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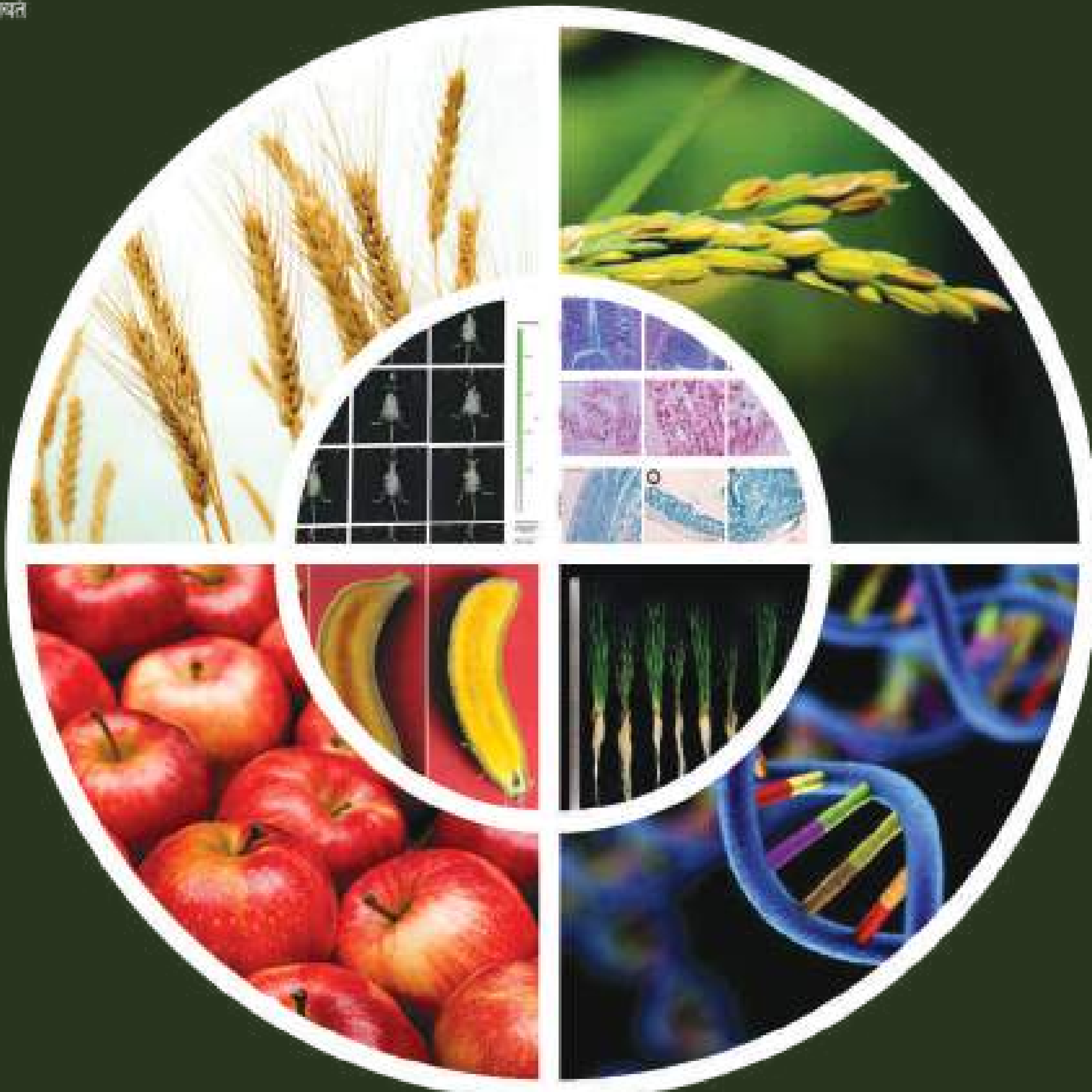
2020-21

DEPARTMENT OF BIOTECHNOLOGY
Government of India



NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE
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Contents

From the Desk of the

EXECUTIVE DIRECTOR

It is my immense pleasure to unveil the 10th Annual Report of National Agri-Food Biotechnology Institute (NABI), Mohali for the year 2020-2021. NABI, an autonomous institute of the Department of Biotechnology, Government of India has now completed ten years of its establishment. The institute was established in February 2010 to foster the transformation of agri-food sector in India and provide innovative and tangible solutions to the major scientific and technological challenges in the areas of agriculture, food and nutrition. It is a premier R&D institute in the country that has undertaken various overlapping research activities to provide sustainable and novel solutions towards quality food and nutrition. The institute has a vision to be a nodal organization for knowledge generation and translational science leading to the development of value-added products based on agri-food biotech innovations. To achieve the goal, NABI has developed various institutional R&D programs for addressing the interlinked predicament of inadequate household food and nutritional security, malnourishment, limited processing capacity and high post-harvest loses. Hence, the institute has identified programs at the inter-phase of agriculture, food and nutrition. Research priorities at the institute encompass seven core areas that include, a) Development of designer crops with high nutrition, increased shelf-life and processing quality; b) Genomics and computational biology for marker and gene discovery; c) Basic biology for crop improvement; d) Functional foods and nutraceuticals for better health; e) Food and GM crops biosafety; f) Nutrigenomics for health and human welfare and g) Post-harvest biotechnology for value addition and increasing shelf life. NABI is also actively engaged in training the valuable human resource and preparing them to address future research challenges. The decade-old journey of NABI has been highly rewarding in fulfilling the scientific objectives of the mission. In continuation, numerous significant research contributions and deliverables have been achieved in the respective areas of research at NABI during this year. A few salient achievements have been summarized in the succeeding paragraphs.

In the last few years, NABI has expended research on several crops such as wheat, banana, rice, tomato, soybean, lathyrus, brassica, sorghum and pea which are of great significance to the country. NABI has considered biofortification of crop plants as one of the major research areas with the potential of impacting food accessibility, and improving nutritional and processing quality through novel biotechnology and breeding approaches. The institute is 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 identified crop plants. For instance, NABI has developed mutant wheat lines containing high amylose that have shown low glycemic index (GI) and improved agronomic traits. These improved genotypes provide nutritional benefits and good processing of high amylose for the consumer acceptance. Quantitative trait loci (QTL) mapping has identified a few major QTLs for high amylose in selected wheat lines which will be further validated using back cross-population and functional genomics tools. 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 loses than the traditional white wheat. During this year, MOU/NDA has been signed with 5 new companies for the commercialization of colored wheat. Institute is also working in the direction to understand the molecular basis of micronutrient accumulation and development of iron (Fe)-rich 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 ten banana (cultivar Grand Naine) events have shown close to 50-folds higher accumulation of pro-vitamin A in fruit-pulp of ratoon crops (2nd generation) and has been considered for the multi-location event selection trails. Work has also been initiated to establish transgene-free genome editing in banana. On the other hand, lathyrus is an important legume crop but not promoted widely for its cultivation owing to the presence of 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. Constructs for editing of three ODAP biosynthetic pathway genes have been developed for stable plant transformation experiments. In the direction of metabolic engineering of triacylglycerol (TAG) biosynthetic pathway in rice, lipases were identified and also characterized for their role in lipid degradation. The yeast mutant study has validated the role of rice derived Oslip 1 gene in the reduction *of TAG content. A CRISPR/cas9 mediated editing of Oslip 1 was performed in rice and mutant lines are being generated for further analysis. Similarly, improvement of nutritional and processing quality in soybean and tomato by using genome editing approach have also been undertaken and mutant lines are being generated for traits analysis.*

Several significant achievements have also been made in the other research areas of the food and nutritional biotechnology division. Obesity has become an important health concern worldwide. The research has been initiated on the use of plant-derived vesicles as a therapeutic reagent in mitigating obesity. The plant-derived exosome-like nanoparticles (PENLs) has been made from ginger and their regulatory role of pre-adipocytes differentiation has been demonstrated. A subsequent study will be performed in the mouse model to validate the function of PENLs in controlling obesity. Previously, the probiotic characterization of the anti-inflammatory potential of indigenous Bifidobacterium strains was demonstrated in the cell lines. This study was extended further on the characterization of Lactobacillus sp. and other lactic acid bacteria which prevented lipopolysaccharides (LPS) induced pro-inflammatory stress in mice model. 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 dietary capsaicin has produced anti-obesity effects in mice. This study will be directed to develop a diet-based strategy for obesity and related complications.

Iso-malto-oligosaccharides (IMOs) are a low-calorie sweetener that has prebiotic and immunomodulatory activity. Initiatives have been taken to prepare health beneficial functional food products by using IMOs. For this purpose, a methodology was optimized for starch extraction from potato peel (waste-utilization) and subsequently, conditions were optimized for lab-scale production of IMOs. Further studies on the up-scaling production of IMOs are in the progress.

NABI is also working on the development of alternative therapeutic formulation for controlling iron deficiency anemia. A novel synbiotic hydrogel encapsulating iron has been developed that releases its contents in the duodenum (major site of iron absorption) and reduces the risk of iron aggregation in the acidic environment of the stomach. NABI is investigating the role of different dietary glycans in the modulation of the gut microbiome and their relation to the human immune system. This study will pave the way to invent new nutraceutical strategies to purposefully manipulate microbial composition to improve human health. The gene constructs have been designed for the chloroplast transformation in the Chlamydomonas reinhardtii. The work is going on for the heterologous production of omega-3 polyunsaturated fatty acids (PUFAs) in the yeast system.

Under the area of food and GM crops biosafety, NABI has developed carbohydrate, aptamer, and gold nanostars-based biosensors against different foodborne pathogens to increase sensitivity and selectivity. These approaches will contribute to the development of detection systems for new foodborne pathogen biosensors. The other application-based research is going on for the development of ultra-sensitive, portable, and rapid biosensors for the detection of heavy metals, pesticides and food contaminants using functional hybrid nanostructure. The increased level of arsenic in water systems poses a severe threat to the life of living organisms. NABI has recently developed an arsenic sensing probe that is highly sensitive, selective, and simple to use (one-step procedure). This spectroscopic and color-based sensing probe can detect arsenic lower down to 0.002 and 0.004 ppb, respectively in water and food samples. In the program liked with post-harvest biology for value addition, NABI has developed a biodegradable and nontoxic coating material from agricultural and food processing by-products (e.g. wheat straw, oat bran and potato peel) and demonstrated an increase in the post-harvest shelf life of fresh fruits. The use of this technology has been shown in apples, pears and banana and now it has been extended to increase the shelf life of peach by 6 days, thereby providing an alternative to animal-derived shellac coating in India. The detailed toxicological studies on coating material indicated that the material is non-toxic, non-inflammatory and safe for human consumption.

A significant progress has been made in the multi-institutional flagship program that deals with "high-resolution QTL mapping for Fe, Zn, grain protein and phytate content and their introgression in high yielding wheat cultivars". This collaborative program has been developed along with seven institutions and provides a common platform, sharing resources and eventually delivering wheat grains with enriched micronutrients and proteins. NABI has also established a bio-incubator with the vision of catalyzing Agri-Food Biotechnology Innovations and entrepreneurship for sustainable food and nutritional security. NABI is providing the platform so as to generate a translational ecosystem to scale up technological innovations and handhold entrepreneurs/start-ups. In the next 5 years, NABI plans to incubate multiple companies and develop a model of a self-sufficient incubator.

Human resource development is one of the important mandates at NABI. The institute currently has nearly 134 students who are working in different capacities such as Ph.D. students, junior research fellows, senior research fellows, project assistants and post-doctorate research associates. The institute also provides research training to graduate and post-graduate students in the areas of agriculture, food and nutritional biotechnology. In the last financial year, institute obtained 03 extramural research grants from different national and international funding agencies. On the scientific output, last year the institute has published more than 65 high impact research publications in various peer reviewed journals. Till date, NABI has filed a total of 9 patents and signed more than 60MoUs. 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. Besides, state-of-the-art research facilities will be developed for doing high-quality research in Agriculture, Food and Nutritional Biotechnology.

Finally, I take this opportunity to place on record our sincere thanks to **Dr. Harsh Vardhan ji***, Hon'ble Union Minister for Ministry of Science & Technology, Ministry of Health & Family Welfare, Ministry of Earth Sciences and President of NABI Society for his keen interest and support to all the activities of the institute.*

The relentless support by the Department of Biotechnology has been a critical factor in shaping up the institutional agenda. I would like to place on record my heartfelt thanks to Dr. Renu Swarup, Secretary, DBT and Dr. A. Vamsi Krishna, Scientist E, DBT for their constant support and help

in bringing excellence in the activities of the institute. I am also thankful to Sh. Vishvajit Sahay, Financial Advisor DBT and Sh. C.P. Goel, Joint Secretary DBT for their constant help in various financial and administrative issues of the institute.

I take this opportunity to acknowledge and thank Prof Deepak Pental and Dr. B. Sesikeran, Chairman of Program Advisory Committees (PACs) and all members of PACs for their suggestions and advice during our intensive scientific discussions. I would also like to place on record my sincere thanks to Dr. R.S. Paroda, Chairman Scientific Advisory Committee (SAC) and all the members of SAC for the scientific advice, suggestions and guidance to improve the ongoing programs at the Institute. I am also thankful to Dr. Siddharth Tiwari, Dr. Koushik Mazumdar, Dr. Nitin Singhal and Sh. Arun Kumar for compilation and editing of this Annual Report. I am grateful to all the scientific, technical and administrative staff of NABI for their untiring efforts and hard work in bringing excellence in science and institutional building.

Over the last few years, research advancements at NABI have shown promising results. Even during the past year of pandemic, NABI has been striving hard to deliver good science while ensuring complete safety of all the staff members and students at the campus. We feel quite safe with 100% vaccinated campus at NABI. We are looking forward for achieving more success with confidence and impacting the country's societal needs through innovation and creativity.

-s/d
Prof. Ashwani Pareek
Executive Director, NABI-Mohali

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

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

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

Human Resource & Scientific Achievements

(From 2010 to 31st March 2021)

Ph.D Awarded: 24	Publications: 331
Patents filed: 09	Conference Attended/Lecture Delivered: 247
Invited Guest Lectures: 49	Conference & Workshop Organized: 09
Trainees: 355	Scientific & Administrative Staff: 39
Research Scholars: 134	MoU Signed: 60

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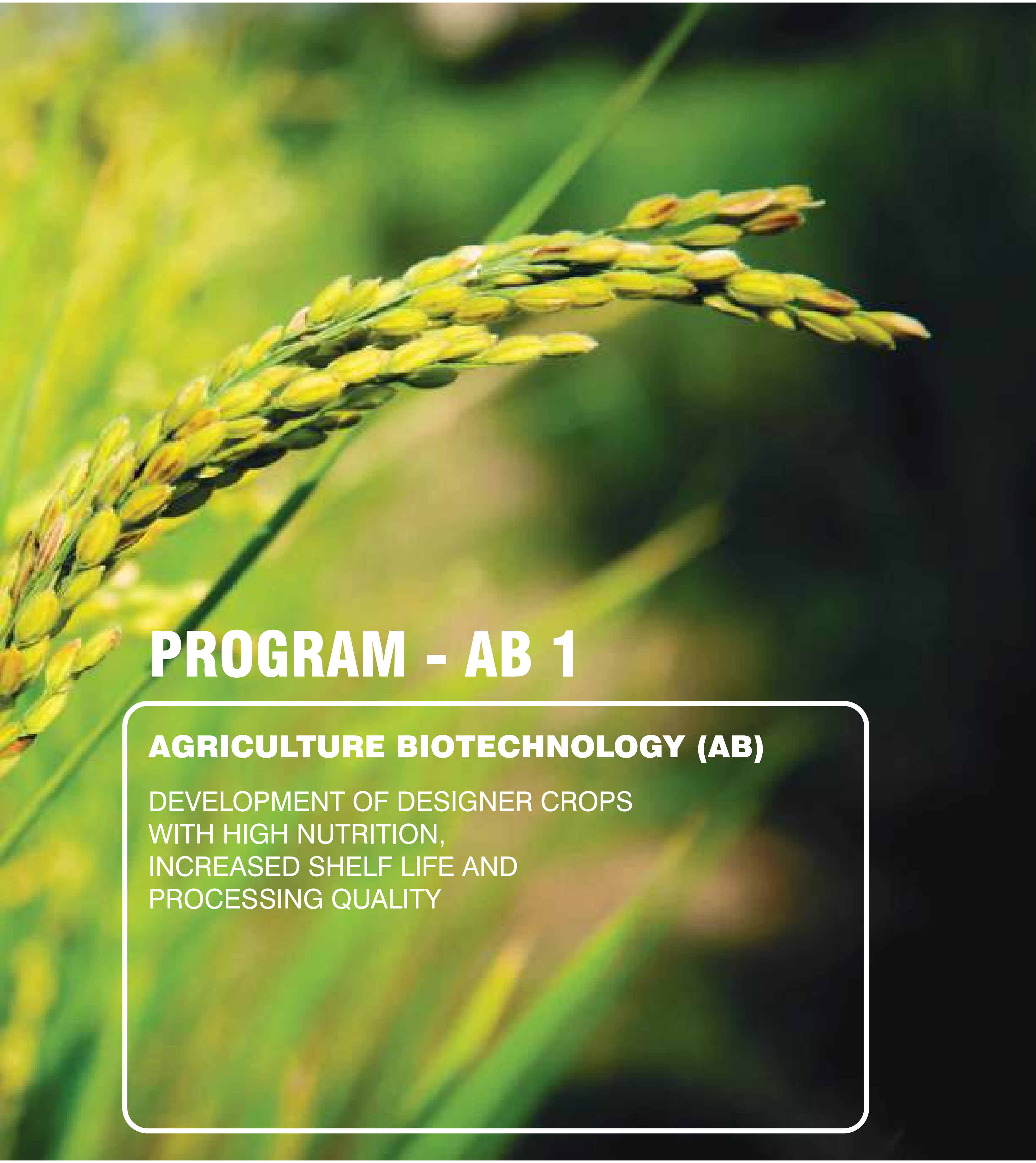
RESEARCH PROGRESS



PROGRAM - AB 1

AGRICULTURE BIOTECHNOLOGY (AB)

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

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

PRINCIPAL INVESTIGATOR
Joy K Roy

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

Wheat (Triticum aestivum L.) flour is processed into several end-use food products, whose processing and nutrition quality are largely determined by storage protein (~12%) and starch (~70%). 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, which is otherwise narrow in wheat germplasm, is induced via non-transgenic approach by random modification of nucleotides in genomes through chemical treatment, ethyl methyl 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. Genomics approaches will be implemented to identify single nucleotide polymorphisms (SNPs) which can be used along with microsatellites on a diverse wheat germplasms, mutant population, and biparental mapping populations to identify markers for QTLs (quantitative trait loci). Candidate QTL regions will be further saturated using SNPs to identify causal genes. Validation of the associated genes will be done using functional genomics tools. In the long term, pyramiding will be done by combing high amylose/resistant starch with other important biomolecules such as high grain protein content.

RESEARCH PROGRESS

1. 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 several biparental segregating populations (F1s, F2s, F6s, F7s). It also includes a set of 101 mutant lines (M10) showing variation in amylose content. A wheat germplasm for high iron and zinc containing wheat genotypes were included in the last rabi season.

2. Low glycemic wheat lines: Two low glycemic wheat lines were multiplied at large scale in the village, Tasoli, Mohali in the last Rabi season, 2020-21.

3. Genetics of high amylose variation: *Development of a recombinant inbred line (RIL) population:* In this study, a recombinant inbred line (RIL) mapping population (named hereafter NABI-HAS-RIL1) was developed by crossing a high amylose mutant line ('TAC 75') with ~64% amylose with a recipient high yield wheat variety 'WH 1105' with ~22% amylose. The segregating population of about 350 F5 progenies was advanced to F6 using spike to row sowing method in the last Rabi season (2020-21). From this larger set of the population, a sub-set of 180 F6 lines was advanced to F7 population.

Amylose estimation on 'NABI-HAS-RIL1': The frequency distribution of amylose data on the 188 F6 RILs from two locations (E1, Mohali and E2, MP), grown in 2019-20 showed high variation. The minimum and maximum value for starch amylose content ranged from 10% to 84% (E1) and 6% to 80% (E2). The histograms for the frequency distribution of starch amylose content in two environments showed a bimodal pattern of distribution for trait value towards both the parents. Pearson's correlation analysis for amylose content in two environments suggested a strong significant positive correlation $r2=0.98$ for the trait at p-value 0.001. Further, the amylose content in the sub-set of 96 F7 progenies (multiplied at NABI in the last Rabi season, 2020-21) ranged from 6 to 79% and the data revealed wide continuous variations. The combined environment ANOVA analysis at p-value 0.001 suggested no significant difference in

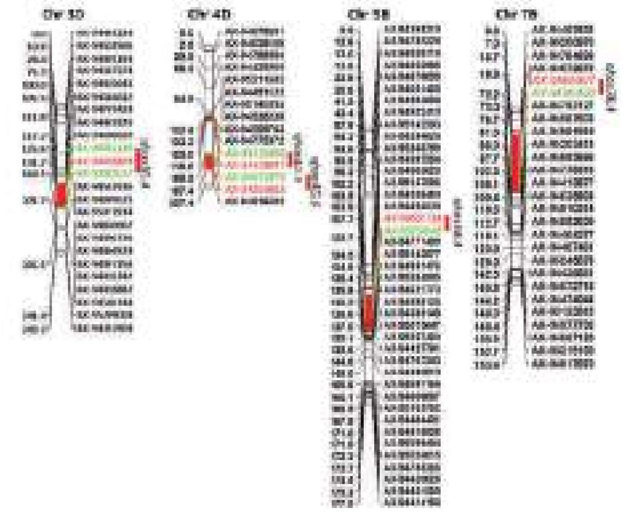


Figure 1. QTL maps of amylose starch on 4 chromosomes (3D, 4D, 5B, 7B) in wheat. The number on the left side of chromosomes indicates chromosome positions in centiMorgan (cM) and the right side indicates SNP names. The colored SNP names are the linked markers with QTLs.

the measured amylose content for both the environments (E1 and E2). High heritability 99% for high amylose starch and high genetic advance 24.5 was found in the RIL population. In the present study, we found high genetic advance of Percent mean value 65.2 for starch amylose content, indicating that the trait is governed by additive gene action.

Construction of linkage maps using SNPs data of 'NABI-HAS-RIL1': The genetic map of the RIL (F6) population using 1,293 high quality SNPs showed the distribution over all the 21 wheat chromosomes. The linkage groups were numbered according to the assigned chromosomes of wheat reference genome. The cumulative length of the map spanned over 3,903.4 cM. The chromosomal genetic distance ranged from 114.8 cM (6A) to 280.4 cM (5D). The inter marker distance between the 21 linkage groups ranged from 1.4 cM (5B) to 11.3 cM (6D). Chromosomes 5B and 6D had the maximum (10%) and minimum (2%) number of SNP markers, respectively. Out of 1,293 SNPs, 532, 410, and 351 SNPs were distributed over the A, B, and D genomes with map length of 1,358.3 cM, 1,560.1 cM, and 1,730.3 cM, respectively (Figure 1). The positions of the markers identified on the genetic map were further used for QTL mapping analysis.

QTL mapping of amylose: Genome wide QTL mapping for high amylose starch using composite interval mapping (CIM) approach, identified a total of 151 QTLs individually representing 99 and 52 QTLs from E1 and E2 respectively. The observed phenotypic variance explained by these QTLs ranged from 1% to 73% in E1 and 1% to 71% in E2. The 99 QTLs detected in E1 were distributed among all the 21 wheat chromosomes. Of them seven QTLs detected on chromosome 3D, 4D, 5B, and 7B were major effect QTLs explaining PVE from 10% (qhas3D.4) to 73.6% (qhas7B.2) at a LOD score from 4.8 (qhas5B.5) to 10 (qhas7B.2). Out of the seven major effect QTLs detected in E1, six QTLs (qhas3D.4, qhas4D.5, qhas4D.6, qhas4D.7, qhas5B.4, and qhas7B.2) identified with negative additive effect indicating the contribution of alleles from 'TAC 75' for increasing trait value. However, the QTL qhas5B.5 suggested positive additive effect indicating the allelic contribution of 'WH 1105' towards trait value. Apart from the QTLs identified in E1, the 52 QTLs detected in E2 were found on all chromosomes except on 5A and 5B. Of them five QTLs (qhas3D.4, qhas4D.5, qhas4D.6, qhas4D.7, and qhas7B.1) explained PVE from 10% (qhas3D.4) to 71.6% (qhas7B.1) at LOD score from 3.5 (qhas4D.7) to 6.6 (qhas7B.1). All the five major effect QTLs detected in E2 showed the negative additive effect suggesting the major allelic contribution coming from donor parent 'TAC 75'. Combined analysis of the 151 QTLs identified in two environments (E1 and E2) identified four common QTLs (qhas3D.4, qhas4D.5, qhas4D.6, and qhas4D.7) were major effect QTLs explaining PVE $\geq$ 10% at LOD score $>$ 3.

QTL x Environmental interaction (QEIs): QTL x environment interactions for high amylose starch identified five QEIs (qhas2B.4, qhas2D.2, qhas7A.6, qhas7B.4, and qhas7D.5) by using the combined phenotypic data for two environments (E1 and E2). Out of five, the locus qhas2D.2 was identified in both environments during single environment as well in multi-environment (MET) analysis. However, none of the five QEIs identified explain phenotypic variance due to environmental influence.

Epistasis (QTL x QTL interaction): The epistatic effect for high amylose starch was estimated as the total effect introduced by dual interacting QTLs pyramiding effect (AA). In the present study at LOD threshold 10 we identified 24 pairs of digenic epistatic QTLs exhibiting significant epistatic effects. Among the 24 pairs of digenic epistatic QTLs twelve pairs of epistatic loci identified on chromosomes 3B/3B, 2B/6D, 5B/6D, 3B/3D, 3B/4D, 5D/6D, 3D/4D, 1A/AB, 3A/5B, 4D/6B, 2B/2D, and 5A/6B showed increased amylose content by significant additive interactions between the loci coming from donor parent 'TAC 75'. The phenotypic variance explained by these epistatic QTLs was $>$ 50%. Another twelve pairs of epistatic loci found on chromosome 1A/5B, 5B/5D, 1A/4A, 1A/5D, 1A/7D, 1A/6D, 1A/1A, 1A/2B, 4A/4D, 4B/4D, 4D/5A, and 2D/4D were 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'.

ACTIVITIES FOR NEXT YEAR

- 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.

1.2 Improvement of nutritional and processing quality in wheat

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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

1. 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.

2. Understanding effect of phosphate deficiency on colored wheat lines: Wheat plants of four different genotypes black wheat (NABI MG-11), purple wheat (NABI MG-10), blue wheat (NABI MG-9) and white wheat (cv. PBW621)(Figure 1) were grown in three replicates. To check the effect of the P-deficiency on the colored wheat genotypes, 15 days old seedlings were subjected to Pi limiting conditions. Our phenotypic data indicated that Pi deficiency treatment increased the root biomass including root length, volume and dry weight and decreased shoot biomass including shoot length, shoot volume and dry weight (Figure 2). This increase in the root biomass was in the order of white < blue <purple < black. The decrease in the shoot biomass was in the order of black< purple=blue < white. Colored wheat genotypes showed lower reduction total P and free phosphate (Pi) contents and was in the order of white wheat>blue >purple >black. Further, all the colored wheat varieties showed significantly higher shoot total anthoccynin content (TAC) content than white wheat. The Pi-deficiency increased the antioxidant activity of the genotypes. Highest antioxidant activity was observed in black wheat followed by purple, blue and white. Our data suggests that the Pi-deficiency resulted in the increased expression of multiple phosphate transporters - PHT1 family genes that includes TaPHT1.5, TaPHT1.9, TaPHT1.10, TaPHT1.11 and TaPHT1.13 and other phosphate starvation responsive (PSR) genes such as TaIPSI and TaSPX1 in the roots (Figure 3a). Our expression results showed that Pi-deficiency impacted the expression levels of genes including TaCHS, TaDFR, TaUFGT, TaF3'H involved in anthocyanin biosynthesis, TaMATE34 and TaMRP4 in transport and its related transcription factors i.e. TaMyb10 and TaMYC1. The highest fold change in expression was observed for the TaMyb4, TaUFGT and TaMATE 34 genes in the black wheat genotype (Figure 3a). Under P deficiency significantly higher number of anthocyanins was observed in comparison to +Pi conditions (Figure 3b). Besides this, Pi deficiency also triggered the accumulation of unique anthocyanins which were not observed in +Pi conditions. Our study, suggest that under Pi deficiency black wheat had highest number of anthocyanin peaks (n=9) as compared to n=6 observed in control condition. Interestingly, two P-deficiency induced anthocyanin i.e. cyanindin-3-o-glucoside, and peonidin-3-o-rutinoside were common for black, blue and purple wheat indicating their regulation is genotype non-specific and induced for stress mitigation in colored germplasm. Besides this, some anthocyanins accumulation after the stress was genotype specific. For example, delphinidin-3-o-sambubioside is found to be unique in blue, delphinidin-3-o-rutinoside in purple. While black wheat had both of these. As black wheat is combination of purple and blue color, it seems that stress induced accumulation of these anthocyanins is regulated tightly based on the genotype and its color. Overall, the present study has provided information about the role of accumulation of anthocyanins such as cyanidin, peonidin and delphidine derivatives in colored wheat under Pi deficiency that may help in generating phosphate stress tolerant wheat genotypes.

Figure 1. Seeds of differet colored wheta lines.

3. Investigation of colored wheat-grass as a new nutraceutical: The white wheat line (PBW621) and colored wheat lines- purple (NABIMG-10), blue (NABIMG-9), black (NABIMG-11) were

Figure 2. Growth of different wheat genotypes under +Ph and -Ph conditions for 15 days. Bl=Black wheat, P=Purple wheat, B=Blue wheat and W=white wheat. (+Pi) = phosphate sufficient condition; (-Pi)= phosphate deficient condition.

used to raise the wheat-grass. Wheat-grass was harvested on the 12th day, its juice was extracted and lyophilized to powder (WJLP) (Figure 4). Different biochemical tests were perfomed to check the nutritional evaluation. Total chlorophyll content was highest in black, followed by purple, blue and then white wheat-grass (Figure 6A). Further, total anthocyanin content (TAC) indicated that black had the highest amount of anthocyanin content, followed by purple, blue, and then white wheat-grass (Figure 6B). Total phenolic content indicated that colored wheat genotypes had highest total phenolic content specifically in black wheatgrass. Soluble phenolics were found to be higher in colored wheat in comparison to insoluble phenolics (Figure 6C). Ultra performance liquid chromatography (UPLC) analysis of individual phenolic compounds gave insights that all wheat-grass extracts (white, blue, purple and black) had the highest content of di-hydroxy benzoic acid (DHB) in free as well-bound form. Further, antioxidant capacity was estimated via ABTS and DPPH assays. It indicated the highest antioxidant activity by black followed by purple and blue wheat-grass powder (Figure 6D). The EAAI indicated that quality of protein is in black wheat-grass samples is complete/equilalent to egg protein as compared to moderate found in white wheat. Hence, these samples could be used as quality protein efficient diet. Elemental analysis was performed using Inductive Coupled Plasma-Mass Spectroscopy (ICP-MS). Minerals estimated (Mg, Fe, Li, Na, Al, K, Ca, V, Cr, Mn, Cu, Co, Ni, Zn, Se, Rb, Sr, Cd, Cs, Ba, Pb and U) in wheat-grass showed highest concentration of K followed by Na, Mg, Ca, and Fe. Overall, the present study demonstrated that, wheat-grass can be considered as nutraceutical due to high content and/or quality of chlorophyll, phenolics, protein and K. The colored wheat gives additional nutraceutical potential to wheat-grass in the order of < blue <purple < black due to better quantity of chlophyll and anthocyanins, quality of phenolics, quantity and quality of protein and antioxidant activity with black wheat outperforming all of them.

4. Understanding effect of chapatti cooking on amino acids in colored wheat: The determination and quantification of amino

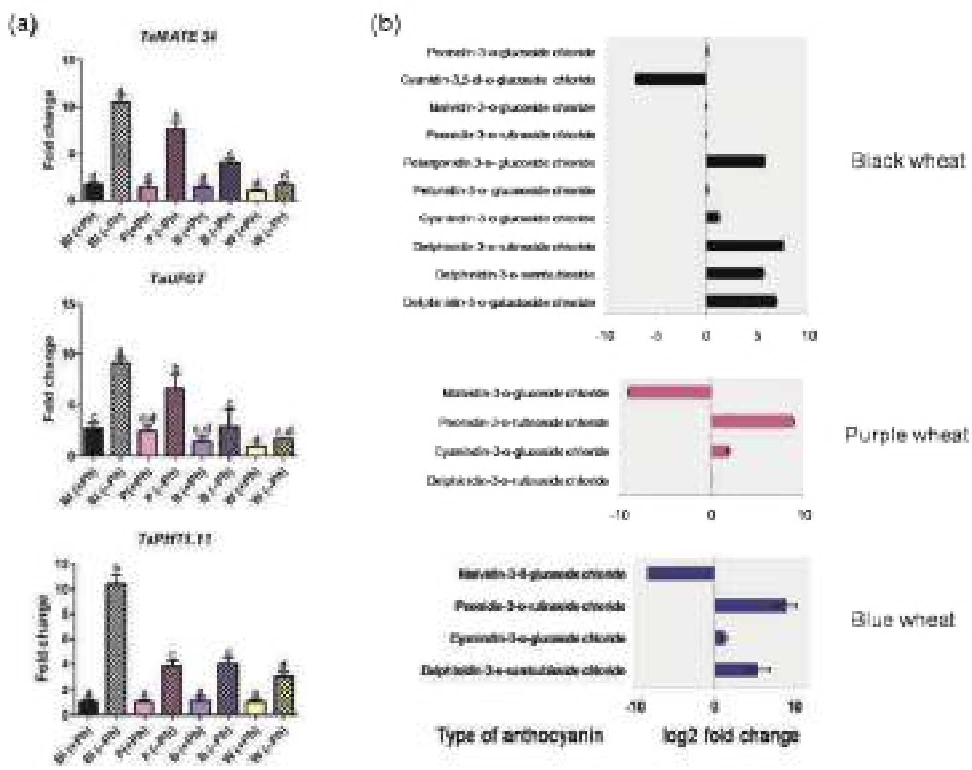

Figure 3. (a) Quantitative real-time PCR expression analysis of anthocyanin transporter; TaMATE34, anthocyanin biosynthesis gene; TaUFGT and phosphate transporter; TaPHT1.11 showed highest expression in black wheat. (b) Different accumulated anthocyanins response ratio (–Ph/+Ph) calculated from UPLC based separation of anthocynin extract from shoots. Bl=Black wheat, P=Purple wheat, B=Blue wheat and W=white wheat. (+Ph) = phosphate sufficient condition; (-Ph) = phosphate deficient condition. Different letters above the graphs indicate statistically significant value ($P<0.05$).

Figure 4. Seeds, grass and juice of different wheat lines. A- white wheat; B- blue wheat; C- purple wheat; D- black wheat. The color of wheatgrass was darkest for black> blue> purple> white.

acid profile in black, purple, blue and white colored wheat flour and chapatti samples was done using ultra-high-performance liquid chromatography. The protein content of wheat flour (9.17-10.18%) and chapatti samples (7.04-8.55%) followed the order: black> blue> purple> white. UPLC chromatogram (Figure 5) indicated that glutamic acid was the most abundant amino acid with the concentration ranging from 38.70 - 43.04 mg/g and 29.98 mg/g - 35.35 mg/g in flour and chapatti samples, respectively. Black and blue wheat flour and chapatti samples had higher total amino acid content, and nutrition index in comparison other flour and chapatti samples. The EAAI (essential amino acid index) values of flour (69.01-74.49%) and chapatti samples (44.65-50.73%) were less than 100 suggesting that these samples are deficient in EAA. Lower EAAI values of chapatti samples was due to cooking losses. Lysine was found to be the most limiting amino acid in all wheat samples. Asp was most vulnerable to cooking as all chapatti samples showed >20% losses in its content upon cooking (Table 1). The average percent reduction in amino acid content in black, blue, purple and white wheat chapati after cooking were 11.41, 12.4, 19.0, and 23.8%, respectively. Variation in the amino acid losses after cooking was associated with relative concentration and types of anthocyanins present in different flour samples. Black flour showed minimum cooking losses. The higher retention of amino acids in colored wheat might be due to the masking and protective effects of anthocyanins against heating and oxidative damages of amino acids and proteins. Hence, incorporation of anthocyanin rich colored wheat in diet can pose several nutritional and health advantages to the consumers, especially of the South-Asian countries where wheat is mainly consumed in the form of chapattis.

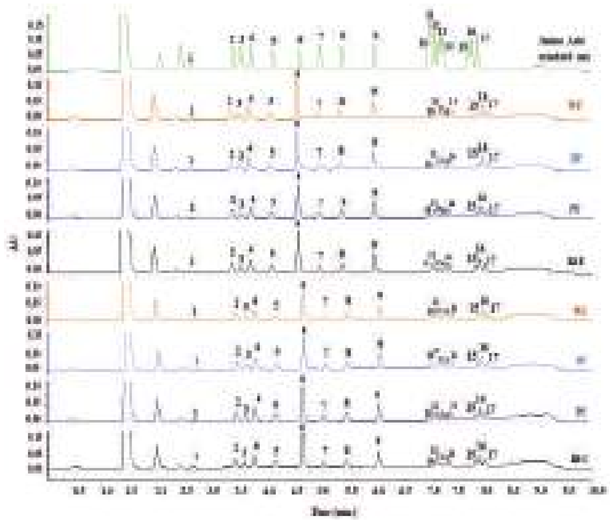

Figure 5. Chromatographs of 17 amino acids in different colored flour and chapatti samples1 = Histidine, 2 = Serine, 3 = Agrinine, 4 = Glycine, 5 = Aspartic acid, 6 = Glutamic acid, 7 = Threonine, 8 = Alanine, 9 = Proline, 10 = Cysteine, 11 = Lysine, 12 = Tyrosine, 13 = Methionine, 14 = Valine, 15 = Leucine, 16 = Isoleucine, 17 = phenylalanine; WF = white flour, BF = Blue flour, PF = purple flour, BlF = black flour, WC = white chapatti, BF = blue chapatti, PF = purple chapatti, BlC = black chapatti

5. Effect of storage on the food product (vermicelli) prepared from the colored wheat: Vermicelli of different colour whole wheat flours (white, purple and black) was made using a single screw vermicelli making machine, cut into desired lengths and

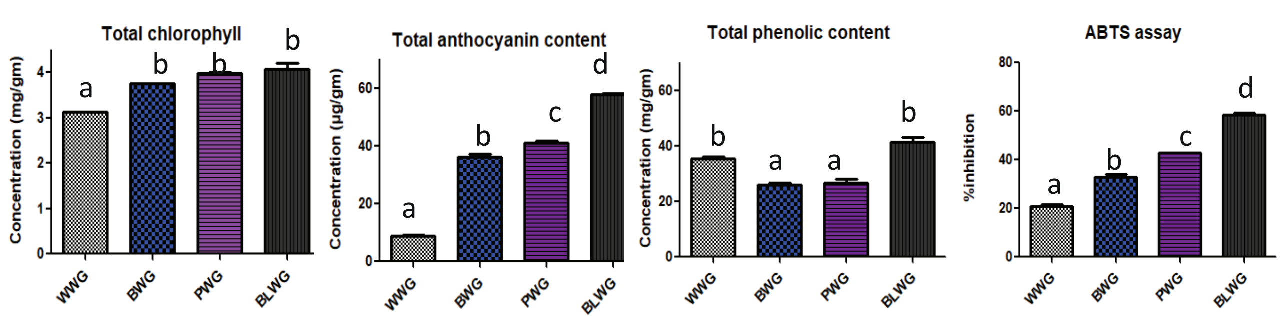

Figure 6. Total chlorophyll (A), anthocyanin (B), phenolic contents (C) and ABTS activity (D) of dofferent wheat grass juice lyophilised powder (WJLP). ABTS assay was carried out on acidified methanolic extract of WJLP samples. WWG- white wheat grass; BWG- blue wheat grass (C); PWG- purple wheat grass; BLWG- black wheat grass. Different letters above the graphs indicate statistically significant value (P<0.05).

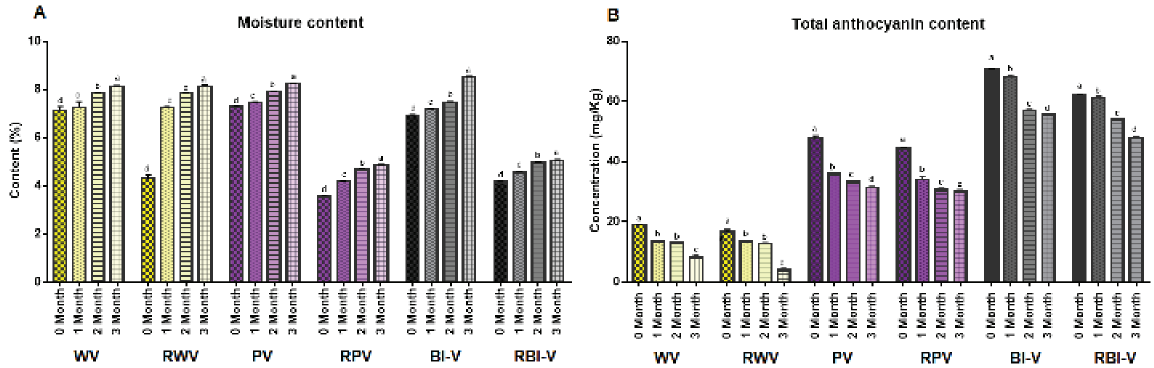

Figure 7. Moisture content (A), total anthocyanin content (B) of different color vermicelli's. Means with different superscripts in column differ significantly ($p \leq 0.05$). WV-white wheat vermicelli, RWV-roasted white wheat vermicelli, PV-purple wheat vermicelli, RPV-roasted purple wheat vermicelli, BV-black wheat vermicelli and RBV-roasted black wheat vermicelli.

dried in an oven at 60°C until the moisture content reached 6-9%. After drying, few vermicelli samples were roasted. The normal dried vermicelli (V) and roasted vermicelli (RV) samples were packed in low-density polyethylene bags (LDPE) at room temperature (35–37 °C) for a period of 3 months. Samples were withdrawn periodically and total anthocyanin content, moisture, and antioxidant activity were assessed at 0, 1, 2 and 3 months of storage. Over the course of three months storage period, a steady increase in vermicelli moisture content was noticed in all the samples (Figure 7A). The TAC showed reduction. The rankings of the vermicelli for TAC losses after 3 months of storage were white > purple > black for normal vermicelli as well as roasted vermicelli with the corresponding percentages losses at 40%, 22%, 15%, and 39%, 29%, 12%, respectively. During the storage period, the TAC content of black normal and roasted vermicelli was found to be the least affected. We observed that after passage of time, antioxidant activities was still higher in the colored wheat vermicelli in the order of black > purple > white (figure 2A, B). After 3 months of storage duration, black vermicelli possesses highest antioxidant activity while white vermicelli possesses the lowest activity. Hence, replacement of white wheat with anthocyanin rich colored wheat in diet can pose several nutritional and health advantages to the consumers, especially in India where vermicille is regularly consumed.

SALIENT ACHIEVEMENTS

- MOU/NDA has been signed with 5 new companies for colored wheat commercialisation.
- Anthocyanins present in the anthocyanin biofortified wheat might be a potential phytochemical that could help it in better adaptation against phosphate deficiency.
- Data showed that colored wheat-grass has nutraceutical properties
- Food products prepared from colored wheat have better storage life, higher retension of antioxidant activity and lower amino acids loses than white wheat.

ACTIVITIES FOR NEXT YEAR

- Development of improved colored wheat lines and commercialization
- Understanding other phytochemical diversity in colored wheat
- Evaluation of anti-daibetic and phyto-estrogenic potential of colored wheat
- Clinical trials of colored wheat.

