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Conference Report: "Next Generation Proteome Mining and Nano-Bio Interactions for Better Plant Health"

A three-day conference entitled "Next Generation Proteome Mining and Nano-Bio Interactions for Better Plant Health" was organized by the Department of Botany, University of Delhi from 27thto 29th December, 2021 under the umbrella of the Delhi University Botanical Society. This conference was conceptualized because of the fact that (a) biology works at nanoscales (b) in proteomics, there are bottlenecks that can be overcome by working on nano-scale. We could not organize the regularly conducted physical workshop because of the prevailing pandemic conditions.

The inaugural lecture was delivered by Subhra Chakraborty, Director, National Institute of Plant Genome Research. She delivered a lecture on chitin mediated defense machinery with respect to *Fusarium oxysporum*. A cumulative proteomics, transcriptomics and metabolomics approach was used to decipher chitosan responsive network leading to novel insights into host specific immune signaling. This information may facilitate cellular therapeutic targets for fusarium associated diseases.

Session-I entitled "Nano Proteomics: Next Generation Proteome Mining" was chaired by Suman Lakhanpaul, Head Department of Botany and Chairperson, Delhi University Botanical Society, University of Delhi, India. The session was co-chaired by Ravi Gupta, Assistant Professor, Kokmin University, Seoul, South Korea. Ankita Shehrawat, Assistant Professor, Maitreyi College, University of Delhi was the rapporteur for the session. The session had lectures by Gagan Deep Jhingan, Director, V-proteomics, Green Park, Delhi, India and Ravi Gupta. Both the speakers discussed the currently available mass spectrometry hardware and software which may be used for proteomics analysis. A lecture was also delivered by Renu Deswal, Convenor of the conference & Professor, Department of Botany, University of Delhi, India. She provided insights about nano-proteomics, providing an overview about its potential uses in nano fabrication, nano electrophoresis, nano chromatography and nano fluidics. She also discussed about protein corona as an example to understand the mechanism of action of Nanoparticles in biological systems and its applications in proteomics.

Session-II entitled "Nanomaterials for Better Plant Health and Performance" was chaired by Prashant Mishra, Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology, Delhi and co-chaired by Partima Solanki, Special Centre for NanoScience, Jawaharlal Nehru University, Delhi, India. Bhavana Sharma, Assistant Professor, Kirori Mal College, University of Delhi was the rapporteur for the session. Prashant Mishra along with Partima Solanki elaborated on different nano-sensors being developed to help in proteome analysis in the field of medicine and agriculture. Ankur Vashisht, Bharat Biotech, Hyderabad, India presented his research work about nano-herbal drug delivery system. The last lecture of the day was delivered by Kalpana Bhargava, Additional Director, High Energy Material Research Lab, Pune. She discussed how "nano-ceria" may be used as a remedy for high altitude maladies faced by defense forces posted in Siachin and Leh, Ladakh.

The second day started with the Session-III entitled "Applications of Metabolomics for Safety Assessment and Nano Bio-integration" which was chaired by Renu Deswal and co-chaired by Jagadish Gupta Kapuganti, Staff Scientist V, National Institute of Plant Genome Research, New Delhi, India. The session was reported by Ashish Choudhary, University of Delhi. The invited speakers Niroj Sethy, Scientist, Defense Institute of Physiology and Allied Sciences (DIPAS), Defense Research Development Organization (DRDO), Delhi and Robin Joshi, Assistant Professor, CSIR-IHBT, Palampur, Himachal Pradesh, India, introduced the tools and techniques being used for metabolomics analysis right from sample preparation to setting up columns/equipment for separation of metabolites, as well as analysis of data and software being used to study humans as well as high altitude Himalayan plants. Jagadish Gupta Kapuganti, discussed about the role of alternate oxidases in Nitric oxide (NO) signaling during hypoxia. Extensive information on tools and techniques being utilized for NO analysis were shared.

