	36,750	58,41,893	35,09,904	5,59,645	2,10,000	1,05,83,442	1,05,90,192		40,26,255
47	M.K.Bhan Fellowship		1,94,35,000	28,155	1,94,63,099		22,50,000	4,93,786			27,43,786	27,43,786		1,67,19,369
48	DOC Fellowship		1,254		1,254						-	-		1,254
49	Newton Bhabha Ph. D Placement Programme	1,03,500			1,03,500				1,03,500		1,03,500	1,03,500		-
50	Agreement for Industry Research with M/s Cremica Food Industry Limited (EP 02)	56,883			56,883					56,883	56,883	56,883		-
51	Genetically Enhanced Pearl Millet with High Grain Iron Density for Improved Human Nutrition in India (EP 04)	53,544	69,891		1,23,435			1,17,975		5,460	1,23,435	1,23,435		-
52	Technology for sugar free food product by using prebiotic sweetener, isomalto - oligosaccharides (EP 03)	2,35,892			2,35,892		22,123	1,76,302	37,467		2,35,892	2,35,892		-
53	Agreement for Industry Research with M/s Panchkula Healthcare Labs (EP 01)	26,832			26,832					26,832	26,832	26,832		-
54	Extramural Project		6,76,907		6,76,907						-	-		6,76,907
	Total	4,35,87,022	8,08,06,313	4,10,175	14,32,95,628	2,22,83,447	3,78,12,864	2,04,71,779	21,17,898	22,61,740	6,25,25,258	8,38,08,627	78,59,282	5,05,87,808

For National Agri-Food Biotechnology Institute

Date: 05/08/2022
Place: Mohali

(DR. MUNISH SINGLA)
Finance Officer

डॉ. मुनीष सिंगला / Dr. Munish Singla
वित्त अधिकारी / Finance Officer
राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान
National Agri-Food Biotechnology Institute
भारत सरकार / Govt. of India
जैवप्रौद्योगिकी विभाग / Dept. of Biotechnology
मोहाली, पंजाब / Mohali, Punjab-140306

(SUNEET VERMA)
Manager Finance

सुनीत वर्मा / Suneet Verma
वित्त प्रबंधक / Manager (Finance)
राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान
National Agri-Food Biotechnology Institute
भारत सरकार / Govt. of India
जैवप्रौद्योगिकी विभाग / Dept. of Biotechnology
मोहाली, पंजाब / Mohali, Punjab-140306

(PROF. ASHWANI PAREEK)
Executive Director

प्रो. अश्वनी पारीक
Prof. Ashwani Pareek
कार्यकारी निदेशक / Executive Director
राष्ट्रीय कृषि-खाद्य जैव प्रौद्योगिकी संस्थान
National Agri-Food Biotechnology Institute
जैव प्रौद्योगिकी विभाग, भारत सरकार
Dept. of Biotechnology, Govt. of India
मोहाली (पंजाब) – 140306, भारत
Mohali-140306, Punjab, India

M/s Goyal Parul & Co.
Chartered Accountants
(CA VIJAY KUMAR)
Partner
Membership No. 92042

Page 7 of 14

SCHEDULE-4
SECURED LOANS & BORROWINGS

(Amount in Rs.)

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

SCHEDULE-5
UNSECURED LOANS & BORROWINGS

(Amount in Rs.)

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

SCHEDULE-6
DEFERRED CREDIT LIABILITIES

(Amount in Rs.)

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

For National Agri-Food Biotechnology Institute

M/s Goyal Parul & Co.
Chartered Accountants

(DR. MUNISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

(CA VIJAY KUMAR)
PARTNER
Membership No.506042

Dated: 05/08/2022
Place: Mohali

SCHEDULE-7
CURRENT LIABILITIES & PROVISIONS

(Amount in Rs.)