1.3 Functional genomics strategies for improving micronutrient transport and its bioavailability in wheat

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Micronutrients including iron (Fe) in grains are not only required for germination and seedling development but also provide primary nutritional sources for humans, especially for people consuming cereals as staple foods. However, Fe-deficiency impair the physiological and biochemical processes of the plant affecting several indispensable functions, including photosynthesis, respiration redox reactions. Therefore, plants must recruit the mechanisms that could entail the necessary uptake and mobilization of Fe forms from the rhizosphere to the plant tissue. Transcriptional changes in response to Fe deficiency have previously reported in wheat by our group, for the stage when photosynthesis and growth were severely impacted, confounding primary and secondary responses. Subsequently, we specifically uncover the transcriptional landscape of wheat roots during the early stages of the Fe deficiency response (4 and 8 days) and after Fe resupply. This work will lead to identification of multiple early response metal-transporters, transcription factors (TFs) and Fe-sensors involved in the nutrient homeostasis. An attempt has also been made to explore the role of miRNA mediated regulation of Fe-homeostasis related genes in different wheat tissue under Fe-deficiency condition. Overall, we have characterized multiple transporter gene families/TFs, initiated genome editing tools to target identified genes and identify specific miRNA so as to explore new avenues to enhance the Fe-uptake and its loading in the wheat grains.

RESEARCH PROGRESS

Comparative transcriptomic dissection at the early stages of iron deficiency in wheat roots:

1. 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 1). Typically, a significant reduction in SPAD values was observed at the 8d post –Fe. Reduction in root size was in accordance with the previously observed phenotype (Kaur et al., 2019. JExB).
2. Transcriptome 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-regulated. 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.
3. Our analysis suggested that few genes were induced genes during Fe deficiency show downregulation under resupply conditions. These changes in the expression level were high in the 8 d of Fe deficiency compared to the 4 d. Most of the genes for this category are involved in the biosynthesis and efflux of phytosiderophores (PS) including ZIFL, NAS and NAAT. Interestingly, YSL transporters belonging to the oligo-peptide sub-family were observed to specifically respond at the transcriptional level to the changing regimes of Fe. Therefore, we went on to analyze expression of all the wheat YSL genes, which were

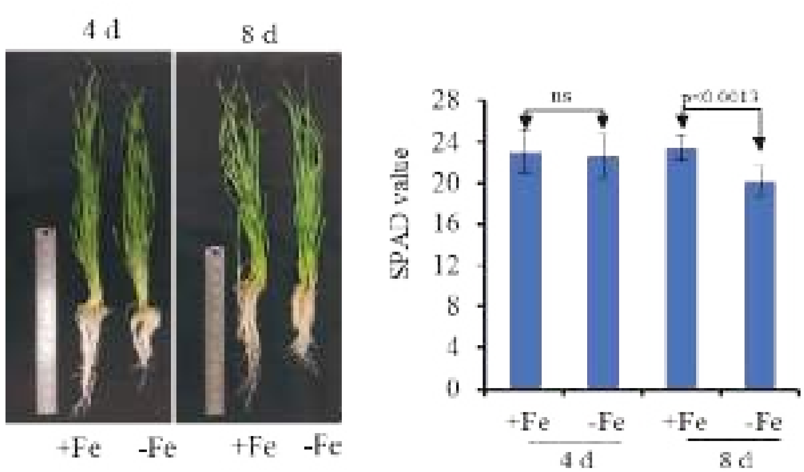

Figure 1. Phenotypic effect of short-term nutrient deficiency stress. Five-day wheat seedlings grown under sufficient Fe (80 μM; +Fe) condition and were subjected to Fe-deficient (-Fe) condition. For control the wheat seedlings were subjected to sufficient condition. Post deficiency of 4 and 8 days (d) the morphological changes were noted and Chlorophyll content (SPAD-units) was measured. Values at the indicated days of treatment are given as average±SD (n = 12).

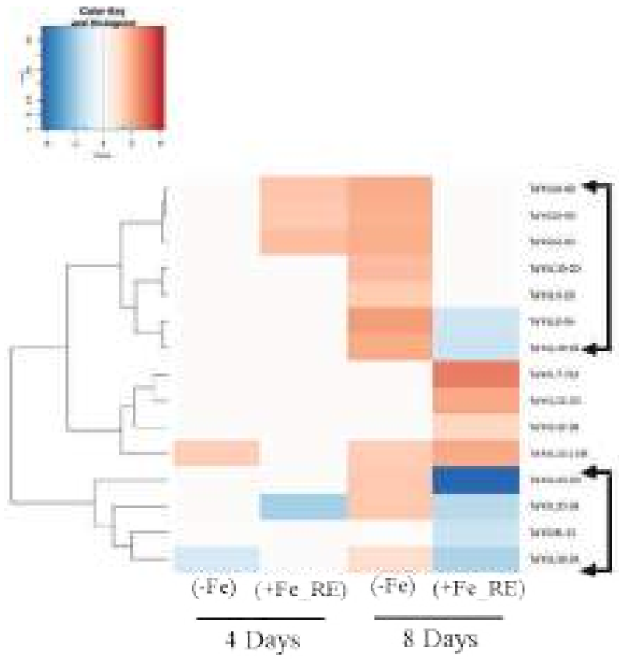

Figure 2. The heatmap analysis of the wheat YSL transporters showing differential regulation under the Fe-deficiency conditions. Multiple YSL transporters responds to the varying regies of the Fe in the roots.

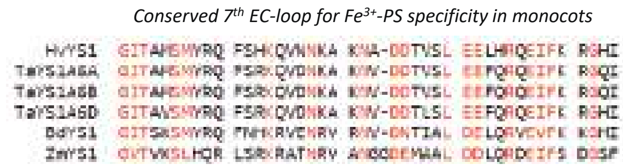

Figure 3. Domain alignment of the 7th extracellular (EC) loop from multiple crop species. The shaded portions show the conservation of the amino acids in the sequence.

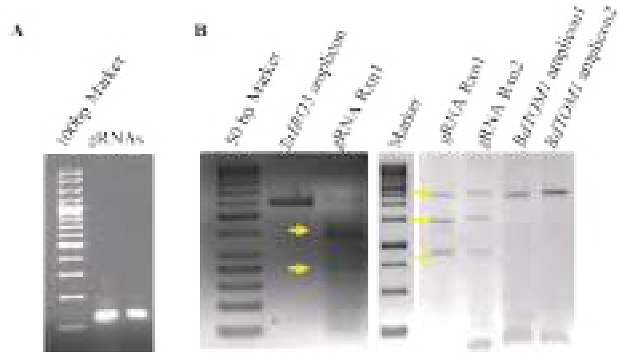

Figure 4. Synthesis of guide RNAs (gRNAs) and In-vitro target validation of the gRNAs for the TaIRO3 and TaTOM1 for genome editing in hexaploid wheat. (A) Synthesis of representative gRNA by using in-vitro transcription method as resolved on the 2 % agarose-gel using 100 bp marker. The band ~130 bp show the synthesis of the gRNA. (B) In-vitro validation designed gRNA using Cas9 and template DNA as a reaction mixture (Rxn) for the TaIRO3 genes (left panel) and for BdTOM1 (BdZIFL7) (right panel). The Rxn1 and Rxn2 indicates two different gRNA targeted for the TOM1 amplicons(1&2) (~2.3 kb) from Brachypodium. The gRNA was designed as described earlier and was checked for their target function using Cas9, gRNA and target amplicon in the ratio of 10:10:1. The validated gRNA was cloned in binary vector for generating the CRISPR constructs.

previously reported to respond for Fe-deficiency (Kaur et al., 2019, JExB; Kumar et al., 2019, FIGS). Our analysis suggests that YS1A, YSL12, YSL13, YSL19 and YSL20 show expression response co-related with the Fe concentrations in the rhizosphere at either of the time points (Figure 3). Interestingly, the Fe-dependent expression of TaYS1A was intriguing. The sequence alignment revealed that the 7th extracellular (EC)-loop for TaYS1A show the presence of unique protein sequences those were specific for Fe3+-PS binding as described for the HvYS1A and ZmYS1A.

4. To understand the regulatory events occurred during this early time points of Fe deficiency, the TFs gene families were also categorized. Our data indicates differential gene regulation of TFs such as AP2-EREBP, WRKY, bHLH and MYB genes. Among all the time points analyzed, the highest number of TFs encoding genes (371) were differentially regulated post 8 d of nutrient deficiency. Interestingly, a greater number of genes were perturbed during the resupply of Fe, with as many as 1192 genes encoding TFs show differential changes.

5. For the functional assays of genes, we choose TF referred as IRO3 and an effluxer of PS referred as zinc-induced facilitator-like protein ZIFL4 (TaTOM2; Sharma et al., 2020, BMC Mol. Biol). We utilized CRISPR/Cas9 mediated genome editing of the target genes to assess their functional role. A minimum of two-three gRNAs were designed for the conserved regions of the hexaploid wheat homeologs. The designed gRNA was validated for their target-template by using in-vitro methods (Figure 4). As reflected in the Figure 4, under the in-vitro condition gRNAs for the wheat *IRO3* and *Brachypodium BdTOM1 (BdZIFL7*; homolog for wheat ZIFL4.2) show editing capability. We chose to perform genome editing of the wheat homolog *TaZIFL4.2*(TOM1) in *Brachypodium* for quick assessment. We also

Table 1. Type and quantity of sequencing data of sRNA library derived from wheat tissue subjected to iron starvation.

SAMPLE	TOTAL READS	TOTAL BASES (bp)	UNIQUE READS	UNIQUE BASES (bp)	Mapping %
Control Shoot	8998461	214867209	2284826	54583936	79.51
Starvation Shoot	6444310	156769903	1746110	42083683	74.01
Control Root	10019096	232393586	2030006	47737568	66.94
Starvation Root	9293575	217931998	2023989	47778909	68.60

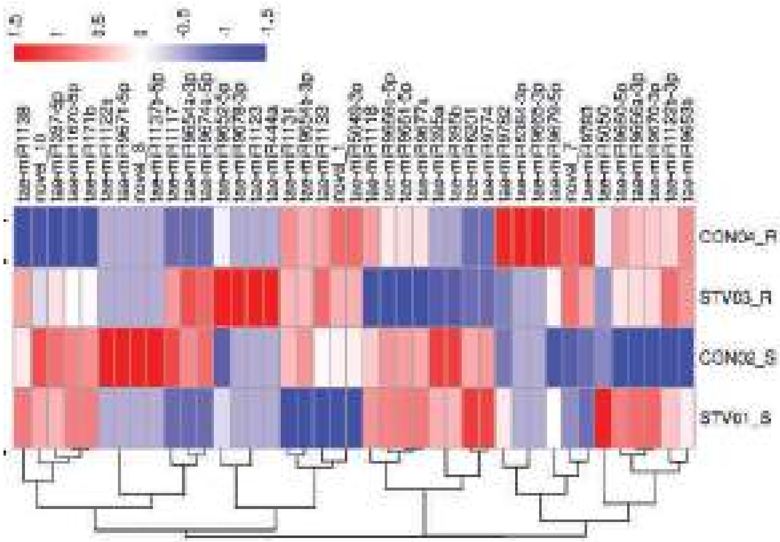

Figure 5. Cluster heat-map analysis of the differentially expressed sRNA (miRNA) in the roots and shoots of the control and Fe-deficient seedlings. The control roots (CON04_R), -Fe roots (STV03_R), control shoots (CON02_S) and -Fe shoots (STV01_S). The scale indicates the expression changes at the log fold level. The expression of known and unique miRNAs in each sample were statistically analyzed and normalized by TPM. TMM was used to normalize the read count value and the DEGseq was used to calculate the differential expression level. If the P-value was < 0.01 and with the normalized sequence count log2 (Fold change) >1 the specific miRNA was considered to be differentially expressed.

acquired wheat TILLING (JIC, Norwich) lines to further validate the role of TOM proteins in wheat. Therefore, the gene editing cassette was designed using these gRNA in binary construct capable of genome editing.

System analysis of distinct miRNA in hexaploid wheat tissue for their regulatory role in modulating Fe-deficiency responses: Plant endogenous small RNAs such as microRNAs have functional roles in diverse fundamental processes as controllers of vegetative and floral organ development and regulation of various biotic and abiotic stress responses including regulation of genes involved in nutrient uptake and transport under nutrient stress conditions. Specifically, micro-RNAs mediated regulation of Fe-homeostasis genes have been reported earlier in rice, Arabidopsis and maize. No such reports of the miRNA are known in hexaploid wheat for governing Fe-homeostasis related genes. Following are the major observations were made during this course of this study.

1. Small RNA (sRNA) sequencing was done from the roots and shoots of wheat subjected to Fe-deficiency condition. Sequencing was done with the pooled RNA samples of wheat tissues subjected to Fe-deficiency (-Fe) for the period of 3, 6, 9 and 12-days post deficiency condition. This was done to identify the early and late-stage responsive miRNAs those which are differentially expressed compared to control roots (sufficient Fe). The raw sequencing reads of sRNA libraries were generated by Illumina HiSeq™ pipeline. Q30, Q20 scores and GC content were calculated followed by data filtering to remove low quality reads. The details of the sequencing parameters are mentioned in the Table 1. The most of the predicted miRNAs show varying length distribution pattern with highest percentage for the 24 bp length among all the total sRNA. For examples, in shoot 25.41 and 26.51 % distribution was observed for 24 bp length for shoots under deficiency and in control respectively. Similarly, in roots 17.72 and 15.9 % 24 bp length distribution was observed for the sRNAs. Although predominant in both the tissue, the 24 bp length of sRNA was predominant in the shoots compared to the shoots.

2. Screening of Fe deficiency responsive small RNAs from hexaploid wheat resulted in identification of multiple root and shoot specific response under Fe-deficiency condition. A heat-map analysis of the differentially expressed miRNA are presented as Figure 5. As shown in the heat-map roots specific miRNA include tae-miR1138 tae-miR397-5p, tae-miR167c-5p, tae-miR171b, tae-miR1117, tae-miR9654a-3p, tae-miR9674a-5p, tae-miR9652-5p; whereas, in shoots tae-miR1138, tae-miR9652-5p tae-miR6201, tae-miR9774, tae-miR9782, tae-miR9679-5p, tae-miR5050, tae-miR9660-5p, tae-miR9666a-3p, tae-miR9670-3p, tae-miR1122b-3p and tae-miR9653b were also differentially expressed. This suggested the tissue specific response of the small RNA encoded miRNA.

SALIENT ACHIEVEMENTS

- Multiple genes encoding for transporter and transcription factors those able to sense the root Fe-status were identified and are the best targets for the forward and reverse genetics studies.
- Yeast complementation and expression studies demonstrated a functional TaYS1A Fe-transporter with putative Fe-binding site for the transport.
- Optimization and validation of the gRNA constructs for the wheat genes targeted at the conserved regions of the wheat homoeologs was done.
- Candidate miRNAs were identified those which are differentially expressed by the fluctuation of the Fe- in the roots and shoots.

ACTIVITIES FOR NEXT YEAR

- Expression validation of the miRNA and their putative target site to decipher their role in Fe-homeostasis.
- Generating genome edited hexaploid wheat lines for the TOM and IRO3 genes to validate their function for Fe-regulation and related responses.
- Employing wheat TILLING lines (Cadenza and Kronos) for a parallel validation of the targeted gene of interest those which are involved and mobilization of Fe from roots to grains.

1.4 Genetic transformation of banana for quality improvement

PRINCIPAL INVESTIGATOR
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RESEARCH FELLOWS
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Lakhani Hiralben
Mithilesh Kumar

The BIRAC supported banana biofortification project was initiated in Nov. 2012 with the objective to utilize the experience and achievements 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. In the first phase of the project, QUT has provided the best pro-vitamin A (PVA) gene constructs, containing the Asupina banana derived *phytoene synthase* (APsy2a) gene under the control of Ubiquitin (Ubi) and 1-Aminocyclopropane-1-carboxylic acid oxidase (*ACO*) promoters for the generation of genetically engineered (GE) Indian banana cultivars. Parallelly, additional efforts have also been laid by us to design our own indigenous gene construct Ubiquitin>1-Deoxy-D-xylulose-5-phosphate synthase (*NEN-DXS2*) for PVA enhancement in banana cultivars.

Several rounds of genetic transformation experiments were performed by using these three constructs namely, *ACO>APsy2a* and *Ubi*>APsy2a derived from QUT, Australia and *Ubi>NEN-DXS2* developed by NABI. Nealy 400 events of two cultivars, Grand Naine and Rasthali were generated and confirmed by PCR. These putative GE plants were transferred into the net-house for growth and fruit analysis by following the DBT Biosafety Guidelines. Plants showing PVA ≥20 *µ*g/g DW (threshold value) were selected for further growth and analysis in next-generation (ratoon) to check the stability of trait (PVA). Based on the data generated by the analysis in main as well as ratoon crops, the banana project task force committee has recommended BIRAC to further support this project for the 2nd phase of the activities from Feb. 2021 to Jan. 2024 for three years. The major objective of project extension would be to select the best promising GE Grand Naine cultivar events (lines) by testing in different agroclimatic conditions for further biosafety approvals and commercial release. The progress made during the previous year is summarized below.

RESEARCH PROGRESS

1. Transfer and Evaluation of Indian Banana with Pro-Vitamin A (PVA) Constructs: The most promising ten events were identified for multi-location event selection trials. These events have been generated by QUT gene constructs (5 events) and NABI gene construct (5 events). The PVA contents in main as well as ratoon crops of these ten events are summarized in Table 1. We noted 10 – 5 fold high PVA content in ratoon crops developed with QUT gene constructs compared to non-transformed control plants. Whereas 54 - 21 fold high PVA was recorded in ratoon crop generated with NABI-DXS construct compared to non-transformed control plants. The visual observations of promising events have shown dark yellow to golden colour of the fruit-pulp, while the fruit-pulp of non-transformed control plants appeared creamy-white (Figure 1).

2. Metabolic engineering for enhanced biosynthesis of pro-vitamin A in Indian banana fruit: The research work aims to develop and apply CRISPR/Cas genome editing tool for the modification of certain genes for pro-vitamin A (PVA) biofortification 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

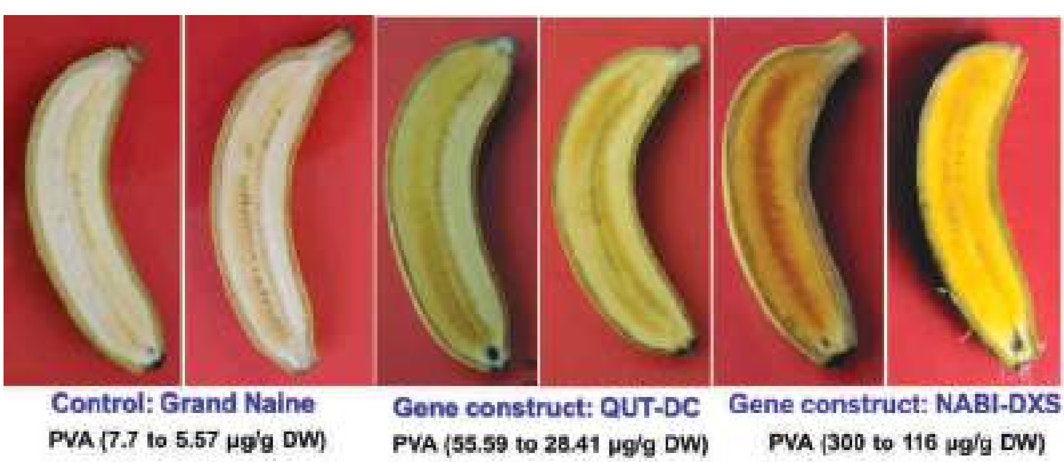

Figure 1. Visual observation of PVA content in ripe-fruit-pulp.

Table 1. Pro-vitamin A (PVA) estimation in main and ratoon crops of 10 promising lines.

No.	Unique ID	Gene construct name (QUT/NABI)	MAIN CROP		RATOON CROP	
			PVA (µg/g DW)	Fold change wrt control	PVA (µg/g DW)	Fold change wrt control
1	NABI-GN321	QUT-DC-32	30.45	6.20	29.31	5.26
2	NABI-GN346	QUT-DC-32	26.96	5.49	30.41	5.46
3	NABI-GN416	QUT-DC-32	25.80	5.25	28.41	**5.10**
4	NABI-GN440	QUT-DC-34	34.41	7.01	32.92	5.91
5	NABI-GN423	QUT-DC-34	22.06	4.49	55.59	**9.98**
6	NABI-GN544	NABI-DXS	114.58	23.34	254.00	45.60
7	NABI-GN545	NABI-DXS	152.23	31.00	300.00	**53.86**
8	NABI-GN547	NABI-DXS	100.69	20.51	116.00	**20.83**
9	NABI-GN552	NABI-DXS	157.24	32.02	176.00	31.60
10	NABI-GN553	NABI-DXS	126.23	25.71	153.00	27.47
11	NABI-GN	Control	4.91		5.57	

carotenoid cleavage dioxygenase; *CCD*) in the carotenoid biosynthesis pathway. As expected, the mutation in these genes will improve the PVA accumulation and stability in banana fruit. In addition, these findings will also help us to understand the basic regulatory mechanism for the accumulation and degradation of β-carotene in banana fruit-pulp. Previously we mutated *phytoene desaturase* (PDS) and *lycopene epsilon-cyclase* (LCYε) genes through CRISPR/Cas9 in commercial cultivars Grand Naine and Rasthali. Editing of LCYε in Grand Naine genome has demonstrated up to six-fold (23.80 ± 1.26 μg/g) higher β-carotene deposition in the fruit-pulp as compared to the unedited control plants. In continuation to genome editing work, we had performed bioinformatics and gene expression analysis of *CCD* genes. The *CCD4* gene was identified for its role in β-carotene degradation in banana fruit-pulp and used for designing the CRISPR/Cas9 construct.

1. Genome-wide mining and in-silico characterization of CCD genes from banana: We identified six *CCD (MaCCD1, MaCCD4, MaCCD7, MaCCD-like, MaCCD8a* and *MaCCD8b*) genes in the banana genome (http://banana-genome.cirad.fr/) by using homologs from arabidopsis, rice, and maize as the query sequences. Phylogenetic analysis showed the clustering of MaCCD proteins into five different classes (CCD1, CCD4, CCD7, CCD8, and CCD-like). Each class is represented by single homolog (MaCCD1, MaCCD4, MaCCD7, and MaCCD-like), except CCD8 (MaCCD8a and MaCCD8b) in banana (Figure 2).

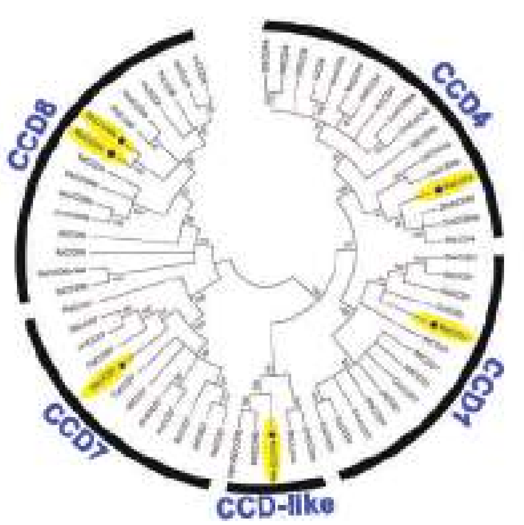

Figure 2. Phylogenetic analysis of the banana CCD (MaCCD) proteins divided into five different classes.

2. MaCCDs expression profiling in different tissues of contrasting cultivars (Rasthali and Nendran): The expression profiling of all six MaCCDs was carried out in various tissues of Rasthali (low PVA containing variety) and Nendran (high PVA containing variety). Based on the higher expression of MaCCD1, MaCCD4 and MaCCD8a in fruit tissues (peel and pulp), they were further selected for the expression analysis in different fruit development stages of Rasthali and Nendran.

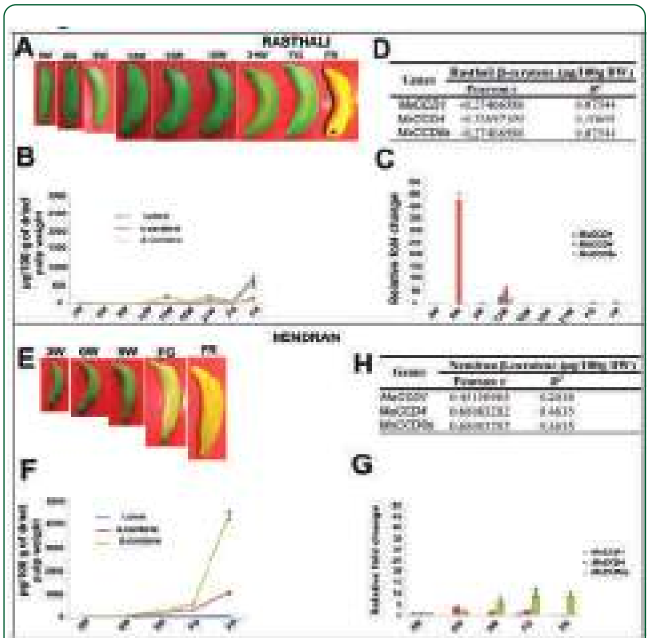

Figure 3. Correlation study between carotenoid accumulation and expression of *MaCCD* genes at different fruit development stages of cultivars Rasthali and Nendran.

3. Correlation analysis of carotenoid accumulation and MaCCDs expression in fruit developmental stages: The fruit pulp of Rasthali and Nendran were collected at three weeks (W) interval from fruit emergence to harvest stages for the carotenoid estimation and gene expression analysis (Figure 3). Rasthali fruit took 27W (Figure 3A), whereas Nendran fruit reached the ripening stage in 15W (Figure 3E). Nendran pulp accumulated a higher amount of α- and β-carotene than in Rasthali, but the difference was more prominent at the ripening stage of the fruit (Figure 3B, F). The expression patterns of *MaCCD1, MaCCD4,* and *MaCCD8a* in the different fruit development stages of Rasthali (Figure 3C) and Nendran (Figure 3G) were correlated with β-carotene accumulation. The expression of *MaCCD4* was increased to 432-fold at the 6W stage as compared to the 3W stage of Rasthali (Figure 3C), while it did not change significantly during the different fruit development stages of Nendran (Figure 3G). A strong negative Pearson correlation (-0.3269) was found between the expression of *MaCCD4* and β-carotene accumulation in fruit-pulp of the Rasthali (Figure 3D). These observations instigate us to investigate the role of the *CCD4* in the β-carotene accumulation.

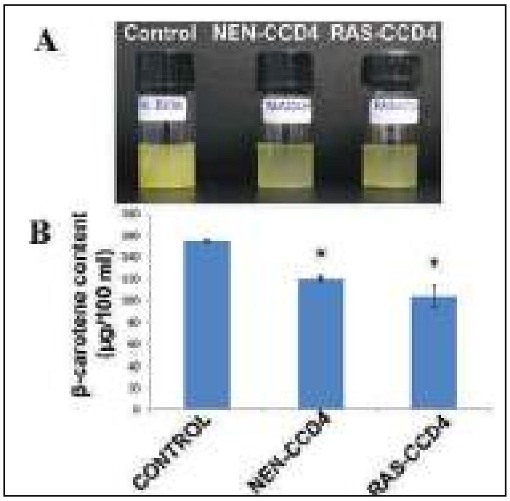

Figure 4. Functional characterization of Nendran and Rasthali derived *CCD4* genes in pAC-BETA bacterial complementation study.

4. Functional validations suggest high activity of *CCD4* derived from Rasthali (*RAS-CCD4*): Furthermore, CCD4 gene was cloned from Rasthali (*RAS-CCD4*) (NCBI accession MF440662) and Nendran (*NEN-CCD4*) (NCBI accession MF440663) and used for the pAC-BETA mediated bacterial complementation assay to check their role in β-carotene degradation. The visual observation suggested that the co-transformed cells accumulated light yellow color as compared to pAC-BETA control (Figure 4A). The lower β-carotene content was found in cells co-transformed with *RAS-CCD4* (98.30 ± 4.84 μg/100 ml) and *NEN-CCD4* (120.50 ± 2.44 μg/100 ml) as compared to pAC-BETA control (155 ± 0.94 μg/100 ml) (Figure 4B). These observations suggest that RAS-CCD4 has higher activity than *NEN-CCD4* for β-carotene degradation.

5. Enzyme assay to determine substrate-specific cleavage activity of *RAS-CCD4*: Subsequently, the *RAS-CCD4* gene was cloned into the pET28a expression vector (Novagen, USA) to ascertain the substrate-specific enzymatic activity. The recombinant RAS-CCD4 protein was purified (Figure 5A) using a Ni-NTA column (Qiagen, Germany) and subjected to in-vitro enzyme-substrate assay. Among the carotenoid substrates (β-carotene, α-carotene, 10-apo-β-carotenal, and β-cryptoxanthin), *RAS-CCD4* was active only against β-carotene and 10-apo-β-carotenal, which can be seen by the β-ionone peak in GC-MS spectrum (Figure 5B). Hence, *RAS-CCD4* is accountable for the low content of β-carotene in Rasthali.

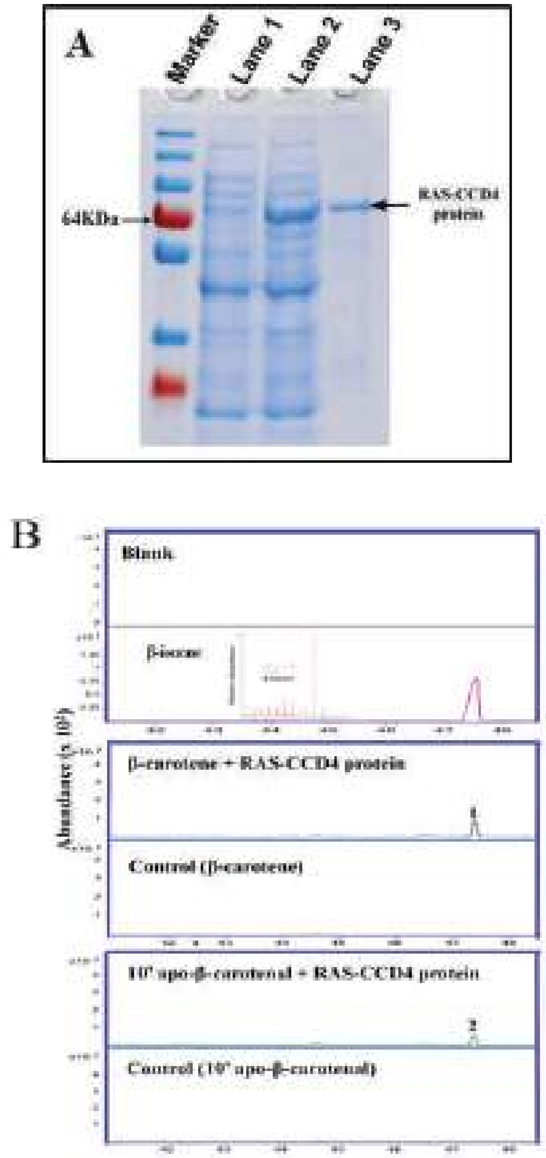

Figure 5. Purification and validation of enzymatic activity of RAS-CCD4

- The full-length *CCD4* gene sequences have been cloned from Rasthali and Nendran and also submitted to the GenBank data libraries under accession numbers MF440662 (*RAS-CCD4*) and MF440663 (*NEN-CCD4*).

SALIENT ACHIEVEMENTS

- The banana biofortification project has been extended for three years with financial support from BIRAC for the multi-location event selection trials.
- Based on the PVA content in fruit-pulp of main as well as ratoon crops, a total of 10 promising events are identified for subsequent multi-location event selection trials.
- Analysis of CCDs in banana revealed the promising role of *CCD4* in the β-carotene degradation in fruit-pulp of cultivar Rasthali.

ACTIVITIES FOR NEXT YEAR

- The standard operating procedures (SOPs) will be followed for a multi-location event selection trial by following DBT biosafety guidelines.
- Optimization of efficient protoplasts isolation protocol from Rasthali ECS.
- Protoplasts will be used for transient expression of the CRISPR/Cas9 construct to edit the *CCD4* gene in the banana genome.

1.5 Application of genomics approaches for reducing ODAP content in Lathyrus spp.

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Lovenpreet Kaur

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 the soybean in terms of protein content, its cultivation is not promoted widely 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 homoarginine. Global warming is leading to a reduction in the area of cultivable land worldwide, this crop needs promotion as it can be a hardy crop with guaranteed yield in problem soils. The major impediment to the promotion and wide acceptability of this crop is the presence of neurotoxin. Breeding efforts in the past lead to the development of a number of low ODAP cultivars, but 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 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.

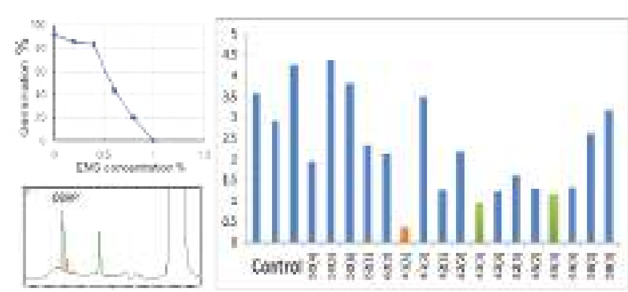

Figure 1. Generation of mutant population of Lathyrus. Mutagen concentration 0.6% was used for mutagenesis. An HPLC method was used for screening the mutants for low ODAP content. Right panel shows the screening of mutant seeds.

RESEARCH PROGRESS

1. Developing an EMS mutant population of Lathyrus: We developed an Ethyl methane sulphonate (EMS) mutant population of Lathyrus. EMS treatment at 0.6% v/v which showed 45% germination rate was used to treat approximately 8000 seeds. The mutant seeds were germinated and the seedlings were transplanted in the field and M2 seeds are harvested.

2. Screening of EMS mutant population to identify low ODAP mutants: M2 seeds that we have currently are being analyzed by HPLC to identify mutants with reduced ODAP levels. 4-6 seeds per M2 lines are used for analysis. Seedling tissue at 4 days was collected and lyophilized. The lyophilized tissue was used for ODAP extraction and HPLC analysis. We have screened more than fifty lines so far and more are on the way.

3. Genome editing of Lathyrus: We made constructs using single guide RNAs and double guide RNAs. We verified the efficacy of the constructs 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 3 genes in the ODAP biosynthetic pathway. These constructs are being used for developing transgenic Lathyrus plants and are at various stages of transformation.

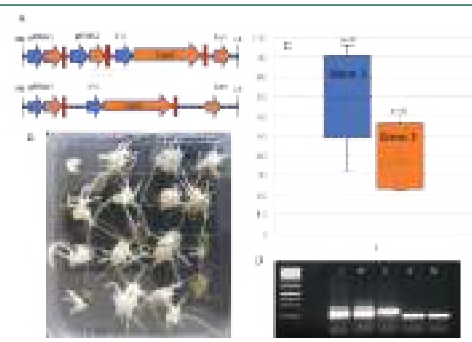

Figure 2. A. Schematic diagram of gRNA constructs single and double guide RNAs. B. Hairy root system C. Editing percentage in the hairy root tissue in various lines. D. Editing analysis by PCR of the double guide RNA transformed hairy roots. Lane 3 is vector control hairy roots and others are showing editing of target gene with product size less than that of control (indicative of deletion).

SALIENT ACHIEVEMENTS

- Development of a mutant population and establishment of a screening pipeline for low ODAP mutants.
- Constructs for editing of three ODAP biosynthetic pathway genes developed and being used in whole plant transformation experiments.

ACTIVITIES FOR NEXT YEAR

- Generating transgenic Lathyrus plants with biosynthetic pathway gene mutations
- Identification and characterization of low ODAP EMS mutant plants.

1.6 Molecular and functional analysis of seed specific Dof transcription factor(s) in rice

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Prafull Salvi

RESEARCH FELLOWS
Kajal Mittal
Nishu Gandass

Rice is the most important food crops in the world, especially in the developing countries as it feeds more than 50% of the world population. One of the biggest challenges for scientific community to ensure the food security and food safety for the growing human population. The improvement in the rice gain quality is one of the very important research topics worldwide. The nutritional value of rice could be raised by manipulating the metabolic pathway of seed storage protein (SSP) to increase the essential amino acid and/or its protein contents which would fairly support the nutritional intake of poor families. Therefore, engineering the regulatory aspect of metabolic pathway associated with SSP could be an important strategy for improving nutritional quality in rice. Transcription factors (TFs) are key regulators of gene expression, operate as imperative switches of almost all biological processes, and are therefore of great research interest. The transcription factor like rice seed b-Zipper 1 (RISBZ1) and DNA binding with One Finger (DOF) proteins, particular class of zinc finger domain are a group of transcription factors (TFs) that have been implicated in synergistic regulation of metabolic processes associated with seed storage protein. Besides, these DOF-TF are also known to regulate diverse biological process such as photosynthesis, vascular development, flower induction, lipid metabolism in the seed, light and hormone signalling, secondary metabolism and stress responses. Interestingly, these Dof TF's not only regulate the transcription of target gene, but they also interact with other regulatory proteins such as bZIP and MYB TFs and display functional versatility. The protein–protein interactions further influence the expression of associated genes of respective transcription factors. Thus, these TF play versatile role in plants, and we have chosen the DOF-TF predominantly expressed in seed to explore their role and regulation in rice nutrient value. We have cloned seed specific DOF-TF encoding gene from rice and generated different constructs for their molecular and functional analysis. We have shown the interaction between RPBF and RISBZ1, which has a role in transactivating the seed storage protein encoding genes in rice. We have found that RPBF-RISBZ interact in the nuclear as well as in cytosolic region. The RPBF-RISBZ interaction in the cytosolic region represent the plausible regulatory role beyond DNA binding or transcriptional regulation that can be utilized to determine the unexplored regulatory framework of grain filling in rice. Collectively, the project aims to delineate the molecular and functional role of seed-specific Dof transcription factor(s) in rice that are associated with seed development, maturation and grain quality which can be used as candidate molecular target for improving grain quality in rice.

RESEARCH PROGRESS

1. We assessed the RPBF and RISBZ interaction using Insilco tool (https://string-db.org/) which clearly revealed their positive interaction (Fig. 1).

2. We have studied the Protein-Protein Interaction (PPI) between RPBF and RISBZ1, which has a role in transactivating the seed storage protein encoding genes in rice. We cloned two genes encoding RPBF and RISBZ1 TFs into the pENTR/D-TOPO vector, sequenced and subsequently subcloned in pSITE-nEYFP-C1 (CD3-1648) and pSITE-cEYFP-N1 (CD3-

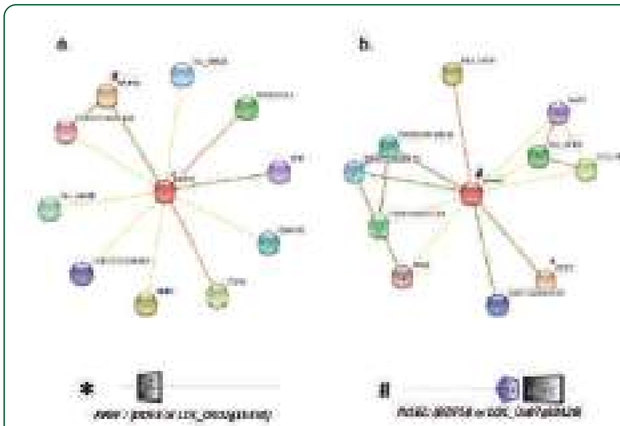

Figure 1. Protein-Protein interaction network of RPBF generated using STRING database.

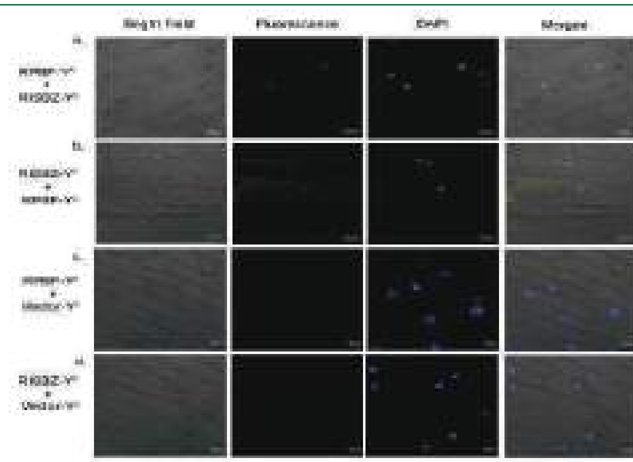

Figure 2. BiFC assay of RPBF and RISBZ for PPI study in onion epidermal cells.

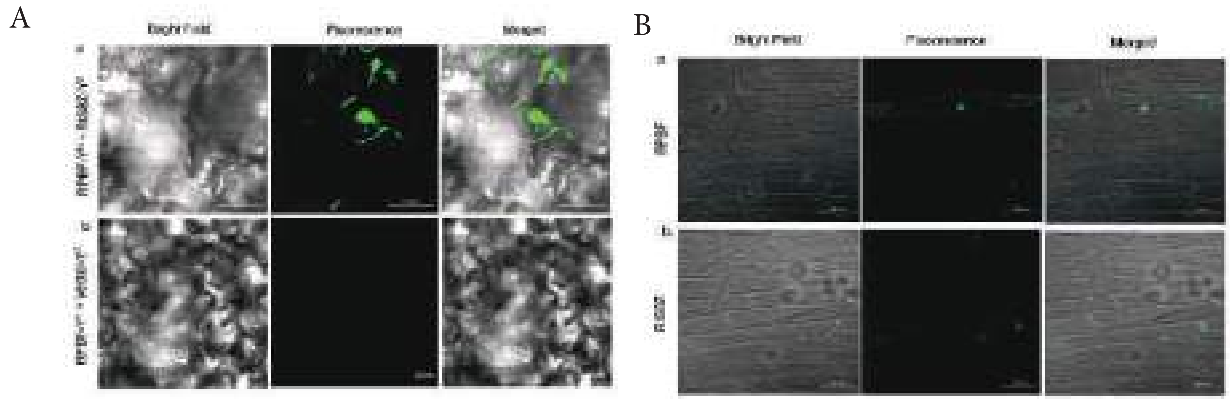

Figure 3. (a) BiFC assay for PPI study of RPBF and RISBZ using agroinfiltration and nicotiana benthaniana (b) subcellular localization of RPBF and RISBZ in onion epidermal cells.

1651), and pMDC84 (CD3-743) destination vector using gateway cloning. The confirmed clones were transformed in Agrobacterium strains like EHA-105α for agro-infection. Our results revealed that both RPBF and RISBZ interact in the nucleus and cytosolic region (Fig. 2).

3) To study the subcellular localization of RPBF and RISBZ, we cloned both genes into destination vector pMDC84 using gateway cloning. In the destination vector, the RPBF and RISBZ were cloned under the control of the CaMV 35S promoter with a C-terminal eGFP tag. The confirmed clones were transformed to EHA-105α and used for Agrobacterium-mediated subcellular localization study in onion epidermal cells. Both RPBF and RISBZ were localized in the nucleus and cytosol of onion epidermal cells (Fig. 3).

SALIENT ACHIEVEMENTS

- Insilco analysis of protein-protein interaction of RPBF-RISBZ
- Molecular cloning and generation of BiFC constructs to study protein-protein interaction.
- Optimization of easy and efficient BiFC protocol to study the in vivo protein-protein interaction of seed-specific transcription factors, RISBZ and RPBF
- In planta interaction study of RPBF with RISBZ protein using agrobacterium mediated infection in onion epidermal cell.

ACTIVITIES FOR NEXT YEAR

- Screening and validation of DOF-TF (RPBF) transgenic plants and their multiplication.
- Generation of promoter-gus reporter lines using agrobacterium mediated genetic transformation of rice.
- Protein expression and purification for RPBF protein.
- Sequence-function correlation analysis of RPBF using In-silco approach.
- Cloning and rice transformation of other *DOF-TF* (LOC_Os10g35300) predominantly expressed in rice seed.

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

PRINCIPAL INVESTIGATOR
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RESEARCH FELLOW
Kshitija Sinha

Rice Bran Oil (RBO) is abundantly rich in essential fatty acids, proteins, vitamins, minerals and antioxidants like oryzanol which makes it the most healthy edible oil. 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. To date, many conventional physical, chemical and biological methods have been used to reduce bran lipid spoilage but all these conventional methods have 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. Minimal emphasis has been made on understanding the causative genes involved in the rancidity process and its underlying function, expression, and molecular mechanisms involved in triggering bran lipid oxidative and hydrolytic rancidity. 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.