Session-IV entitled "Environmental Nano Technology" was chaired by K.S. Rao, Department of Botany, University of Delhi, India and Manoj

Shrivastava, Principal Scientist, Centre for Environment Science and Climate Resilient Agriculture, ICAR, Delhi was the co-chair for the session. Siddhartha Kaushal was the rapporteur for the session. The first lectured was delivered by Manoj Shrivastava, in which he provided insights about Engineered Nanoparticles (ENPs) dynamics in Soil-plant continuum. His lecture was followed by a lecture by Karunakar Kar, Assistant Professor, School of Life Sciences, Jawaharlal Nehru University, Delhi, India. He elaborated about the formation of amyloid fibrils due to protein aggregation which leads to several complications including onset of about 60 neurodegenerative diseases. His group is working on plant-based natural nano formulations for preventing protein aggregations as a tool for potential cure of the diseases caused by protein aggregations. The last lecture was delivered by Ramovtar Meena, Assistant Professor, School of Environmental Sciences, Jawaharlal Nehru University, Delhi, India. His work mainly focused on the green synthesis of nanoparticles by utilizing organic specialists like microorganisms and plants extracts from Tulsi and green tea. The potential application of the green synthesized nanoparticles was shown as anticancer drug, antibacterial agent, waste water treatment, environmental pollutant degradation (Dye degradation).

The valedictory session was chaired by Dinabandu Sahoo, Professor, Department of Botany, University of Delhi. The valedictory lecture was delivered by Suman Kundu, former Director, University of Delhi, South Campus, Delhi, India. He discussed the novel plant

hamyoglobin proteome from *Arabidopsis thaliana*. The crystal structural characteristics of the globins provided remarkable insights into their potential function, evolution, and ligand binding properties.

The lectures were interesting and educative. The conference generated interest in the listeners and this was evident from the discussions that were followed by each lecture.

A poster presentation session was organized on the second day of the conference. A total of 8 posters were presented by students from different universities namely, Indian Institute of Technology, Delhi, University of Delhi, IHBT-Palampur, Himachal Pradesh, Multanimal Modi College, Uttar Pradesh, University of Kashmir, Mohanlal Sukhadia University, Udaipur and MIT-World Peace University, Maharashtra. The final announcement of the best poster award was made by Dinabandu Sahoo. Manglesh Kumari, CSIR-IHBT, Palampur, Himachal Pradesh was awarded the best poster award. The title of her work was "Mapping the Picrorhiza kurroa proteome and towards understanding its adaptation strategies to an altitudinal gradient in Western Himalaya". Prof. Girish Mishra, Treasurer, Delhi University Botanical Society thanked all the participants speakers and organizing committee members for the support. He also thanked the abstract assessment and poster evaluation committee members - Prof. Sandip Das, Prof. Yashwanti Mudgil and Dr. Priya Panjabi, Department of Botany, University of Delhi. He concluded the conference by thanking Department of Biotechnology, India for providing funds for the conference.

Abstracts of Papers Presented during the Conference on "Next Generation Proteome Mining and Nano-BioInteractions for Better Plant Health"

System Level Understanding of Organellar Control of Chitosan-triggered Immunity

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Key words: chitosan triggered immunity, extracellular matrix, fungal pathogen, fusariosis, Fusarium oxysporum

Impending changes in the global climate coupled with increased frequency of high complexity diseases have resulted in challenges related to food and nutrition. Fusarium oxysporum, a medically and agronomically important multi-host fungal pathogen is known to be associated with neuronal stress in humans and vascular wilt in plants, while Fusarium-mediated killing of worm has recently been described. Chitin oligosaccharides, the deacetylated form of chitin, act as archetypal general elicitors and typical fungal MAMPs that induce defence responses in a broad host range, including plants, insects, mice and humans suggesting the shared occurrence of chitin-mediated defence machinery in higher eukaryotes. Although chitosan is a known MAMP implicated in defence, the precise mechanism of chitosan-triggered immunity (CTI) remains unknown. Extracellular matrix (ECM) is the unique organelle that perceives stress signals and reprograms molecular events while nucleus, the regulatory hub serves as modulator of such signalling events dictating cell fate decisions. To elucidate regulatory framework of Fusarium-associated disease and immune response, we analysed the gene and protein expression during infection, integrated temporal expressions and network analysis with genetic inactivation data in worm and plant. Cumulative data led to the discovery of chitosan-responsive networks that cause significant extracellular matrix (ECM) and guard cell remodelling and translate ECM cues into cell fate decisions during fusariosis. Finally, this study for the first time provides novel insights on host-specific immune signalling that impinge upon the surveillance mechanism of innate immunity in multi-host pathogen response and facilitated discovery of cellular therapeutic targets for Fusarium-associated disease.