Particulars	Current Year	Previous Year
A)CURRENT LIABILITIES		
1. Sundry Creditors		
a) For goods/Equipment	2,21,45,356	1,99,61,202
b) For Securities deposited by others	28,73,854	26,82,098
c) Earnest Money Deposit	11,59,340	19,18,122
d) Caution Money deposited by Students	1,30,000	1,25,000
e) NABI Biotech Club		16,217
f) Staff Welfare Fund	4,32,400	2,11,553
g) Nobel Prize Series		1,10,21,760
2. Interest accrued but not due on:		
a) Secured Loans/Borrowings	-	-
b) Unsecured Loans/Borrowings	-	-
3. Statutory Liabilities		
a) Overdue	-	-
4. Other Current Liabilities		
a) Manpower (Salary) Payable	34,97,806	25,24,523
b) Other Expenses Payable	26,90,457	23,79,766
c) TDS Payable	6,18,601	5,85,407
d) Fellowship Payable	34,86,300	48,31,020
e) GST Payable	4,20,005	3,03,749
f) Punjab Development Tax Payable	8,000	7,400
g) RITES Limited	9,77,997	
h) Interest refundable to CFI	28,34,347	93,09,262
TOTAL(A)	**4,12,74,463**	**5,58,77,079**
B) PROVISIONS		
1. Gratuity	20,81,149	33,34,773
2. Leave Encashment	43,02,264	47,11,251
TOTAL(B)	63,83,413	80,46,024
TOTAL(A+B)	**4,76,57,876**	**6,39,23,103**

For National Agri-Food Biotechnology Institute

(DR. MUNISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

M/s Goyal Parul & Co.
Chartered Accountants

(CA VIJAY KUMAR)
PARTNER
Membership No.506042

Dated: 05.08.2022
Place: Mohali

डॉ. मुनीष सिंगला / Dr. Munish Singla
वित्त अधिकारी / Finance Officer
राष्ट्रीय कृषि-खाद्य जैव प्रौद्योगिकी संस्थान
National Agri-Food Biotechnology Institute
भारत सरकार / Govt. of India
जैवप्रौद्योगिकी विभाग / Deptt. of Biotechnology
मोहाली, पंजाब / Mohali, Punjab-140306

सुनीत वर्मा / Suneet Verma
वित्त प्रबंधक / Manager (Finance)
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मोहाली, पंजाब / Mohali, Punjab-140306

प्रो. अश्वनी पारीक
Prof. Ashwani Pareek
कार्यकारी निदेशक / Executive Director
राष्ट्रीय कृषि-खाद्य जैव प्रौद्योगिकी संस्थान
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Deptt. of Biotechnology, Govt. of India
मोहाली (पंजाब) - 140306 भारत
Mohali-140306, Punjab, India

NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE

NABI Campus, Knowledge City, Sector 81, PO Manauli, SAS Nagar, Mohali

SCHEDULE-8 FIXED ASSETS

S.No.	Description	Depreciation Rate	GROSS BLOCK: Cost/Valuation as at beginning of the year	Additions during the year	Additions during the year	Deductions/Sold off during the year	Cost/Valuation at the year end	DEPRECIATION: As at the beginning of the year	Depreciation during the year	Total at the year end	NET BLOCK: As at the Current Year End	As at the Previous Year End
			1st April 2021	UPTO 30.09.21	AFTER 30.09.21	2021-22	31st March 2022	1st April 2021	2021-22	31st March 2022	31st March 2022	31st March 2021
A)	FIXED ASSETS CREATED FROM GOVT. GRANTS											
I	LAND		1				1				1	1
	a) Free Hold	0.00%	1	-	-	-	1	-	-	-	1	1
	b) Lease Hold	0.00%	-	-	-	-	-	-	-	-	-	-
II	BUILDINGS											
	a) On Freehold Land	10.00%	1,53,65,79,801	11,79,000	14,000	-	1,53,77,58,782	58,07,85,281	10,28,84,129	68,83,11,410	85,24,04,172	1,00,48,65,278
	b) On Leasehold Land	10.00%	-	-	-	-	-	-	-	-	-	-
	c) Ownership Premises	10.00%	-	-	-	-	-	-	-	-	-	-
	d) Other Superstructures	10.00%	-	-	-	-	-	-	-	-	-	-
III	PLANT, MACHINERY & EQUIPMENT EQUIPMENTS	15.00%	58,58,18,217	60,72,443	7,84,41,257	-	67,44,47,157	33,11,05,700	4,36,17,190	37,47,23,875	29,97,20,180	25,58,30,668
IV	VEHICLES	15.00%	20,44,875	-	-	-	20,44,875	10,01,311	1,56,335	11,57,646	8,87,229	10,43,564
V	FURNITURE & FIXTURES	10.00%	3,79,12,868	3,33,000	3,56,373	-	3,86,02,175	1,22,72,218	26,18,177	1,48,90,395	2,37,41,779	2,56,40,565
VI	COMPUTER/PERIPHERALS	40.00%	6,60,48,991	5,08,512	7,03,299	-	6,73,42,986	4,60,58,167	88,20,698	5,48,19,865	1,24,13,881	1,91,58,424
VII	NSM EQUIPMENTS	0.00%		1		-	1		-	-	1	-
VIII	LIBRARY BOOKS	40.00%	8,95,646			-	8,95,646	8,58,529	14,850	8,73,370	22,276	37,116
IX	OFFICE EQUIPMENT	10.00%	60,89,345	49,409		-	61,38,886	27,94,991	3,34,381	31,29,372	30,09,432	32,94,214
	TOTAL OF CURRENT YEAR (A)		2,33,85,80,736	8,11,64,809	7,95,00,219	-	2,50,13,47,344	98,08,42,677	15,53,60,056	1,06,88,88,733	1,26,13,38,601	1,35,08,60,059
B)	Fixed Asset Created from Projects Grants:											
	Equipments	15%	7,22,11,284	6,68,054	13,36,643	18,607	7,42,18,809	1,76,32,189	85,41,203	2,61,73,392	5,80,44,667	5,45,79,095
	Furniture & Fixtures	10%	1,47,498	-	-	18,342	1,28,917	72,037	11,562	83,599	45,378	1,15,022
	Computers/Peripherals	40%	30,11,317	1,93,44,534	82,55,626	40,747	3,16,72,690	25,33,047	68,81,707	94,14,754	1,93,38,236	6,79,730
	Library Books	40%	4,559	-	-	-	4,559	3,481	499	3,982	600	1,167
	Office Equipments	10%	-	-	-	-	-	-	-	-	-	-
	Buildings	10%	1,73,69,713	-	-	-	1,73,69,715	1,07,13,632	13,36,856	1,20,70,478	12,99,237	66,56,083
	TOTAL OF FIXED ASSETS PROCURRED FROM PROJECTS (B)		9,27,46,325	3,10,33,263	1,17,42,269	1,07,696	10,53,94,211	3,07,15,268	1,99,90,757	5,07,06,025	6,46,88,186	6,20,31,057
	TOTAL (A+B)		2,43,13,49,061	2,21,77,781	9,13,22,488	1,07,696	2,63,66,41,555	93,13,57,945	17,53,50,813	1,11,07,24,758	1,32,59,16,797	1,89,38,91,116
	PREVIOUS YEAR											
	a) Expenditure on Assets/Fixed Assets		-	-	-	-	-	-	-	-	-	-
	b) Expenditure on Plan Activities		-	-	-	-	-	-	-	-	-	-
	TOTAL OF PREVIOUS YEAR		-	-	-	-	-	-	-	-	-	-
C)	CAPITAL WORK-IN-PROGRESS											
	a) Equipment		4,92,37,373	1,14,89,875		4,92,37,373	1,04,89,875	-	-	-	1,04,89,875	4,92,37,373
	TOTAL OF CURRENT YEAR (CWIP) (C)		4,92,37,373	1,04,89,875	-	4,92,37,373	1,04,89,875	-	-	-	1,04,89,875	4,92,37,373
	GRAND TOTAL (A+B+C)		2,37,24,86,434	3,26,67,577	9,13,22,488	4,93,45,069	2,64,71,31,430	93,13,57,945	17,53,50,813	1,11,07,24,758	1,53,64,06,672	1,44,11,28,489