RESEARCH PROGRESS

1. Identification of lipases: In order to identify the rice lipase genes, we performed data mining by using Rice Genome Annotation Project (RGAP). We obtained 73 putative lipase sequences out of which we shortlisted 18 lipases. RNA extracted from the bran of germinating rice seeds at different stages were used for expression analysis via qRT-PCR(fig.1). Three lipases namely Oslip 1, Oslip 12, Oslip 13 from the expression analysis were selected for functional characterisation.

2. Functional Characterization: The final constructs of the yeast expression vector (pYES2) carrying Oslip 1, Oslip 12, Oslip 13 were transformed independently in the yeast cells by the lithium acetate method. The cells were grown in 100ml of broth and were harvested and subjected to lipid extraction. On comparing the TAG content, we observed Oslip1, Oslip12 and Oslip13 have significantly reduced content of TAG in comparison with yeast lipase mutant (TGL). Wild type Saccharomyces cerevisiae (Sc) is served as the positive control.

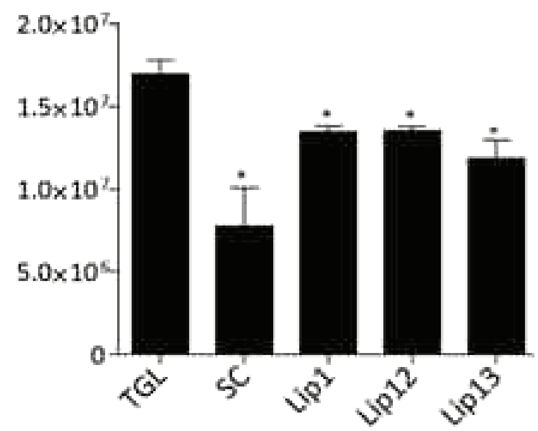


Figure 2. Analysis of the TAG content of the yeast cells

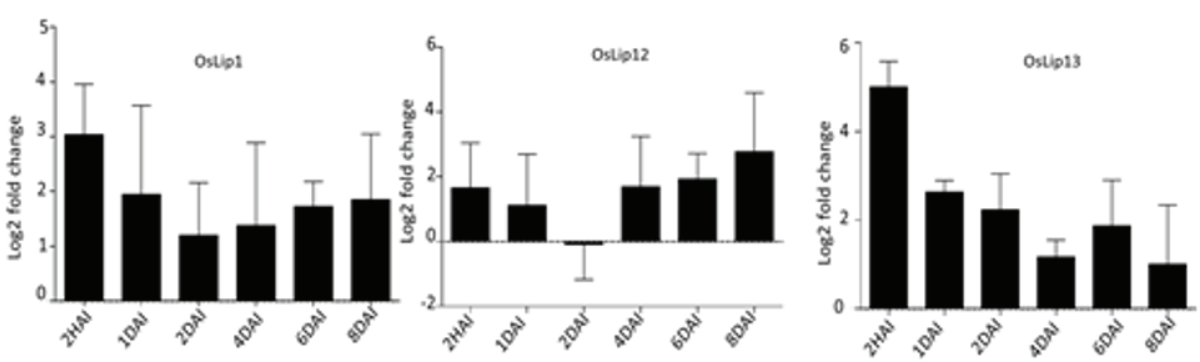


Figure 1. Expression analysis of lipases

wt	ATCTCTTCTGGAAGCAGCTTGATGTAGGTTATCCAGGCAT
XS84	ATCTCTTCTGGAAGCAGCTTGATGTAGGTT-TCCAGGCAT
XS86	ATCTCTTCTGGAAGCAGCTTGATGTAGGTTAATCCAGGCAT

Figure 3. Sequences of positive CRISPR/Cas9 edited

3. CRISPR/cas9 mediated editing of Oslip 1: We prepared CRISPR constructs of the Oslip 1 gene, transformed them in Agrobacterium tumefaciens (LBA4404). In order to study the role of the genes in rice, we used Taipei-309. The calli induced from the mature embryos of rice grains were infected by Agrobacterium containing the CRISPR constructs. Several positive plants were obtained which exhibited both deletion and insertion types of gene editing of a single base. T1 seeds collected from these plants have been germinated in order to get Cas9-free edited homozygous lines for further analysis.

SALIENT ACHIEVEMENTS

- Identification of rice bran lipases expressed during the time of germination.
- Functionally characterize lipase function in lipid degradation
- Sucefully editing the Oslip 1 gene.

ACTIVITIES FOR NEXT YEAR

- Evaluating the stability of rice bran lipid obtained from edited plants in different storage conditions.
- CRISPR/Cas9 mediated editing of multiple lipases in rice and checking its effects on bran lipid stability.

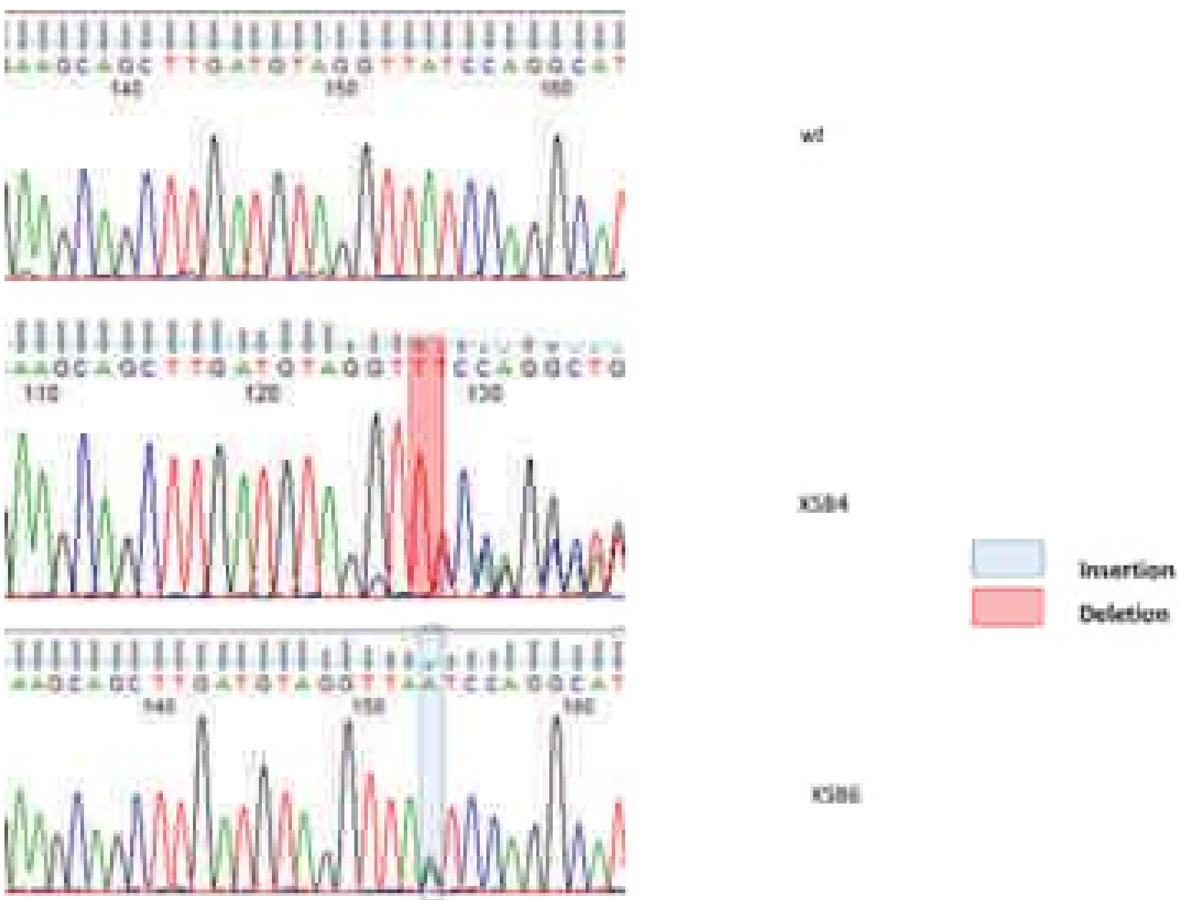


Figure 4. Representative sequencing chromatogram of positive Oslip 1 edited plants

1.8 Genetic improvement in nutritional quality and yield in tomato (*Solanum lycopersicum* L.)

PRINCIPAL INVESTIGATOR
Rupesh Deshmukh

RESEARCH FELLOWS
Shivani Sharma
Sanskriti Vats

Tomato is one of the most widely grown vegetable crops having tremendous nutritional potential. Tomato has numerous uses since it is consumed as raw, cooked as well as processed food. To fulfil year around demand, tomato is being cultivated worldwide under field as well as protected greenhouse conditions. Significant progress has been made to increase tomato productivity which managed to close the gap between production and global consumption. However, while increasing the tomato production, nutritional aspects are largely ignored. At present, issues like nutritional quality, processing quality and shelf life of fruits are the major concerns for tomato improvement programs. Considering the versatile uses of tomato, more particularly the raw fruit consumption make it important component for balanced diet. To explore the nutritional potential, genetic improvement of tomatoes seems to be a more sustainable approach. Developing high yielding cultivars having improved nutritive value will be helpful to address increasing malnutrition in India. Globalisation has popularised many processed tomato product in India however, there is continuously increasing gap between the production of processing quality tomatoes and industrial demand. Very limited tomato varieties in India can produce processing quality fruits. Therefore market price and demand get significantly affected. Similarly, shelf life of the tomato is also major factor deciding farmer's profit. Short shelf life of tomato fruit is one of the major causes for yearly post-harvest losses in India and most of the developing countries. Considering the challenges, present project simultaneously exploring different approaches was planned to improve nutritional and processing quality and shelf life of tomato. To achieve the desired improvement in tomato cultivars, three approaches including genome wide association study (GWAS), mutation breeding and genome editing are being employed. Identification of genomic loci and respective donors for these traits will be performed by exploring available genomic resources through GWAS. Similarly, novel traits and desired improvement in high yielding cultivars expected to achieve through induced mutagenesis. As a part of Ramalingaswami Fellowship program, high-end multiplex genome editing approach is being used for tomato improvement.

Figure 1. Field view of tomato germplasm at reproductive stage. The germplasm set has 192 genotypes evaluated for one cropping season and 48 newly added genotypes including wildtypes, elite lines and popular varieties collected from different Institutes and Universities.

Figure 2. Representative pictures of genome edited tomato plants showing dwarf determinate tomato plant generated by the knock-out of Self pruning gene. (Left: control, Right: Determinate genome edited tomato plant).

The multiplex genome editing program will not only help in crop improvement but also for the technological adaptation. For the genome editing, three types of genes were selected. Firstly, gene which have been well characterised are selected to translate the available knowledge into desired trait improvements. Second category includes genes with limited information but demonstrated to influence the target traits. Lastly the third category, where candidate genes were selected based on the available knowledge. Selection of three categories of genes make the program more promising in achieve desired crop improvement as well as for the technology adaptation.

RESEARCH PROGRESS

Activity I: To identify GWAS loci for seed number, locule number, soluble solids content, longer shelf life of fruits, oxalate content in seeds, vitamins, minerals and other agronomical traits including stress. Phenotypic evaluation of 192 tomato genotypes was completed for the first year and now in the second year, the set is being grown at Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Himachal Pradesh (Figure 1). This supposed to be third year evaluation however last year we failed to receive the fruits due to pandemic restrictions. DNA was extracted for all 192 genotypes and addition 48 genotypes including high yielding cultivars, wild accessions and mutants are being performed. Similarly, genotyping-by-sequencing analysis is being performed.

Activity II: To develop mutant population using fast neutron and EMS approach and screen for nutritional and marketable fruit quality traits. Mutant populations developed in genetic background of Pusa Ruby and Arka Vikas are respectively at M4 and M3 generation. Collaborating centers have selected some mutant lines and now those are being evaluated for the stability and confirmation.

Activity III: To perform genome-editing for plant architecture, shelf-life and fruit quality traits. Single and multi-target constructs were developed and subsequently transformed to two different tomato cultivars. Transgenic lines expressing genome editing constructs were confirmed with PCR assay, qPCR and segregation analysis. Desired mutations at some of the targets are confirmed with sanger sequencing and sebsequenty evaluated with segregating progenies and amplicon sequncing (Figure 2).

SALIENT ACHIEVEMENTS

- Developed several multi-target genome editing constructs
- Multi-target genome editing in tomato is well adopted
- A diverse set of tomato with extensive phenotyping data is developed
- Developed mutant population in two different genetic background.

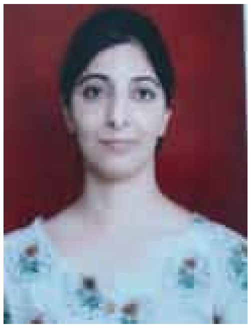
ACTIVITIES FOR NEXT YEAR

- Genome wide association study for fruit quality related trains will be performed
- Extensive phenotypic evaluation of transgene free genome edited tomato lines will be done.
- Cross using selected mutants and genotypes from the GWAS set will be made.

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

PRINCIPAL INVESTIGATOR
Humira Sonah

RESEARCH FELLOWS
Himanshu Yadav
Virender Kumar



Soybean (Glycine max) is rich in edible oil and protein content. Soybean oil is widely used as cooking oil, biodiesel, and has many industrial uses whereas protein is excellent source for animal feed and food products for human consumption. Soybean seed contains about 36–40% crude protein and 18–21% oil. The nutritional and economical values largely depend on the seed composition. These traits are mostly governed by the same loci, which makes it challenging to identify the candidate genes. Moreover, the stability and shelf life of soybean is restricted due to the high percentage of polyunsaturated fatty acids. Modification in the fatty acid composition can improve its stability as well as nutritional quality. Similarly, the presence of antinutritional compounds, allergens and off-flavor are the major constrains affecting the nutritional potential. More particularly, these factors are major concern for the under-nutrition vegetarian population in India. Improving nutritional quality by reducing undesired components and optimising the desired composition will be helpful to develop food grade soybean. Considerable efforts exploring different approaches are required to improve the nutritional quality of soybean. In the present investigation, two different approaches were used a) Genome-wide association study to identify the loci governing nutritional quality traits b) CRISPR/Cas9 based genome editing approach to enhance the nutritional quality in soybean. The main aim of this project is to integrate genomics and breeding research for the development of superior cultivars and to expand the utilization of soybean crop in India.

RESEARCH PROGRESS

1. Genome-wide association study to identify the loci governing nutritional quality traits: Phenotyping of soybean germplasm is being performed. Genotyping of soybean germplasm has been performed using the GBS approach. The GBS data was analysed using inhouse scripts and online tools. The raw reads were processed and mapped to reference genome. The mapped reads were used to identify SNPs and InDels. A high quality 40,000 SNPs were selected and is being used for the genome-wide association studies (GWAS). GWAS was performed for the soybean flower color to confirm the efficacy of GBS and phenotyping evaluation. As expected, GWAS loci for flower color was identified on chromosome 13 (Figure 1) which confirm the reliability of the genotypic and phenotypic data. The loci were further explored for the candidate gene identification. GWAS analysis for other seed composition traits is in progress.

2. CRISPR/Cas9 based genome editing approach to enhance the nutritional quality in soybean: CRISPR/Cas9 based genome editing approach is being used to improve the soybean quality. Genome edited construct was developed and transformed to soybean genotype. The genome edited plants are being raised and will be evaluated using different approaches to confirm the transgene and the desired mutations.

SALIENT ACHIEVEMENTS

- Phenotypic characterization for some of the traits has been done.
- GBS data has been analysed and is being used for GWAS analysis
- Soybean transformation was optimized
- Development of genome edited lines.

ACTIVITIES FOR NEXT YEAR

- Phenotypic evaluation of seed quality-related traits
- Genome-wide association study for seed quality-related traits
- Evaluation and confirmation of genome edited lines.

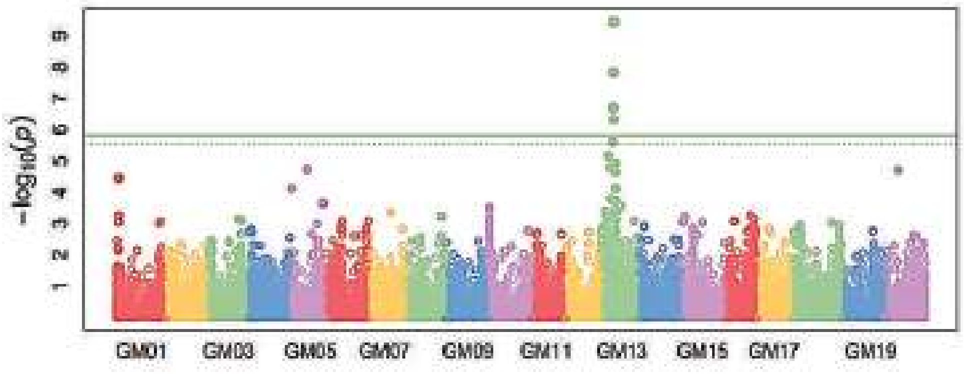
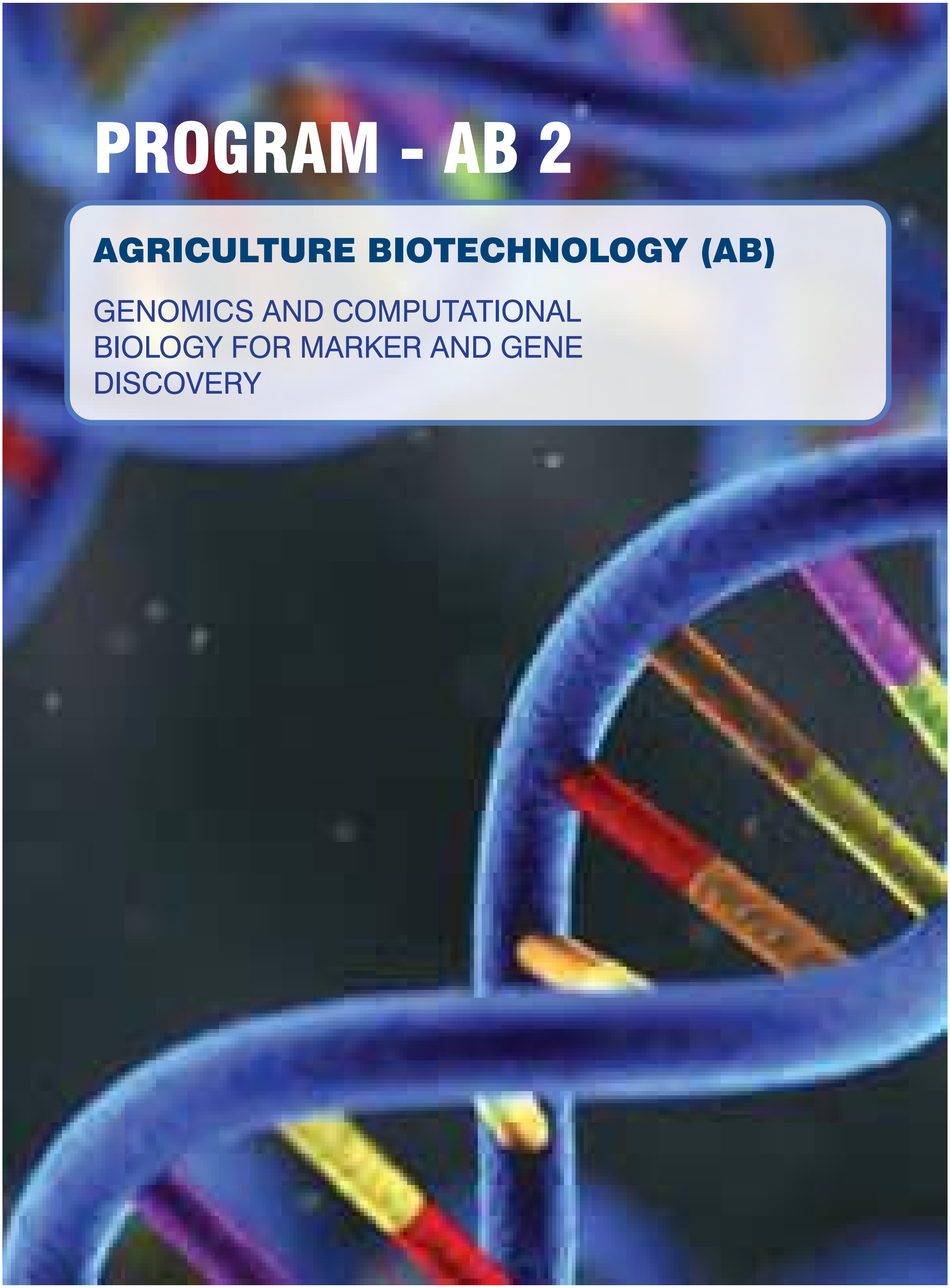


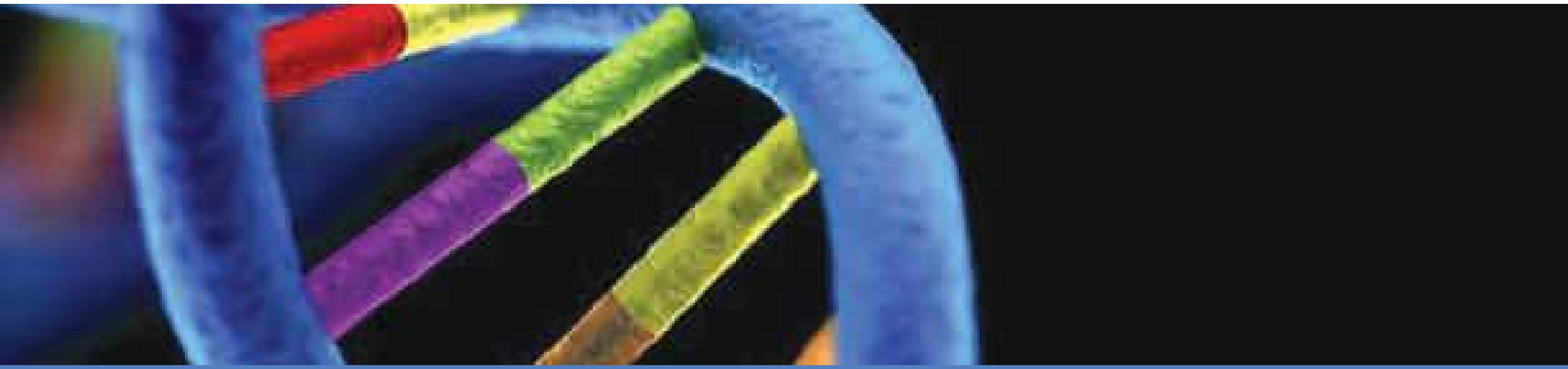
Figure 1. Manhattan plots showing loci governing flower color trait in soybean



PROGRAM - AB 2

AGRICULTURE BIOTECHNOLOGY (AB)

GENOMICS AND COMPUTATIONAL BIOLOGY FOR MARKER AND GENE DISCOVERY



2.1 Genomics assisted breeding for disease resistance against necrotrophic gens of *Brassica* crops (patho)

PRINCIPAL INVESTIGATOR
Sivasubramanian R

RESEARCH FELLOWS
Deepak Duhan
Shivani Gajbhiye

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 above-ground 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

1. Prospecting for resistance genes in the landraces and wild germplasm of *Brassica* species: High-throughput PAMP-triggered immunity (PTI) assays (96-well format) using secreted peroxidase activity was standardized in the lab. The method uses a simple colorimetric test to measure the activity of secreted peroxidases from leaf discs treated with mock solution (distilled H_2O) and culture filtrate of *A. brassicae*. We used the readouts from this assay as an endophenotype of disease resistance since field infections of *A. brassicae* are not possible. PTI assay was used to screen a panel of 261 *Brassica jun-*

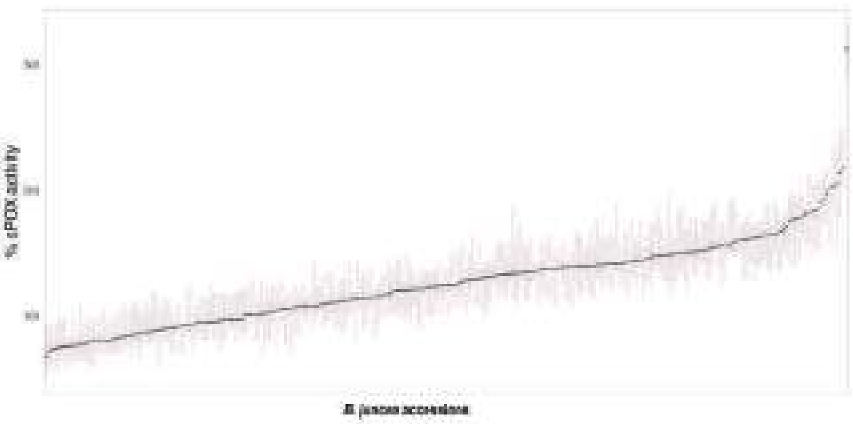


Figure 1. Natural variation in 261 B. juncea accessions' PTI response to A. brassicae culture filtrate as measured by the secreted peroxidases (sPOX) activity.

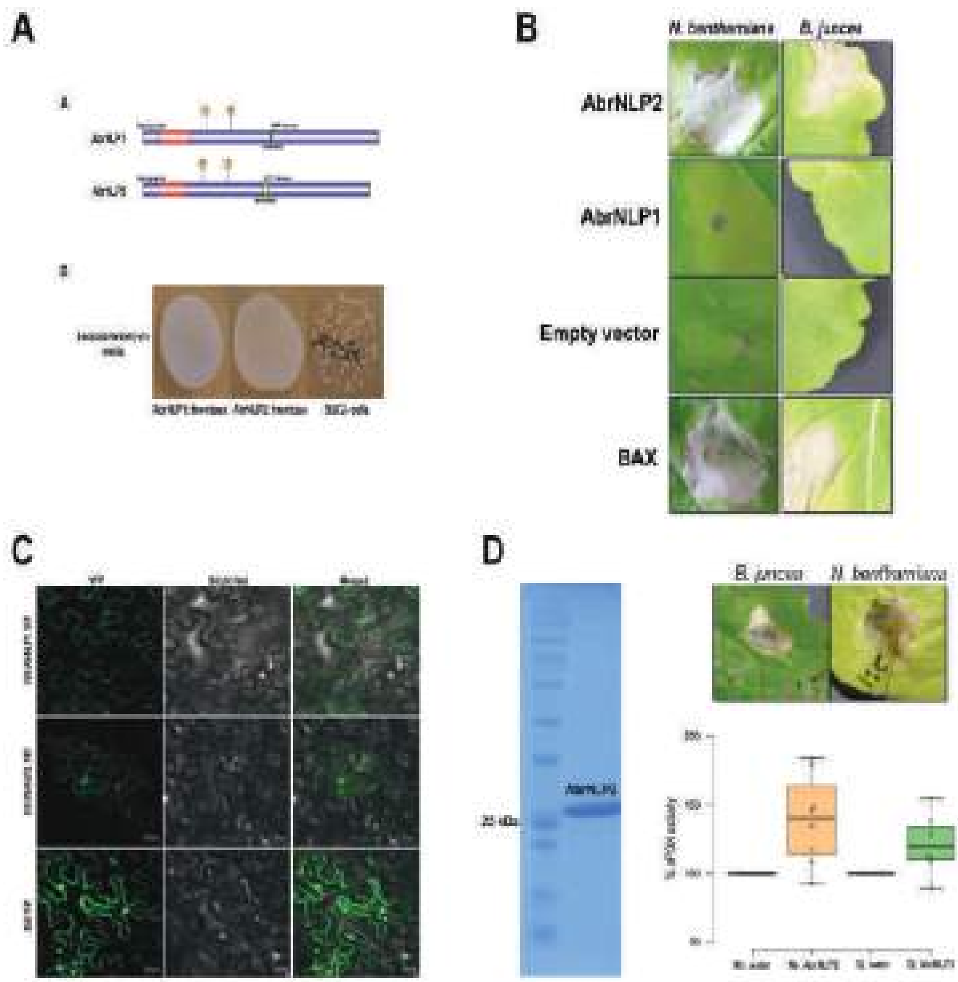


Figure 2. Functional characterisation of AbrNLPs. A) Secretory nature of AbrNLPs as demonstrated by a yeast secretion trap assay; B) Necrosis inducing ability of AbrNLPs in host (*B. juncea*) and non-host species (*N. benthamiana*); C) Differential subcellular localization of AbrNLPs in *N. benthamiana*; D) Induction of necrosis as well as PTI responses in host and non-host species by purified AbrNLP2 protein.

cea accessions to check the variability of PTI responses to A. brassicae culture filtrate (CF). We found quantitative variation in the response to *A. brassicae* CF in terms of % POX activity (Figure 1). Further, association mapping was carried out using the %sPOX activity as the phenotype and SNPs derived from the transcriptomes of the *B. juncea* accessions. We found 28 SNPs in 6 genomic loci to be significantly associated with the trait. Functional validation of the genes underlying the loci are underway.

2. Characterisation of the pathogenic effectors and their interacting partners in the host: We identified two necrosis-and-ethylene inducing peptides-like proteins (NLPs) from *A. brassicae* viz. AbrNLP1 and AbrNLP2 and functionally characterised them. Although both AbrNLPs were found to be secretory in nature, they localised differentially inside the plant. AbrNLP2 was found to induce necrosis in both host and nonhost species, while AbrNLP1 could not induce necrosis in both species. Additionally, AbrNLP2 was shown to induce pathogen-associated molecular pattern (PAMP)-triggered immunity in both host and nonhost species (Figure 2). Overall, we have shown that AbrNLPs are functionally and spatially (subcellular location) distinct and may play different but important roles during the pathogenesis of *A. brassicae*.

SALIENT ACHIEVEMENTS

- Phenotyping of 261 accessions of *B. juncea* for their response to A. brassicae culture filtrate (CF).
- GWA mapping of PTI responses of *B. juncea* in response to *A. brassicae* CF.
- Identification and functional characterisation of necrosis-and-ethylene inducing peptides-like proteins (NLPs) of *A. brassicae*.

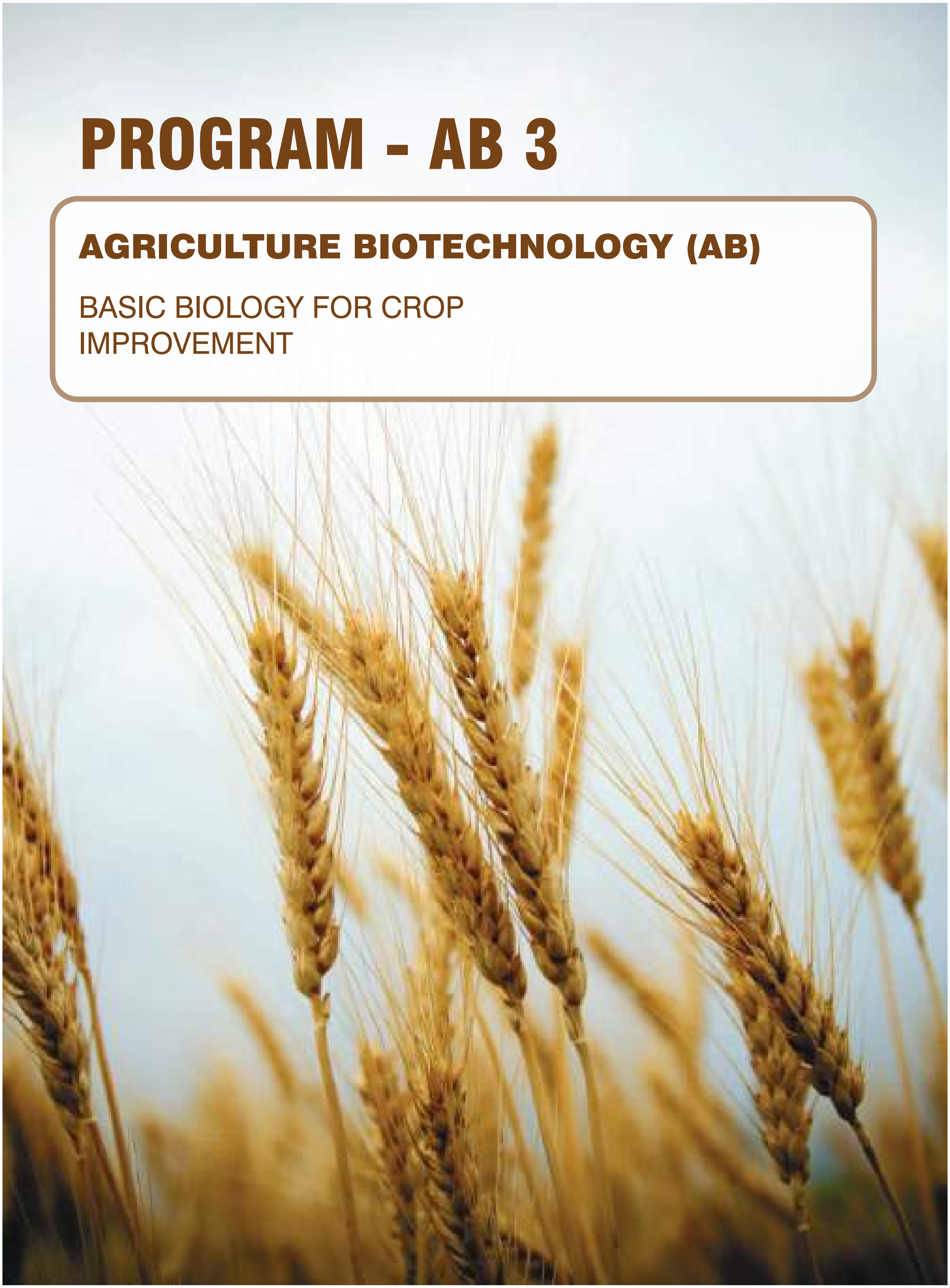
ACTIVITIES FOR NEXT YEAR

- Functional validation of candidate genes identified in GWA mapping.
- Characterization of other secreted effectors of *A. brassicae* and identification of interacting partners in the host.

PROGRAM - AB 3

AGRICULTURE BIOTECHNOLOGY (AB)

BASIC BIOLOGY FOR CROP
IMPROVEMENT



3.1 Functional characterization of wheat inositol pyrophosphate kinase

PRINCIPAL INVESTIGATOR
Ajay K Pandey

RESEARCH FELLOW
Anuj Shukla

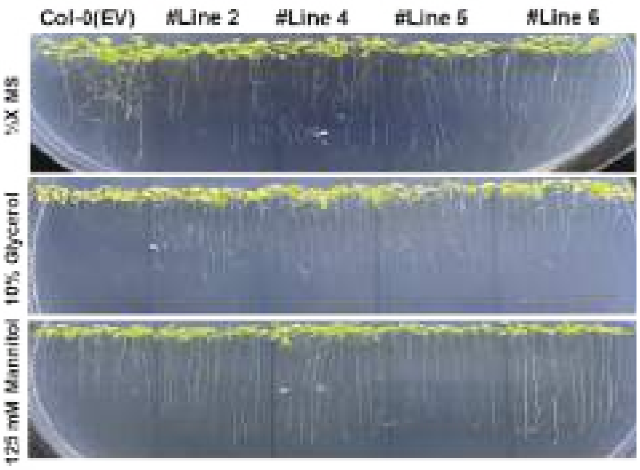
Inositol phosphates (InsPs) are a well-known family of eukaryotic water-soluble signalling molecules that are conserved mainly in their function. This family is characterized by the presence of phosphate either at the single or all the 6-carbon inositol ring backbone. The full phosphorylated InsPs ($InsP_6$; *myo*-inositol-hexakisphosphate) species can be again phosphorylated to generate high energy Inositol pyrophosphates (PP-InsPs). In summary, to date, the studies could reveal the function of plant VIH only in pathogen defense and phosphate-homeostasis. No other potential role has been investigated or reported for these genes in Arabidopsis or other crop plants. Previously, we have identified two functionally active VIH genes from hexaploid wheat, capable of utilizing InsP7 as a substrate to generate InsP8. Hexaploid wheat, an important crop around the globe and its productivity can be affected when exposed to abiotic stress. In the current work, we have done expression studies, physiological investigations accompanied by forward and reversed genetic approaches; we provide evidence that wheat VIH2 could impart tolerance to drought in transgenic Arabidopsis. Our study could provide novel insight into the possible function of plant VIH towards stress tolerance.

RESEARCH PROGRESS

Previously, we identified two VIH encoding genes from hexaploid wheat and using GUS-promoters of VIH genes its was observed that pVIH2 (promoter of VIH2-3B) respond to drought mimic conditions. So, we attempted to characterized functionally the wheat VIH2-3B function in Arabidopsis by using gain and loss of function approaches.

1. TaVIH2-3B in Arabidopsis display robust growth of the transgenic plants: The biological functions of TaVIH2 were analyzed by overexpressing the cDNA of TaVIH2-3B in Columbia (Col-0) Arabidopsis thaliana. In total, seven transgenic lines were pre-selected based on TaVIH2 expression that was analyzed by western analysis. Four transgenic lines (#Line2-3, #Line 4-3, #Line 5-2 and #Line 6-1) were selected for further characterization. We observed that at the vegetative stage, TaVIH2-3B transgenic Arabidopsis show robust growth. Plants (14 days old seedlings) showed enhanced rosette area cover and increased number of leaves as compared to the controls (Col-0 and Col-0Ev (empty vector). These transgenic Arabidopsis also displayed enhanced branching with an overall increase in the length of the main shoot axis and leaf size as compared to the controls. These results suggest that the expression of TaVIH2-3B in Arabidopsis impacts the overall growth of the plant.

2. Wheat VIH2-3B imparts resistance to water-deficit stress: To check the functional activity of the TaVIH2-3B the generate overexpressing transgenic lines, the Arabidopsis seedlings were exposed to drought-like conditions using mannitol (125 mM) and glycerol (10 %). No significant difference in the root growth pattern on the ½ MS plates was observed in all the Arabidopsis seedlings (Figure 1; left panel). Inhibition of the root growth was observed for the control Arabidopsis suggesting their sensitivity to the presence of both the mannitol and glycerol (Figure 1; left panel). In contrast, TaVIH2-3B overexpression in Arabidopsis was able to escape the detrimental root growth that was significantly higher compared to its control (Figure 1; right panel). Further, drought stress experiments were carried out for all the plants by exposing 7 days old plants to 14 days of water withholding (drought). These experiments were carried out for the mutant, wild-type and overexpressing plants together in the same pot for ensuring that they are inter-rooted and exposed to the same soil moisture conditions. After 14 days of drought, the relative soil moisture content was observed to be as low as 35% in the pots. This caused a dramatic withering of both control and transgenic Arabidopsis plants. However, when the plants were re-watered, high survival rates (~65 %) were observed in the transgenic plants, whereas no or very low (3%) survival efficiency was observed in control (Figure 2). No survival was observed for the vih2-3 mutant plants, indicating



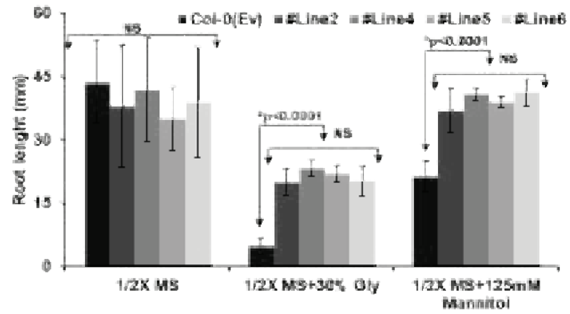
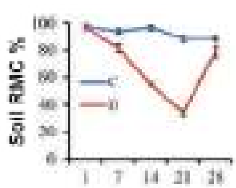


Figure 1. Drought-mimic stress for VIH2-3B Arabidopsis transgenic lines (Left panel) Transgenic Arabidopsis and control seedlings were subjected to drought mimic conditions with glycerol-10 % and mannitol-125 mM. Ten seedlings were used for each transgenic line for each treatment. These experiments were repeated in three experimental replicates with a similar phenotype. (Right panel) Root length of treated seedlings (in mm) for all the lines. Twenty seedlings were used for the measurement of root length for each line.

their sensitivity to drought conditions. This indicates that the transgenic Arabidopsis overexpressing TaVIH2 escapes the effect of drought and improves survival rate by imparting drought tolerance.

3. Transcriptomics data suggest that VIH2-3B stimulate genes related to drought stress: In order to understand the basis of robust phenotype and drought resistance observed in the transgenic Arabidopsis plants when complemented with TaVIH2-3B, we used the transcriptomics approach. Transcriptomics changes in 25 days old seedlings of control and two transgenic plants (#Line4 and #Line6) were analyzed. PCA of normalized expression abundances revealed a high level of correlation among biological replicates (n=3) in each transgenic line. PCA also indicates a distinct cluster for overexpressing transgenic lines and controls. Interestingly, a high number of genes were constitutively activated in the transgenic Arabidopsis belong to the dehydration response element-binding (DREB) protein, including Integrase-type DNA-binding superfamily proteins and glycine-rich proteins. Upon analysis of the GO terms, the highest number of genes for "stress-related" and "cell-wall related activities" were enriched in the biological process and cellular component categories. Strikingly, multiple genes involved in cell-wall biosynthesis, modification and degradation were also up-regulated in the transgenic plants (Figure 3). In addition to that, distinct clusters of genes involved in Abscisic acid (ABA) biosynthesis were also significantly up-regulated among the different lines of transgenic Arabidopsis (Figure 3). Notably, drought-marker genes encoding 9-cis-epoxycarotenoid dioxygenase (AtNCED6 and AtNCED9) involved in ABA biosynthesis were also up-regulated. Multiple DREB encoding genes and cytochrome P450 (CYPs) related family genes (CYP71A23, CYP94B3, CYP71B12, CYP96A2, CYP702A1, CYP707A3, CYP82C2, CYP76G1, CYP705A4, CYP71B10, CYP706A2, CYP81D11) were also differentially regulated in the transgenic Arabidopsis (Figure 2). Our expression response was also



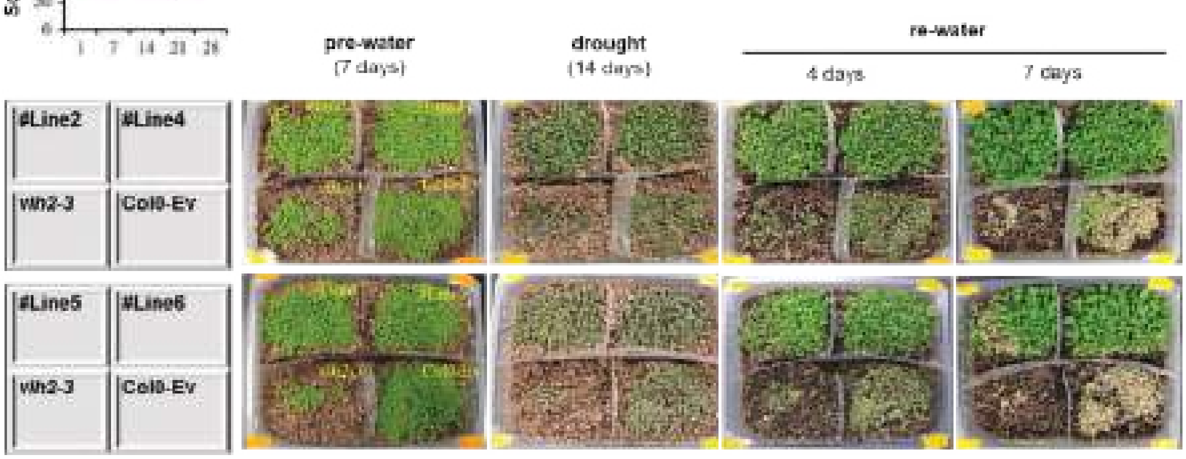


Figure 2. Drought response of VIH2-3B Arabidopsis transgenic lines. Drought treatment of soil-grown plants. Seedlings were pre-grown for the period of fourteen days were subjected to drought for additional fourteen days. The plants were then re-watered for a period of seven days, and % survival rates were calculated. C- indicates controls and D indicate drought condition. This experiment was repeated twice. Representative pictures were taken post seven days of re-watering.