Nano Proteomics: From Nano-Analytical Platforms to Protein Corona

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Keywords: Brassica juncea, nano-analytical platforms, nanoproteomics, protein corona

With the advent of protein mass spectrometry, proteomics underwent technological upgrades for almost three decades though, it is still limited by the inability to identify very low abundant proteins. In contrast, nano-based proteomics, commonly known as nano proteomics offers deeper mining of proteomes besides added advantages like protein enrichment, protein detection, study of protein-protein interaction, among others without using labor-intensive protocols. Nano-analytical platforms offer real-time multiplexing, high sensitivity, high specificity, short run time, and analysis of very small sample volume/concentration. This talk focuses on the introduction of nano proteomics, some examples of nano analytical tools, and NP protein corona (PC) formed on the surface of AuNPs (gold nanoparticles) using leaf isolates from *Brassica juncea*. Protein corona determines the fate, distribution, and effects of nanoparticles inside living systems including plants and animals yet PC formed in plants is poorly studied. We studied temporally evolved (2 h–36 h) AuNP-PC using the techniques like UV-Visible spectroscopy, gel-based proteomics, Zeta Nanosizer, and Nano LC-MS/MS. Optical scanning and proteomic analysis revealed the formation of two regimes (Regime I from 2 to 8 h, representing soft corona & regime II from 16 to 36 h,

representing hard corona). Notably, approximately 1/3rd hard corona proteins were from energy-yielding pathways showcasing the potential of AuNPs to manipulate the yield in *Brassica juncea*.

Recent Advancements in Proteome Analysis Tools

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Key words: Data-dependent acquisition, data-independent acquisition, targeted data acquisition, parallel reaction monitoring, proteomics

Recent years have witnessed a huge advancement in proteomics technologies, especially in mass spectrometry and data analysis tools. Moreover, a switch from gel-based proteomics to gel-free or shotgun proteomics has been observed in the last few years. Shotgun proteomic technologies are more automated, robust and generate a huge amount of data to provide in-depth coverage of cell/tissue proteomes. Further, with the introduction of isobaric mass tags, multiplexing of a varied number of samples is possible which avoids instrumental errors to provide the precise changes in the proteome upon any internal or external stimuli. Besides, the development of different data acquisition modes such as data-dependent acquisition (DDA), data-independent acquisition (DIA), and targeted data acquisition (TDA) allow the identification of even small changes in the proteomes with a high degree of precision. In particular, TDA modes including selected/multiple reaction monitoring, SRM/MRM) or most parallel reaction monitoring, PRM have provided a platform for systemic identification of stress-specific biomarkers in different biological samples. The development of low-protein enrichment techniques has further boosted the identification of biomarkers and other proteins which are usually not identified during whole-cell proteome analysis. In essence, these advancements are fostering the development of detailed proteome maps to provide an in-depth understanding of different biological responses at the protein levels.

Advances in Nanomaterials Based Biosensors for Pathogen Detection and Protein Based Nanodevices

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Keywords: Biosensor, field effect transistors, memristors, nanomaterials, pathogen

Advancement of nanomaterials have led to the development of novel biosensors which have their applications in the field of healthcare, agriculture, food safety, environmental monitoring and water quality management. Rapid, sensitive and culture free detection of pathogens using biosensors are needed not only in medical technology but also to support sustainable agriculture and food safety. Biosensors have tremendous potential for rapid detection and discrimination of pathogens, where tests are not limited in the hands of highly trained personnel and specialized laboratories, and can be conducted in fields using simple devices. Various nanomaterial-based platform technologies for electrical, mechanical and optical biosensors have been developed. We will discuss electrical (Impedance and FET based) and optical (SERS based) sensors for bacterial detection. Vancomycin functionalized WO₃ thin film-based impedance sensor for efficient capture and highly selective detection of Grampositive bacteria was developed. Further, MoS₂/TiO₂ hybrid nanostructure-based field effect transistor has been fabricated for highly sensitive, selective and rapid detection of Gram-positive bacteria. Using a smartphone-based silver nanorods (AgNRs) array sensors were fabricated using glancing angle deposition method for detection of live and dead bacteria as well as antibiotic resistant bacteria. The colorimetric and water wettability features were tested which showed drastic changes. These changes were detected using in house developed mobile app 'colorimetric detector'. For pathogen detection in agriculture and food safety low cost and highly sensitive

recognition elements such as MIPs are needed. Briefly, we will discuss protein based nanoelectronics for the development of field effect transistors (FETs) and memristors.