For National Agri-Food Biotechnology Institute

Dated: 05/09/2022
Place: Mohali

(DR. MUNISH SINGLA)
Finance Officer

(SUNEET VERMA)
Manager Finance

(PROF. ASHWANI PAREEK)
Executive Director

M/s Goyal Parul & Co.
Chartered Accountants

(CA VIJAY KUMAR)
Partner

डॉ. मुनीश सिंगला / Dr. Munish Singla
वित्त अधिकारी / Finance Officer

सुनीत वर्मा / Suneet Verma
वित्त प्रबंधक / Manager (Finance)
राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान
National Agri-Food Biotechnology Institute
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जैवप्रौद्योगिकी विभाग / Deptt. of Biotechnology
मोहाली, पंजाब / Mohali, Punjab-140306

प्रो. अश्वनी पारीक
Prof. Ashwani Pareek
कार्यकारी निदेशक / Executive Director
National Agri-Food Biotechnology Institute
Deptt. of Biotechnology, Govt. of India
Mohali-140306, Punjab, India

SCHEDULE-9
INVESTMENTS FROM EARMARKED/ENDOWMENT FUNDS

(Amount in Rs.)

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

SCHEDULE-10
OTHER INVESTMENTS

(Amount in Rs.)

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

For National Agri-Food Biotechnology Institute

M/s Goyal Parul & Co.
Chartered Accountants

(DR. MUNISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

(CA VIJAY KUMAR)
PARTNER
Membership No.506042

Date: 05/08/2022
Place: Mohali

SCHEDULE-11
CURRENT ASSETS, LOANS & ADVANCES

(Amount in Rs.)

Particulars	Current Year	Previous Year
A) CURRENT ASSETS		
1. Inventories	-	-
2. Sundry Debtors	-	-
3. Cash balances in hand	-	-
4. LIC Group Gratuity Scheme	1,27,20,717	1,03,75,011
5. LIC Leave Encashment Scheme	1,69,61,880	1,12,38,912
6. Bank balances:		
a) With Scheduled Banks:		
-On Current accounts	-	-
-On Fixed Deposit accounts	9,97,10,563	17,62,75,125
-On Savings accounts		
(i) State Bank of India A/c	1,88,02,521	73,42,600
TOTAL(A)	**14,81,95,681**	**20,52,31,648**
B) LOANS, ADVANCES AND OTHER ASSETS		
1. Loans		
2. Advances and other amounts recoverable		
a) On Capital Account		
b) Advances		
(i) Deposit with M/s RITES Ltd.	-	22,003
c) Recoupable form Govt. Agencies & others		
(i) NIPER		5,900
(ii) Kuber Roller Floor Mills		5,523
(iii) Bharat Rattan (Dr. B. R. Ambedkar)	11,050	-
(iv) CIAB		12,19,752
(v) Anton Parr India Pvt. Ltd.		29,500
(vi) M/s Micro Education and Social Welfare Society (NGO)	28,000	-
(vii) Bionixs India	11,800	-
(viii) M/s Balmer Lawrie & Co. Ltd.	15	-
(ix) M/s Chandigarh Scientific Agencies	29,500	-
(x) M/s Pyramid Builders		13,882
(xi) M/s Navprayas	15,000	-
(xii) M/s Prism Automation Pvt. Ltd.	17,700	-
(xiii) Superintendent Model Jail, Sector 51, Chandigarh	18,000	-
(xiv) NICSI	10,97,155	-
(xv) Fellows/Students	771	-
d) Advance to Employees		22,720
e) Others(specify)		
(i) Security for Gas Connection to M/s Chahal Gas	26,250	26,250
(ii) Deposit with PSPCL	3,59,941	44,581
(iii) TDS/TCS Receivable	1,79,820	43,265
(iv) PSEB Elelct Security for Main Campus	11,12,090	11,12,090
3.Income accrued:		
a) On investments from earmarked/endowment funds		-
b) Interest On Saving A/c and Fixed Deposits	2,57,463	13,98,266
c) On loans & advances	-	-
TOTAL(B)	**31,64,555**	**39,43,732**
TOTAL(A+B)	**15,13,60,236**	**20,91,75,380**