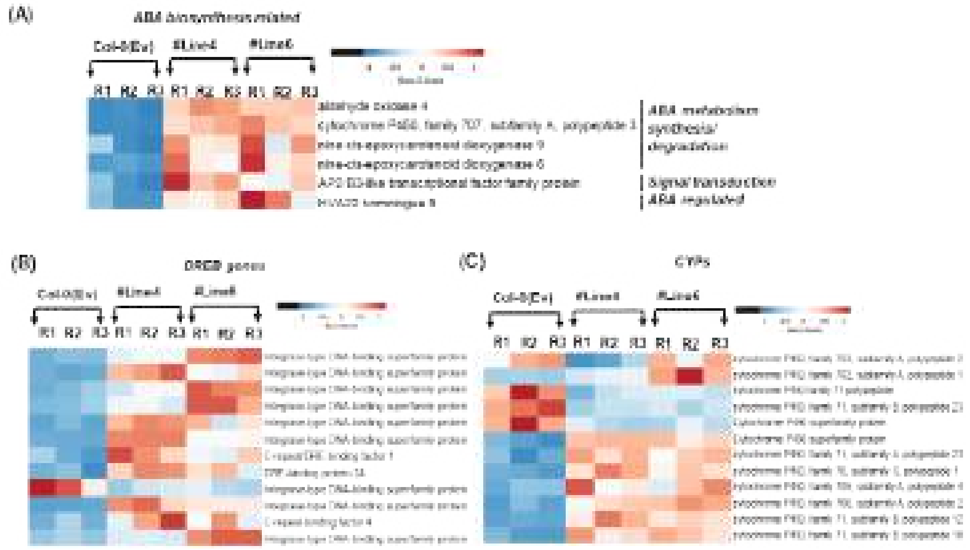


Figure 3. Hetamap expression analysis of gene families in Col-0(Ev) overexpressing TaVIH2-3B Arabidopsis (#Line4 and 6). (A) Heatmaps for expression patterns (as Z-scores) for genes DE in both transgenic lines w.r.t. Col-0(Ev) encoding for ABA biosynthesis genes; (B) DREB encoding genes; (C) cytochrome P450 (CYPs) genes. Increasing intensities of brown and blue colours represent the comparatively low and high expression for each gene, as depicted by the colour scale. Normalized expression counts were used to plotting the expression as Z-scores using heatmap. R1, R2 and R3 represent the biological replicates for the RNAseq analysis of the individual lines.

validated by the qRT-PCR analysis. Overall, we conclude that a distinct cluster of genes involved in drought and ABA stress were significantly up-regulated in these transgenic plants and thus may impart tolerance to stress.

SALIENT ACHIEVEMENTS

- Wheat VIH proteins encodes for a functionally active PPIP5K like activity by utilizing IP7 as a substrate.
- VIH2 promoter respond to mimic stress condition and is able to impart resistance to drought induced condition
- RNAseq analysis show that Arabidopsis overexpressing VIH2 lines show high accumulation of transcripts encoding for cell-wall biosynthesis related genes.

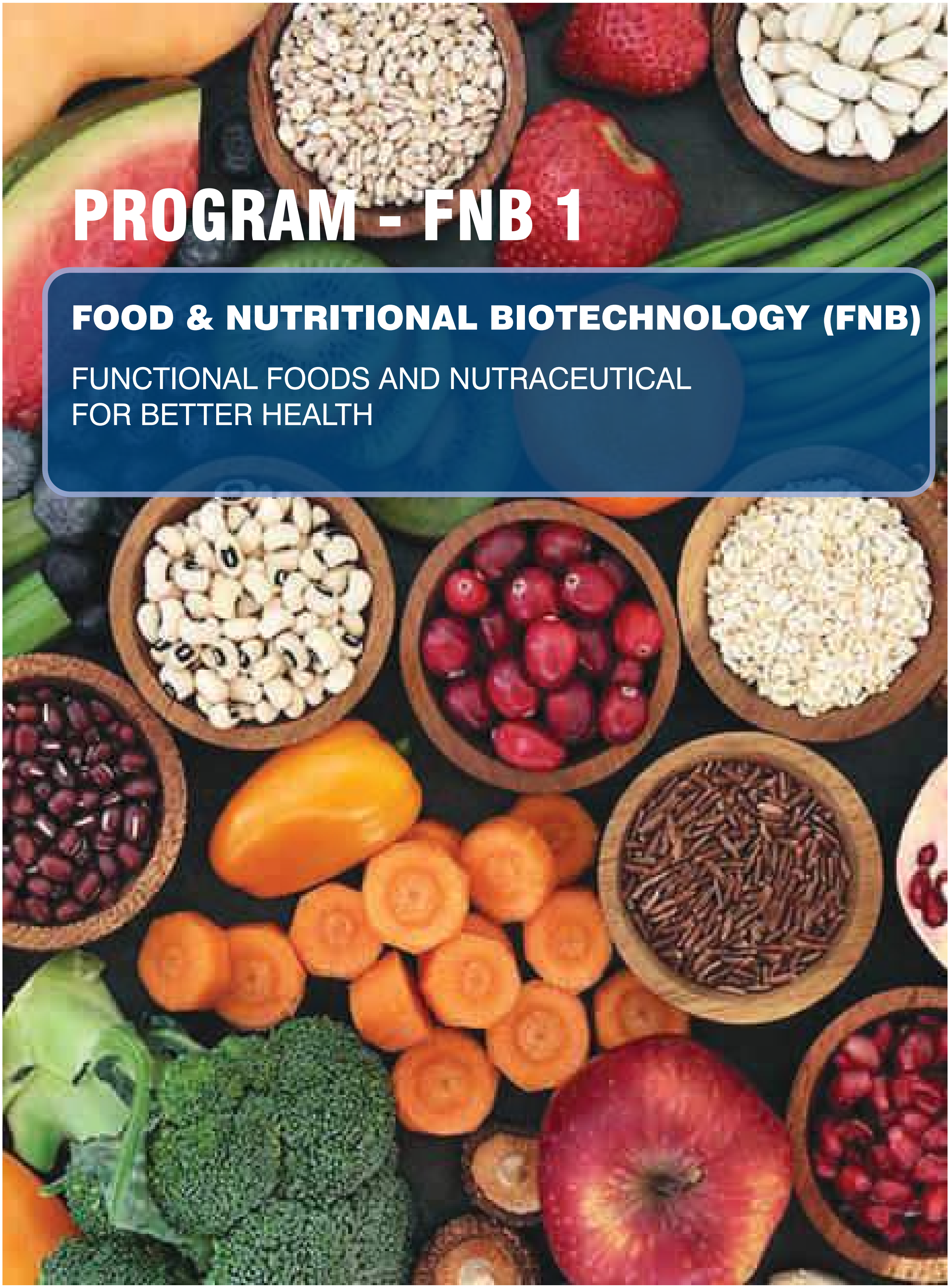
ACTIVITIES FOR NEXT YEAR

- We propose to study the biochemical changes that may occur in the VIH2 overexpressing Arabidopsis lines
- Comparative enzymatic activity will be determined for the different homoeologs of the VIH2/1.

PROGRAM - FNB 1

FOOD & NUTRITIONAL BIOTECHNOLOGY (FNB)

FUNCTIONAL FOODS AND NUTRACEUTICAL
FOR BETTER HEALTH



1.1 Effect of plant-derived exosome-like nanoparticles (PENLs) isolated from ginger rhizome on differentiation of pre-adipocytes: Implications for obesity

PRINCIPAL INVESTIGATOR
Vikas Rishi

RESEARCH FELLOWS
Diksha Choudhary
Binduma Yadav

Eukaryotic cells secrets membrane-derived heterogeneous nano-sized vesicles in the extracellular environment, first described in the late 1960s. It was not until 2011 that the generic term ‘extracellular vesicles’ (EVs) was proposed to define all lipid bilayer-enclosed extracellular structures. Not only mammalian cells but lower eukaryotes and some prokaryotes also secret extracellular vesicles. These vesicles help in cell-to-cell communication both with neighboring and distant cells. Based on size these extracellular vesicles are classified as microvesicles (100-1000 nm), and exosomes (50-100 nm). Exosomes comprise the lipid membrane and cytosolic molecules like nucleic acids, proteins, and metabolites (Figure 1). These regulatory molecules have sparked interest in these vesicles. Recently, it has been reported that exosomes participate in cell-cell communication by transferring their bioactive contents to distant cells, and they are suggested to regulate the expression levels of genes in recipient cells. Like animal cells, plant cells are known to extrude exosomes like nanoparticles to their extracellular environment. Plant-derived exosomes like nanoparticles (PELNs) also carry lipid, proteins, and nucleic acids as in mammalian exosomes. PELN’s exosomes can be extracted from seeds, leaves, fruits, etc. Recent studies suggested that PELN’s affect the mammalian digestive system. Exosomes extracted from carrot, ginger, grapefruit, and grape are analysed for concerning pathways involved in interspecies communication. There are pieces of evidence suggesting oral

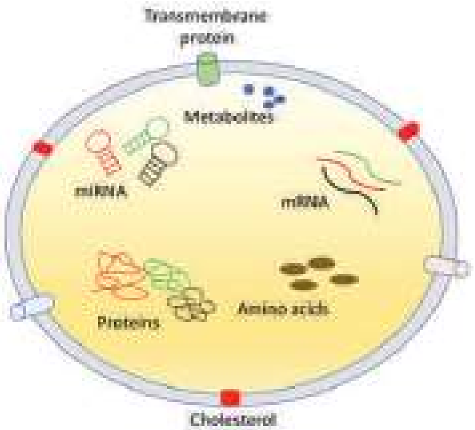


Figure 1. Exosome carrying cargo of nucleic acids, proteins, and metabolites.

Figure 2. Ginger rhizome used for isolating extra vesicles nanoparticles.

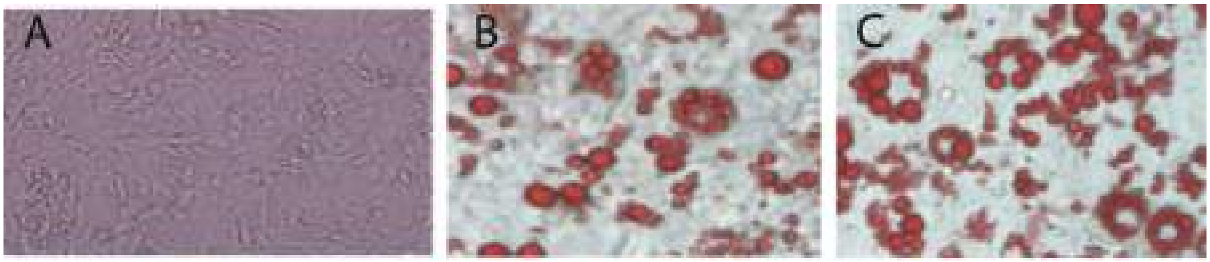


Figure 3. Differentiation of NIH 3T3-L1 cells by exosomes derived from already differentiated cells. (A) Undifferentiated cells. (B) Differentiated cells when exposed to hormone cocktail. (C) Cells that were not exposed to hormone cocktail but were treated with exosomes-derived from (B).

uptake of plants or their extracts leads to cross-kingdom transfer of plant miRNAs into mammals. PELN's miRNAs produce a beneficial effect by regulating mammalian miRNA expressions and act as active signaling molecules to regulate mammalian genes. Diverse exosomes vesicles secreted by plant cells

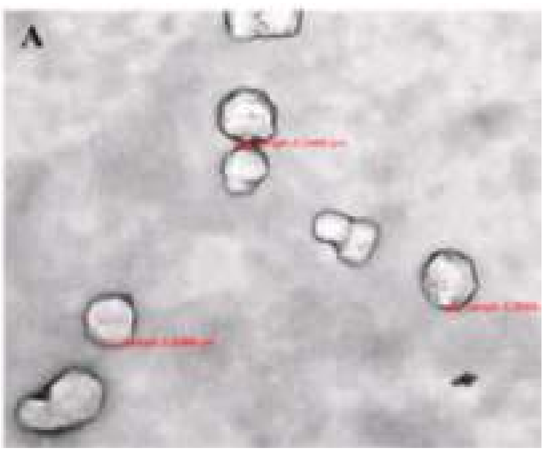


Figure 4. Morphology of ELNs derived from ginger observed under transmission electron microscope (TEM).

have recently received attention as they potentiate and help in stem cell proliferation in the intestine and colon, activate anti-inflammatory cytokines, are involved in Wnt signaling, and ameliorate inflammatory bowel diseases like colitis by activating the AMP-Activated Protein Kinase pathway. One research area where studies involving exosomes application are scarce is adipogenesis, defined as cascading regulatory events which result in conversion of undifferentiated preadipocytes into differentiated fat-laden adipocytes leading to obesity. Obesity is considered to be a morbidity and is affecting a large proportion of the world population including India. We decided to explore if PENL's from ginger can effect hormone-induced differentiation of preadipocyte NIH-3T3 cell line. The study's long-term implications will be the application of plant-derived vesicles as a therapeutic reagent in mitigating obesity.

RESEARCH PROGRESS

NIH 3T3-L1 pre-adipocytes cells are an excellent model to study the process of differentiation. These cells spontaneously differentiate and start accumulating lipids under the influence of hormone cocktail (MDI hormone cocktail (0.5mM 3-iso-butyl-1-methylxanthine, 1μM dexamethasone and 1μg/ml insulin). Oil accumulated in the presence and absence of exosome treatment is quantified using Oil Red O stain. Results obtained from such studies can easily be extrapolated to in vivo systems. One of the most interesting observations that was made relates to the differentiation of NIH 3T3-L1 cells that were not exposed to hormones but treated with exosomes derived from fully differentiation cells. These exosome-treated cells spontaneously differentiate as shown by the accumulation of lipids (Figure 3). These results show that exosomes released from differentiated cells have all the information required for inducing differentiation in otherwise undifferentiated cells proving that exosomes are mediators of the cell to cell communication

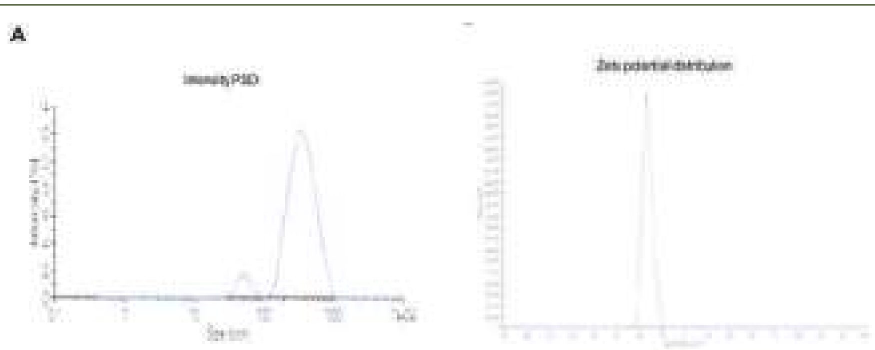


Figure 5. Exosomes like nanoparticles (ELNs) isolated from ginger. (A) size of ELNs from ginger (250 nm). (B) Zetapotential of ginger ELN's (-33.2 mV).

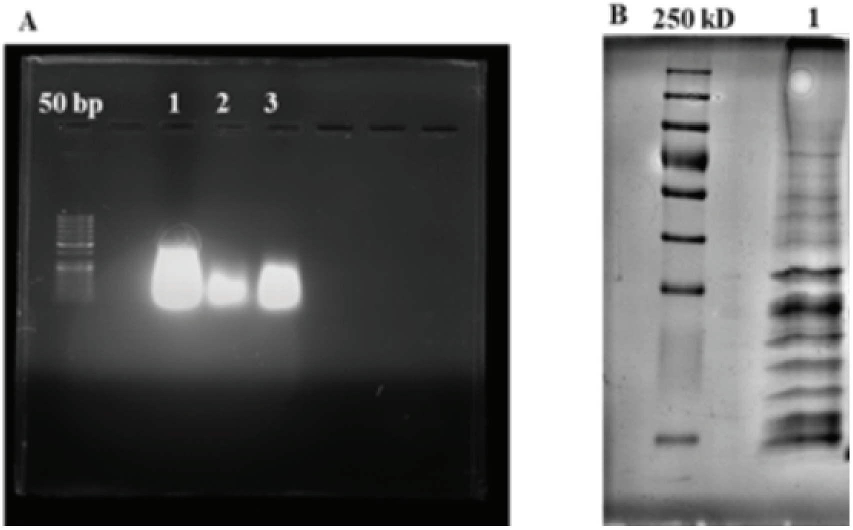


Figure 6. Biomolecule analysis of ginger exosomes. (A) Agarose gel showed RNA from three independent exosomes isolations. (B) Coomassie blue staining of protein gels showed various proteins in ginger exosomal extract.

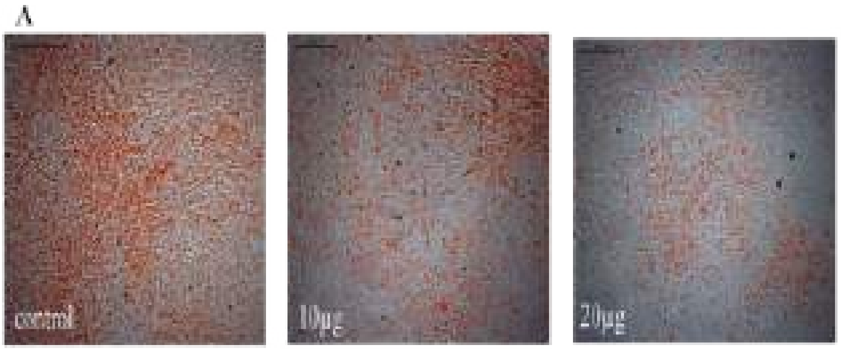


Figure 7. Inhibitory effect of ginger exosomes on lipid accumulation on NIH 3T3-L1 pre-adipocytes. (A) Differentiated NIH-3T3-L1 cells were incubated with 10, and 20, μg/mL of ginger exosomes for 24 hrs. Cells were stained with Oil Red O. Stained intracellular oil droplets were eluted with isopropanol and quantified spectrophotometrically at 510 nm.

and are involved in gene regulation in the recipient cells. The results suggest that during adipogenesis, initial environment signals are all that are required to induce differentiation and cells continue to differentiate even when the signals are no more in play. The results prompted us to probe for nutritional interventions that may stop or reverse the process of cell-cell signaling involved in adipogenesis.

In the present study, we have chosen ginger rhizome as a source of exosomes to test their anti-adiposity activities on NIH 3T3-L1 cells. Ginger health benefits among others e.g., lowering blood pressure, and cancer, also include weight loss, prompting us to choose the spice as an exosome source. Ginger exosomes were purified using an ultracentrifuge following the protocol published previously. Quality, quantity, and physical parameters of exosomes were analyzed using transmission electron microscopy (TEM) (Figure 4) and Dynamic light scattering (DLS) (Figure 5). Biochemical analyses of the components of ginger exosomes were carried out by gel electrophoresis. For the RNA component, total RNA was isolated from purified exosomes using the TRIzol method. The quality of the isolated RNA was checked using agarose gel electrophoresis (Figure 6A). Total protein was estimated using the BCA protein quantification method. SDS-PAGE was used to visualize the protein profiling of exosomal proteins (Figure 6B). In order to observe the effects of ginger ELNs on lipid accumulation in 3T3-L1 adipocytes, cell were treated with 10 and 20 μg/mL of ginger exosomes for 24 hrs before they were induced to induced to differentiate by hormonal treatment (Figure 7). It is clearly demonstrated that ginger exosomes inhibit the differentiation of 3T3 cells.

SALIENT ACHIEVEMENTS

- The observed results demonstrated that the extravesical population in ginger have a regulatory role in pre-adipocyte differentiation.
- Our results show that ginger-derived nanoparticles inhibit lipid accumulation in 3T3 pre-adipocytes.

ACTIVITIES FOR NEXT YEAR

- In future, transcriptomic and proteomic analysis of the cells treated with exosomes will be carried out to decipher the underlying mechanism of low lipid accumulation.
- In- vivo study of effects of plant-derived exosomes will be tested in mouse model.

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

PRINCIPAL INVESTIGATOR(S)
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RESEARCH FELLOWS
Ruchika Bhatia
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The shift in dietary habits from the traditional to the westernized diets results in gut microbial dysbiosis. Higher abundance of pathogenic Gram negative bacterial associated molecules such as lipopolysaccharides (LPS) causes leaky gut with increased intestinal permeability towards microbial pathogens and their toxins, which can cause obesity, diabetes, metabolic syndrome and many other diseases. Diet based strategies, i.e., using plant based bioactives such as polyphenols, antioxidants, dietary fibers and probiotics from various sources are being investigated for the prevention of metabolic complications as there could be very low or no adversities on health over long term usage. In our previous reports we have reported on the isolation and probiotic characterization of anti-inflammatory *Bifidobacterium* strains (i) isolated from human adults and infants; (ii) their protective action against inflammation using in vitro macrophage cell lines; (iii) protection against gut inflammation caused by dextran sodium sulphate (Singh et al., 2020) and (iv) protection against diet induced pre-metabolic syndrome (unpublished). Here we report on the characterization of *Lactobacillus* sp. and other lactic acid bacteria which could curtail LPS induced pro-inflammatory stress in murine macrophages in vitro; LPS induced inflammation in C57/BL6 mice model and preventive efficacy against diet induced metabolic complications in SD rat model.

RESEARCH PROGRESS

Development of synbiotics for the prevention of chronic disease: Protection against inflammation: Total 36 Lactic acid bacterial strains were isolated from various fermented food samples, vegetables and human infants. Out of these, four strains namely LAB3, 8, 20, 31 and 39 were shortlisted for the further studies based on their ability to reduce inducible nitric oxide production by the murine macrophages treated with LPS (anti-inflammatory property) and desirable probiotic attributes. In vitro studies on the inhibition of pro-inflammatory cytokine pathway and in vivo protection from systemic and colonic inflammation caused by LPS by the LAB3, 8, 20, 31 and 39 in C57BL/6 mice were evaluated.

Prevention of LPS-Induced Inflammation in C57BL/6 mice: Male C57BL/6 mice (Age: 2 months; Body weight range 20-25 g) were fed with standard diet and water *ad libitum*. The mice were randomly divided into 10 groups and were fed with re-

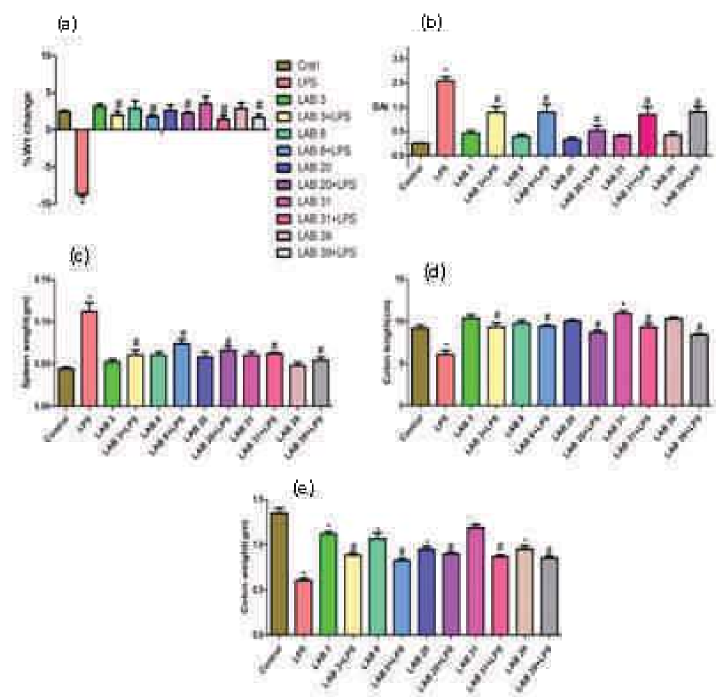

Figure 1. Protection from LPS-induced inflammation by the lactic acid bacteria: Weight change (a); disease activity index (b); Spleen weight (c); spleen weight; colon length (d); colon weight (e). Data was analyzed by one-way ANOVA followed by Tukey's Post-hoc test (P ≤ 0.05). *significant relative to control; #significant relative to LPS treated animals; N = 5 for control and per-se groups; N = 5 for LPS and intervention groups.

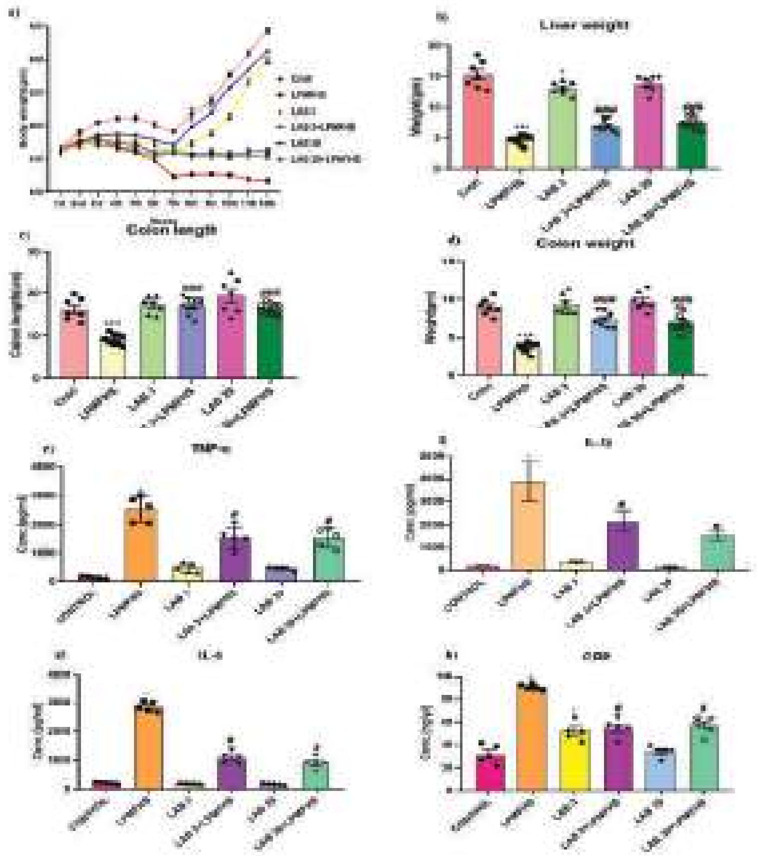

Figure 2. Lactic Acid bacteria supplementation protected against diet induced metabolic complications: Body weight(a); Colon weight (b); Colon length (c); Liver weight (d); TNF-α (e); IL-1β (f); IL-6 (g); CRP (h). Data was analyzed by one-way ANOVA followed by Tukey's Post-hoc test (P ≤ 0.05). *significant relative to control; #significant relative to LPMHS treated animals; N = 6 for control and per-se groups; N =8 for LPMFHS and intervention groups.

spective strains suspended in sterile PBS using oral gavage at a concentration of dose 1 $\times 10^{10}$ CFU/mouse per day for 2 weeks. Just before 24 h of sacrifice, inflammation was induced by injecting a single dose of LPS (10 mg/kg bodyweight) through intraperitoneal route in all the mice except those in normal control and per-se groups. Blood samples were collected by cardiac puncture 24 h after the LPS challenge. Serum obtained from the blood samples were stored at − 80°C and used for biochemical analysis. The viable number of *lactobacilli* in the feces and adhering to the ileal mucosa after 24 h LPS injection were evaluated. There was a decrease in the body weight and colonic length and an increase in the weight of colon and spleen in the LPS treated mice relative to the normal ones was observed. LPS caused loose stool consistency, watery discharge in feces, lower levels of acetate, propionate and butyrate in the feces and high inflammation due to higher levels of pro-inflammatory markers in the serum relative to the control mice. Furthermore, LPS treatment elevated the expression of toll like receptor; inflammatory genes and decreased the expression of tight junction proteins such as zona occludin, claudin, occludin relative to the control group. All these negative effects were alleviated by the LAB3, 8, 20, 31 and 39 in a strain dependent manner (Fig. 1). The protective effects were much better for Lab 3 and Lab 39 and hence these strains were further evaluated in diet induced pre-metabolic syndrome model.

Prevention of Diet-Induced Pre-metabolic syndrome: Male Sprague Dawley rats (100-200g), were acclimatized for a week under standard experimental conditions (temperature 22 ± 2°C; humidity 55 ± 5%; 12 h light and dark cycles) at Animal Experimentation Facility (AEF) of NABI, Mohali (Approval number: NABI/2039/CPCSEA/IAEC/2019/14). Weight matched rats were randomized into 6 experimental groups each comprising of 8-10 rats to evaluate the effect of bacterial supplementation. The rats were orally gavaged with 2 ×1010 CFU/rat of either Lab 3 or Lab 39 strains suspended in sterile PBS daily for 14 weeks. The entire experiment was conducted for three months. Blood samples were collected and serum was prepared and stored at − 80°C until further use. In LPMFHS treated mice there was decrease in the colonic length, weight, spleen and liver as compared to normal rats. LPMFHS groups have shown loose stool in feces as compared to treatment groups. LPMFHS treated rats showed higher levels of triglyceride, cholesterol, LDL and lower level of HDL relative to control rats. The rats also showed increased levels of LPS, CRP, IFN, TNF-α, IL-6, Leptin, insulin and decrease level of adiponectin as compared to the normal rats while Lab 3, and 39 fed rats mitigated these deleterious changes (Fig. 2). Per se supplementation with the bacterial strains did not elevate their levels compared to normal rats.

SALIENT ACHIEVEMENTS

- LAB 3, and 39 are the prioritized bacterial strains out of 36 strains as they prevented LPS induced inflammation in mice and diet-induced deleterious metabolic alterations in rats. These strains will be further evaluated for the prevention of chronic low grade inflammation associated with metabolic diseases.

ACTIVITIES FOR NEXT YEAR

- All the prioritized strains will be further evaluated for the prevention of chronic low grade inflammation associated with metabolic diseases.

1.3 Development of novel cobiotic formulations for metabolic disorders

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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 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 (in revision); Kumar et al., 2020). 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). In the present study, 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.

RESEARCH PROGRESS

Studies on Transient Receptor Potential (TRP) channel mediated modulation of adipogenesis & obesity (browning) by dietary molecules: Capsaicin and exogenous mucin admin-

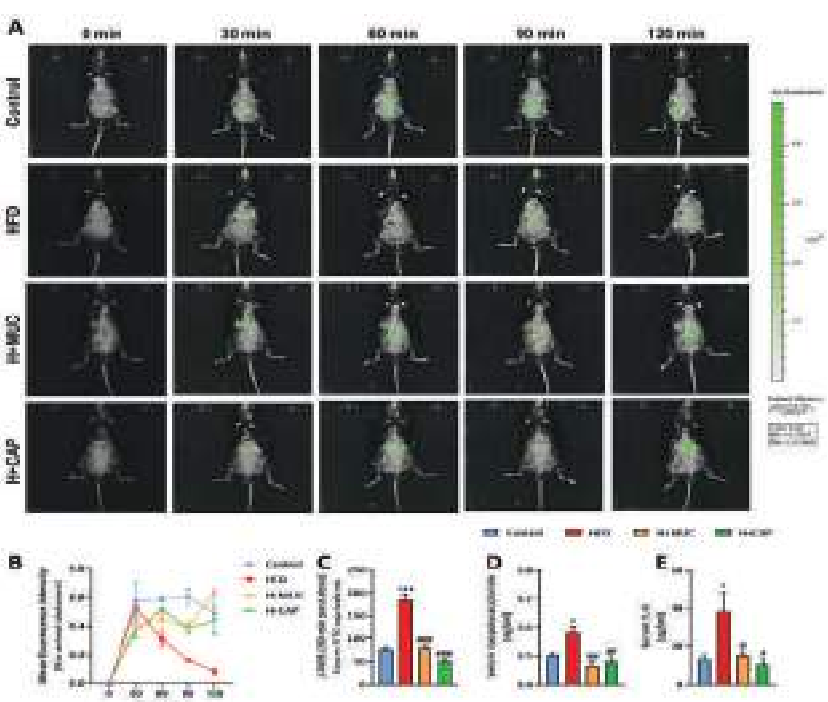

Figure 1. Effect of high fat diet and interventions on intestinal permeability, endotoxemia and inflammation. A) FITC-dextran In vivo fluorescent imaging – representative figure,d B) FITC fluorescence intensity quantification in abdominal region at different time points, and C) Serum FITC fluorescence measurement at 90min, D) Serum LPS and E) Serum IL-6 levels. Mice were divided into 4 groups – Control (normal pellet diet-fed), HFD (high fat diet-fed), H+MUC (HFD + porcine stomach mucin (1g/kg/day p.o.), H+CAP (HFD + capsaicin 2mg/kg/day p.o.). Food and water were given ad libitum. Treatments were given for 12 weeks. During week 11 and 12, intestinal permeability was tested. Single dose (n=3 each) of FITC-dextran (200mg/kg p.o., mol. wt. 3-5kDa) was given, animals were anaesthetized for live imaging at 0 (before treatment), 30, 60, 90 and 120 min after treatment. In vivo fluorescence intensity (n=3 each) was measured using ImageJ software, serum FITC levels were measured at 480nm (ex.) - 520nm (em.). After sacrifice, serum LPS (n=6 each) and IL-6 (n=5 each) were measured using commercial kits. All data is represented as mean ± SEM. Intergroup variations were assessed using One-way ANOVA with Bonferroni's multiple comparisons test. *$p<0.05$, **$p<0.01$, *** $p<0.001$ versus Control; #$p<0.05$, ##$p<0.01$, ###$p<0.001$ versus HFD.

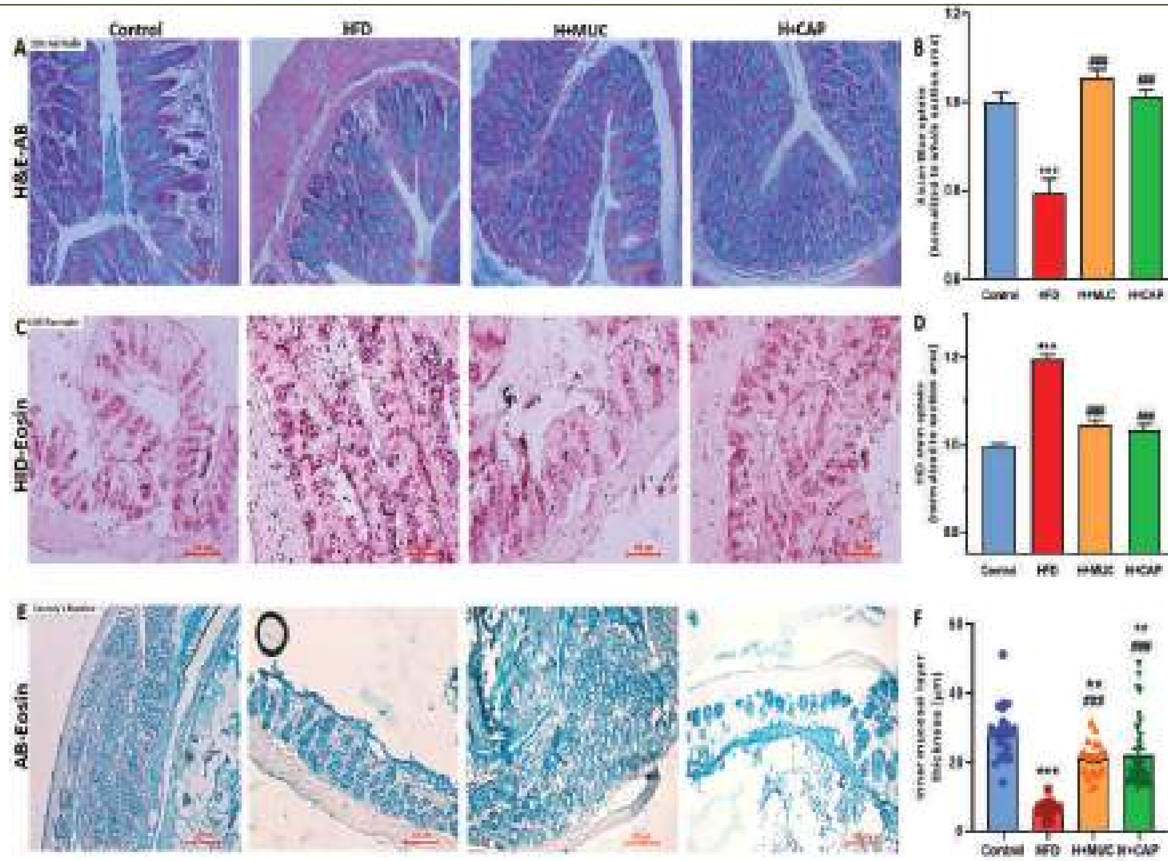

Figure 2. Effect of high fat diet and interventions on colonic mucus production. Histology – representative images of A) Hematoxylin-Eosin-Alcian blue (H&E-AB) staining and B) Alcian blue intensity, C) High iron diamine-Eosin (HID-Eosin) staining and D) HID staining intensity, E) Alcian blue-Eosin (AB-Eosin) staining (in Carnoy-fixed sections) for inner mucus and F) Average inner mucus layer thickness. Mice were divided into 4 groups – Control (normal pellet diet-fed), HFD (high fat diet-fed), H+MUC (HFD + porcine stomach mucin (1g/kg/day p.o.), H+CAP (HFD + capsaicin 2mg/kg/day p.o.). Food and water were given ad libitum. Treatments were given for 12 weeks. After sacrifice, colon tissues were fixed (formalin or Carnoy's fixative), subjected to serial dehydration (ethanol for 2h each: 25, 50, 70, 90%, twice 100%), xylene treatment (1h twice) and paraffin embedding. 5μm sections were cut. For staining, paraffin was removed using xylene (5min twice), sections were serially rehydrated (ethanol: 100% twice, 90, 70, 50, 25% and PBS, 2min each) and treated with respective stains (H&E: 2min each, AB: 15min, HID: 24h) followed by washing (5min, thrice after each stain) in water. After serial dehydration and xylene treatment, sections were mounted using DPX medium. Microscopy was performed for imaging. Respective staining intensities (normalized to counter-stained whole section area) and mucus layer thickness were measured using ImageJ software. All data is represented as mean ± SEM. Intergroup variations were assessed using One-way ANOVA with Bonferroni's multiple comparisons test. **$p<0.01$, *** $p<0.001$ versus Control; ###$p<0.001$ versus HFD.

istration prevented HFD-induced weight gain and other metabolic complications. Similar to exogenous mucin, capsaicin prevented HFD-induced gut permeability, inflammation and endotoxemia. In vivo fluorescent imaging showed major decrease in mean fluorescent intensity in target abdominal area with time in HFD group, indicating higher rates of diffusion of FITC-dextran into circulation through gut barrier. In mucin and capsaicin groups, the levels of intensity remained comparable to Control throughout the experiment (Fig.1A-B). Serum fluorescence measurement in 90 min samples also reflected the same phenomenon, with a significantly higher fluorescence level in HFD group whereas both interventions significantly prevented it, with levels similar to Control (Fig.1C). Serum LPS (endotoxemia) and IL-6 (inflammation) levels were significantly higher in HFD group, while they were significantly reduced in both interventions (Fig.1D-E). Alcian blue (AB) and High Iron Diamine (HID) stains were used to study colonic morphology and mucus production (Fig.2A, C). ImageJ analysis of intensity (normalized to whole section area) of AB stain revealed that total mucin staining in HFD group was significantly lower than control. Mucin and capsaicin groups showed levels of AB uptake similar to control, indicating a preventive effect of both interventions on high fat diet-induced alterations in mucus production (Fig.2B). HID stain, specific for sulfomucins, showed an opposite pattern, where HFD group showed higher HID uptake compared to Control. Both interventions reversed this effect (Fig.2D). AB staining of sections from undisturbed colon samples in Carnoy's fixative, was used to measure inner mucus layer thickness (Fig.2E). Average thickness was found significantly lower in HFD group compared to Control. While average thickness in both, mucin and capsaicin, groups was also significantly less than Control, both showed remarkable improvement compared to HFD (Fig.2F).

These summarized results in association with our gut metagenome analysis, in-vitro fermentation studies, calcium imaging in TRPV1 transfected HEK cells suggested that mucus-microbe interactions are crucial to capsaicin effects, and TRPV1 must be involved via both direct and indirect effect of capsaicin, provoking a neuro-enteric communication (Fig. 3).

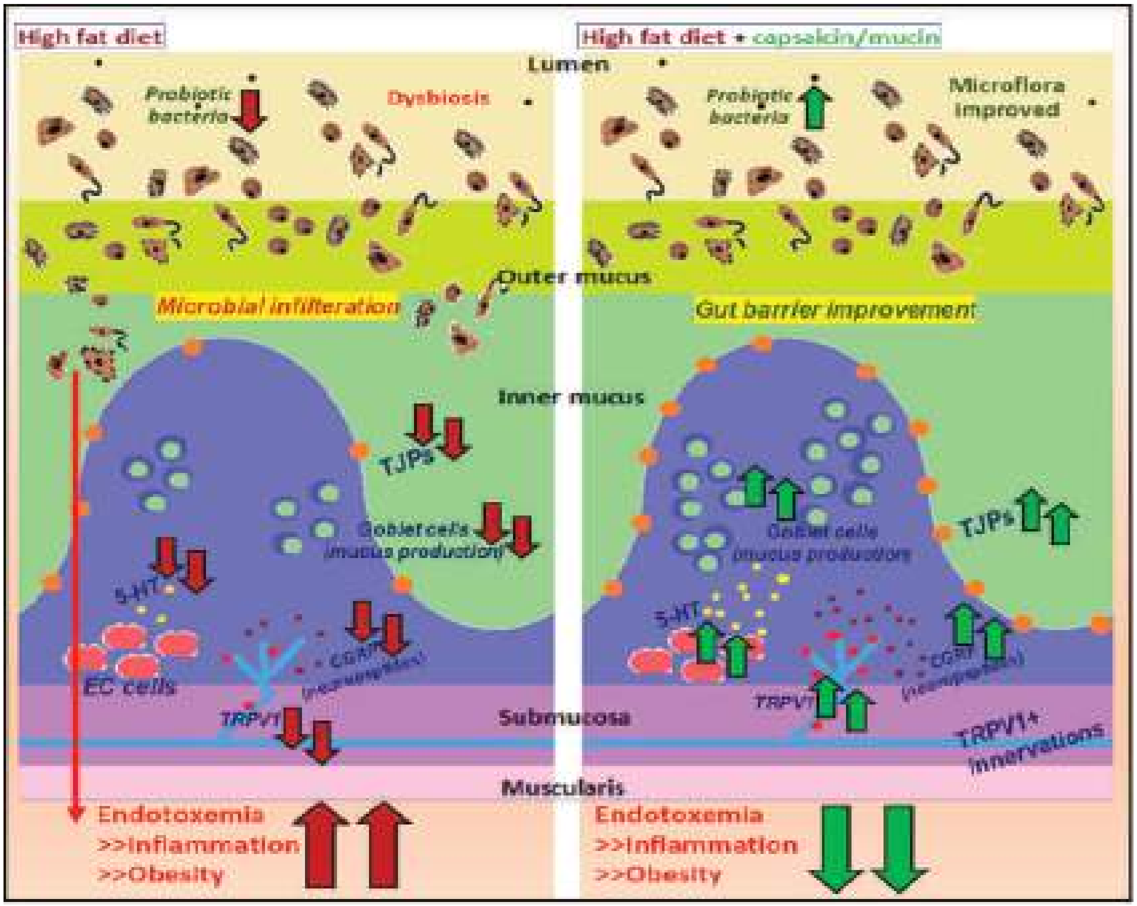

Figure 3. Schematic diagram of proposed involvement of mucin: microbe interaction in anti-obesity action of capsaicin.

SALIENT ACHIEVEMENTS

- 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.
- There is a potentially significant role of gastrointestinal TRPV1 and mucin in diet-induced obesity. We can harness this finding to develop diet-based strategy for obesity and related complications.

ACTIVITIES FOR NEXT YEAR

- TRPV1 sensory neurons co-express with TRPA1 in gastrointestinal tract. It is important to understand the role of TRPA1 in mucin homeostasis.
- Whether under different dietary conditions, microbial specific metabolites can activate these sensory neurons.

1.4 Production, characterization and biological significance of health beneficial of prebiotics

PRINCIPAL INVESTIGATOR
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RESEARCH FELLOWS
Rohit Maurya

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 (g/kg level) of IMOs. Further, initiatives will be made to prepare health beneficial functional food products based on IMOs.

RESEARCH PROGRESS

Starch was isolated from potato peel by extraction with water at room temperature; the yield obtained was nearly 7% from wet potato peel. Further, the protocol for lab-scale production of isomalto-oligosaccharides (IMOs) from starch was optimized by three step processes (i) initial liquefaction of starch by termamyle (ii) saccharification of liquefied starch to malto-oligosaccharides by fungamyle (iii) α-trans-glycosylation of malto-oligosaccharides by α-trans-glucosidase. Further, in house production of α-transglucosidase (average MW ~110 kDa) with high purity was achieved via cloning of synthesized α-glucosidase gene, followed by transformation into *E. coli* BL21(DE3), overexpression and purification of the protein. Further, the produced α-trans-glucosidase was immobilized with magnetic nanoparticle for better reusability. The study suggested the magnetically immobilized enzyme (α-transglucosidase) has reusability of 6 cycles with nearly 60% activity. MALDI-TOF-MS analysis suggested the highly abundant molecular ions with degree of polymerization (DP) in the range of 2-7 (Fig. 1). Further studies on the optimization of yield by response surface methodology for the up-scaling production of IMOs are in under progress.

SALIENT ACHIEVEMENTS

- In our study, starch was extracted from food-processing by-product (potato peel). Further, the process for lab scale enzymatic production was optimized to produce IMOs with DP 2-7. The detailed study for the process optimization and upscaling production is under progress.

ACTIVITIES FOR NEXT YEAR

- Detailed study for the process optimization using response surface methodology.
- Purification of IMOs and optimization of protocol for upscaling production.

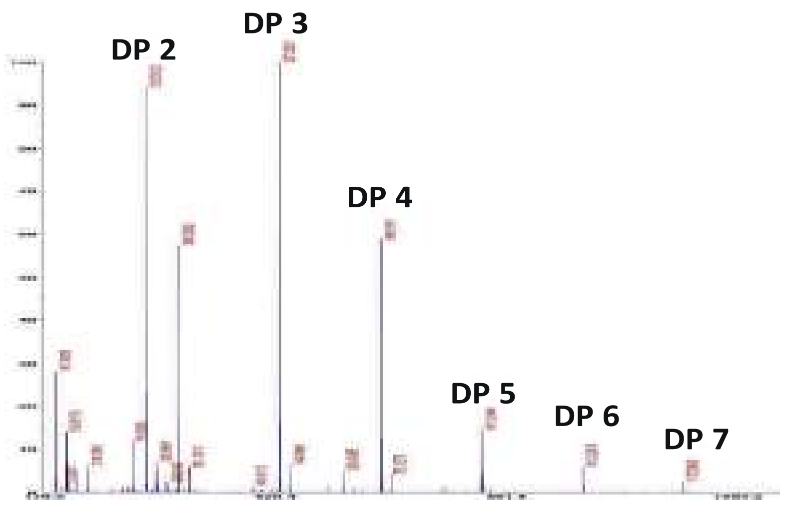

Figure 1. MALDI-TOF-MS spectrum of IMOs.

1.5 Development of therapeutic formulations for nutritional and lifestyle disorders

PRINCIPAL INVESTIGATOR
Nitin Kumar Singhal

RESEARCH FELLOWS
Poonam Sagar
Geetika Bajaj

Two major health issues that are India's major concerns are iron deficiency anemia and type 2 diabetes mellitus. These are long term metabolic/lifestyle disorders and are affecting a large fraction of the Indian population. We are working towards the identification and execution of different therapeutic approaches for these major disorders. (1) Several side effects and incidence of inflammatory response have been reported caused by the already available iron supplements. This occurs due to the translocation major fraction of unabsorbed iron from the small intestine to the colon. To avoid this a pH sensitive, synbiotic hydrogel encapsulating iron and probiotic has been developed that releases its contents in the duodenum (major site of iron absorption) and reduces the risk of iron aggregation in the acidic environment of stomach. This is responsible for higher absorption and lower inflammatory response. The prepared formulation has been tested for its efficacy in improving the diet induced anemic mice model's iron status. The prepared hydrogel enhanced haemoglobin levels in the treated group as compared to a non-treated anemic group within two weeks of the treatment. The prepared formulation was also able to enhance the gene expression status of hepcidin, ferritin, DMT1, DcytB, and ferroportin. Lower local inflammation response was observed in the groups treated with hydrogel than ferrous sulphate and non-encapsulated iron-dextran. Thus, based on the observed results, this formulation can be utilised as a new alternate supplement to treat iron deficiency anemia.

RESEARCH PROGRESS

Development of oral iron supplement-side effects and incidence of inflammatory response: In this study, sodium alginate based hydrogel co-encapsulating probiotic and iron were developed for iron deficiency anemia oral therapy. The hydrogel was characterized using transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM), and atomic force microscopy (AFM). These pH responsive hydrogels swell and release their contents at intestinal pH, but stay protected in the stomach's acidic environment. The formulation showed efficient in vitro and in vivo iron bioavailability in Fe depletion-repletion studies. The sustained release of encapsulated bacteria and iron facilitates Fe^{2+} absorption by duodenal enterocytes and improves IDA. In addition, enhanced iron absorption resulted in a lower inflammatory response than ferrous sulfate or iron dextran alone (Figure 1). Co-supplementation of iron and probiotics in encapsulated form is responsible for the sustained release of iron, and bacteria in the gastrointestinal tract is responsible for its enhanced efficiency in restoring iron homeostasis. The increased iron bioavailability and the lower inflammatory response indicate the potential of this hydrogel formulation as an efficient iron supplement for oral consumption.

(2) **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. T2DM is the most common (accounting for more than

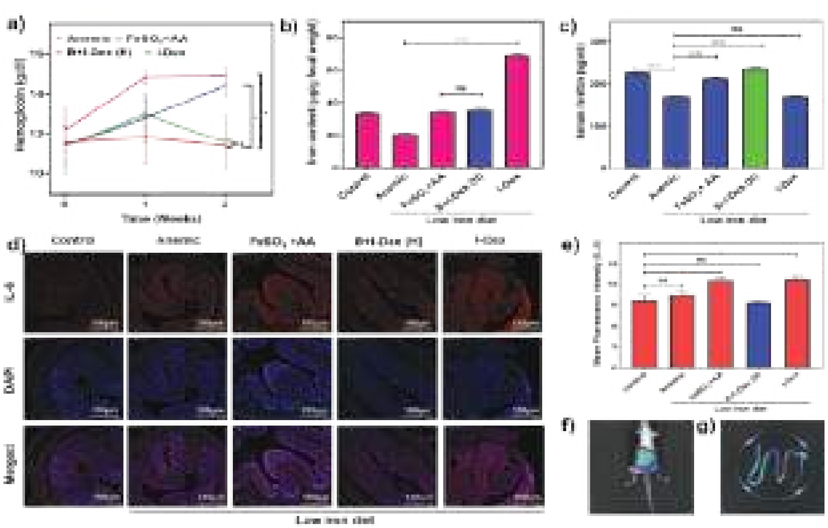

Figure 1. In vivo efficacy of the hydrogel to replenish iron status in iron deficient mice: (a) hemoglobin levels (g/dl) (b) fecal iron content (μg/g fecal weight) (c) serum ferritin levels estimated through ELISA (ng/ml). (d) Mean fluorescence intensity of IL-6 calculated from immunofluorescence (f-g) In-vivo and ex-vivo bio distribution studies of the prepared hydrogel.

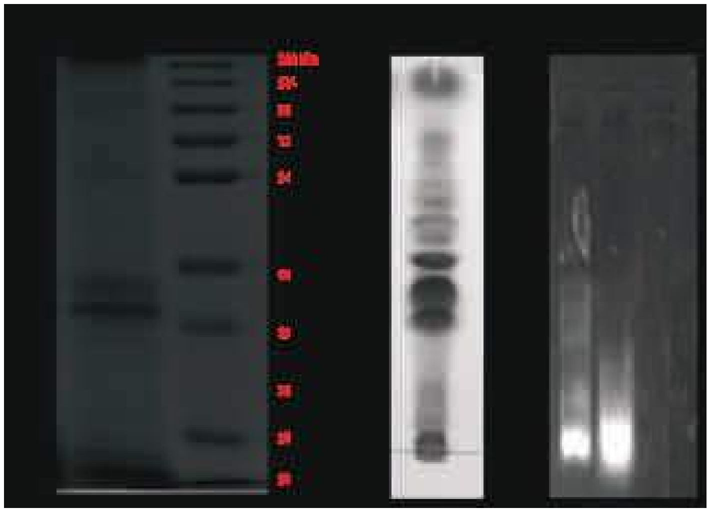

Figure 2. (A) Total protein of exosome was run on 10% SDS-PAGE. (B) TLC of total lipids isolated from exosomes developed using (10% $CuSO_4$ in 8% phosphoric acid). (C) Exosomal RNA treated with/without RNAase was run on 1.2% agarose gel and stained with ethidium bromidel.

90% of all cases) than gestational and type 1 diabetes. 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, e.g. 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 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.

We have successfully isolated exosomes from medicinal plants (ginger). The average size and zeta potential of the vesicles were found out to be 162.4 ± 7.5 nm and -31.2 ± 1.7 mV, respectively. Transmission Electron Microscope imaging of these vesicles also confirmed their cup-shaped morphology and size, 131.06 ± 37.08 nm. Further, proteins were isolated from these vesicles and were run on 10% SDS-PAGE gel. RNA and lipids were also isolated from these small vesicles and were subsequently run on 1.2% agarose gel with/without RNAase and on a TLC plate, respectively. Hydrodynamic size, zeta potential, TEM imaging followed by the presence of proteins, RNA and Lipids in these small vesicles further confirmed the identity of these small vesicles as exosomes (Figure 2).

SALIENT ACHIEVEMENTS

- Successful synthesis of hydrogel and its characterization was performed by scanning electron microscopy (SEM), pH sensitivity and release behavior.
- The formulation was able to increase haemoglobin levels in mice after two weeks of treatment than non-encapsulated formulation.
- Iron content in the fecal samples represents higher iron in the non-encapsulated group representing less iron absorption than hydrogel beads.
- Successful isolation of exosomes from Ginger as confirmed by the Transmission Electron Microscope, Zetasizer and presence of Proteins, RNA and Lipids in these small vesicles.

ACTIVITIES FOR NEXT YEAR

- Toxicity analysis of isolated exosomes on different cell lines.
- 2-NBDG uptake analysis in Insulin Resistant HepG2 cells treated with exosomes.
- RT-PCR followed by western blot of important markers up/downregulated in insulin resistane cells with/without exosome treatment.
- In-vivo efficacy evaluation of ginger exosomes in HFD and Streptozotocin induced mice model of T2DM.

1.6 Glycogenomic approaches to understand molecular interactions of gut microbes and glycans

PRINCIPAL INVESTIGATOR
Ravindra Pal Singh

RESEARCH FELLOWS
Raja Bhaiiyya
Jayashree Niharika

Glycans (e.g. pectin, hemicelluloses and cellulose) are main components of plant cell walls, and are covalently attached to proteins or lipids in all eukaryotic cell surfaces. These molecules are crucial for many key biological processes including wide variety of cellular processes ranging from cell to cell communication to immunity. Amidst other functions, dietary glycans modulate gut microbiome and immune system. Gut microbiome consists of 100 trillion microbial cells that exert several beneficial roles including earlier development, maintaining gut homeostatic, modulating immune system and providing nutrition and energy to cells and tissues of the host. Contrary, glycan-binding proteins called lectins are highly specific for sugar moieties and directly play numerous roles in biological recognition phenomena. Despite several physiological cues of dietary glycans have been discovered in last several decades, this field is lacking systematic evaluation of a well characterized glycan (in term of DPs and structures) on modulating gut microbiota and immune system along with unravelling their molecular interactions. Having this lacuna, we are currently focusing on pectin of different fruits, and β-glucans obtained from variety of sources to understand their microbiome and immunomodulatory actions at molecular levels. The study applies approaches of synthetic glycobiology, biochemical, proteomics, bioinformatics and microbiology. Synthetic glycobiology and proteomic adopt to synthesize a define glycan, and monitoring total protein expression under given conditions respectively. Bioinformatics is used for comparative genomics analysis and to know how a glycan utilizing locus widespread admits human gut bacteria before it validates in *in vitro* condition. Structure activity relationship of receptor/lectin–glycan interaction is chased by biophysical experiments and structural biology. Sympathetic these gaps are crucial for developing nutritional based therapeutic intervention for the better health.We have been working on digestion of β-glucans

A

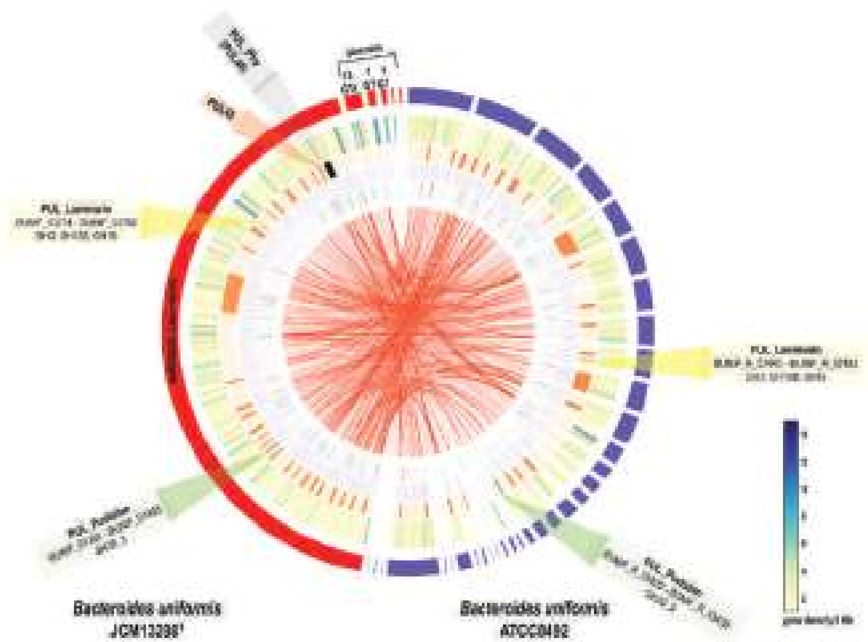

Figure 1. (A) A comparative genome analysis of the *B. uniformis* JCM13288T with the *B. uniformis* JCM 5828 (ATCC 8492). The tracks (outside to inside) represent (i) density of protein-coding genes per 5 kb, (ii) location of PULs, (iii) CAZymes, (iv) transposases and (v) the synteny between the two genomes. The PULs responsible for laminarin utilization are marked in yellow (GH3, GH158, and GH16) and green (GH30_3) and the PUL responsible for porphyran is marked in black. The *B. uniformis* JCM13288T has five plasmid-like sequences that contain genes for several glycosyl transferases. (B) Comparison of synteny and integrative conjugative element of the *B. uniformis* JCM13288T and *Bacteroides plebeius* DSM 17135, highlighting the presence of extra PUL (PUL45) adjacent to porphyran utilization locus without disrupting its function.

B

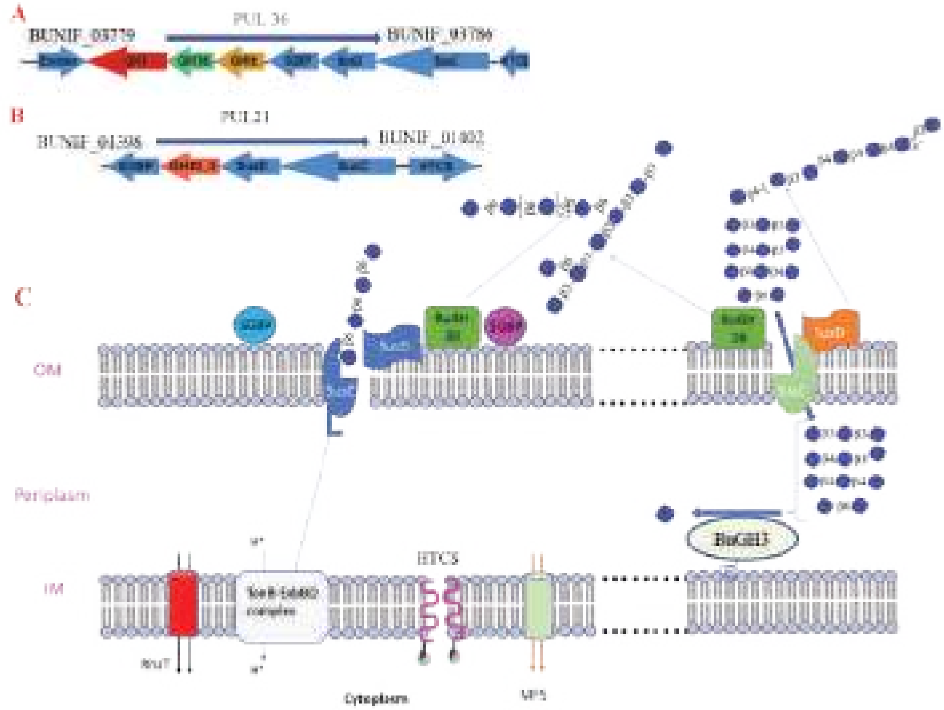

Figure 2. (A) β-1, 3- linked glucan utilization locus, (B) β-1, 6- linked glucan utilization locus and (C) possible role of the BuGH3MLG (BuGH3) for utilizing oligosaccharides generated from enzymatic degradation of laminarin (A), pustulan (B) and barley β glucan (BβG).

by human gut commensal bacteria, and generation of pectin oligosaccharides in last year. Specific details are:

RESEARCH PROGRESS

1. We screened out a bacterial strain (JCM13288T) belonging to *Bacteroides uniforms* that showed a robust growth on β-glucans such as laminarin, barley β-glucan and lichenan. We subsequently identified β- glucans utilizing locus in the strain that can degrade a diverse range of β-1-3 and alternative β-1-3/1-4 linked polysaccharides. Notably, we identified a porphyran utilizing locus that seems to be transformed from a marine bacterium- such as *Bacteroides plebeius* DSM 17135. Please see figure 1.

2. The locus associated glycoside hydrolases, including an enzyme belonging to newly identified GH family 158, were cloned and functionally characterized. For the first time, GH158 was used to generate several β-1, 3- oligosaccharides. Those were then purified through gel filtration column and characterized by matrix-assisted laser desorption/ionization- electrospray ionization, thin layer chromatography and nuclear magnetic resonance.

3. Those β-1, 3- oligosaccharides were found to promote the growth of several human gut associated bacteria including *Bifidobacteria, Blautia* and *Roseburia*. Overall results of the study indicate that drilling the molecular mechanism of dietary glycans utilizing by the human gut bacteria is crucial for developing nutritional based therapeutic intervention for the better health. This particular finding is published in the Gut Microbes Journal (Singh RP et al. 2020 Gut Microbes. 12:1, 1-18.).

4. We recently identified a novel mechanism for utilization of mixed linked β- glucans via an alternative strategy in the human gut *Bacteroides uniformis* JCM13288T. The *B. uniformis* lacks polysaccharide utilization locus (PUL) archetypal organisation for efficient utilization of pustulan (β1-6 type glucan) and mixed linkage (β1-4/ β1-3) glucans. Particularly, the *B. uniformis* JCM13288T coordinates between PUL associated glycoside hydrolase (GH)-16 and distantly localised bespoke GH 3 (BuGH3MLG) for efficient utilization of mixed linkage (β1-4/ β1-3) glucans. BuGH3MLG found to cleave the β1-4, β1-3 and β1-6 glucan-oligosaccharides. Such promiscuous enzyme is important for industrial purpose. Please see Figure 2.

SALIENT ACHIEVEMENTS

- Different molecular mechanisms for utilizing diverse β-glucans are identified and functionally characterized. Thus, indentifying molecular mechanisms will pave a way to invent new nutraceutical strategies to purposefully manipulate the microbial composition to improve health.
- Different β- 1-3 glucan-oligosaccharides are generated by enzymatic method for highlighting importance of the enzyme for commercial purpose

ACTIVITIES FOR NEXT YEAR

- Molecular mechanism of β- glucans depolymerization by *Blautia* strains will be determined. Some of enzymes involved in this mechanism will be used to generate short to mid-ranged β- 1-3 glucans.
- Different pectin will be extracted from citrus fruits and will be chemically characterized.

1.7 Production of high value products viz. nutraceuticals and therapeutic proteins using sustainable algae system

PRINCIPAL INVESTIGATOR
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RESEARCH FELLOW
Lata Pawar

The therapeutic protein-based drugs are an important class of medicines and they currently have privilege of unprecedented recognition for their therapeutic potential to treat a wide variety of fatal diseases, including cancers, acute infections, genetic disorders, etc. Therefore, the development of therapeutic protein based drugs is one of the fastest growing pharmaceutical sectors in health-care industry. In order to develop a low cost protein expression platform, microalgae are the ideal candidates which are often termed as "solar powered protein factories". The robustness of chloroplast of *Chlamydomonas reinhardtii*, eukaryotic green algae, as protein production platform has been demonstrated to produce wide range of recombinant proteins. Apart of tremendous cost advantage, production of therapeutic proteins in algae has several other advantages over traditional mammalian expression system, such as high scalability, absence of viral and other pathogens, scope for oral delivery (as algae are placed in GRAS category) and production of prokaryotic toxins which would otherwise be not possible in other eukaryotic host. In addition, the algae are the rich source of other value-added products. The concept of algal biorefinery to produce multiple products from a single alga is now gaining importance. Economic viability of the algal technology can be further improved if multiple products are extracted from the algal biomass. Algal polysaccharides exhibit many industrially and pharmaceutically important activities, and get secreted into the growth medium, which leaves the biomass available for extraction of other products. In this regard, we tried to extract and characterize the exopolysaccharide from the algae isolated from the barely explored high altitude lake of the Himalayan region. The successful implementation of the research idea has significant extrapolation in developing platform for the production of prebiotics and vaccine, in addition to the other by-products with commercial importance.

RESEARCH PROGRESS

1. Humanized antibody production in algae: Construct preparation and chloroplast transformation: For the proof of concept to produce protein of interest in algae, amino acid sequence of antibody Cetuximab, used for the treatment of squamous cell carcinoma, was retrieved from drug bank (www.drugbank.ca/drugs). The coding sequence was optimized according to the codon usage of *Chlamydomonas reinhardtti* chloroplast (www.kazusa.or.jp/codon). The codon optimized gene was fused genetically to Exotoxin A to express Ab-toxin conjugate (Fig. 1). A chloroplast transformation vector has been constructed to target the psbA locus as site of transgene integration. The psbA flanking sequence was used for homologous recombination for transgene integration. The constructed vectors have psbA gene under different promoters and UTRs, for the restoration of photosynthetic growth in CC4147 strain of *C. reinhardtti* (a non-photosynthetic psbA deletion mutant) and for the selection of positive transformants (Fig. 2). The chloroplast transformation was performed using plasmid coated gold particles on biolistic PDS-1000/He particle delivery system. The Chloroplast of *C. reinhardtti* has been successfully transformed as confirmed by successful rescue of photosynthetic growth as selective marker. The positive transformants has been selected for homoplasmicity after eight successive generation. The chloroplast transformation was performed using plasmid coated gold particles on biolistic PDS-1000/He particle delivery system. The Chloroplast of *C. reinhardtti* has been successfully transformed as confirmed by rescue of photosynthetic growth as selective marker. The positive transformants has been selected for homoplasmicity after eight successive generation.

Protein expression analysis: The positive transformed and untransformed control was grown under low light conditions and samples were extracted at different time intervals for protein analysis. The western blot analysis showed the expression of protein of interest using anti-strep-II tagged antibody. The further attempts are being under progress to enhance the expression of transgene by using different regulatory elements.

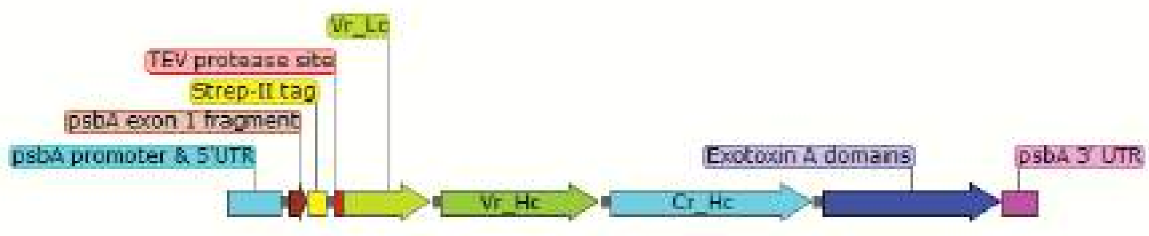


Figure 1. The gene of interest fused to psbA promoter and UTRs.

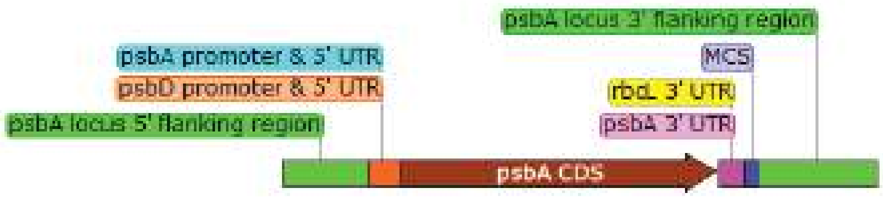


Figure 2. Representative picture of chloroplast transformation vectors designed with endogenous *psbA* gene under different promoters (either *psbA* or *psbD*) and UTRs (either *psbA* or *rbcL*).

2. Exopolysaccharide extraction and characterization:

Algal isolation and identification: The microalgae were isolated from the water samples of high-altitude brackish water lake of the north western Himalayas. The microalgae were purified by repeated colony isolation and the colonies with slimy appearance were then selected for further experiments. The screening of the high EPS algae isolates was carried out by growing them in liquid medium for 10 days in shake flask at 100 rpm and the secreted EPS was measured by phenol sulfuric acid method. The high EPS producing algal isolate was then identified through 18S rRNA gene.

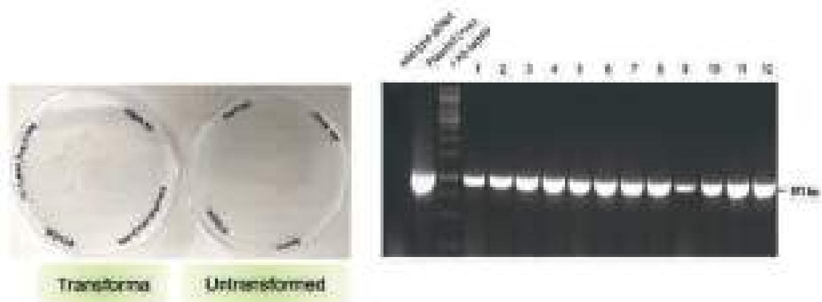


Figure 3. The image showing appearance of photosynthetic active positive transformants on minimal media after DNA coated gold particles bombardment, while the control plate shows no growth on minimal media. The gel images showing PCR based selection of transformed homoplasmic lines and integration of gene of interest.

Media optimization for enhanced EPS production: The media optimization was performed using Plackett-burman statistical designs. The 12-run design with made it possible to gain an insight to the individual main effect of each medium component viz. NH_4Cl, $CaCl_2$, $MgSO_4.7H_2O$, K_2HPO_4, CH_3COOH and Hunter trace elements. Among all the medium constituents, NH_4Cl and CH_3COOH were shown to have strong positive effect. Further to optimize the concentration of two major factors, viz. NH_4Cl and CH_3COOH, the response surface methodology (RSM) with central composite design (CCD) having 5 center points was employed to attain maximum precision in final optimized values (Fig. 5). Final composition of the optimized medium was 480.3 mg/L 20 mg/L - $CaCl_2.2H_2O$, 100 mg/L - $MgSO_4.7H_2O$, 600 mg/L - K_2HPO_4, 1.29 ml/L - CH_3COOH and 1.0 ml/L HTE. Optimized medium showed an increase in the production of EPS to 2.17-fold from 212 mg/L in basal media to 460 mg/L in optimized medium.

3. Chemical characterization of EPS:

Monosaccharide analysis of EPS was performed by using GC-MS analysis of BSTFA + 1% TMCS derivatized carbohydrate samples. The galactose (33.43%), glucose (22.1%) and mannose (15.84%) were the most abundant monosaccharide species, while the least abundant were galacturonic acid (1.34%) and galactosamine (0.93%). The 1H NMR showed typical signals of polysaccharide in the range of 1.0 to 5.5 ppm. The characteristic peaks for the polysaccharides are in the regions between 1.0 ppm to 4.5 ppm and indicate the presence of different functional groups in the isolated EPS (Fig. 6a). In FT-IR analysis of the isolated EPS(Fig.6b), a broadly stretched peaks at 3090 cm-1 suggests the presence of free hydroxyl group (-OH). As evidenced by -OH stretching, EPS form strong interaction with water molecule, thus explaining high viscosity of

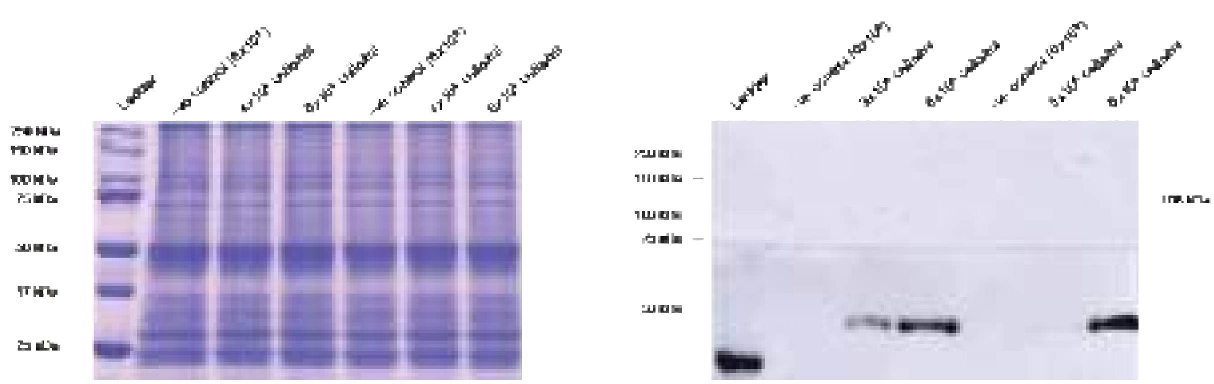


Figure 4. The gel images showing analysis of protein of interest. The SDS-PAGE gel showing total protein of the cells harvested at different cell densities (the equal cell numbers were lysed and equal protein were loaded in each well). The western blot image of corresponding SDS-PAGE gel showing detection of protein of interest (Ab) through HRP conjugated anti-Strep-II tag antibody.

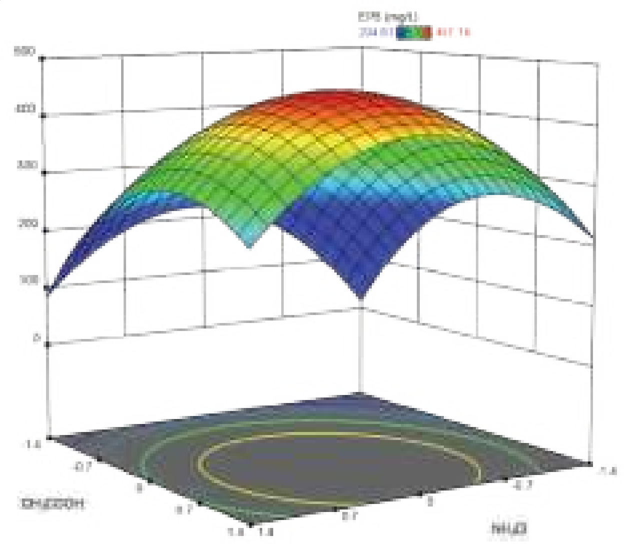


Figure 5. 3D response surface plot showing the effect of NH4Cl and CH3COOH concentration on EPS production.

aqueous solution of isolated EPS. The presence of sulphate group is indicated by the presence of S=O stretching vibrations at 1033 cm-1. In addition, the other peaks in the 700-1400 cm-1 region display the characteristic absorption of the polysaccharides and signifies the vibration of C-O-C glycosidic linkage and the stretching vibrations of C-O-H side groups.

5. Physical characterization of EPS: The molecular weight of the EPS was determined through gel permeation chromatography (Fig. 7a). The size exclusion chromatographic and MALS analysis showed that the EPS had an average Mw of about 1.52×10^5 Da. The values of Mn and Mz were 6.57×10^4 Da and 3.00×10^5 Da, respectively, with the polydispersity of 2.315 (Mw/Mn) (Fig. 7b). The rheological characteristics of the isolated EPS were investigated in aqueous solution at four different EPS concentrations, and the results indicate the non-Newtonian nature of the EPS aqueous solution (Fig. 8a). The EPS isolated in the present study was able to retain emulsion as; 98.1% and 93.6% in almond oil (polarity index 20.3), 95.2% and 88.8% in xylene (polarity index 5.3), 90.9% and 82.8% in toluene (polarity index 5.2), 88.7% and 84.1% in decane (polarity index 4.4) and 81.1% and 68.5% in n-hexane (polarity index 4.3) after 15 min and 30 min, respectively (Fig.8b).

6. Gold Nanoparticles (AuNPs) synthesis and characterization: We used the isolated EPS for a single pot green-synthesis of AuNPs and analyzed them for their stability. We observed a well-defined absorbance band for citrate- and polysaccharide-capped AuNPs with peak maxima at 521 and 525 nm, respectively. The size and morphological parameters of the synthesized AuNPs was analysed using TEM. In both the cases, almost spherical shaped particles were obtained with size range of 10-15 nm (for citrate-AuNPs) and 30-40 nm (for polysaccharide-AuNPs) (Fig. 9a and 9b). We also analyzed the stability of synthesized AuNPs at pH range from 2-12. Excitingly, the polysaccharide-AuNPs displayed exceptional stability at a range of pH (2-12) without any signs of aggregation (Fig. 9c and 9d). The colour of citrate-AuNPs was changed at 12 as well as 2 pH, while no obvious colour change was observed for polysaccharide-AuNPs at pH 2, 6, 8, 10, and 12 (Fig. 8c and 8d). This confirmed that the citrate-AuNPs were not capable to withstand pH changes, while the surface charges and stability was maintained in case of polysaccharide-AuNPs. The stability of AuNPs in solution with higher ionic strength is also equally important. Interestingly, the polysaccharide-AuNPs can withstand the ionic concentration of 750 mg/mL NaCl, whereas the citrate-AuNPs got aggregated even with the 25 mg/mL of NaCl. The absorbance spectrum of citrate-AuNPs was shifted immediately after the addition of 25 mg/mL NaCl. In contrary, the polysaccharide-AuNPs was stable even in presence of 750 mg/mL NaCl (Fig. 9e and 9f). The AuNPs are one of the most explored metal nanoparticles that exhibit diverse technological applications including but not limited to drug delivery (Bansal et al. 2020), catalysis (Doan et al. 2020), energy storage (Dong et al. 2020), and pollutant removal (Chatterjee et al. 2020). Interestingly, in majority of aforementioned applications, the AuNPs should be able to tolerate a range of conditions (e.g., varying

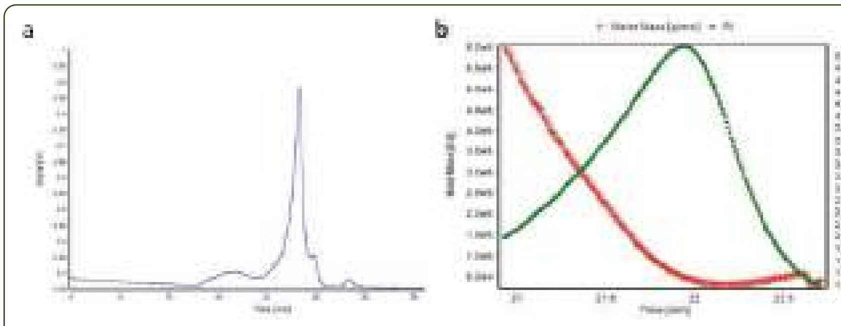


Figure 6. Chemical characterization of isolated EPS

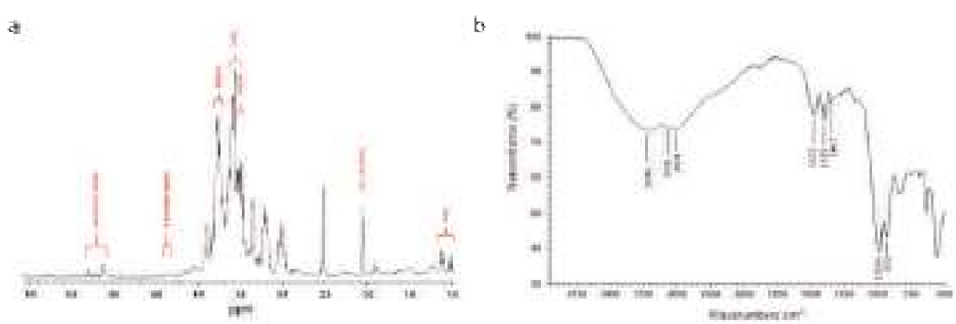


Figure 7. The molecular mass determination of isolated EPS using GPC.

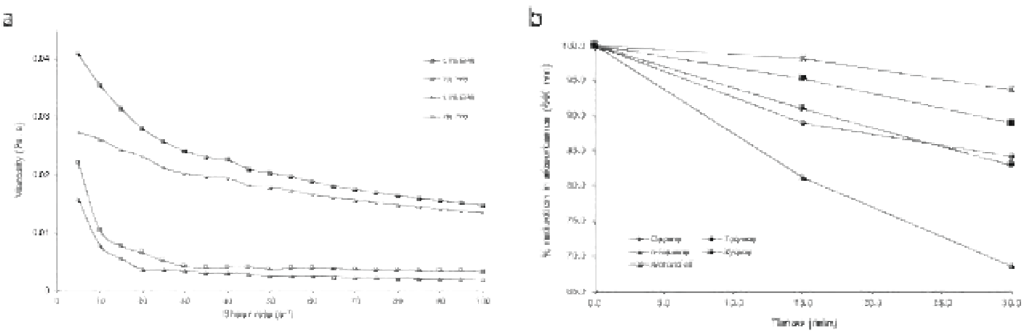


Figure 8. The rheological characteristic and the emulsification assay of isolated EPS.

pH and ionic strength) for real life applications. For such cases, AuNPs with extended stability capabilities is needed to realize its usefulness for all type of experimental conditions. It is evident from our results that the polysaccharide-capped AuNPs exhibited far superior stability as compared to that of most commonly used citrate-capped AuNPs. The isolated EPS gives one pot simple approach to synthesize AuNPs with advantages such as (1) exceptionally improved stability in harsh conditions, (2) biocompatibility due to polysaccharide capping, and (3) controlled size range.

SALIENT ACHIEVEMENTS

- Vectors and transformation constructs have been successfully prepared for the Chlamydomonas reinhardtii chloroplast transformation.
- The exopolysaccharide from high altitude algal species has been characterized and its application in green synthesis of nanoparticles is demonstrated.

ACTIVITIES FOR NEXT YEAR

- Deciphering the role of rice hybrid proline rich protein in systemic acquired resistance.

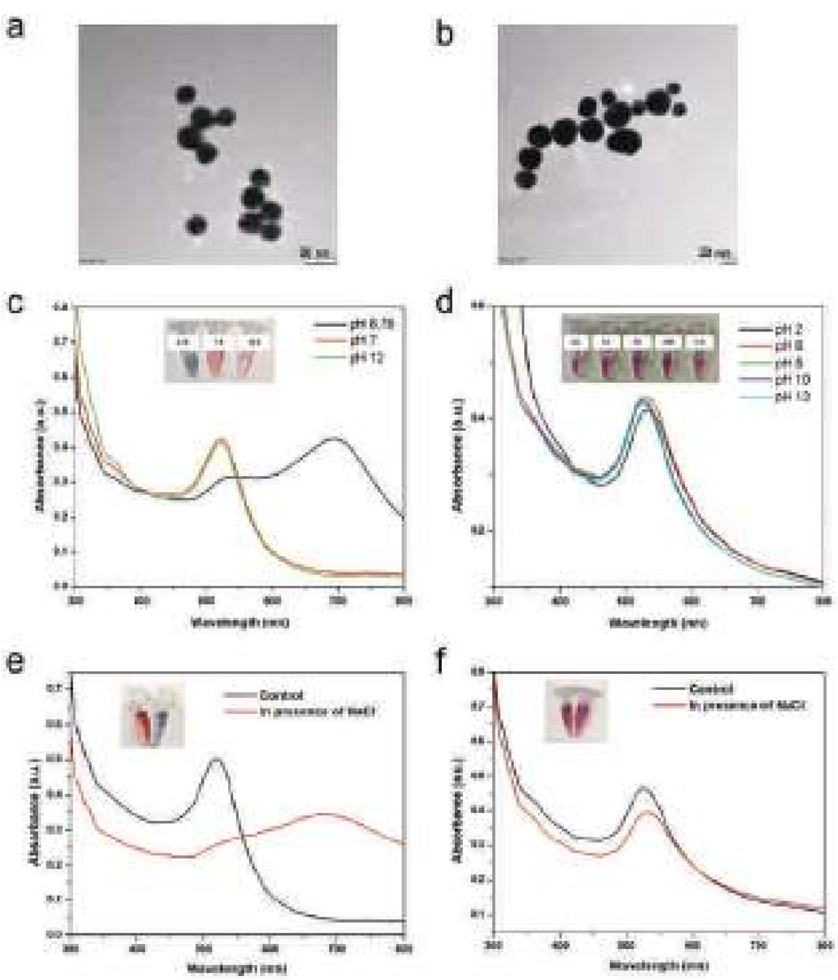
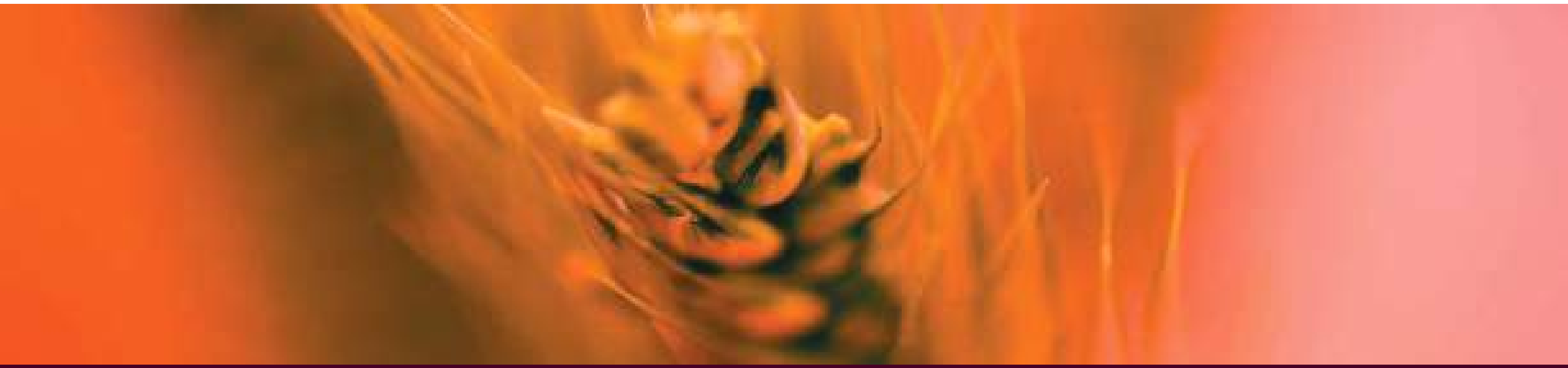


Figure 9. The rheological characteristic and the emulsification assay of isolated EPS.

PROGRAM - FNB 02

FOOD & NUTRITIONAL BIOTECHNOLOGY (FNB)

FOOD AND GM CROPS BIOSAFETY



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

PRINCIPAL INVESTIGATOR
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RESEARCH FELLOWS
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Ritika Gupta
Sunaina Kaul
Priyanka Garg

According to the World Health Organization (WHO) reports, the presence of bacteria, viruses, and parasites in food are responsible for diseases ranging from Diarrhoea to Cancer. For 40% of foodborne diseases, Carriers are children under 5 years, with annual deaths reported to be 1, 25, 000. Major causes of food and waterborne disease are bacterial contamination with the growth of *Salmonella, E. coli, Campylobacter, Listeria* and *Vibrio cholera* etc. Productivity loss of US$ 95.2 billion has been estimated by World Bank, 2018 in low and middle-income countries. While cost for treating these illnesses have been accounted for an annual amount of US$ 15 billion. To maintain the balance between healthy lives and monetary productivity, it is essential to develop a technique for bacterial detection. The traditional plate counting technique takes approximate 18 hours for bacterial load detection. Sensitive techniques such as Fluorescence in situ Hybridization (FISH), quantitative Real-Time PCR (qRT-PCR), Surface Enhanced Raman Spectroscopy (SERS), Enzyme-Linked Immuno-Substrate Assay (ELISA) and Electrochemistry have come up as detection tools. These techniques though sensitive, are expensive and require skilled workers for their operation. Quick identification of bacterial species in food is a proximate requirement for ensuring food safety. Progression in nanotechnology has empowered to incite new diagnostic tools for sensitive and fast discovery of pathogens and toxins. Nanomaterials like gold nanoparticles (AuNPs), gold nanostars (AuNst), graphene oxide (GO) have unique optical properties and surface chemistry which make functionalization very convenient. We have developed carbohydrate, aptamer, and gold nanostars-based biosensors against different foodborne pathogens to increase sensitivity and selectivity. These approaches will contribute to the development of new foodborne pathogen biosensors.