For National Agri-Food Biotechnology Institute

(DR. MUNISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

M/s Goyal Parul & Co.
Chartered Accountants

(CA VIJAY KUMAR)
PARTNER
Membership No.506042

Dated: 05/08/2022
Place: Mohali

डॉ. मुनीश सिंगला / Dr. Munish Singla
वित्त अधिकारी / Finance Officer
राष्ट्रीय कृषि खाद्य जैव प्रौद्योगिकी संस्थान
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मोहाली, पंजाब / Mohali, Punjab-140306

सुनीत वर्मा / Suneet Verma
वित्त प्रबंधक / Manager (Finance)

प्रो. अश्वनी पारीक
Prof. Ashwani Pareek
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National Agri-Food Biotechnology Institute
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Mohali-140306, Punjab, India

Page 12 of 21

SCHEDULE-12
INCOME FROM SALES/SERVICES

(Amount in Rs.)

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

SCHEDULE-13
GRANTS/SUBSIDIES

(Amount in Rs.)

Particulars	Current Year	Previous Year
(Irrevocable Grants & subsidies received)		
1. Central Government	16,43,00,000	14,25,00,000
2. State Government	-	-
3. Government Agencies	-	-
4. Institutional /welfare bodies	-	-
5. International Organisations	-	-
6. Others (to be specified)	-	-
TOTAL	16,43,00,000	14,25,00,000

SCHEDULE-14
FEES/SUBSCRIPTIONS

(Amount in Rs.)

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

SCHEDULE-15
INCOME FROM INVESTMENTS

(Amount in Rs.)

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

For National Agri-Food Biotechnology Institute

M/s Goyal Parul & Co.
Chartered Accountants

(DR. MUNISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

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Dated: 05.08.2022
Place: Mohali

डॉ. मुनीष सिंगला / Dr. Munish Singla
वित्त अधिकारी / Finance Officer
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Prof. Ashwani Pareek
कार्यकारी निदेशक / Executive Director
National Agri-Food Biotechnology Institute
Deptt. of Biotechnology, Govt. of India
Mohali-140306, Punjab, India

SCHEDULE-16
INCOME FROM ROYALTY/PUBLICATIONS, ETC.

(Amount in Rs.)

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

SCHEDULE-17
INTEREST EARNED

(Amount in Rs.)

Particulars	Current Year	Previous Year
1) On Term Deposits		
a) With Scheduled Banks:		
i) Actual Received	10,14,506	79,74,859
ii) Accrued as on 31.03.2022	2,57,463	13,98,266
b) With Non-Scheduled Banks:	-	-
2) On Savings Accounts:		
a) With Scheduled Banks:	2,67,559	2,29,138
b) With Non-Scheduled Banks:	-	-
c) With Serb Account	1,53,464	1,95,137
d) With Project Account	391	3,767
3) On Loans		
a) Employees/staff		2,362
4) Interest on Debtors & other Receivables		
a) Interest on refund of Income Tax	108	360
b) Interest on PSPCL Security	34,297	37,200
c) Interest on Leave encashment scheme with LIC	4,67,550	
TOTAL	**21,95,338**	**98,41,089**