RESEARCH PROGRESS

Carbohydrate based biosensors: (a) The current approach critically compares the affinity of AuNPs for bacteria/lectin detection based on different sizes of AuNPs, length of Poly (ethylene glycol) 2-mercaptoethanol ether acetic acid (PEG), and diversity of glycans. Here, AuNPs were synthesized by varying the ratio of gold to citrate. Two different sizes of AuNPs were synthesized respectively of 20 nm and 40 nm. Two AuNPs were stabilized by functionalization with PEG with two different chain lengths, i.e., PEG_{1000} & PEG_{5000}. Further, synthesized glycans like mannose monopod (MM), mannose tripod (MT), galactose monopod (GM), galactose tripod (GT) were functionalized onto the surface of pegylated AuNPs to design novel biosensors for specific bacterial detection. We have successfully synthesized multivalent carbohydrates. The amine-terminated mannose and galactose monopod was achieved by modifying the anomeric position of the mannose and galactose respectively, and respective tripod glycan was synthesized by click chemistry between synthesized glycan azides and alkyne. Further, synthesized glycans were functionalized onto the surface of pegylated AuNPs (20 nm and 40 nm) to design novel biosensors for specific bacterial detection. The resultant nanoglycoclusters showed significant binding with respective bacteria/lectins. The tripod glycan showed higher affinity compared with the corresponding monovalent ligands. Mannose tripod showed a significant increase in binding with Concanavalin A (ConA) and *E.Coli* concerning its corresponding monopod. The resultant nanoglycocluster was incubated with specific and nonspecific bacteria to observe specific aggregation under a transmission electron microscope (TEM). The synthesized nanoglycocluster were tested with different foodborne bacteria, proving the specificity of mannose for *E.Coli* and galactose for *L.monocytogenes*. Since receptor for galactose is peanut agglutinin (PNA) type of lectin which is present on the surface of *L.monocytogenes*. The probe was also used to detect the presence of *E. coli* in real-time samples (meat).

(b) Another approach was used to design a fluorescence-based bacterial detection system by preparing fluorescent silica-coated gold nanoparticles (SCGNPs). Fluorescent nanoparticles were synthesized by conjugating tetra carboxy phenyl porphyrin (TCPP) with silica-coated gold nanoparticles. Incorporating porphyrin with SCGNPs exhibits longitudinal surface plasmon resonance in the NIR region and higher fluorescent intensity, then used to detect bacteria in food samples. Fluorescent nanoparticles will further be functionalized with multivalent carbohydrates by which nanoparticles can bind to bacteria via carbohydrate-lectin interaction. Multivalency further increases the sensitivity as it elevates the binding efficiency of nanoparticles with bacteria. In addition, the synthesized nanoparticles, when they absorb light, it releases heat in the vicinity. Taken together, this method provides microscopic and spectroscopic detection and ultimately killing of bacteria. This study will confirm the effect of nanoparticles modified with site-directed recognition elements on bacterial culture and real samples.

Citrate capped gold nanoparticles were prepared and characterized by UV-Vis spectroscopy for particle confirmation, dynamic light scattering (DLS) for charge and hydrodynamic size, and TEM (transmission electron microscopy) for external morphology. Further, the prepared gold nanoparticles were coated with a silica layer. Around 35-50 nm of the thickness of the silica coat was observed in TEM. With silica coating, it has been seen that the increase in hydrodynamic size was also observed with MPTPMS coating. Furthermore, the charge of particles observed was -39.93 mV as silane groups mask the exposed negative citrate groups of gold nanoparticles. Furthermore, to conjugate TCPP onto silica-coated gold nanoparticles via EDC/NHS chemistry, PEI was used. PEI attachment leads to red shifting of absorption maxima. UV spectra showed the successful conjugation of TCPP onto Au@SiO2-PEI particles. Absorption maxima at 441nm and four peaks in the range of 500-700 nm in Au@SiO2-PEI-TCPP represent a short band and four Q bands of TCPP. After PEI attachment, zeta potential changed from negative to positive. The positive charge represents amino groups on the surface of Au@SiO2-PEI, which further was exploited to link carboxyl groups of TCPP. The negative charge of Au@SiO2-PEI-TCPP represents free carboxyl groups of TCPP. From fluorescence spectra, it has been observed that Au@SiO2-PEI-TCPP particles could excite at 600nm and emit at 653nm and 718nm as free TCPP. This probe would be further utilized to conjugate specific multivalent sugar such as galactose and mannose for specific detection of bacteria.

Aptamer based biosensor: Aptamer (ssDNA molecule) based detection has gained interest due to its simple, cost-effective synthesis, high specificity, and sensitivity. We synthesized aptamers against the *E.coli* by cell-SELEX (Systematic evolution of ligands by exponential enrichment) method. The labeled aptamer was conjugated to graphene coated gold nanoparticles. This probe was used for the colorimetric detection of E. coli. Furthermore, this proposed sensor will be fabricated as a convenient tool to detect multiple bacteria (multiplexing) as a kit. The best selected aptamer was used that sensitively and specifically identify *E.coli*. Thus, exploiting the AuNPs property, a colorimetric nanosensor was designed. To detect *E.coli*, cell dilution from 10^8 cells/mL to 10^1 cells/mL was taken. Aptamer nanoprobe complex bind to the target cells decreasing the distance between Gold nanoparticles, leads to aggregating of the particles. A visible color change of AuNPs from red to blue on aggregation was observed. However, with GO coated CTAB-AuNPs, the limit of detection increase upto 10^2 cells/mL visually and 10 cells/ml by UV-Vis Spectroscopy. The assay was also performed in a glass capillary. Nanoprobe and bacterial cells were added from both the ends, mixed with a micropipette and allowed to react. The reaction leads to visible color change from red to blue with the increase in cell number due to the aggregation of nanoparticles. This capillary method proves this detection procedure could be possible in a microfluidic channel device.

Bacteria sensing using gold nanostars: This study was planned to check the efficiency of the spikes of gold nanostars to lyse and kill the bacteria with further species-specific detection via reaction of released cellular enzymes with specific chromogenic substrates. To check the efficiency of nanospikes (approx. 100 nm), gold nanostars will be used to rupture the bacterial membrane and detect the species corresponding to their respective colored substrates. Gram positive bacteria (*S.aureus*) having a thick peptidoglycan layer of 40-80 nm and Gram negative (*E.coli, S.typhimurium, P.aeruginosa*) possessing a membrane of 7-8 nm will be used as model organisms. In this study, differently shaped gold nanoparticles were synthesized eg. Gold nanospheres, Gold nanostars and characterized via

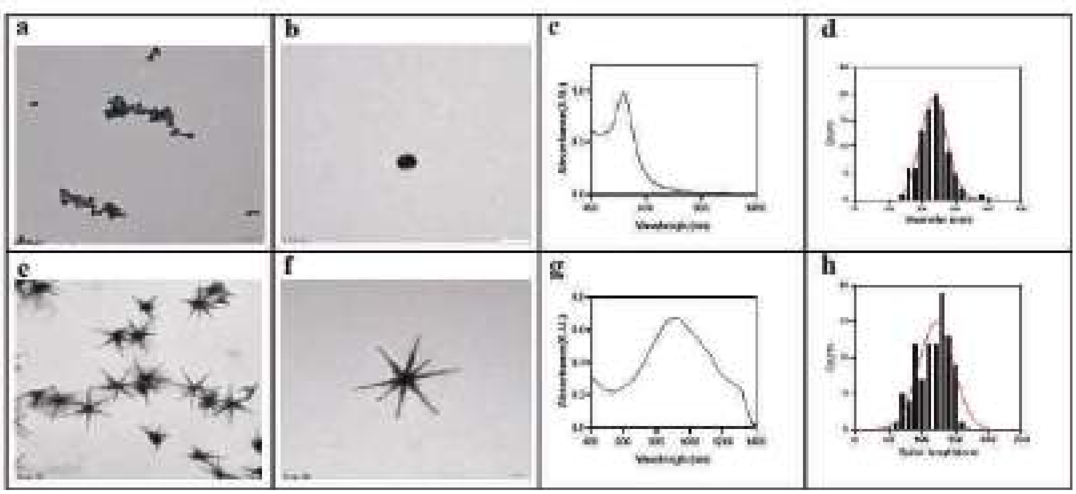


Figure 1. TEM images of gold nanoparticles (a, b) and gold nanostars (e, f). UV spectrum obtained for gold nanoparticles(c) and gold nanostars (g) and size distribution curves of gold nanoparticles(d) and gold nanostars(h).

UV-vis spectroscopy and TEM (Figure 1). The concentration of gold nanoparticles and Triton-X required for the desired bacterial lysis was standardized. Further, the use of nanostars in bacterial detection was accomplished by bacterial lysis. The enzyme released from the bacteria was detected through chromogenic substrates. Yellow and purple were the colours obtained after the chromogenic substrates, p-nitrophenol and magenta caprylate reacted with *E.coli* and *S.typhimurium* lysates respectively.

Bacterial lysates obtained after probe sonicating were first optimized for colour generation by reaction with chromogenic substrates. The substrates used for enzyme assay were taken from the literature. A range of concentrations of substrates 0.25-12mM for p-Nitrophenol for magenta caprylate was used to react with a specific amount of bacterial lysate. Saturation of the absorbance obtained confirmed the substrate concentration required for the desired colour change to be observed.

SALIENT ACHIEVEMENTS

- LOD of bacteria for different glycan ranged from 2.8×10^7 CFU/ml (for MM) to 1.4×10^4 CFU/ml (for MT).
- The presence of *E.coli* was detected in the meat sample up to 10^4 CFU/mL using glycoconjugate gold nanoparticles.
- Successfully detected *E.coli* using Graphene Oxide coated Gold Nanoparticles.
- The designed assay was also found to be successful in a capillary tube that mimics microfluidics devices.
- The successful synthesis and characterization of gold nanostars and nanoparticles with desired aspect ratio were done.
- Significant lysis was observed with a combination of triton and gold nanostars, and species-specific identification was made possible by reaction of chromogenic substrates with bacterial lysates.
- Successful preparation and characterization of silica-coated gold nanoparticles conjugated with TCPP.

ACTIVITIES FOR NEXT YEAR

- Synthesis of multivalent carbohydrates with greater glycan diversity.
- Development of biosensor kit for specific detection of bacteria.
- To develop an assay for multiple bacterial detection.
- To develop a kit for bacterial detection in food samples.
- To study the bacterial killing aspect by gold nanostars.
- To check the efficacy of bacterial killing by gold nanostars on skin based bacterial infections (in vivo).
- Conjugation of multivalent carbohydrates with fluorescent SCGNPs.
- Food borne bacterial detection using the prepared biosensor.

2.2 Development of ultrasensitive, portable, and rapid biosensor for the detection of pesticides and food contaminants using functional hybrid nanostructures

PRINCIPAL INVESTIGATOR
Vanish Kumar

RESEARCH FELLOWS
Kalyan Vaid
Jasmeen Dhiman

The excessive use of chemicals in agriculture and industrial waste release increased the level of several contaminants (e.g., heavy metals and pesticides) in food and water system. Moreover, the toxic chemicals such as pesticides and herbicides are used extensively in agriculture, public areas (to avoid vector-borne diseases), animal husbandry (to pest control), and public aesthetics (e.g., storage facilities and walkways, and parks). The extended use of these materials increased their level in outer environment, food, and water. Moreover, due to use of contaminated raw material and improper production methods the level of food contamination (such as heavy metals, plastics, and microbes) is also increasing. Notably, the level of these contaminants has reached alarming level in many parts of the country. In particular, the increased level of arsenic (As) in water systems poses severe threat to the life of living organisms. The United States Environmental Protection Agency (USEPA) has prescribed a maximum allowable contaminant level of As at 50 ppb in drinking water, further reduced to 10 ppb in 2001. The intake of contaminated products can lead to the occurrence of many complications in human beings including short term and chronic health issues. Unfortunately, due to unavailability of cost effective, sensitive, specific, and user friendly sensing device, it is very difficult for a common man to quantify the level of these contaminants in their daily products. However, high end contaminants testing machinery has been installed in many cities of India but limited awareness and expensive testing charges makes these machinery unavailable for everyone. Herein, to meet the demand of simple, sensitive, specific, portable, and cost effective sensor we used metal organic frameworks for spectroscopic and paper based sensing of arsenic in water and food samples. The Mo in synthesized MOFs can easily show color change with As(V) through the formation of Mo-blue complex that tends to be accompanied by structural alterations in MOF. The synthesized purple colored mixed metal MOF is highly susceptible for Mo-As interactions due to easy availability of high number of Mo. This bimetallic MOF exhibited high sensitivity and specificity by taking imme-

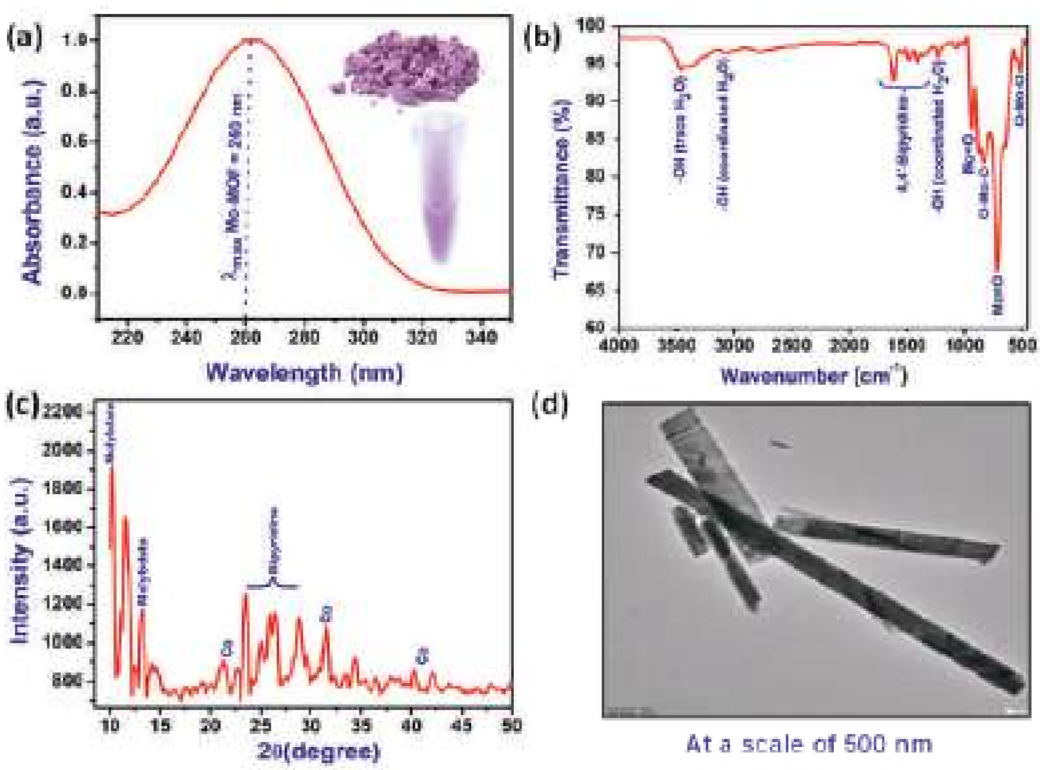


Figure 1. Characterization of synthesized Co/Mo-MOF using (a) UV-vis spectroscopy, (b) FTIR, (c) XRD, and (d) TEM.

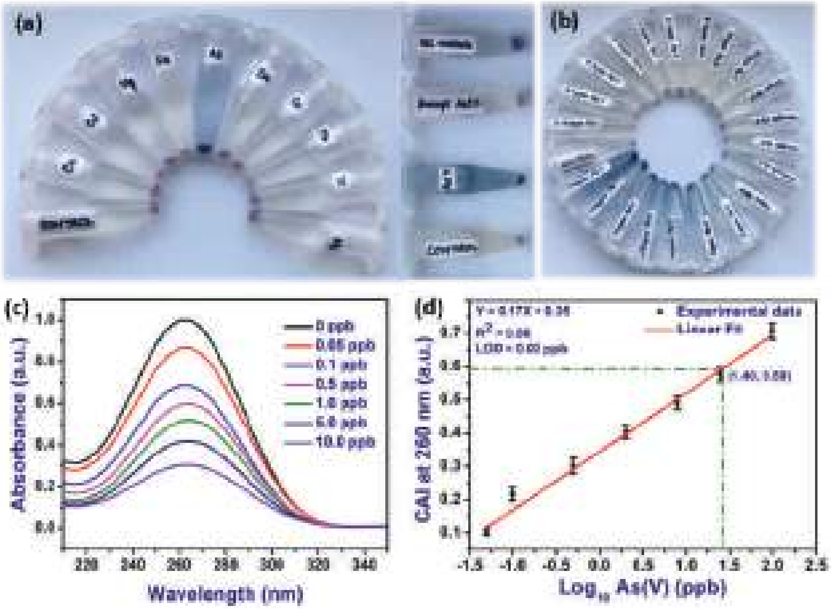


Figure 2. Interactions of As(V) and other metal ions with Co/Mo-MOF (a) Effect of different metal ions on the original color of Co/Mo-MOF, (b) change in the color of Co/Mo-MOF in presence of different concentrations of As(V) (0.05 ppb-500 ppm), (c) UV-vis spectra showing effect of different concentrations of As(V), and (d) linear fit for the calculated absorbance intensities (CAI) at 260 nm of Co/Mo-MOF vs As(V) concentration gradient. The green-colored dotted line shows the measurement of As(V) in spiked ground water sample.

diate color change to blue (with precipitation) in the presence of As(V). We were also able to detect the gross levels of As ions (e.g., as sum of As(III) and As(V)) in aqueous samples.

RESEARCH PROGRESS

1. Synthesis and characterization of mixed metal Co/Mo-MOF: The MOF in the present study was prepared via hydrothermal methodology and characterized with several techniques including, TEM, XRD, and UV-vis and FTIR spectroscopy (Figure 1).

2. Spectroscopic sensing of arsenic: The synthesized MOF was mixed with the arsenic containing solutions, which led to the development of blue color due formation of Mo:As complex. The developed sensing probe is very specific and sensitive for the arsenic detection (Figure 2).

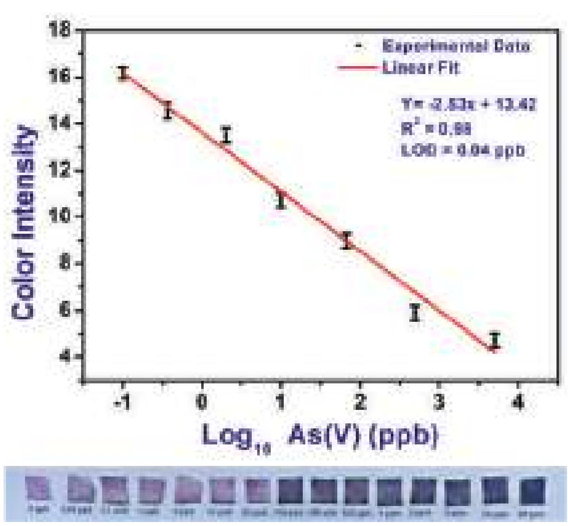


Figure 3. The effect of different concentrations of As(V) on the color/color intensity of Co/Mo-MOF impregnated paper sensors.

3. Paper sensor for arsenic: The developed MOF was then coated on the paper for the fabrication of paper-based sensing strip. The paper sensing strip changed its color from purple to blue in presence of arsenic only in 15 min. The change in the color intensity can be monitored through naked eyes/dedicated instruments/smartphone applications. The developed sensor is highly sensitive and can detect arsenic lower down to 0.002 ppb (by spectroscopic methodology) and 0.004 ppb (through paper sensing strip), respectively (Figure 3).

SALIENT ACHIEVEMENTS

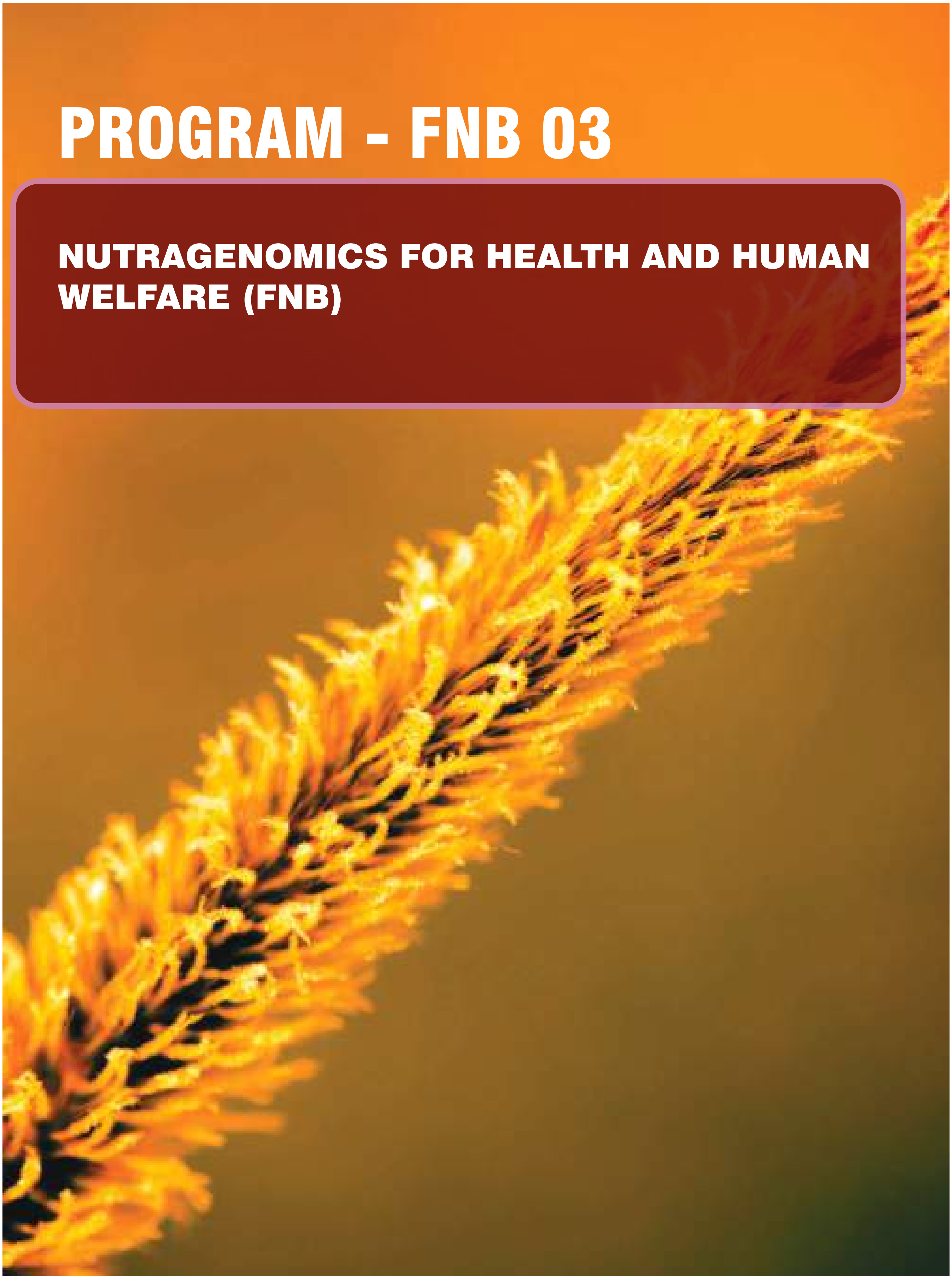
- The developed arsenic sensing probes are highly sensitive, selective, and simple (one step procedure).
- The spectroscopic and color-based sensing probe can detect arsenic lower down to 0.002 and 0.004 ppb, respectively in water and food samples.
- The color changing capacity (from purple to blue) of Co/Mo-MOF works well for a linear range of As(V) ranging from 0.05 ppb to 1000 ppm.

ACTIVITIES FOR NEXT YEAR

- The gold nanoparticles/polyaniline, graphene, and metal organic frameworks will be used for the development of electrochemical sensors for heavy metal contaminants and pesticides.

PROGRAM - FNB 03

NUTRAGENOMICS FOR HEALTH AND HUMAN WELFARE (FNB)



3.1 Role of DNA methylation in preadipocyte differentiation and its modulation by nutritional intervention

PRINCIPAL INVESTIGATOR
Vikas Rishi

RESEARCH FELLOWS
Binduma Yadav

Recently, the world is in the grip of the growing obesity epidemic. The number of people with obesity, and its associated co-morbidities has soared leading to dire health consequences with a critical challenge to the healthcare system. Although associations between DNA methylation and gene expression were established four decades ago, the causal role of DNA methylation in gene expression remains unresolved. Different strategies to address this question were developed; however, all are confounded and fail to disentangle cause and effect. This study proposes the role of DNA methylation per se in the differentiation of 3T3-L1 cells that will help in exploring the true extent, nature, and diverse contribution of DNA methylation at different genomic regions/hotspots. DNA methylation is broadly involved in transcriptional regulation across a vast number of physiological and pathological conditions. For nearly half a century, it has been widely documented that the presence of methyl groups on the fifth carbon of cytosines in the context of CpG nucleotides within promoters is associated with transcriptional repression. In a previous study, it was proposed that transcription factors operate in complex networks through thousands of genomic binding sites, many of which bind several transcription factors and thus are hotspots. The extent and mechanisms of crosstalk between the transcriptions factor at the hotspots remain unclear. We are trying to explore the methylation-dependent binding of a transcription factor at the hotspots. With the combination of advanced proteomics and genomic approaches, we will explore how cooperativity between transcription factors at the level of hotspot enhances their activity and reprogramme transcription phenomena in a methylation-dependent manner. McrBc restriction enzyme digestion of 3T3L1 genomic DNA demarcate that global CpG methylation status increases as the cell differentiation progresses. The overexpression vector used in this study increased the expression of proteins manifold compared to other commercially available vectors.

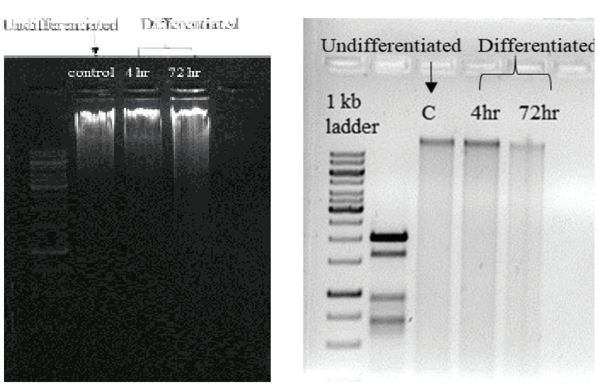


Figure 1. Genomic DNA extraction from un- Differentiated and differentiation of 3T3L1 cells.

RESEARCH PROGRESS

NIH 3T3-L1 pre-adipocytes cells differentiate into adipocytes when exposed to a mix of three hormones i.e., 3-iso-butyl-1-methylxanthine, dexamethasone, and insulin. Differentiating cells are characterized by accumulating fat. It is a well-characterized system often used to study gene regulation networks during adipogenesis. Although adipogenic transcription factors (TFs) are well-known studies involving methylation-dependent DNA binding activities of these TFs are lacking. DNA methylation is characterized by the addition of methyl group to cytosine in CpG dinucleotide. This chemical event precludes the binding of many TFs to the promoter of the genes. Therefore by measuring the methylation status of a genome one can have an insight into gene regulation associated with methylation. To address if the global methylation status of the genome changes when 3T3 cells differentiate, we used the McrBc restriction enzyme. McrBc digest only methylated genome and is used in screening the global methylation status of a genome.

SALIENT ACHIEVEMENTS

- McrBc digestion of genome DNA of NIH 3T3-L1 cells shows massive methylation after 72 hrs of differentiation induction suggesting a direct link between methylation and differentiation.

ACTIVITIES FOR NEXT YEAR

- In the future high-resolution bisulfite sequencing will reveal the role of individual cis-element methylation status on TF binding and differentiation.

3.2 Recombinant production of omega-3 polyunsaturated fatty acids of bacteria from high altitude lakes of Indian Himalayas

PRINCIPAL INVESTIGATOR
Hena Dhar

RESEARCH FELLOWS
Varnika Rana
Anchal Chaudhary
Rishav Sharma

Health promoting omega-3 polyunsaturated fatty acids (ω3LC-PUFAs) including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are primarily available from fish and fish oil. Other than this traditional source, microbes from cold and marine environments or from gastrointestinal tracts of fish could be developed as the sustainable and economical source of these health beneficial ω3LC-PUFAs. Moreover, microbial oil has advantage over fish oil due to growth of microbes over broad range of substrates, its cultivation under controlled conditions and easy manipulation for enhanced production. Bacteria synthesize ω3LC-PUFAs *de novo* via anaerobic polyketide synthase (PKS) pathway unlike aerobic pathway which is basically found in animals and some eukaryotic microorganisms for synthesis of long chain fatty acids from short chain fatty acids using desaturase and elongase enzymes. Such bacteria are generally psychrotrophs and peizophiles, therefore, ω3LC-PUFA production is thought to be associated with adaptation mechanism to thrive in cold, saline and high pressure environment. In view of this, these physiological conditions along with manipulation of media components like carbon and nitrogen sources could be useful in enhancing the EPA production. Accordingly, the study aims at optimizing the culture conditions including temperature, salt concentration, growth time, and media components in order to enhance the production of EPA from a bacterium isolated from gastrointestinal tract.

RESEARCH PROGRESS

Omega-3 polyunsaturated fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to be associated with several health benefits. In order to develop a sustainable source of these beneficial fatty acids other than the traditional source fish which has several limitations, a number of EPA-producing isolates were isolated from the gastrointestinal tract of various freshwater fishes as well as water and sediment samples of high altitude lakes of Indian Himalayas. In total, 15 bacterial isolates capable of producing EPA obtained from the gastrointestinal tract of various freshwater fishes were selected for further studies. All of these bacteria belonged to various species of the genera *Shewanella* based on 16S rRNA gene sequencing. These bacteria were then characterized based on various morphological, physiological and biochemical parameters. All of these isolates were Gram-negative rods, motile and facultative anaerobes. The isolates showed variation in their enzyme production profile- only seven could produce arginine dihydrolase and amylase, five were found positive for gelatinase test, while, only three showed protease production. Whereas, all were capable of producing lipase enzyme but

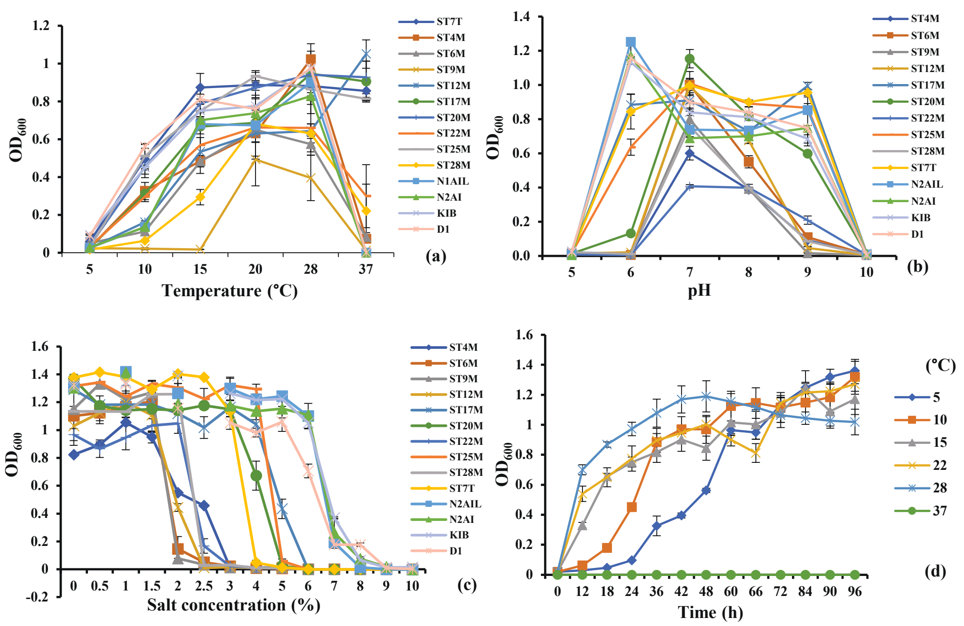


Figure 1. Effect of pH (a), NaCl concentration (b) and temperature (c) on cell growth of EPA producing Shewanella spp. Growth curve (d) of an EPA producing strain Shewanella sp. KIB at different temperatures.

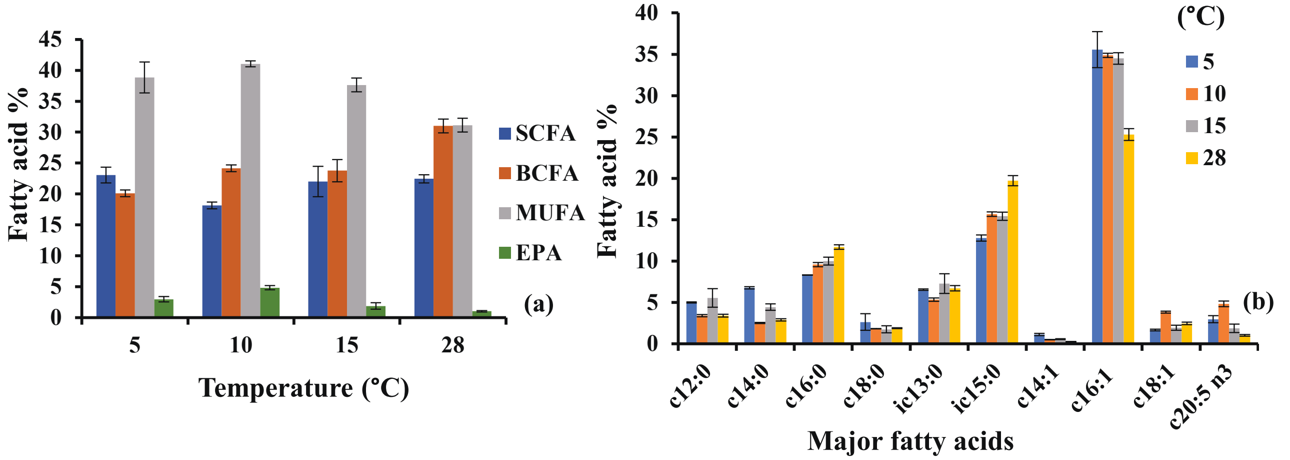


Figure 2. Relative whole cell fatty acid proportion (a) and major fatty acids (b) of strain KIB grown in temperature range of 5-28°C in Luria Bertani broth medium. Straight chain fatty acids (SCFA), branched chain fatty acids (BCFA), monounsaturated fatty acids (MUFA), eicosapentaenoic acid (EPA).

none of them showed cellulase activity. All of the isolates were psychrotrophs as they could grow in the temperature range of 10-28°C, while four isolates could grow at 5°C also and six could grow up to 37°C (Figure 1a,d). Five isolates could grow in narrow range of pH 7-8, seven in pH range of 6-9, while the remaining three could grow in pH range of 7-9 (Figure 1b). All of the isolates could grow well in the absence of NaCl, among which four were able to grow up to 6% salt concentration (Figure 1c). Further, fatty acid profile of these isolates at 15°C in Luria Bertani medium showed EPA-production ranging from 0.05% to 4.1% of total fatty acid (TFA). Among these, the selected five isolates tested for EPA-production at different tem-

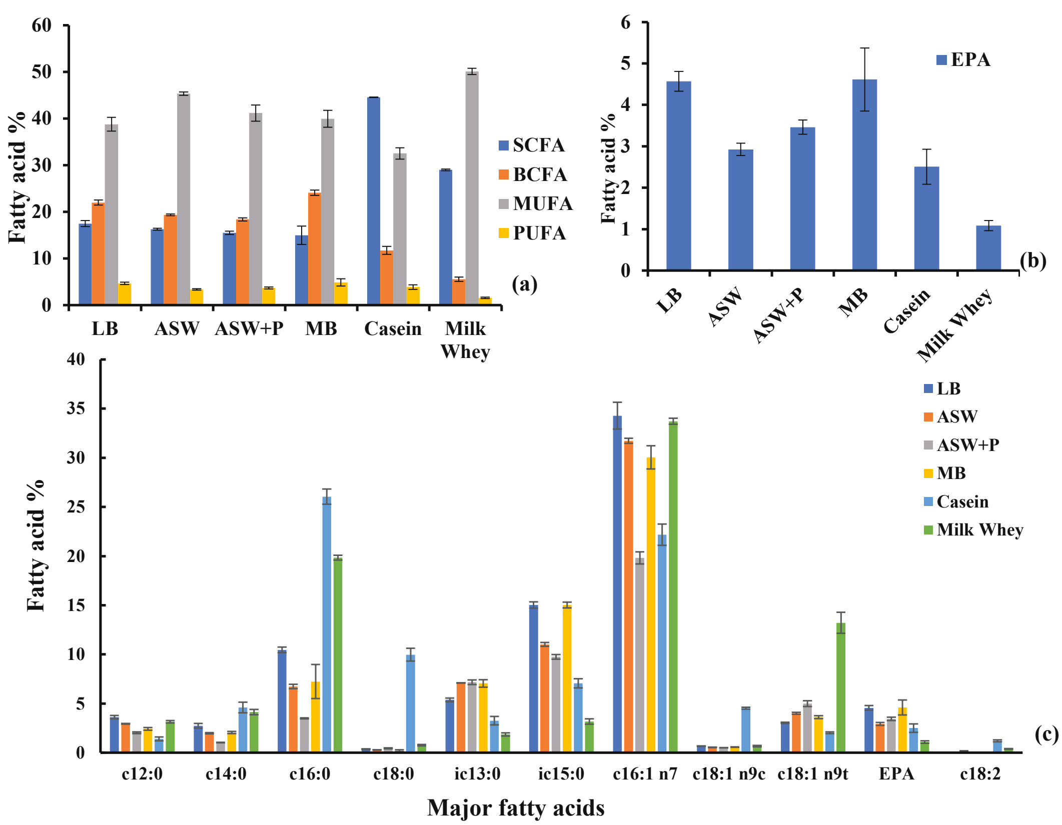


Figure 3. Effect of various media components on whole cell fatty acid composition (a), EPA production (b) and proportion of major fatty acids (c) of Shewanella sp. KIB. LB: Luria Bertani broth, ASW: artificial sea water broth, ASW+P: artificial sea water plus proline, MB: Zobell's marine broth, casein: Casein marine broth and Milk whey: Milk whey marine broth. SCFA: Straight chain fatty acids, BCFA: branched chain fatty acids, MUFA: monounsaturated fatty acids, EPA: eicosapentaenoic acid .

peratures showed increase in EPA-production from 0.649-1% to 3.7-4.8% of TFA when temperature decreased from 28°C to 10°C. Further, optimization of EPA-production of one of the selected bacterium Shewanella sp. KIB was carried out using different media components and at different physiological conditions. For this strain, the major fatty acid was 9-hexadecenoic acid (c16:1 n7) followed by iso-pentadecanoic acid (ic15:0) and hexadecanoic acid (c16:0) at all the temperatures. Increase in temperature from 10 to 28°C led to increase in both branched chain fatty acids (BCFA) and straight chain fatty acids (SCFA), while the percentage of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) get decreased. EPA (c20:5 n3) was found to increase in proportion from 1% to 4.8% with decrease in temperature from 28°C to 10°C along with decrease in percentage of total saturated fatty acids particularly the major fatty acids c16:0 and ic15:0 (Figure 2). In contrast, among monounsaturated fatty acids (MUFAs), the major proportions c16:1 n7 and c14:1 (tetradecanoic acid) were found to increase with decrease in temperature, while, other MUFAs get decreased. Various media compositions were used to elucidate the fatty acid composition of strain KIB at optimum temperature of 10°C. The maximum production of EPA was found in complex media MB and LB from 3.5 to 6 mg g-1, while the bacterium could produce EPA in the range of 0.3-2.3 mg g-1 in the other studied media. In addition to the complex hydrolysed components like peptone, yeast extract and tryptone, some raw components like milk whey and casein were also used to check the fatty acid composition of the strain KIB. The replacement of milk whey in milk whey marine broth in place of peptone and yeast extract in MB, led to increase in both SCFA and MUFA particularly c16:0 and c18:1 n9t, but decrease in BCFA particularly ic13:0 and ic15:0, and EPA (Figure 3a,b). Whereas, in case of casein marine broth, where unhydrolyzed casein was used in place of peptone in MB, SCFA were substantially increased particularly c18:0 and c16:0, but the other fatty acids including BCFA, MUFA and EPA were decreased (Figure 3a,c). Interestingly, the bacterium could produce increased proportion of c18:1 n9c, c18:2 and c18:0 in casein marine broth in contrast to almost negligible production of these in other media. But, both milk whey and casein could produce less quantity of EPA as compared to other media with the least production in milk whey.

SALIENT ACHIEVEMENTS

- Fatty acid profile of bacteria from gastrointestinal tract was studies at different fermentation conditions
- Optimization of culture conditions has improved the production of EPA from 1% to 7.1% by Shewanella sp. KIB.

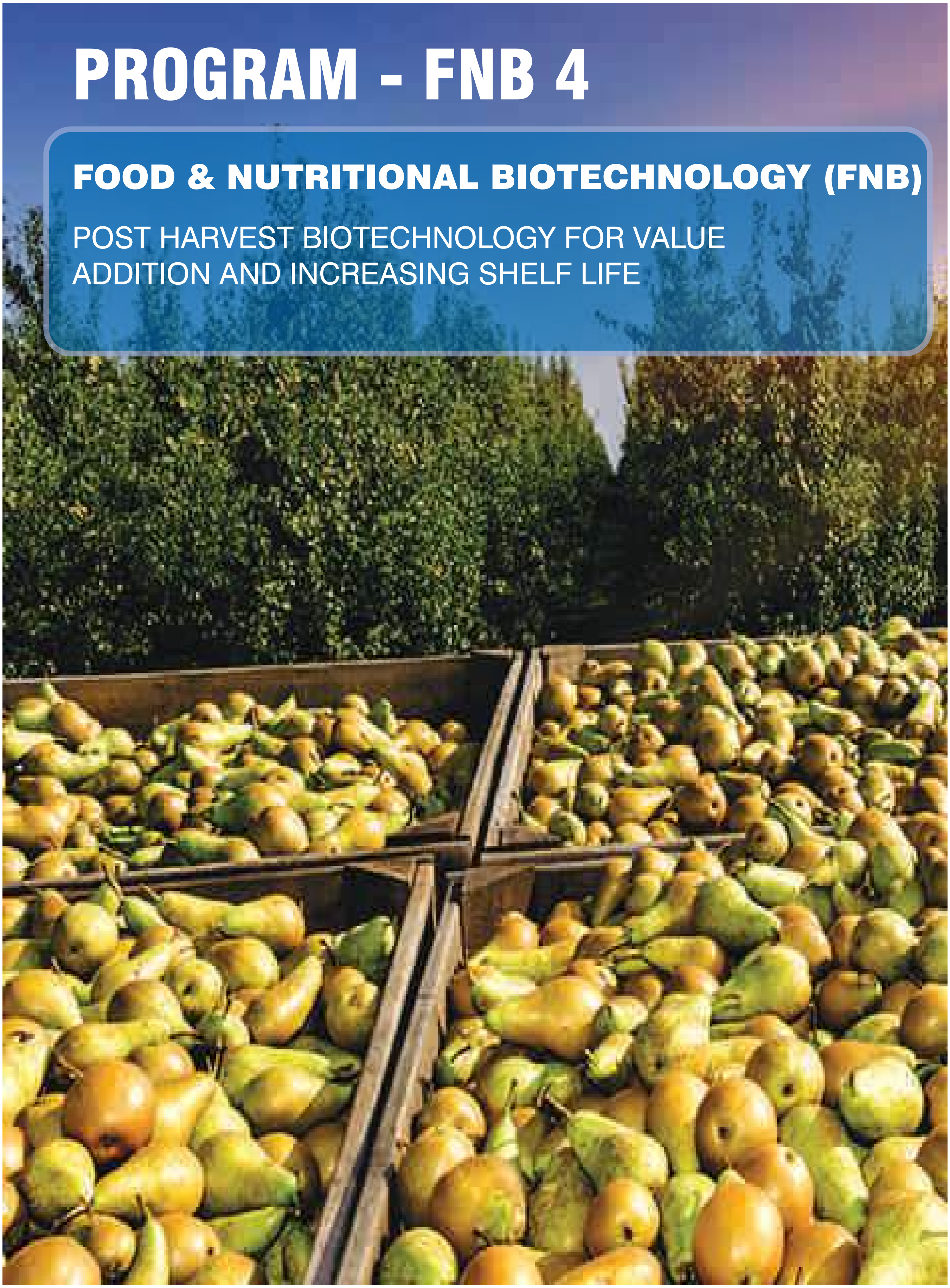
ACTIVITIES FOR NEXT YEAR

- Heterologous production of EPA by cloning PUFA synthase gene cluster in Yarrowia lipolytica and its process optimization.

PROGRAM - FNB 4

FOOD & NUTRITIONAL BIOTECHNOLOGY (FNB)

POST HARVEST BIOTECHNOLOGY FOR VALUE ADDITION AND INCREASING SHELF LIFE



4.1 Development of edible coating materials for the post-harvest shelf life improvement of fresh fruits

PRINCIPAL INVESTIGATOR
Koushik Mazumder

RESEARCH FELLOWS
Usman Ali
Swati Kanwar

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, oat bran and potato peel). Further, novel strategies were adopted to structurally modify the polysaccharides (oat bran β-glucan and potato peel starch) with several fatty acids to prepare hydrophobic derivatives to improve their functional properties. These hydrophobic fatty acid-polysaccharide esters were further blended with hydrophilic wheat straw polysaccharide (arabinoxylan) to prepare composite formulations for shelf life improvement of the coated fresh fruits during transportation and storage

RESEARCH PROGRESS

The efficacy of AX-SABG (AX: wheat straw arabinoxylan and SABG: oat bran β-glucan stearic acid ester) coating (1-2%) was examined on fresh peaches (*Sharbati* variety) at 22°C for 6 days. The study revealed AX-SABG (1-2%) coated peaches had nearly 1.1-1.2 fold reduced weight loss compared to uncoated peaches during storage. AX-SABG (1-2%) coating was also effective in maintaining significantly higher hue value in the range of 54.4-55.1 compared to uncoated peaches (hue value 49.7), similarly AX-SABG (1-2%) coated peaches had ripening index in the range of 12.8-14.8 suggesting 1.3-1.5 fold reduction in ripening index compared to uncoated peaches (Fig. 1). The results suggested AX-SABG coating material (1-2%) had ability to extend the post-harvest quality of highly perishable peaches up to 6 days under recommended ambient storage (22°C). The effect of AX-SABG and shellac coating on volatile compounds during storage was investigated which indicated the concentrations of total volatile compounds were significantly higher (45.3-99.4 μg/kg) in AX-SABG (1-2%) coated peaches compared to the significantly lower concentration (24.4-27.1 μg/kg) in shellac coated (1-2%) peach. The higher concentrations of volatile compounds in AX-SABG coated peach could be linked to the barrier properties of coating against respiration, transpiration and metabolic activity of fruit during storage.

Acute toxicological study was conducted with *Swiss Albino* mice model for 14 days. During the study 6 mice in each

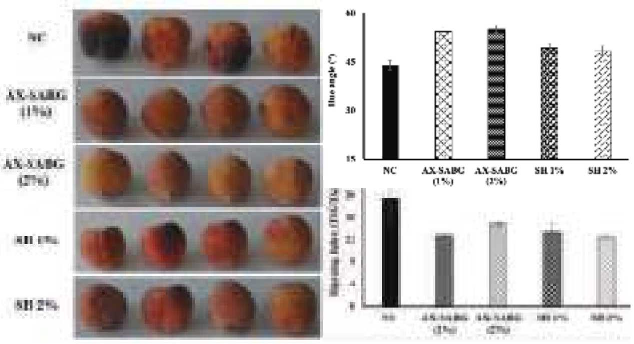


Figure 1. Change in color (hue angle) and ripening index of peaches after 6 days storage at 22°C (±2) with 85% RH (SH: Commercial Shellac coating).

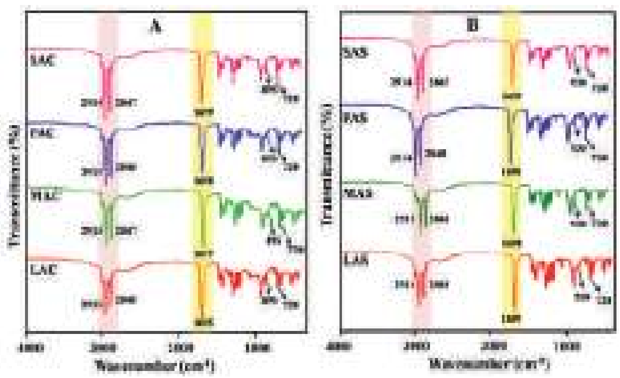


Figure 2. ATR-FTIR spectrum of (A) CFAs and (B) SFAs.

group were fed with control (normal diet) and AX-SABG (AX: arabinoxylan, SABG: β-glucan stearic acid ester) supplemented diet with dosage of 50, 300, 2000, 5000 mg/kg body weight. Acute oral toxicity studies in male mice showed no mortality or treatment related complications or any signs of toxicity. There were no significant changes in mean body weight (bw), average feed intake, average water intake and liver weight in different groups of animals. At tissue level, no treatment related histological changes were observed in any of the treatment groups even at the highest dose of 5000 mg/kg bw suggesting the non-toxic nature of AX-SABG. Thus, LD_{50} value of AX-SABG exceeds 5000 mg/kg bw and falls in GHS category 5. In accordance with Hodge and Sterner scale, the coating material is practically non-toxic. Overall, the histological investigation confirmed that the coating material is non-toxic, non-inflammatory and safe for human consumption.

Study 2: AX and cellulose was extracted from wheat straw with a yield of 17% and 41% respectively whereas 7% yield of starch was obtained from wet potato peel (Table 1). The compositional sugar of the AX, cellulose and starch as alditol acetate derivatives indicated the presence of arabinose and xylose as major constituent sugars in AX whereas glucose was identified as major sugar residues in cellulose and starch. Cellulose and starch were esterified with 1% NaOH-fatty acid chloride to prepare cellulose and starch fatty acid esters (CFAs and SFAs). FTIR spectra were collected for the structural characterization of CFAs and SFAs and the results suggested that fatty acid substituent have been linked directly onto cellulose and starch in CFAs and SFAs respectively (Fig. 2). The degree of substitution (DS) values of CFAs and SFAs obtained from elementary analysis were in the range of 2.1-2.8. The measurement of CI also revealed that AX-CFAs and AX-SFAs composite films had significantly lower values in the range of 4.2-12.1% and 3.7-6.9% compared to the values obtained for cellulose and AX-cellulose and Ax-starch films. Further, films were prepared by blending AX-cellulose, AX-starch, AX-CFAs and AX-SFAs in the ratio of 60:40 (w/w). 20 % glycerol and 3% Tween-80 were incorporated in the film forming emulsion as plasticizer and emulsifier respectively. The AX-cellulose and AX-starch films exhibited remarkably higher WVP values as hydrophilic polymer matrix is quite compatible with water vapor and offers little resistance to water transfer. However, in all cases incorporation of CFAs and SFAs to AX significantly decreased the WVP due to increase in film's hydrophobicity. Our results further indicated that WVP decreased as the chain length of fatty acids in AX-CFAs and AX-SFAs films increased from lauric acid (C12) to stearic acid (C18) which could be associated with increased film's hydrophobicity and greater laminar structures. All AX-CFAs and AX-SFAs composite films were found to be thermally more stable than AX-cellulose and AX-starch films respectively. Incorporation of SAC to AX films lead to films with best properties, improved thermal stability, reduced the WVP to about 76% compared to native AX-cellulose film. Similar observation was found in AX-SAS film resulted nearly 49% reduction in WVP compared to native AX-starch film. Further, the efficacy of AX-SAC and AX-SAS composite film forming material will be evaluated for the post-harvest quality improvement of perishable fruit crops (guava) in our future studies.

SALIENT ACHIEVEMENTS

- In our study polysaccharides from agricultural and food processing by-products (e.g. wheat straw, oat bran and potato peel) were extracted to produce edible coating materials for the shelf-life improvement of fruit crops. The studies of the post-harvest qualities of peach suggested AX-SABG coating formulation (1-2%) significantly improved the quality during 6 days storage. The detailed toxicological studies on AX-SABG coating material indicated that the coating material is non-toxic, non-inflammatory and safe for consumption. Further studies were conducted to develop new coating materials based on cellulose and starch from wheat straw and potato peel respectively. It was observed AX-SAC and AX-SAS coating material lead to best functional properties for fruit coating purpose and further studies will be conducted to determine their efficacy for the post-harvest quality improvement of perishable fruit crops.

ACTIVITIES FOR NEXT YEAR

- Efficacy evaluation of the AX-SABG coating for the quality improvement of apple under cold storage.
- FSSAI approval of AX-SABG coating material.
- Efficacy evaluation of AX-SAC and AX-SAS coating materials for the post-harvest quality improvement of perishable fruit crops (guava).

NEW INITIATIVE

1.1 Towards generating high yielding, nutritionally improved stress tolerant rice

PRINCIPAL INVESTIGATOR
Prof. Ashwani Pareek

RESEARCH FELLOWS
Manjari Mishra

EMS induced mutagenesis to raise abiotic stress tolerant and environmentally acceptable mutant of Pusa Basmati 1121 (PB1121): Developing high yielding and salinity tolerant genotype of rice is the need of hour and major challenge before all of us due to occurrence of unfavorable environmental conditions such as drought, salinity etc and therefore loss of arable land. Recently, ethyl methane sulfonate (EMS) induced mutagenesis is proving to be an advantageous over other methods due to having benefits of higher point mutation, less chromosomal aberrations and easier screening of mutants (Zhang et al., 2011). Therefore, considering the high grain quality of PB1121 and importance of mutagenesis to create genetic variability, we have done EMS induced mutagenesis to develop high yielding and stress tolerant mutant of PB1121 rice. The mutant population (M0) is growing in field under natural conditions. The subsequent generations of these mutants will be screened for abiotic stress tolerance at both seedling and mature stage.

Molecular and functional assessment of PLATZ family transcription factors and LHY gene(s) in rice: The PLATZ (plant AT-rich protein and zinc-binding protein) transcription factor family is a class of plant-specific zinc-dependent DNA-binding proteins. PLATZ has essential roles in seed endosperm development, as well as promoting cell proliferation duration in the earlier stages of the crops. PLATZ constitutes a multigene family having15 members in rice. The LHY genes perform important functions in maintaining in plants circadian clock. The circadian clock plays essential roles in diverse plant biological processes, such as flowering, phytohormone biosynthesis and abiotic stress responses. The OsLHYs and OsPLATZs will be checked for their differential regulation in presence of different abiotic stresses to find out the potential candidate gene having potent role in abiotic stress adaptation in rice. The candidate genes will be further used for rice genome editing through CRISPR-based technique.

Figure 1. PB1121 mutant population growing in field under natural climatic conditions.

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. Hasthi Ram	Inspire Faculty	10-08-2017
15.	Dr. Pramod Kaitheri Kandoth	Ramalingaswami Fellow	17-08-2017
16.	Dr. Rupam Kumar Bhunia	Inspire Faculty	01-01-2018
17.	Dr. Rupesh Deshmukh	Ramalingaswami Fellow	23-04-2018
18.	Dr. Vanish Kumar	Inspire Faculty	24-04-2018
19.	Dr. Humira Sonah	Ramalingaswami Fellow	07-05-2018
20.	Dr. Sivasubramanian	Inspire Faculty	03-10-2018
21.	Dr. Ravindra Pal Singh	Ramalingaswami Fellow	01-06-2019
22.	Dr. Prafull Salvi	Inspire Faculty	01-04-2019
23.	Dr. Karambir Kaur	Project Scientist	13-08-2019
24.	Dr. Kali Dass	Project Scientist	22-08-2019
25.	Dr. Sanjay Kumar	Project Scientist	17-02-2020
26.	Dr. Anjil Kumar Srivastava	Ramalingaswami Fellow	08-03-2021
27.	Dr. Vadthya Lokya	Project Scientist	27-03-2021

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 Vatsa	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

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	Library Assistant	19-12-2012

National Post-Doctoral Fellows

S.no	Name	Area of Research	Date of Joining
1.	Dr. Abishek Bhandawat	Agri-Biotechnology	09-04-2018

Research Scholars

S. No	Name	Area of Research	Awarding University-Institute
Student awarded Ph.D degree:			
1	Sh. Vishnu Shukla	Characterization of wheat phosphate transporter & their involvement during iron and phosphate interaction	Panjab University, Chandigarh

S no.	Name of the Student	Position at Present	Date of Joining
1.	Ms. Navneet Kaur	Project Fellow	30-08-2013
2.	Ms. Nishtha Sharma	Senior Research Fellow	01-09-2015
3.	Ms. Shimayali Kaushal	Senior Research Fellow	21-01-2016
4.	Sh. Vishal Singh	Senior Research Fellow	23-02-2016
5.	Ms. Neha Thakur	Senior Research Fellow	16-03-2016
6.	Sh. Vijay Kumar	Senior Research Fellow	22-03-2016
7.	Sh. Nitesh Priyadarshi	Senior Research Fellow	19-08-2016
8.	Ms. Afsana Parveen	Senior Research Fellow	31-08-2016
9.	Ms. Raminder Kaur	Senior Research Fellow	01-09-2016
10.	Ms. Shahirina Khan	Senior Research Fellow	21-11-2016
11.	Sh. Anil Kumar	Senior Research Fellow	28-11-2016
12.	Ms. Poonam Sagar	Senior Research Fellow	21-06-2017
13.	Ms. Vinita Sharma	Senior Research Fellow	24-07-2017
14.	Sh. Siddhanth Chaturvedi	Senior Research Fellow	11-09-2017
15.	Ms. Swati Kanwar	Senior Research Fellow	13-09-2017
16.	Ms. Sunaina Kaul	Senior Research Fellow	22-09-2017
17.	Ms. Ritika Gupta	Senior Research Fellow	20-02-2018
18.	Sh. Vibhu	Senior Research Fellow	10-10-2018
19.	Ms. Jasleen	Senior Research Fellow	10-10-2018
20.	Mrs. Ruchi Bansal	Senior Research Fellow	11-12-2018
21.	Ms. Surbhi Kumawat	Senior Research Fellow	15-01-2019
22.	Mrs. Flowerika	Senior Research Fellow	15-05-2019
23.	Ms. Ruchika Bhatia	Senior Research Fellow	24-08-2019
24.	Mrs. Gazaldeep Kaur	Senior Research Fellow	30-01-2020
25.	Ms. Nitika Rana	Senior Research Fellow	13-11-2020
26.	Sh. Mohammad Saba Rahim	Senior Research Fellow	01-01-2021
27.	Mrs. Shika Sharma	Senior Research Fellow	11-03-2021
28.	Mrs. Anita Kumari	Junior Research Fellow	02-05-2018
29.	Ms. Varnika Rana	Project Fellow-I	18-06-2018
30.	Dr. Himanshu Sharma	Research Associate-III	05-10-2018

31.	Dr. Arti Chauhan	Research Associate-I	15-10-2018
32.	Ms. Renu Sharma	Project Assistant-II	16-10-2018
33.	Sh. Atul Munish Chander	Research Associate-I	12-11-2018
34.	Dr. Natasha	Research Associate-I	15-03-2019
35.	Sh. Usman Ali	Research Associate-II	27-03-2019
36.	Sh. Mithlesh Kumar Sardar	Project Field Assistant	29-03-2019
37.	Ms. Mitaly Bansal	Research Associate-III	01-04-2019
38.	Sh. Akarsh Kushwaha	Project Fellow	29-04-2019
39.	Ms. Satveer Kaur	Junior Research Fellow	01-05-2019
40.	Sh. Gurvinder Singh	Field Assistant	02-05-2019
41.	Sh. Hemraj	Field Assistant	02-05-2019
42.	Ms. Shivani Sharma	Junior Research Fellow	03-05-2019
43.	Dr. Jitendra Kumar	Research Associate-I	06-05-2019
44.	Mrs. Lakhani Hiralben L.	Junior Research Fellow	20-05-2019
45.	Sh. Pargat Singh	Field Assistant	21-05-2019
46.	Mrs. Aanchal Chaudhary	Junior Research Fellow	31-05-2019
47.	Ms. Prabhjot Kaur	Junior Research Fellow	06-06-2019
48.	Ms. Lovenpreet Kaur	Junior Research Fellow	07-06-2019
49.	Dr. Aancal Aggarwal	Research Associate	14-06-2019
50.	Sh. Kalyan Vaid	Junior Research Fellow	24-06-2019
51.	Sh. Koushik Shah	Research Associate-I	22-07-2019
52.	Sh. Akshay Verma	Project Fellow	16-08-2019
53.	Sh. Gaurav Raturi	Junior Research Fellow	30-08-2019
54.	Ms. Sunaina	Project fellow-I	02-09-2019
55.	Sh. Deepak Duhan	Project fellow-I	02-09-2019
56.	Ms. Manorma Negi	Project Fellow-I	09-09-2019
57.	Ms. Tanika Thakur	Project fellow-I	09-09-2019
58.	Ms. Kirti Chawla	Project fellow-I	13-09-2019
59.	Dr. Ashish Kumar	Research Associate-I	03-10-2019
60.	Dr. Pankaj Kumar Singh	Research Associate-III	19-10-2019
61.	Dr. Lakshay Mahajan	Laboratory Animal Veterinarian	21-10-2019
62.	Mrs. Shivani Sharma	Project Fellow-I	21-10-2019
63.	Ms. Jasmeen Dhiman	Project Fellow-I	21-10-2019
64.	Sh. Nirbhay Kumar	Project Fellow-I	21-10-2019
65.	Sh. Himanshu Yadav	Project Fellow-I	01-11-2019
66.	Sh. Dalwinder Singh	Research Associate-I	18-11-2019
67.	Dr. Pragaynshu Khare	Research Associate-II	18-11-2019
68.	Ms. Gunashri Ganesh Padalkar	Junior Research Fellow	10-12-2019
69.	Ms. Navpreet Kaur	Junior Research Fellow	16-12-2019
70.	Ms. Nitika	Junior Research Fellow	01-01-2020
71.	Sh. Amit Kumar	Junior Research Fellow	16-12-2019

72.	Sh. Anmol Singh	Project Fellow-I	20-12-2019
73.	Ms. Nikita	Project Fellow-I	24-12-2019
74.	Ms. Kriti Sharma	Junior Research Fellow	01-01-2020
75.	Ms. Vandana Thakral	Junior Research Fellow	14-01-2020
76.	Sh. Kiran Kishore Khandare	Junior Research Fellow	20-01-2020
77.	Sh. Raja Bhaiyya	Junior Research Fellow	21-01-2020
78.	Ms. Pragati	Junior Research Fellow	21-01-2020
79.	Ms. Sreeja S S	Junior Research Fellow	14-02-2020
80.	Ms. Pallavi	Junior Research Fellow	17-02-2020
81.	Ms. Anjali	Junior Research Fellow	28-02-2020
82.	Ms. Kirti Devi	Junior Research Fellow	05-03-2020
83.	Dr. Shivaraj S.M	Research Associate-I	07-03-2020
84.	Ms. Kshitija Sinha	Junior Research Fellow	09-03-2020
85.	Ms. Shivani Gajbhiye	Junior Research Fellow	17-03-2020
86.	Ms. Nikita Saini	Project Fellow-I	08-07-2020
87.	Ms. Vandita Tiwari	Junior Research Fellow	20-08-2020
88.	Ms. Shivani Jaiswal	Junior Research Fellow	01-09-2020
89.	Ms. Raksha	Project Fellow-I	07-09-2020
90.	Ms. Geetika Bajaj	Junior Research Fellow	14-09-2020
91.	Sh. Tushar Matta	Junior Research Fellow	17-09-2020
92.	Ms. Pooja Kanwar Shekhawat	Project Fellow-I	14-10-2020
93.	Dr. Ravneet Kaur Boparai	Research Associate-III	23-10-2020
94.	Dr. Ankita Mishra	Research Associate-I	18-11-2020
95.	Ms. Nishu Gandass	Project Fellow-I	24-11-2020
96.	Dr. Shikha Sharma	Project Associate-II	01-12-2020
97.	Sh. Rabishankar Ojha	Project Fellow-I	13-01-2021
98.	Ms. Priyanka Garg	Junior Research Fellow	13-01-2021
99.	Sh. Rajdeep Jaiswal	Research Associate-I	18-01-2021
100.	Sh. Mandlik Rushil Ramesh	Junior Research Fellow	19-01-2021
101.	Sh. Rishav Sharma	Project Fellow-I	22-01-2021
102.	Sh. Naveen Kumar	Junior Research Fellow	25-01-2021
103.	Ms. Simranjit Kaur	Project Fellow-I	12-02-2021
104.	Ms. Jayshree Niharika Panda	Junior Research Fellow	13-02-2021
105.	Ms. Riya Joon	Junior Research Fellow	26-02-2021
106.	Ms. Kajal	Junior Research Fellow	01-03-2021
107.	Dr. Dharavath Ravinder Naik	Research Associate-II	09-03-2021
108.	Sh. Yatin	Project Assistant	27-03-2021
109.	Sh. Hardeep Singh	Project Associate-I	27-03-2021
110.	Ms. Lata Pawar	Junior Research Fellow	30-03-2021

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 Madhawanf	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. Deep Shikha Tyagi	PhD Research Scholar	28-09-2020
24.	Ms. Mona	PhD Research Scholar	28-09-2020

Trainees

S. No	Name	Designation	Date of Joining
1.	Harish B. Jadhav	Trainee	27-01-2021
2.	Chitrakshi Shandilya	Trainee	27-01-2021
3.	Sakshi Rathee	Trainee	27-01-2021
4.	Srishti Baliyan	Trainee	27-01-2021
5.	Pallavi	Trainee	28-01-2021
6.	Jashandeep Kaur	Trainee	01-02-2021
7.	Neeti Rastogi	Trainee	01-02-2021
8.	Kajal	Trainee	01-02-2021
9.	Shubhi Mishra	Trainee	04-02-2021
10.	Pratishtha Chaudhary	Trainee	08-02-2021
11.	Ayushi Satawne	Trainee	16-02-2021
12.	Manisha	Trainee	16-02-2021
13.	Manik Bathla	Trainee	01-03-2021
14.	Riya Rani	Trainee	01-03-2021
15.	Apoorv Bhardwaj	Trainee	01-03-2021
16.	Shivam Singh Dogra	Trainee	01-03-2021
17.	Manmehar Kaur	Trainee	03-03-2021
18.	Pooja Aggarwal	Trainee	03-03-2021
19.	Pallavi	Trainee	04-03-2021
20.	Nidhi Kaushal	Trainee	04-03-2021
21.	Mahima Arora	Trainee	08-03-2021
22.	Richika Arora	Trainee	08-03-2021
23.	Eshita Jain	Trainee	09-03-2021
24.	Prerana	Trainee	10-03-2021
25.	Komal Koul	Trainee	13-03-2021
26.	Ankush Kumar	Trainee	13-03-2021
27.	Sukhmandeep Kaur	Trainee	13-03-2021
28.	Archna Sharma	Trainee	17-03-2021

RESEARCH PUBLICATIONS

1. Sharma H, Kumar P, Singh A, Aggarwal K, Roy J, Sharma V and Rawat S (2020). Development of polymorphic EST-SSR markers and their applicability in genetic diversity evaluation in Rhododendron arboreum. Molecular Biology Reports. https://doi.org/10.1007/s11033-020-05300-1
2. Bhandawat A, Sharma V, Rishi V and Roy J (2020). Biolistic Delivery of programmable nuclease (CRISPR/Cas9) in bread wheat. In Biolistic DNA Delivery in Plants. http://doi.org/10.1007/978-1-0716-0356-7_17
3. Sharma H, Bhandawat A, Rahim MS, Kumar P, Choudhoury MP and Roy J (2020). Novel intron length polymorphic (ILP) markers from starch biosynthesis genes reveal genetic relationships in Indian wheat varieties and related species. Molecular Biology Reports.https://doi.org/10.1007/s11033-020-05434-2
4. Kumar V, Mahajan N, Khare P, Kondepudi KK, Bishnoi M (2020). Role of TRPV1 in colonic mucin production and gut microbiota profile. European Journal of Pharmacology. 2020; 888:173567.
5. Uttam P, Kumar V, Kim KH, and Deep A (2020). Nanotwinning: Generation, properties, and application. Materials & Design: 108752. Doi: 10.1016/j.matdes.2020.108752
6. Sharma, S, Khare P, Kumar A, Chunduri V, Kumar A, Kapoor P, & Garg M (2020). Anthocyanin rich biofortified colored wheat prevents high fat diet induced alterations in mice: Nutrigenomics Studies. Molecular Nutrition & Food Research, 1900999. https://doi.org/10.1002/mnfr.201900999
7. Vikrant K, Kim KH, Kumar V, Giannakoudakis DA and Boukhvalov DW (2020). Adsorptive removal of an eight-component volatile organic compound mixture by Cu-, Co-, and Zr-metal-organic frameworks: experimental and theoretical studies. Chemical Engineering Journal: 125391. Doi: 10.1016/j.cej.2020.125391.
8. Kumar A, Sharma S, Chunduri V, Kaur A, Kaur S, Malhotra N and Sonah H (2020). Genome-wide identification and characterization of heat shock protein family reveals role in development and stress conditions in Triticum aestivum L. Scientific Reports, 10(1), 1-12.
9. Negi S, Jaiswal G, Das K, Mazumder K, Elumalai S and Roy J (2020). Torrefaction: a sustainable method for transforming of agri-wastes to high energy density solids (biocoal). Rev Environ Sci Biotechnol. https://doi.org/10.1007/s11157-020-09532-2
10. Singh RP, Prakash S, Bhatia R, Negi M, Singh J, Bishnoi M, Kondepudi KK (2020). Generation of structurally diverse pectin oligosaccharides having prebiotic attributes. Food Hydrocolloids. doi: https://doi.org/10.1016/j.foodhyd.2020.105988
11. Madhawan A, Sharma A, Bhandawat A, Rahim MS, Kumar P, Mishra A, Parveen A, Sharma H, Verma SK and Roy J (2020). Identification and characterization of long non-coding RNAs regulating resistant starch biosynthesis in bread wheat (Triticum aestivum L.). Genomics 112: 3365-3074
12. Rahim MS, Bhandawat A, Rana N, Sharma H, Parveen A, Kumar P, Madhawan A, Bisht A, Sonah H, Sharma TR and Roy J (2020). Genomic selection in cereal crops: methods and applications. In: osal S. S. Wani S. H. (eds) Accelerated Plant Breeding, 51-88.
13. Chawla K, Sinha K, Neelam Kaur R and Bhunia RK (2020). Identification and functional characterization of two acyl CoA: diacylglycerol acyltransferase 1 (DGAT1) genes from forage sorghum (Sorghum bicolor) embryo. Phytochemistry, 176:112405.
14. Vellingiri K, Vikrant K, Kumar V and Kim KH Kim (2020). Advances in thermocatalytic and photocatalytic techniques for the room/low temperature oxidative removal of formaldehyde in air. Chemical Engineering Journal: 125759. doi: 10.1016/j.cej.2020.125759
15. Kumar V, Mirzaei A, Bonyani M, Kim KH, Kim HW and Kim SS (2020). Advances in electrospun nanofiber fabrication for polyaniline (PANI)-based chemoresistive sensors for gaseous ammonia." TrAC Trends in Analytical Chemistry: 115938. doi: 10.1016/j.trac.2020.115938
16. Singh R, Bansal Y, Sodhi RK, Khare P, Bishnoi M, Kondepudi KK, Medhi B, Kuhad A. (2020). Role of TRPV1/TRPV3 channels in olanzapine-induced metabolic alteration: Possible involvement in hypothalamic energy-sensing, appetite regulation, inflammation and mesolimbic pathway. Toxicol Appl Pharmacol. 2020 Jul 9;402:115124. doi: 10.1016/j.taap.2020.115124.
17. Kaur N, Awasthi P and Tiwari S (2020). Fruit crops improvement using CRISPR/Cas9 system. In: Vijai Singh, Pawan K. Dhar (eds) Genome engineering via CRISPRCas9 system. Elsevier, San Diego, CA. PP 131-145, https://doi.org/10.1016/B978-0-12-818140-9.00012-X
18. Deol PK, Khare P, Singh DP, Bishnoi M, Kondepudi KK, Kaur IP (2020). Pharmabiotic beads with improved encapsulation and gut survival for management of colonic inflammation associated gut derangements. J Drug Target. 2020 Jun 12:1-10. doi: 10.1080/1061186X.2020.1775838.
19. Kumar V, Vaid K, Bansal SA, Kim KH (2020). Nanomaterial-based immunosensors for ultrasensitive detection of pesticides/herbicides: current status and perspectives. Bi-

osensors and Bioelectronics: 112382, doi: 10.1016/j.bios.2020.112382

20. Sharma H, Bhandawat A, Kumar P, Rahim MS, Parveen A, Kumar P, Madhawan A, Rishi V and Roy J (2020). Development and characterization of bZIP transcription factor based SSRs in wheat. Gene. https://doi.org/10.1016/j.gene.2020.144912
21. Singh DP, Arya A, Kondepudi KK, Bishnoi M, Boparai RK (2020). Prevalence and associated factors of overweight/obesity among school going children in Chandigarh, India. Child Care Health Dev. doi: 10.1111/cch.12794.
22. Chander AM, Singh S, Sharma S, Chaudhry V, Rajarammohan S, Mantri SS, Bishnoi M, Bhadada SK and Kondepudi KK (2020). Draft genome sequence of Bifidobacterium pseudocatenulatum Bif4, isolated from healthy infant feces. Microbiol Resour Announc 9:e00561-20. https://doi.org/10.1128/MRA.00561-20.
23. Khanna S, Walia S, Kondepudi KK and Shukla G (2020). Administration of indigenous probiotics modulate high-fat diet-induced metabolic syndrome in Sprague Dawley rats. Antonie Van Leeuwenhoek. doi: 10.1007/s10482-020-01445-y
24. Koul B and Tiwari S (2020). Microbe-mediated genetic engineering for enhancement of nutritional value in food crops. Microbial Biotechnology: Basic Research and Applications. Springer Singapore https://doi.org/10.1007/978-981-15-2817-0_2
25. Thapak P, Bishnoi M and Sharma SS (2020). Amelioration of diabetes-induced cognitive impairment by Transient Receptor Potential Vanilloid 2 (TRPV2) channel inhibitor: Behavioral and mechanistic study. Neurochem International 2020;139:104783.
26. Rani P, Kumar V, Singh PP, Matharu AS, Zhang W, Kim KH, Singh J, and Rawat M (2020). Highly stable AgNPs prepared via a novel green approach for catalytic and photocatalytic removal of biological and non-biological pollutants. Environment International: 143, 105924, doi: 10.1016/j.envint.2020.105924
27. Bansal SA, Kumar V, Karimi J, Singh AP and Kumar K (2020). Role of gold nanoparticles in advanced biomedical applications. Nanoscale Advances: 2, 3764-3787. Doi: 10.1039/D0NA00472C
28. Alok A, Kaur J and Tiwari S (2020). Functional characterization of wheat myo-inositol oxygenase promoter under different abiotic stress conditions in Arabidopsis thaliana. Biotechnol Letters. https://doi.org/10.1007/s10529-020-02967-1
29. Sharma M, Rahim MS, Kumar P, Mishra A, Sharma H and Roy J (2020). Large scale identification and characterization of phenolic compounds and their marker-trait association in Wheat. Euphytica. https://doi.org/10.1007/s10681-020-02659-x
30. Wang J, Yeckel G, Kandoth PK, Wasala L, Hussey RL, Davis EL, Baum TJ and Mitchum MG (2020). Targeted suppression of soybean BAG6 induced cell death in yeast by soybean cyst nematode effectors. Molecular Plant Pathology, 21: 1227-1239
31. Kumar V, Majhi SM, Kim KH, Kim KW and Kwon EE (2020). Advances in In2O3-based materials for the development of hydrogen sulfide sensors. Chemical Engineering Journal: 126472, doi: 10.1016/j.cej.2020.126472
32. Akhtar A, Bishnoi M and Sah SP (2020). Sodium orthovanadate improves learning and memory in intracerebroventricular-streptozotocin rat model of Alzheimer’s disease through modulation of brain insulin resistance induced tau pathology. Brain Res Bull. 2020; 164:83-97.
33. Rahim MS, Chauhan A, Madhawan A, Mishra A, Parveen A, Garg M, Bishnoi, Kumar V, Oak MD, Singhal NK and Roy J (2020). Development and evaluation of chapatti quality of high amylose wheat mutants on the basis of physicochemical, textural and sensory characteristics. LWT-Food Science and Technology. DOI https://doi.org/10.1016/j.lwt.2020.110051
34. Bhandawat A, Sharma H, Pundir N, Madhawan A and Roy J (2020). Genome-wide identification and characterization of novel non-coding RNA-derived SSRs in wheat. Molecular Biology Reports. https://doi.org/10.1007/s11033-020-05687-x
35. Rahim MS, Mishra A, Katyal M, Thakur S, Sharma M, Kumar, P, Parveen A, Jaiswal V, Singh N, Kumar V, Gupta RK, Sharma H and Roy J (2020). Marker- trait association identified candidate starch biosynthesis pathway genes for starch and amylose-lipid complex gelatinization in wheat (Triticum aestivum L.). Euphytica. DOI:10.1007/s10681-020-02688-6
36. Singh C, Sharma A, Bishnoi M, Kondepudi KK, Singh RP (2020). Nutrigenomics approaches to control metabolic diseases and challenges to personalized nutritional intervention: In Advances in Agriculture Biotechnology, DOI:10.2174/1573401313666170614094410
37. Kaur R., Bhunia R.K and Rajam MV (2020). MicroRNAs as potential targets for improving rice yield via plant architecture modulation: Recent studies and future perspectives. Journal of Bioscience 45:116. DOI https://link.springer.com/article/10.1007/s12038-020-00084-9
38. Mourya R, Bishnoi M and Kondepudi KK (2020). Plant Polyphenols and gut bacteria: Role in obesity-induced metabolic endotoxaemia and inflammation: In Advances in Agriculture Biotechnology. DOI: 10.1007/978-981-15-2874-3_10
39. Vaid K, Dhiman J, Sarawagi N, and Kumar V (2020). Experimental and computational study on the selective interaction of functionalized gold nanoparticles with metal ions: sensing prospects. Langmuir: 36:41, 12319–12326, doi: 10.1021/acs.langmuir.0c01472
40. Taak P, Tiwari S and Koul B (2020). Optimization of regeneration and Agrobacterium-mediated transformation of Stevia (Stevia rebaudiana Bertoni): a commercially important natural sweetener plant. Scientific Reports, 10:16224. https://doi.org/10.1038/s41598-020-72751-8
41. Singh RP, Rajarammohan S, Thakur R and Hassan M (2020). Linear and branched □-Glucans degrading enzymes from versatile Bacteroides uniformis JCM 13288T and their roles in cooperation with gut bacteria. Gut Microbes. 12:1(1)18. DOI https://doi.org/10.1080/19490976.2020.1826761
42. Sinha K, Kaur R, Singh N, Kaur S, Rishi V and Bhunia RK (2020). Mobilization of storage lipid reserve and expression analysis of lipase and lipoxygenase genes in rice (Oryza sativa var. Pusa Basmati 1) bran during germination. Phytochemistry, 180:112538. DOI https://doi.org/10.1016/j.phytochem.2020.112538
43. Bhunia RK, Sinha K, Chawla K, Randhawa V and Sharma TR (2020). Functional characterization of two type-1 diacylglycerol acyltransferase (DGAT1) genes from

rice (Oryza sativa) embryo restoring the triacylglycerol accumulation in yeast. Plant Molecular Biology. DOI https://doi.org/10.1007/s11103-020-01085-w

44. Khare P, Maurya R, Bhatia R, Mangal P, Singh J, Podili K, Bishnoi M, Kondepudi KK (2020). Polyphenol rich extracts of finger millet and kodo millet ameliorate high fat diet-induced metabolic alterations. Food Functional DOI: 10.1039/d0fo01643h
45. Bhardwaj SK, Bhardwaj N, Kumar V, Bhatt D, Azzouz A, Bhaumik J, Kim KH and Deep A (2020). Recent progress in nanomaterial-based sensing of airborne viral and bacterial pathogens. Environment International, DOI: 10.1016/j.envint.2020.106183
46. Reddy MS, Thakur B, Mukherjee A, Pandey AK, Marmeisse R and Fraissinet-Tachet L (2020). Multi-metal tolerance of DHHC palmitoyl transferase-like protein isolated from metal contaminated soil. Exotoxicology. DOI:10.1007/s10646-020-02301-5
47. Singh S, Bhatia R, Khare P, Sharma S, Rajarammohan S, Bishnoi M, Bhadada SK, Shyam SS, Kaur J, Kondepudi KK (2020). Anti-inflammatory bifidobacterium strains prevent dextran sodium sulfate induced colitis and associated gut microbial dysbiosis in mice. Scientific Reports. DOI: https://doi.org/10.1038/s41598-020-75702-5
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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. A novel anti-inflammatory multi-strain synergistic synbiotic formulation to prevent/ameliorate IBD and Ulcerative Colitis. Full patent Application Number: 02011021331

Participation in National/ International Conferences/ Workshops

1. Dr. kanthi Kiran delivered a talk on the topic "Functional Foods and Nutraceuticals" held on 20th April 2020 at School of Health Sciences, University of Petroleum and Energy Studies, Dehradun.
2. Dr. kanthi Kiran delivered a talk on the topic "Gut: A bio-refinery in improving health" during a short term course on "Trends and prospects in biorefinery" held on 10th – 14th June 2020 at NIT-Jalandhar.
3. Dr. kanthi Kiran delivered a lecture on the topic "Nutraceuticals for healthy living" held on 02nd August 2020 at AMI-Chandigarh Webinar series.
4. Dr. Koushik Mazumder delivered an invited lecture on the topic "Edible coatings to enhance post-harvest quality of horticultural produce" in the National Conference "Polymer Nanocomposites: Fundamentals and Applications held on 30th August 2020 at Department of Basic and Applied Sciences, NIFTEM, Sonipat, Haryana.
5. Dr. Siddharth Tiwari delivered a lecture on the topic "Genetic engineering in crop plants for traits improvement" in the Webinar series on biotechnology organized by the Department of Bio and Nano Technology at Guru Jambheshwar University of Science and Technology, Hisar, Haryana on 1st September 2020.
6. Dr. Koushik Mazumder delivered an invited lecture on the topic "Carbohydrate based edible coatings to enhance post-harvest quality of perishable fruit crops" in International Webinar Series on the occasion of National Nutrition Month held on 06th September 2020 at Department of Home Science, University of Calcutta, Kolkata.
7. Dr. kanthi Kiran delivered an invited lecture on the topic "Millets -The nutricereals for nutritional security" held on 16th October 2020 at World Food Day Lecture, Pandemic Times, University of Portugal.
8. Ms. Saloni Sharma delivered an oral presentation on the topic "Colored wheat as a novelty crop: Understanding potential health benefits." in the international conference on Biotechnology and Healthcare (BIOHE) held during 26th -27th October 2020 at PJTSAU, Hyderabad.
9. Dr. Vikas Rishi delivered an invited talk on CRISPR/Cas technology under DBT sponsored lecture series to Star Colleges of Punjab and West Bengal under the aegis of 'Science Setu' 2021.
10. Dr. kanthi Kiran delivered an invited lecture on the topic "Nutraceuticals for healthy life" held on 16 October 2020 at World Food Day Lecture, Sri Guru Granth Sahib World University, Fatehgarh Sahib, Punjab.
11. Ms. Nishtha Sharma, Ms. Aanchal Aggarwal, Ms. Binduma Yadav, Mr. Koushik Shah, Ms. Raminder Kaur and Dr. Vikas Rishi presented a poster on the topic "Delivery of b-ZIP transcription factor inhibitor protein into 3T3-L1 cells to study time bound preadipocyte differentiation process" in EMBL- Transcription and Chromatin conference held during 28th - 30th October 2020 at Virtual EMBO workshop, Heidelberg, Germany.
12. Mr. Koushik Shah, Ms. Raminder Kaur and Dr. Vikas Rishi presented a poster on the topic "Regulatory landscape of four seed-specific bZIP transcription factors in hexaploid wheat" in the "EMBL conference: from functional genomics to system biology" held during 16th -19th November 2020.
13. Dr. Siddharth Tiwari delivered a lecture entitled "CRISPR Mediated Technologies" 2020 at Bengaluru Tech Summit (BTS) 2020 held on 20th November 2020.
14. Dr. Siddharth Tiwari delivered a lecture on the topic "Biotechnological approaches for nutritional improvement in banana fruit" in the national virtual conference on 'Current trends and challenges in plant biochemistry and biotechnology' organized by the Society for Plant Biochemistry and Biotechnology (SPBB), New Delhi and Birla Institute of Technology and Science (BITS) Pilani at K. K. Birla Goa Campus during 20th - 21st November 2020.
15. Ms. Binduma Yadav and Dr. Vikas Rishi presented a poster on "Effect of short-chain fatty acids on epigenetic regulation of preadipocyte differentiation" in the workshop "New Horizon in Biotechnology" organised by CSIR- National Institute for Interdisciplinary Science and Technology and the Biotech Research Society during 20th - 24th November 2019.
16. Dr. Siddharth Tiwari delivered a lecture on the topic "CRISPR technology: application in the nutritional improvement of plants" a webinar on (Plexus) "Interdisciplinary aspects of CRISPR-Cas9 technology" organised by the Department of Biomedical Science, Shaheed Rajguru College of Applied Sciences for Women (SRCASW), University of Delhi.
17. Dr. Prafull Salvi delivered an oral presentation on the topic "Understanding the molecular role of raffinose family oligosaccharide's associated with seed vigor and abiotic stress responses" for the young scientist award-2020 at international plant physiology virtual conference-2020 "Prospects of plant physiology for climate proofing agriculture" held during 6th - 7th December, 2020.
18. Dr. Siddharth Tiwari delivered a lecture on the topic "Role of bioinformatics and molecular methodologies in genetic engineering of plants" on 21st January 2021 in the UGC sponsored two-week online interdisciplinary refresher course on quantitative biology organised by the Human Resource Development Centre, Guru Jambheshwar University of Science & Technology, Hisar, Haryana.
19. Dr. Vanish Kumar delivered an invited lecture on the topic "Nano-biotechnology based approach-

es for engineering smart sensors" at E-Talk series on
20. "Nanotechnology-Smart Materials: Research: Commercialization" held on 28th January, 2021 at Maharaja Agrasen University, Baddi.
21. Dr. Siddharth Tiwari delivered a lecture on the topic "Development of nutritional rich banana using transgenic and genome editing approaches" in the "3rd National seminar on current trends in life sciences (NSCTLS-2021)" organized by the Amity University, Gwalior, Madhya Pradesh in association with The National Academy of Sciences, India (NASI), Bhopal Chapter and Society of Life Sciences (SLSc) on 29th January 2021.
22. Dr. Siddharth Tiwari delivered a lecture on the topic "Quality improvement in food crops by using biotechnological approaches" a webinar on "Current trends in food technology and food processing"(CTFT-FP-2021)" organized by the Institute of Food Processing and Technology (IFPT), University of Lucknow on 30th January 2021.
23. Dr. Siddharth Tiwari delivered a lecture on the topic "Gene editing in Banana" in the webinar on "Gene-editing research in agriculture: key initiatives in India" organized by TIGS and BCIL on 17th February 2021.
24. Dr. Siddharth Tiwari was invited for a personal interview on the topic "Genome Editing" by Aaj Tak Radio (podcost) on 26th February 2021. (link:https://www.aajtak.in/podcast/explainers/audio/genome-editing-may-treat-cancer-aids-1214084-2021-02-26?utm_source=atweb_share)
25. Dr. Mahendra Bishnoi and Dr. Kanthi Kiran delivered an oral presentation on "Anti-inflammatory potential of indigenous lactic acid bacteria in ameliorating LPS induced inflammation in C57BL/6 mice model" at 3rd Chintan symposium held during 2nd – 3rd March 2021 at NABI, Mohali.
26. Dr. Kanthi Kiran presented a poster on the topic "Prevention of dextran sodium sulphate (DSS) induced colitis in mice using putative probiotic and synbiotics" at 3rd Chintan symposium held during 2nd – 3rd March 2021 at NABI, Mohali.
27. Dr. Mahendra Bishnoi and Dr. Kanthi Kiran presented a poster on the topic "Assessment of compatibility of commensal gut bacteria" at 3rd Chintan symposium held during 2nd – 3rd March 2021 at NABI, Mohali.
28. Dr. Mahendra Bishnoi and Dr. Kanthi Kiran presented a poster on the topic "In vitro assessment of millet polyphenol rich extract in ameliorating fatty acid induced triglyceride accumulation" at 3rd Chintan symposium held during 2nd – 3rd March 2021 at NABI, Mohali.
29. Ms. Raminder kaur gave an oral talk on topic "Deciphering the dimerization potential of 300 bZIP proteins in bread wheat" at 3rd Chintan symposium held during 2nd – 3rd March 2021 at NABI, Mohali.
30. Ms. Diksha Choudhary and Dr. Vikas Rishi presented a poster on the topic "Effect of plant derived exosome like nanoparticles on adipogenesisin" at 3rd Chintan symposium held during 2nd – 3rd March 2021 at NABI, Mohali.
31. Ms. Neha Thakur delivered an oral presentation on the topic "Genome-wide identification and analysis of GHMP kinase gene superfamily and functional characterization of glucuronokinase gene from wheat (Triticum aestivum L.)" at 3rd Chintan symposium held during 2nd – 3rd March 2021 at NABI, Mohali.
32. Ms. Roni Chaudhary presented a poster on the topic "Understanding the role of Wuschel related homeobox gene family in somatic embryogenesis of banana (cv. Grand Naine AAA)" at 3rd Chintan symposium held during 2nd – 3rd March 2021 at NABI, Mohali.
33. Mr. Siddhant Chaturvedi (SRF) presented a poster on the topic "Genome-wide identification and expression analysis of TCP transcription factor gene family in banana reveal a significant role of MaTCP during Fusarium infection" at 3rd Chintan symposium held during 2nd – 3rd March 2021 at NABI, Mohali.
34. Dr Siddharth Tiwari was invited in the webinar on the topic "Stakeholders dialogue on enabling policies for harnessing the potential of genome editing in crop improvement" organized by the Trust for Advancement in Agricultural Sciences (TAAS), in collaboration with ICAR, NAAS, BCIL, TIGS, NABI and BIRAC on 17th March 2021.
35. Dr. Siddharth Tiwari delivered an invited talk on the topic "Crop improvement strategies in the 21st century" organized by the Dept. of Botany, School of Bioengineering and Biosciences, Lovely Professional University (LPU), Phagwara on 27th March 2021.
36. Dr. Siddharth Tiwari was invited for an expert lecture in NAHEP (ICAR) sponsored national webinar on "Genome editing in agriculture: current status and prospects" organized by the Department of Agricultural Biotechnology, Assam Agricultural University (AAU), Jorhat.
37. Dr. Karambir Kaur delivered an invited talk on the topic "Efficient sgRNA designing for knocking out genes" and demonstrated practical application in the workshop on "Hands-on workshop on CRISPR-Cas gene-editing tools" held during 23rd – 27th March 2021 at SGT University, Gurugram.
38. Dr Siddharth Tiwari delivered a talk on the topic "Application of genome editing for nutritional improvement in banana" in the workshop on "Hands-on workshop on CRISPR-Cas gene-editing tools" organized by the SGT University, Gurugram on 24th March, 2021.