For National Agri-Food Biotechnology Institute

M/s Goyal Parul & Co.
Chartered Accountants

(DR. MUNISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

(CA VIJAY KUMAR)
PARTNER
Membership No.506042

Dated: 05/08/2022
Place: Mohali

Page 14 of 21

SCHEDULE-18
OTHER INCOME

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Profit on sale/disposal of assets		
a) Owned Assets	-	-
b) Assets acquired out of grants, or received free of cost	-	-
2. Export Incentives realized	-	-
3. Overhead Income from External Projects	16,65,683	35,10,845
4. Miscellaneous Income		
a) Tender Fees	10,130	40,000
b) Sample Analysis	9,96,384	13,376
c) Guest House (Income)	3,64,170	3,74,250
d) RTI Fee	239	20
e) Training Fee	4,02,975	3,45,000
f) Staff Quarters Licence Fee	7,57,287	7,15,620
g) Hostel fee	9,68,947	7,06,905
h) Application fee	26,492	12,900
i) Rental income	3,38,932	15,79,174
j) Technology transfer	5,67,657	3,71,199
k) Misc Income	8,30,865	21,99,709
l) Campus facilities	30,985	52,247
m) Ph. D Fee	3,30,340	4,84,000
TOTAL	72,91,086	1,04,05,245

For National Agri-Food Biotechnology Institute

M/s Goyal Parul & Co.
Chartered Accountants

(DR. MUNISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

(CA VIJAY KUMAR)
PARTNER
Membership No.506042

Dated: 05/08/2022
Place: Mohali

Page 15 of 21

SCHEDULE-19

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

(Amount in Rs.)

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

SCHEDULE-20

ESTABLISHMENT EXPENSES

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Manpower Salaries, Wages and Allowances	4,01,49,267	3,50,49,720
2. Expenses on Employees Retirement & terminal benefits	74,88,434	29,41,486
3. Leave encashment and admin expenses	3,19,546	
TOTAL	4,79,57,247	3,79,91,206

SCHEDULE-21

OTHER ADMINISTRATIVE EXPENSES

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Cartage & Carriage inward	35,600	45,590
2. Honorarium /Sitting Fee	4,24,300	2,40,000
3. Electricity, power and Water charges	3,17,80,150	2,40,31,682
4. AMCs /CMCs	1,43,69,725	87,06,161
5. Vehicles Running & maintenance	5,05,564	4,06,904
6. Postage,Telephone & communication charges	8,07,859	4,96,583
7. Printing & stationery	7,17,142	7,49,694
8. Travelling & conveyance expenses	4,40,049	2,33,104
9. Outsourcing Manpower Exp	2,44,99,604	2,19,03,074
10. Legal, Professional & Statutory fees/charges	9,96,638	3,64,266
11. Advt. & publicity and display Expenses	3,12,725	18,01,612
12. Repair, Operation & Maintenance of Building	43,66,072	30,09,272
13. Office & Admn Expenses	14,81,793	9,24,723
14. Guest House Expenditure	12,99,679	10,11,949
15. Watch & Ward Expenses	36,28,549	24,53,793
16. Hostel Expenses	24,17,557	23,77,483
17. Interest earned refundable to CFI	28,34,347	93,09,262
TOTAL	9,09,17,353	7,80,65,152

For National Agri-Food Biotechnology Institute

M/s Goyal Parul & Co.
Chartered Accountants

(DR. MUNISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

(CA VIJAY KUMAR)
PARTNER
Membership No.506042

Dated: 03/08/2022
Place: Mohali

Page 16 of 21

SCHEDULE-22

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

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Chemical & Consumables	2,16,52,470	1,87,69,845
2. Fellowship	98,24,300	1,15,17,517
3. ICT Services & Consumables, Software , Accessories etc	9,71,067	6,53,435
4. Research Work Expenses	80,068	82,921
5. Field Expenses (Ploughing, RM & Other Job work)	63,28,587	39,99,497
6. Patent related expenses	1,66,704	51,508
7. Workshops & Seminars	1,84,540	9,286
8. Research Publication Expenses	5,23,234	10,15,877
9. Sequencing Expenses	5,97,998	16,92,930
10. Recognition & Membership Fee	6,36,452	6,07,036
TOTAL	**4,09,65,420**	**3,83,99,852**

SCHEDULE-23

INTEREST

(Amount in Rs.)