Extramural Grants & Fundings

S. No.	Name and Designation of PI	Budget Grant (Rs.in Lac)	Funding Agency	Project Title	Period of Project	
					From	To
1.	Dr. Siddharth Tiwari	179.20	BIRAC & DBT, GOI	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.	November 2012	March 2023
2.	Dr. T.R. Sharma (Project Coordinator) Dr. Koushik Mazumder - PI	275.05	BIRAC	Setting up of Secondary Agriculture/Food Processing Entrepreneurial Network in Punjab Phase-I	March 2018	May 2021
3.	Dr. Siddharth Tiwari	55.92	DBT, GOI	CRISPR/Cas mediated genome editing of genes for high pro-vitamin A accumulation and its stability in banana	March 2018	March 2022
4.	Dr. Joy K Roy (PI) Dr. T.R. Sharma (Co-PI)	59.51	DBT, GOI	Genome -Wide mapping of QTLs controlling healthy amylose starch variation in wheat	September 2018	September 2021
5.	Dr. T.R. Sharma (Project Coordinator) Dr. Joy K Roy (PI)	1122.05	PSA, GOI and Bioendev Sweden	Development and validation of technology for production of high energy density bio-coal from rice straw and other agri-biomasses	January 2019	June 2021
6.	Dr. Kanthi Kiran Dr. Mahendra Bishnoi	62.75	DBT, GOI	Development of amaranth core collection using SSR and SNP markers and evaluation of core set for nutritional, yield traits and abiotic stress tolerance	March 2019	March 2024
7.	Dr. Monika Garg	79.83	DBT, GOI	Bio fortified antioxidant rich colored wheat cultivation under organic and agronomic supplementation (Fe, Zn, Protein) strategies to increase farm income	March 2019	March 2021
8.	Dr. T.R. Sharma (Project Coordinator) Dr. Joy K Roy (PI)	337.79	DBT, GOI	High resolution QTL mapping for iron (Fe), zinc (Zn), grain protein, and phytate content and their introgression in high yielding wheat cultivars	March 2019	March 2022
9.	Dr. Hena Dhar	35.67	SERB & DST	Genomics-guided exploration of polyketide synthases for sustainable production of long chain omega-3 fatty acid via metabolic engineering	March 2019	March 2022
10.	Dr. Vanish Kumar	41.20	SERB & DST	Development of bio nanoplatform-based ultrasensitive sensor for the detection of food contaminants with enhanced portability and regenerability.	March 2019	March 2022
11.	Dr. T.R. Sharma Dr. Humaira Sonah Dr. Rupesh Deshmukh Dr. Sivasubramanian	59.04	DBT, GOI	Imparting sheath blight disease tolerance in rice	June 2019	June 2022

12.	Dr. Gulshan Kumar	33.72	SERB, DST, GOI	Development of microalgae-based feed supplement for enhanced bioavailability of essential micronutrients for monogastric animals	October2019	October 2022
13.	Dr. Rupam Kumar Bhunia	25.13	SERB, DST, GOI	Metabolic engineering of triacylglycerol (TAG) biosynthetic pathway to enhance the nutritional quality of forage sorghum and rumen protection of dietary lipids	March 2020	March 2023
14.	Dr. Pramod Kandoth Dr. Ajay K. Panday and Dr. Siddharth Tiwari	32.69	SERB, DST, GOI	Genomic approaches to reduce B-ODABP levels in Lathyurs spp.	January 2020	January 2023
15.	Dr. Rupesh Deshmukh and Dr. Humira Sonah	37.91	SERB, DST, GOI	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)	January 2020	January 2023
16.	Dr. Nitin K Singhal	30.96	SERB, DST, GOI	Development of Glycoconjugates based site directed fluorescent sensor for the detection of bacteria	February 2020	February 2022
17.	Dr. Rupesh Deshmukh and Dr. Humira Sonah	71.98	DBT, GOI	Development of superior haplotype based near isogenic Lines (Haplo-NILs) for enhanced genetic gain in rice	February 2020	February 2023
18.	Dr. Monika Garg Co-PI – Dr. Koushik Majumdar	78.05	DBT, GOI	Characterisation of Genetic Resources: Germplasm Characterization and Trait Discovery in wheat using Genomics Approaches and its Integration for Improving Climate Resilience, Productivity and Nutritional Quality	March 2020	March 2023
19.	Dr. Ajay K. Pandey	33.12	SERB, DST, GOI	Elucidating the role of wheat ferroproteins during cellular iron homeostasis	February 2020	February 2025
20.	Dr. Ravinder Pal Singh (PI), Dr. Mahendra Bishnoi and Dr. Kanthi Kiran Kondepudi (Co-PI)	43.07	DBT, GOI	Novel enzymatic strategy for synthesizing human milk oligosaccharides for promoting health of infants	December 2020	December 2023
21.	Dr. Kanti Kiran Kondepudi	45.93	ICMR	A Nutrigenomics Driven symbiotic formulation development for the prevention of metabolic syndrome	February 2021	January 2024
22.	Dr. Sivadubramanian R	33.12	SERB, DST, GOI	Mining non-host resistance (NHR) genes for engineering Alteraria Blight resistance in Indian Mustard (Brassica juncea)	March 2021	March 2024

14.	Dr. Pramod Kandoth Dr. Ajay K. Panday and Dr. Siddharth Tiwari	32.69	SERB, DST, GOI	Genomic approaches to reduce B-ODABP levels in Lathyurs spp.	January 2020	January 2023
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21ST MARCH 2021

Inauguration of Advanced High Resolution Microscopy Facility

Hon'ble Minister of Health & Family Welfare, Science & Technology and Earth Sciences **Dr. Harsh Vardhan** inaugurating the advanced 'High Resolution Microscopy Facility' at NABI.

Hon'ble Minister of Health & Family Welfare, Science & Technology and Earth Sciences **Dr. Harsh Vardhan** interacting with faculty about the technologies developed by NABI.

Prof. Ashwani Pareek, Executive Director, NABI presenting a memento and shawl to **Dr. Harsh Vardhan**, Hon'ble Minister of Health & Family Welfare, Science & Technology and Earth Sciences.

26TH JANUARY 2021

Republic Day Celebrations at NABI

Prof. Ashwani Pareek, Executive Director, NABI hoisting the National Flag.

A group picture of NABI & CIAB staff during Republic Day

18TH FEBRUARY 2021

11th Foundation Day celebration at NABI

Dr. Renu Swarup, Secretary, DBT was the Chief Guest on the occasion and attended the event virtually.

Dr. Shoba Sivasankar, , Head, Plant Breeding and Genetics, Joint FAO/IAEA Division of United Nations, Austria was the Guest of Honour and delivered foundation lecture on "Induced genetic diversity for enhanced crop performance and adaptation to climate change".

18TH FEBRUARY 2021

11th Foundation Day celebration at NABI

Prize distributions by **Prof. Ashwani Pareek** during the 11th foundation day of NABI.

Inaugural lecture by **Prof. Ashwani Pareek** during the 11th foundation day of NABI.

ACCOUNTS
OF THE INSTITUTE

Goyal Parul & Co.
Chartered Accountants

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

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 March 31, 2021**, the Income and Expenditure account and Receipt & payments 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, 2021 and

b) In the case of Income and Expenditure Account, of the surplus/(deficit) of the Institution for the year ended on that date

For Goyal Parul & Co.
Chartered Accountants

(CA Vijay Kumar)
Partner
Membership No.506042
UDIN: 21506042AAAAFP1326

Place: Mohali
Date: 17.08.2021

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 2021

(Amount in Rs.)

CORPUS/ CAPITAL FUND AND LIABILITIES	Schedule	Current Year	Previous Year
Corpus/Capital Fund	1	1,52,47,93,753	1,63,68,86,499
Reserves and Surplus	2	1	1
Earmarked / Endowment / Project Grants	3	6,15,87,012	8,67,21,643
Secured Loans and Borrowings	4	-	-
Unsecured Loans and Borrowings	5	-	-
Deferred Credit Liabilities	6	-	-
Current Liabilities and Provisions	7	6,39,23,103	7,17,82,299
TOTAL		**1,65,03,03,869**	**1,79,53,90,442**
ASSETS			
Fixed Assets	8	1,44,11,28,489	1,46,18,63,247
Investments- from Earmarked/Endowment funds	9	-	-
Investments - Others	10	-	-
Current Assets, Loans & Advances etc.	11	20,91,75,380	33,35,27,195
TOTAL		**1,65,03,03,869**	**1,79,53,90,442**
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: 17/08/2021
Place: Mohali

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 2021

(Amount in Rs.)

INCOME	Schedule	Current Year	Previous Year
Income from Sales/Services	12	-	-
Grants in aid /subsidies	13	14,25,00,000	15,30,00,000
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	98,41,089	1,04,45,445
Other Income	18	1,04,05,245	1,17,80,531
Increase/decrease in stock of finished goods & work-in -progress	19	-	-
TOTAL(A)		**16,27,46,334**	**17,52,25,976**
EXPENDITURE			
Establishment Expenses	20	3,79,91,206	4,31,42,335
Other Administrative Expenses	21	7,80,65,152	7,97,57,043
Research & Development Expenditure (Incl. Grants, Subsidies etc)	22	3,83,99,852	5,27,62,140
Interest	23	-	-
Depreciation (net total at the year end-corresponding to schedule 8)		18,54,75,249	18,39,42,173
TOTAL(B)		**33,99,31,459**	**35,96,03,691**
Balance being surplus/(deficit) carried to Capital Fund (A-B)		-17,71,85,126	-18,43,77,715
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: 17/08/2021
Place: Mohali

Page 2 of 20

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 31.03.2021

(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	3,16,09,865	3,38,64,535
b) Bank Balances			2. Expenses on Employees Retirement & terminal benefits	1,65,09,385	32,34,213
i) In Current Accounts	-	-			
ii) In Deposit Accounts	29,72,70,444	27,60,53,099	**(B) Other Administrative Expenses**		
iii) In Savings Accounts	1,76,64,321	33,28,367	1. Cartage & Carriage inward	45,590	15,625
			2. Honorarium /Sitting Fee	2,40,000	6,56,937
			3. Electricity, power and Water charges	2,40,31,482	2,11,14,527
(B) Grant-in-Aid			4. AMCs/ CMCs	94,08,576	56,85,408
(a) Grant from DBT	15,75,00,000	30,15,00,000	5. Vehicles Running & maintenance	4,06,904	5,57,555
			6. Postage, Telephone & communication charges	5,14,871	6,35,457
			7. Printing & stationery	7,49,698	10,30,126
(C) Interest Incomes			8. Travelling & conveyance expenses	2,33,106	24,95,566
(a) Interest Income	1,09,76,468	1,00,07,574	9. Outsourcing Manpower Exp	2,19,86,986	2,10,71,452
			10. Legal & Professional charges	3,04,260	3,14,321
			11. Advt. & publicity	18,32,980	11,80,635
(D) Other Incomes			12. Repair & Maintenance Building	20,39,272	26,81,882
1. Tender Fees	40,000	52,151	13. Office & Admn Expenses	9,58,663	15,77,188
2. Sample Analysis	7,853	21,484	14. Guest House Expenditure	10,11,989	12,19,319
3. Guest House Income	1,74,250	6,22,314	15. Watch & Ward Expenses	24,53,793	30,39,250
4. RTI Fee	20	30	16. Hostel Expenses	23,77,483	30,18,009
5. Project Income	35,10,849	20,34,985			
6. Training fee	3,45,000	12,53,276			
7. Staff Quarter Licence fee	7,15,638	7,15,598	**(C) Research & Development Expenditure**		
8. Hostel Licence Fee	7,06,905	11,72,511	1. Chemicals & Consumables	1,87,85,602	3,03,25,901
9. Application fee	41,901	78,781	2. Fellowships	1,62,55,072	1,16,30,798
10. Rental Income	48,53,518	34,55,922	3. Computer Software & Accessories	6,53,435	11,19,649
11. Technology Transfer	1,54,991	1,78,012	4. Research Work Expenses	82,923	83,638
12. Misc. Income	26,95,867	20,23,965	5. Field Expenses	40,10,587	36,39,881
13. Campus facilities	52,247	2,21,894	6. Patent Filing Expenses	51,568	29,690
14. Ph. D Fee	4,84,000	4,59,600	7. Workshops and seminars	9,286	11,08,728
15. Income from Consultancy Services	2,16,608	-	8. Research Publication Expenses	10,15,877	12,00,512
16. LD Charges	6,098	-	9. Sequencing Expenses	17,54,730	38,17,020
17. Sale of Garments	67,985	-	10. Recognition & Membership Fee	6,07,036	6,05,500
18. Staff Car Usage	700	-			
(E) Other Projects Receipt (including interest earned)	8,50,09,209	7,46,75,201	**(D) Non-Recurring Expenditures**		
(F) Other Receipt			1. Development of Main Campus	1,10,22,594	24,89,203
1. Biotech Lab	16,217	-	2. Scientific Equip & Research Asst	9,16,09,866	13,05,70,828
2. Caution Money	20,000	35,000	3. Computers & Books	55,72,028	55,46,176
3. M/s Perkin Elmer	1,800	-	4. Furniture & Fixture	14,67,673	23,19,675
4. Staff welfare fund	54,561	82,677	5. Office Equipment	32,06,287	2,40,720
5. Earnest Money Deposit	10,01,000	1,05,804	6. Library Books & Periodicals	14,990	4,829
6. Nobel Prize Series	-	2,05,42,948	7. Vehicles	-	18,081
7. NITI Aayog	-	95,817			
8. TDS Refund	-	59,728	**(E) Other Payments**		
9. M/s RITES Ltd	1,42,49,228	-	1. External Project Expenses	11,41,47,809	7,15,66,704
10. M/s Media solutions	2,700	-	2. Nobel Prize Series	1,25,21,188	-
11. M/s Orient Overseas Container Line Limited	26,223	-	3. NABI Biotech Club expenses	29,900	67,362
12. TDS Refund received	4,075	-	4. Security Deposits paid	3,90,851	3,24,855
13. Aahar ITPO	34,920	-	5. Interest refunded to CFI	1,00,83,576	98,86,030
14. GST account	28,175	-			
			(F) Loans & Advances		
			1. Advance to Employees	4720.00	40,500
			2. Advance to M/s Pyramid Builders	17,882	-
			3. Amount recoupable from CIAB	10,44,044	99,501
			4. M/s Orient Overseas Container Line Limited	-	26,223
			5. Aahar ITPO	-	34,920
			(G) Closing Balance		
			a) Cash in Hand	-	-
			b) Bank Balances		
			i) In Deposit Accounts	17,62,75,125	29,72,70,444
			ii) In Savings Accounts	73,42,600	1,76,64,321
Grand Total	59,88,37,784	70,27,20,308	**Grand Total**	59,88,37,784	70,27,20,308

In terms of separate report of even date attached

For National Agri Food Biotechnology Institute

(DR. MUNISH SINGLA)
FINANCE OFFICER

MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

Dated: 17/08/2021
Place: Mohali

for Walia Pant & Co.
Chartered Accountants

(SANJAY KUMAR)
PARTNER
Membership No. 286042

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 31.03.2021

SCHEDULE-1
CORPUS/CAPITAL FUND

(Amount In Rs.)

Particulars	Current Year	Previous Year
Balance as at the beginning of the year	1,63,68,86,499	1,62,41,15,289
Add : Contributions towards corpus/capital fund	1,50,00,000	14,85,00,000
Add : Fixed Assets Created out of Project Grants	4,40,97,380	4,86,48,925
Add : Gratuity & LE transferred to LIC	59,95,000	-
Less/(Deduct) : Expenditure over Income transferred from the income & expenditure A/c	-17,71,85,126	-18,43,77,715
BALANCE AS AT THE YEAR -END	**1,52,47,93,753**	**1,63,68,86,499**

SCHEDULE-2
RESERVES AND SURPLUS

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

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: 17/08/2021
Place: Mohali

Schedule-3: EARMARKED/ENDOWMENT/PROJECT GRANTS

Sr. No.	Project Name	Additions				Utilisation/Expenditure							Refund	Net Balance as on 31-03-2021
		a) Opening balance as on 01-04-2020	b) Additions during the Year	c) Accrued Interest/Income Recd. on Investment	TOTAL (a+b+c)	i) Capital Expenditure	ii) Revenue Expenditure: Fellowship	Chemical & Consumable	Contingency Exp/Travel etc	Overhead Exp	TOTAL	Total Expenses		
1	Development and Transfer of Technology from Queensland University of Technology, Australia to India for Bio-fortification and Disease Resistance in Banana (GAP 02)	5,93,834	1,16,30,000	15,609	1,21,93,443	95,303	11,11,538	24,957		-	11,36,495	12,31,851		1,09,61,592
2	Metagenomic and Functional Characterization of Soy-based Fermented Foods of Northeastern Region (GAP 16)	50,879	-		50,879			46,296	7,091	-	50,779	50,779	100	-
3	Genome and Transcriptome Sequencing of Aromatic rices from North Eastern Region (GAP 17)	491			491						-	-		491
4	Utilizing genome editing tools for nutritional improvement in wheat (GAP 18)	9,61,671		7,030	9,68,701		2,00,000	7,34,042	34,659	-	9,68,701	9,68,701		-
5	Connections: A Comprehensive biological relationships resources and tools for automated literature mining (GAP 19)	7,18,830		3,642	7,22,472			3,75,740	42,085	-	4,17,825	4,17,825	3,04,647	-
6	Functional Characterization and Implications of Plant Inositol Pyrophosphate Kinase (GAP 20)	6,49,497			6,49,498		1,26,320	2,03,110	29,168	-	6,37,721	6,37,721	11,777	-
7	Enhanced rice milling and cross cutting valorization of rice milling by product (GAP 21)	4,87,313		1,076	4,88,389			490	60,000	9,15,929	9,76,419	9,76,419		-4,88,030
8	Pharmacological mimicking of cold via cold thermoreceptors (GAP 22)	13,40,531		14,051	13,54,584	2,36,250		9,718		1,79,028	1,88,736	4,24,986		9,29,598
9	Setting up of Secondary Agriculture/ Food Processing Entrepreneurial Network in Punjab-Phase-I (GAP 23)	1,28,35,249	-	1,27,941	1,29,63,190	95,27,452	7,20,000	16,20,000	98,581	-	23,38,581	1,18,66,033		10,96,157
10	CRISPR/Cas mediated genome editing of genes for high provitamin A accumulation and its stability in banana (GAP 24)	45,642	15,12,307	12,943	15,70,892		4,25,523	10,03,885	85,026	1,00,000	15,14,434	15,14,434		47,858
11	Genome-Wide mapping of QTLs controlling healthy amylose starch variation in wheat (GAP 25)	31,32,578	13,79,514	52,200	45,64,292		9,33,047	24,13,846	86,260	-	34,45,453	34,45,453	18,571	11,00,268
12	Development and validation of technology for production of high energy density biocoal from rice straw and other agri-biomasses (GAP 26)	3,89,65,826	-	6,56,118	3,96,21,944	3,27,65,705	66,78,521	1,13,000	9,969		67,99,490	3,95,63,597		40,43,547
13	Utilizing genome editing tools for nutritional improvement in wheat (GAP-27)	-	15,15,040	24,932	15,37,972		4,32,145	4,96,351	25,997	30,006	10,53,935	10,53,935	-	4,84,037
14	Bio fortified antioxidant rich colored wheat cultivation under organic and agronomic supplementation (Fe, Zn, Protein) strategies to increase farm income (GAP 28)	18,80,331	25,85,310	29,078	45,94,719		6,99,000	22,34,043	1,99,934	11,13,089	38,76,687	38,76,687		6,25,006
15	High resolution QTL mapping for iron (Fe), zinc (Zn), grain protein, and phytate content and their introgression in high yielding wheat cultivars (GAP 29)	83,11,182	1,66,34,400	78,510	1,79,24,092		35,92,034	44,77,346	25,978	-	80,47,096	80,47,096	2,56,000	87,21,073
16	Genomics-guided exploration of polyketide synthases for sustainable production of long chain omega-3 fatty acid via metabolic engineering (GAP 30)	10,74,410	4,50,000	24,629	15,49,039		4,51,737	2,86,560	11,597	50,244	8,00,138	8,00,138		7,48,901
17	Development of bioreceptor/platform-based ultrasensitive sensor for the detection of food contaminants with enhanced portability and sustainability (GAP 31)	1,30,450	8,77,070	12,102	10,19,622		4,31,520	2,28,200	10,000	65,406	7,43,126	7,43,126		2,86,496
18	Imparting sheath blight disease tolerance in rice (GAP 32)	14,19,833	11,20,000	15,117	25,58,950	4,62,014	8,24,005	3,59,561	8,556	-	12,35,246	16,97,260		[illegible]
19	Development of microalgae based food supplement for enhanced bioavailability of essential micronutrients for monogastric animals (GAP 33)	2,12,491	7,00,000	9,606	9,22,097		3,42,000	3,88,166	18,311	98,556	8,35,333	8,35,333		86,4[illegible]

20	Metabolic engineering of triacylglycerol (TAG) biosynthetic pathway to enhance the nutritional quality of forage sorghum and sustain protection of dietary lipids (GAP 34)	7,65,666		8,848	7,74,514		2,72,000	3,49,374	23,000	-	7,86,374	7,86,374		28,140
21	Genomic approaches to reduce β-ODAP levels in Lathyrus spp (GAP 35)	9,57,592		12,864	9,70,456		2,72,000	4,00,907	10,000		8,83,907	8,83,907		86,549
22	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)	12,80,620	3,00,000	15,420	15,29,020		1,86,700	8,04,565	49,715	10,621	13,35,841	13,35,041		3,04,617
23	Development of Glycoconjugates based site directed fluorescent sensor for the detection of bacteria (GAP 37)	9,77,871		11,157	10,14,028		3,31,000	5,25,235	18,907	-	8,85,142	8,85,142		1,28,886
24	Development of superior haplotype based near isogenic Lines (Haplo-NILs) for enhanced genetic gain in rice (GAP 38)	24,31,413	3,92,000	68,913	28,92,326	11,600	2,50,016	11,28,530	1,70,993	-	15,42,547	15,37,147		13,55,119
25	Elucidating the role of wheat ferroportins during cellular iron homeostasis (GAP 40)		14,10,829	11,641	14,22,470		34,331	2,27,161		1,04,586	3,66,238	3,66,238		10,56,132
26	Novel enzymatic strategy for synthesizing human milk oligosaccharides for promoting health of infants (GAP 41)		6,73,880	3,217	6,77,097		48,714	3,85,083			4,33,797	4,33,797		2,45,300
27	A metagenomics driven formulation development for the prevention of metabolic syndrome (GAP-42)		5,07,958	2,170	5,10,128		22,581			14,358	36,939	36,939		4,73,189
28	Mining non-host resistance (NHR) genes for engineering Alternaria Blight resistance in Indian Mustard (GAP-43)		11,10,829	1,150	11,11,979						-	-		11,11,979
29	National Super Computer Mission (CDAC)		1,87,00,000		1,87,00,000						-	-		1,87,00,000
30	Skill Vigyan Program		16,20,000		16,20,000						-	-		16,20,000
31	Department of Biotechnology (DBT) JRF/SRF fellowships	1,35,932	17,28,000		18,63,932		16,08,000		48,129	760	16,57,089	16,57,089		2,06,843
32	Council of Scientific & Industrial Research (CSIR) JRF/SRF Fellowships	1,03,832	3,67,800		4,71,632		16,650		1,28,390		1,45,040	1,45,040		3,26,592
33	Indian Council of Medical Research (ICMR) JRF/SRF Fellowships	4,73,400	28,41,888		33,15,288		27,13,416		1,08,338		28,21,754	28,21,754		4,93,499
34	JC Bose Fellowship	13,06,835		29,712	13,36,547		2,75,000		14,02,416		16,77,416	16,77,416		-40,869
35	DST Inspire Fellowship	2,75,981	9,13,316		11,89,326		10,24,955		48,763		10,73,718	10,73,718		1,15,600
36	National Post Doc Fellowship	18,755			18,755		17,014			1,741	18,755	18,755		-
37	Ramalingaswamy Fellowship	12,38,130	82,61,722		94,99,852		36,88,000	4,20,836	31,02,170	50,000	83,10,006	83,10,006		11,89,855
38	DST Inspire Faculty Fellowship	49,15,803	1,49,14,679		1,98,30,482		1,07,48,930	48,57,940	7,13,434	1,40,000	1,57,09,772	1,57,09,772		40,70,710
39	Newton Bhabha Ph. D Placement Programme	1,03,500			1,03,500						-	-		1,03,500
40	Agreement for Industry Research with M/s Chemica Food Industries Limited (EP 01)	3,98,000	3,68,673		7,36,699		3,65,528	2,14,971	59,629	60,000	6,69,816	6,69,816		56,883
41	Genetically Enhanced Pearl Millet with High Grain Iron Density for Improved Human Nutrition in India (EP 04)	-	2,94,364		2,94,364			2,40,820		-	2,40,820	2,40,820		53,544
42	Technology for sugar free food product by using prebiotic sweetener, isomalto-oligosaccharides (EP 03)	-	9,26,259		9,26,259		2,83,677	3,93,698	13,983	-	6,91,358	6,91,358		2,35,892
43	Agreement for Industry Research with M/s Panacea Healthcare Labs (EP 11)	2,52,247			2,52,247		1,69,891	55,524		-	2,25,415	2,25,415		26,832
	Total	3,67,21,610	9,57,86,718	18,14,490	17,27,20,851	4,40,57,380	3,47,25,730	2,51,33,037	55,14,859	19,30,976	6,93,34,604	11,34,20,984	7,20,855	4,15,87,812

Dated: 17/09/2021
Place: Mohali

Finance Officer
National Agri-Food Biotechnology Institute

Suneet Verma
Manager (Finance)
National Agri-Food Biotechnology Institute

Executive Director
National Agri-Food Biotechnology Institute
Deptt. of Biotechnology, Govt. of India
Mohali-140306, Punjab, India

Partner

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.306042

Dated: 17/08/2021
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	1,99,61,202	1,53,72,124
b) For Securities deposited by others	26,82,098	30,72,949
c) Earnest Money Deposit	19,18,122	9,17,122
d) Caution Money deposited by Students	1,25,000	1,05,000
e) NABI Biotech Club	16,217	-
f) Staff Welfare Fund	2,11,553	1,56,992
g) Nobel Prize Series	1,10,21,760	2,45,42,948
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	25,24,523	24,59,084
b) Other Expenses Payable	23,79,766	33,34,580
c) TDS Payable	5,85,407	8,13,615
d) Fellowship Payable	48,31,020	47,37,535
e) GST Payable	3,03,749	2,65,575
f) Punjab Development Tax Payable	7,400	6,200
g) Interest refundable to CFI	93,09,262	1,00,03,576
TOTAL(A)	**5,58,77,079**	**6,57,87,299**
B) PROVISIONS		
1. Gratuity	33,34,773	32,44,334
2. Leave Encashment	47,11,251	27,50,666
TOTAL(B)	80,46,024	59,95,000
TOTAL(A+B)	**6,39,23,103**	**7,17,82,299**

For National Agri-Food Biotechnology Institute

M/s Goyal Parul & Co.
Chartered Accountants

FINANCE OFFICER

MANAGER FINANCE

EXECUTIVE DIRECTOR

CA VIJAY KUMAR
PARTNER
Membership No.506042

Dated: 17/08/2021
Place: Mohali

NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE
NABI Campus, Knowledge City, Sector 81, PO Manauli, SAS Nagar, Mohali
SCHEDULE-4 FIXED ASSETS

Sl. No.	Description	Depreciation Rate	GROSS BLOCK: Cost/Valuation as at beginning of the year (1st April 2020)	GROSS BLOCK: Additions during the year (UPTO 30.09.20)	GROSS BLOCK: Additions during the year (AFTER 30.09.20)	GROSS BLOCK: Deduction during the year (2020-21)	GROSS BLOCK: Cost/Valuation at the year end (31st March 2021)	DEPRECIATION: As at the beginning of the year (1st April 2020)	DEPRECIATION: Depreciation during the year (2020-21)	DEPRECIATION: Total at the year end (31st March 2021)	NET BLOCK: As at the Current Year End (31st March 2021)	NET BLOCK: As at the Previous Year End (31st March 2020)
A)	FIXED ASSETS CREATED FROM GOVT. GRANTS											
I	LAND											
	a) Free Hold	0.00%	1	-	-	-	1	-	-	-	1	1
	b) Lease Hold	0.00%	-	-	-	-	-	-	-	-	-	-
II	BUILDINGS											
	a) On Freehold Land	[illegible]	1,51,83,33,702	18,21,828	1,02,35,311	-	1,53,05,76,851	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	b) On Leasehold Land	[illegible]	-	-	-	-	-	-	-	-	-	-
	c) Ownership Premises	[illegible]	-	-	-	-	-	-	-	-	-	-
	d) Other Superstructures	[illegible]	-	-	-	-	-	-	-	-	-	-
III	PLANT, MACHINERY & EQUIPMENT EQUIPMENTS	[illegible]	[illegible]	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
IV	VEHICLES	[illegible]	[illegible]	-	-	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
V	FURNITURE & FIXTURES	[illegible]	[illegible]	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
VI	COMPUTERS/PERIPHERALS	[illegible]	[illegible]	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
VII	LIBRARY BOOKS	[illegible]	[illegible]	-	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
VIII	OFFICE EQUIPMENT	[illegible]	[illegible]	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	TOTAL OF CURRENT YEAR (A)		[illegible]	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
B)	Fixed Asset Created from Projects Grants:											
	Equipments	[illegible]	[illegible]	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Furniture & Fixtures	[illegible]	[illegible]	-	-	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Computers/Peripherals	[illegible]	[illegible]	-	-	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Library Books	[illegible]	[illegible]	-	-	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Office Equipments	[illegible]	-	-	-	-	-	-	-	-	-	-
	Building	[illegible]	[illegible]	-	-	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	TOTAL OF FIXED ASSETS PROCURED FROM PROJECTS (B)		[illegible]	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	TOTAL (A+B)		[illegible]	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	PREVIOUS YEAR											
	a) Expenditure on Assets/Fixed Assets		-	-	-	-	-	-	-	-	-	-
	b) Expenditure on Plan Activities		-	-	-	-	-	-	-	-	-	-
	TOTAL OF PREVIOUS YEAR		-	-	-	-	-	-	-	-	-	-
C)	CAPITAL WORK-IN-PROGRESS											
	a) Equipment		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-	-	-	[illegible]	[illegible]
	TOTAL OF CURRENT YEAR (CWIP) (C)		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-	-	-	[illegible]	[illegible]
	GRAND TOTAL (A+B+C)		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

For National Agri-Food Biotechnology Institute

Dr. MUNISH SINGLA
Finance Officer

SUMEET VERMA
Manager Finance

Executive Director

For Chartered Accountants
Partner

Dated: [illegible]
Place: Mohali

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.500042

Dated: 17/08/2021
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	1,03,75,011	-
5. LIC Leave Encashment Scheme	1,12,38,912	-
6. Bank balances:		
a) With Scheduled Banks:		
-On Current accounts	-	-
-On Fixed Deposit accounts	17,62,75,125	29,72,70,444
-On Savings accounts		
(i) State Bank of India A/c	73,42,600	1,76,64,321
TOTAL(A)	**20,52,31,648**	**31,49,34,765**
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	1,42,71,231
c) Recoupable form Govt. Agencies & others		
(i) NIPER	5,900	5,900
(ii) Kuber Roller Floor Mills	5,523	-
(iii) INST	-	2,58,958
(iv) CIAB	12,19,752	1,75,108
(v) Anton Parr India Pvt. Ltd.	29,500	-
(vi) M/s Perkin Elmer	-	1,800
(vii) M/s E media solutions	-	2,700
(viii) M/s Balmer Lawrie & Co. Ltd.	-	13,382
(ix) M/s Orient Overseas Container Line Limited	-	26,223
(x) M/s Pyramid Builders	13,882	-
d) Advance to Employees	22,720	42,000
e) Others(specify)		
(i) Security for Gas Connection to M/s Chahal Gas	26,250	26,250
(ii) Deposit with PSPCL	44,581	44,581
(iii) TDS/TCS Receivable	43,265	6,442
(v) PSEB Elelct Security for Main Campus	11,12,090	11,12,090
(vi) Aahar ITPO	-	34,920
3.Income accrued:		
a) On investments from earmarked/endowment funds	-	-
b) Interest On Saving A/c and Fixed Deposits	13,98,266	25,70,845
c) On loans & advances	-	-
4. Claims Receivable		
TOTAL(B)	**39,43,732**	**1,85,92,430**
TOTAL(A+B)	**20,91,75,380**	**33,35,27,195**

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: 17/08/2021
Place: Mohali

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	14,25,00,000	15,30,00,000
2. State Government	-	-
3. Government Agencies	-	-
4. Institutional /welfare bodies	-	-
5. International Organisations	-	-
6. Others (to be specified)	-	-
TOTAL	14,25,00,000	15,30,00,000

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

(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: 17/08/2021
Place: Mohali

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	79,74,859	74,85,491
ii) Accrued as on 31.03.2021	13,98,266	25,70,845
b) With Non-Scheduled Banks:	-	-
2) On Savings Accounts:		
a) With Scheduled Banks:	2,29,138	3,78,405
b) With Non-Scheduled Banks:	-	-
c) With Serb Account	1,95,137	-
d) With Project Account	3,767	-
3) On Loans		
a) Employees/staff	2,362	4,500
4) Interest on Debtors & other Receivables		
a) Interest on refund of Income Tax	360	6,204
b) Interest on PSPCL Security	37,200	-
TOTAL	**98,41,089**	**1,04,45,445**

For National Agri-Food Biotechnology Institute

M/s Goyal Parul & Co.
Chartered Accountants

(DR. MUNISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

EXECUTIVE DIRECTOR

(CA VIJAY KUMAR)
PARTNER
Membership No.506442

Dated: 17/08/2021
Place: Mohali

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	35,10,845	20,34,985
4. Miscellaneous Income		
a) Tender Fees	40,000	63,411
b) Sample Analysis	13,376	27,352
c) Guest House (Income)	3,74,250	6,24,014
d) RTI Fee	20	30
e) Training Fee	3,45,000	12,58,676
f) Staff Quarters Licence Fee	7,15,620	7,19,088
g) Hostel fee	7,06,905	11,72,911
h) Application fee	12,900	24,883
i) Rental income	15,79,174	29,58,429
j) Technology transfer	3,71,199	1,88,149
k) Misc Income	21,99,709	20,27,654
l) Campus facilities	52,247	2,21,259
m) Ph. D Fee	4,84,000	4,59,690
TOTAL	1,04,05,245	1,17,80,531

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: 17/08/2021
Place: Mohali

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	3,50,49,720	3,39,13,122
2. Expenses on Employees Retirement & terminal benefits	29,41,486	32,34,213
3. Provision for Gratuity and leave encashment	-	59,95,000
TOTAL	**3,79,91,206**	**4,31,42,335**

SCHEDULE-21

OTHER ADMINISTRATIVE EXPENSES

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Cartage & Carriage inward	45,590	15,625
2. Honorarium /Sitting Fee	2,40,000	6,56,957
3. Electricity, power and Water charges	2,40,31,682	2,16,69,358
4. AMCs /CMCs	87,06,161	59,79,647
5. Vehicles Running & maintenance	4,06,904	5,53,555
6. Postage,Telephone & communication charges	4,96,583	6,35,937
7. Printing & stationery	7,49,694	10,31,126
8. Travelling & conveyance expenses	2,33,104	24,92,209
9. Outsourcing Manpower Exp	2,19,03,074	2,25,64,705
10. Legel & Professional charges	3,64,266	3,14,321
11. Advt. & publicity and display Expenses	18,01,612	13,83,006
12. Repair, Operation & Maintenance of Building	30,09,272	37,35,182
13. Office & Admn Expenses	9,24,723	13,80,338
14. Guest House Expenditure	10,11,949	12,76,977
15. Watch & Ward Expenses	24,53,793	31,45,688
16. Hostel Expenses	23,77,483	29,18,836
17. Interest earned refundable to CFI	93,09,262	1,00,03,576
TOTAL	**7,80,65,152**	**7,97,57,043**

For National Agri-Food Biotechnology Institute

M/s Goyal Parul & Co.
Chartered Accountants

(DR. MONISH SINGLA)
FINANCE OFFICER

(SUNEET VERMA)
MANAGER FINANCE

(PROF. ASHWANI PAREEK)
EXECUTIVE DIRECTOR

(CA VIJAY KUMAR)
PARTNER
Membership No.596042

Dated: 17/08/2021
Place: Mohali

SCHEDULE-22

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

(Amount in Rs.)

Particulars	Current Year	Previous Year
1. Chemical & Consumables	1,87,69,845	2,92,93,871
2. Fellowship	1,15,17,517	1,16,80,798
3. ICT Services & Consumables, Software , Accessories etc	6,53,435	16,48,623
4. Research Work Expenses	82,921	90,114
5. Field Expenses (Ploughing, RM & Other Job work)	39,99,497	55,10,705
6. Patent Filling Expenses	51,508	29,690
7. Workshops & Seminars	9,286	13,38,728
8. Research Publication Expenses	10,15,877	12,06,913
9. Sequencing Expenses	16,92,930	13,57,199
10. Recognition & Membership Fee	6,07,036	6,05,500
TOTAL	3,83,99,852	5,27,62,140

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: 17/08/2021
Place: Mohali

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. The Land which is allotted free of cost by Govt. of Punjab for setting up of NABI has been taken as 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 2020-21.

F) MISCELLANEOUS EXPENDITURE

There is no deferred revenue expenditure during 2020-21.

G) ACCOUNTING FOR SALES

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

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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 2020-21, recurring grants amounting to Rs. 14,25,00,000/- has been sanctioned for the purpose as shown in Schedule-13. Non-recurring Grants amounting to Rs. 1,50,00,000/- sanctioned by DBT have been shown as addition to Corpus/ Capital Fund (Schedule-1).

Interest earned on Govt. Grant amounting to Rs. 93,09,262/- 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, 2021, pertaining to FY 2020-21, 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 2020-21, 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. 80,46,024/- (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
Dated: 17/08/2021			Partner
Place: Mohali			Membership No. 506042

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 2020-21. It is virtually a copy of cash book / Institute's accounts. The total receipt as shown in receipt & payment account comes to Rs. 28,36,02,939/- which include Rs. 15,75,00,000/- as Recurring and Non-recurring grants from DBT, grant of Rs. 8,90,09,209/- for externally funded projects and Rs. 3,70,93,730/- from other receipts. Total amount of Rs. 41,49,19,978/- has been released as payments during the financial year 2020-21.

2. The Income and Expenditure Account

The Income and Expenditure accounts are prepared on accrual basis. The total income is Rs. 16,27,46,334/- out of which includes Rs. 14,25,00,000/- Recurring Grant from DBT and rest is from Other Resources.

Total expenditure (before depreciation) comes to Rs. 15,44,56,210/- which also includes Rs. 93,09,262/- of interest earned on Grants, which is refundable to Govt., and depreciation of Rs. 18,54,75,249/- has been charged in the current FY 2020-21. A sum of Rs. 17,71,85,126/- 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.

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 2020-21.

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.

Finance Officer
National Agri-Food Biotechnology Institute

Manager (Finance)
National Agri-Food Biotechnology Institute
Govt. of India

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 2021, there is a balance of Rs. 6,15,87,012/- 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. 12,14,490/- 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. 1,03,75,011/- was computed towards liability for gratuity (Schedule-11) out of which an amount of Rs. 70,40,238/- has been paid to LIC during the year and Rs. 33,34,773/- 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. 1,12,38,912/- was worked as liability for leave encashment (Schedule-11) out of which an amount of Rs. 65,27,661/- has been paid to LIC during the year and Rs. 47,11,251/- has been shown as provision for leave encashment (Schedule-7).

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

For M/s Goyal Parul & Co.
Chartered Accountants

Finance Officer

Manager Finance

Executive Director

CA Vijay Kumar

Partner

Membership No.506042

Dated: 17/08/2021

Place: Mohali

20 of 20

NATIONAL AGRI-FOOD BIOTECHNOLOGY INSTITUTE

An Autonomous Institute of the Department of Biotechnology
Government of India

Mohali, Punjab 140306 INDIA

Website: www.nabi.res.in; Tel: +91-172-5221-106