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

For National Agri-Food Biotechnology Institute

M/s Goyal Parul & Co.
Chartered Accountants

(DR. MUNISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

(CA VIJAY KUMAR)
PARTNER
Membership No.506042

Dated: 05/08/2022
Place: Mohali

Page 17 of 21

FORM OF FINANCIAL STATEMENTS

NATIONAL AGRI FOOD BIOTECHNOLOGY INSTITUTE

Knowledge City, Sector 81, PO Manauli, S.A.S. NAGAR, MOHALI

SCHEDULE 24

SIGNIFICANT ACCOUNTING POLICIES

A) ACCOUNTING CONVENTION

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

B) INVENTORY VALUATION

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

C) INVESTMENTS

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

D) FIXED ASSETS

Fixed assets are created out of grants received from DBT and valued at cost of acquisition inclusive of inward freight, duties and taxes and incidental and direct expenses related to acquisition. As per the advice of the audit the Fixed Assets created out of the external funded projects/fellowships have also been taken at cost of acquisition inclusive of inward freight, duties and taxes and incidental and direct expenses related to acquisition for each article.

All the assets allotted/transferred by other organizations without any consideration, have been taken at nominal value of Re. 1.

E) DEPRECIATION

Depreciation on fixed assets including fixed assets created out of projects have been charged as per the rate prescribed in the Income Tax Act-1961 on written down value method. The depreciation on fixed assets created out of project has been charged from the date of its acquisition, during the year 2021-22.

F) MISCELLANEOUS EXPENDITURE

There is no deferred revenue expenditure during 2021-22.

G) ACCOUNTING FOR SALES

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

Page 18 of 21

H) GOVERNMENT GRANTS/ SUBSIDIES

As the Institute is funded by the Department of Biotechnology (DBT), Ministry of Science and Technology, (Govt. of India) and the grants are treated as irrevocable, the same has been accounted for on sanction and receipt basis. During the FY 2021-22, recurring grants amounting to Rs. 16,43,00,000/- has been sanctioned for the purpose as shown in Schedule-13. Non-recurring Grants amounting to Rs. 2,75,00,000/- sanctioned by DBT have been shown as addition to Corpus/ Capital Fund (Schedule-1).

Interest earned on Govt. Grant amounting to Rs. 28,34,347/- has been shown as payable to Govt. (Schedule-7) in compliance to Rule 230 (8) of General Financial Rules 2017.

I) Expenses payable up to 31st March, 2022, pertaining to FY 2021-22, have been shown under expenses payable (Schedule-7). Any expenditure which has not been claimed or for which bill has not been received pertaining to any expenditure relevant to the FY 2021-22, the same will be accounted for in the year of claim.

J) RETIREMENT BENEFITS

The Institute is covered under New Pension Scheme of Government of India and is registered with the agency approved by Ministry of Finance. Institute is regularly depositing the monthly pension contribution (both employee and employer share) with appropriate authority. Provision for Gratuity and leave encashment of Rs. 63,83,413/- (Schedule-7) has been made in the books of accounts.

K) FOREIGN CURRENCY TRANSACTIONS

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

For National Agri-food Biotechnology Institute

For Goyal Parul & Co.
Chartered Accountants

Finance Officer

Manager Finance

Executive Director

CA Vijay Kumar

Partner

Membership No. 506042

Dated: 05/08/2022

Place: Mohali

Page 19 of 21

FORM OF FINANCIAL STATEMENTS

NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE

Knowledge City, Sector 81, PO Manauli, S.A.S. Nagar, Mohali

SCHEDULE 25

NOTES ON ACCOUNTS

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

1. Receipt and Payment Accounts

The Receipt & Payment Account carries the figures of actual receipts & actual payments of the Institute during the financial year 2021-22. It is virtually a copy of cash book / Institute's accounts. The total receipt as shown in receipt & payment account comes to Rs. 28,52,89,230/- which include Rs. 19,18,00,000/- as Recurring and Non-recurring grants from DBT, grant of Rs. 8,07,08,616/- for externally funded projects and Rs. 1,27,80,614/- from other receipts. Total amount of Rs. 35,03,93,871/- has been released as payments during the financial year 2021-22.

2. The Income and Expenditure Account

The Income and Expenditure accounts are prepared on accrual basis. The total income is Rs. 17,37,86,424 /- out of which includes Rs. 16,43,00,000/- Recurring Grant from DBT and rest is from Other Resources.

Total expenditure (before depreciation) comes to Rs. 17,98,40,020/- which also includes Rs. 28,34,347/- of interest earned on Grants, which is refundable to Govt., and depreciation of Rs. 17,93,56,813/- has been charged in the current FY 2021-22. A sum of Rs.18,54,10,408 /- being excess of expenditure over income has been transferred to Corpus/ Capital Fund (Schedule-1).

3. Fixed Assets

Fixed assets are created from grant from DBT and valued at cost of acquisition inclusive of inward freight, duties and taxes and incidental and direct expenses related to acquisition. Fixed Assets created out of the external funded projects/fellowships have also been taken at cost of acquisition inclusive of inward freight, duties and taxes and incidental and direct expenses related to acquisition for each article. Fixed assets transferred to other organizations are reduced as per their written down value. Also, fixed assets received from CDAC, free of charge, under National Supercomputing Mission have been taken at nominal value of Re.1 and corresponding accounting effect has been given in Schedule-2

4. Depreciation

Depreciation on fixed assets including fixed assets created out of projects has been charged as per the rate prescribed in the Income Tax Act-1961 on written down value method. The depreciation on fixed assets created out of projects has been charged from the date of its acquisition during the year 2021-22.

5. Current Assets, Loans and Advances

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

20 of 21

6. Land

The Government of Punjab has provided approx. 35 acres of land in Knowledge City at Sector-81, Mohali to the Institute, free of cost, for setting up of NABI Campus. Therefore, the cost of NABI land has been taken as nominal value of Re. 1 and corresponding accounting effect has been given in Schedule-2.

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

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

8. Externally Aided Project

As on 31st March 2022, there is a balance of Rs. 5,05,87,799/- in the externally funded project accounts. The balance will be spent in accordance with the terms and conditions of the projects. An interest of Rs. 6,10,275/- has been credited to the externally funded projects as shown in Schedule-3.

9. Gratuity & Leave Encashment

a) A Group Gratuity Scheme has been taken from Life Insurance Corporation of India (LIC). An amount of Rs. 1,27,20,717/- was computed towards liability for gratuity (Schedule-11) out of which Rs. 1,06,39,568/- has been paid to LIC upto the financial year 2021-22 and Rs. 20,81,149/- has been shown as provision for gratuity (Schedule-7).

b) A Group Leave Encashment Scheme has been taken from Life Insurance Corporation of India (LIC). An amount of Rs. 1,69,61,880/- was worked as liability for leave encashment (Schedule-11) out of which an amount of Rs. 4,67,550/- has been credited by LIC as interest and Rs.1,24,20,218/- (including other charges) has been paid to LIC upto the financial year 2021-22, and Rs. 43,02,264/- has been shown as provision for leave encashment (Schedule-7). An amount of Rs.2,28,025/- has been paid by LIC towards leave encashment in r/o the employee resigned during the FY 2021-22.

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

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

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

For National Agri-food Biotechnology Institute

Finance Officer

Manager Finance

Executive Director

Dated: 05/08/2022

Place: Mohali

For M/s Goyal Parul & Co.
Chartered Accountants

CA Vijay Kumar

Partner

Membership No.506042

21 of 21

NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE

Sector 81, Mohali, Punjab 140306

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