Tuning PLGA PEGylated Drug Delivery Systems Using Various Formulation Parameters to Improve their Physio-biological Attributes for Improved Phytomedicinal Nano-therapy

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Keywords: Double emulsion, drug delivery, formulation, nanoparticles, PLGA

Phytomedicine has been utilized since ancient times. The medicinal properties of certain plants have been explored for medical purposes. Advancement and better understanding of biophysiological processes in human physiology enhanced the utilization of such phytomedicines more effectively. Nevertheless, bioavailability of phytomedicine still remains a challenge and can be explored extensively. To circumvent this problem, nanoparticles have proven to be very useful. The encapsulation of such medicinal plant-driven compounds within nanoparticles and delivering them to the site of target has been observed. To further enhance the activity of the encapsulated nanoparticles, it is equally important to control the biopharmaceutical delivering properties such as size and surface charge of the NP of the loaded nanoparticles. A better understanding of both the phytomedicinal drug and the carrier nanoparticles may enhance the therapeutic ability of the phytomedicine without compromising its natural therapeutic potential.

Nanomaterials-based Smart Sensor for Soil Nutrient Monitoring

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Keywords: Nanotechnology, Nutrients, Plant Growth, Zinc, Copper

In present time nanotechnology plays important role in the various field of science and technology. It can help in enhancing the ability of plants to absorb nutrients to improve their growth. Plants extract nutrients from soil, which are classified as macronutrients and micronutrients. Nutrients such as zinc (Zn), copper (Cu), iron (Fe), boron (B), manganese (Mn), and chlorine (Cl), are plant nutrients which are needed in small amounts for the growth of plants. Macro-nutrients like sulphur (S), calcium (Ca), nitrogen (N), potassium (K), phosphorous (P), and magnesium (Mg) are essential nutrients and are used in higher amounts for plant growth. For better plant growth balanced concentration of soil nutrients is very important. Nanotechnology can monitor the soil concentration of nutrients for improvement of plant growth. In this direction we have developed nanomaterials-based sensor for monitoring the concentration of Zn and Cu ions [1]. For this, CdSe quantum dots were synthesized at high temperature followed by surface modification with MPA and L-cysteine and applied these for the detection of Cu and Zn ions, respectively (Figure). Green synthesized carbon dots were synthesized and used for the detection of Cu ions in water samples(Figure)[2]. The developed sensors are able to determine the concentration of Zn and Cu and can be used to balance these nutrients concentration for better growth of plants.

Cerium Oxide Nanozymes Modulate the 'Exercise' Redox Biology of Skeletal Muscle

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Key words: Cerium nanozyme, nanomaterial, quantitative proteomics, reactive oxygen species

Exercise is a double-edged sword for the skeletal muscle. Small amount of Reactive oxygen species (ROS) generated during mild exercise, is essential for normal force generation; whereas large quantity of ROS generated during intense exercise may cause contractile dysfunction, resulting in muscle weakness and fatigue. One of the key questions in skeletal muscle physiology is 'could antioxidant therapy improve skeletal muscle endurance? A question which resulted in contradictory experimental findings till this date. This work has addressed this 'very question' using a synthetic, inorganic, anti oxidant nano-material viz., 'cerium oxide nanozyme' (CON). It was introduced in the rat by intramuscular injection, and the skeletal muscle endurance was evaluated

Intra muscular injections of CON,concurrentwith exercise, enhanced muscle mass, glycogen and ATP content, type-I fibre ratio, thus resulting in significantly higher muscle endurance. Electron microscope studies confirmed the presence of CON in the vicinity of muscle mitochondria. Following exercise there was an increase in the number and size of muscle mitochondria in the CON treated muscle, as compared to the untreated group with only exercised muscle. Quantitative proteomics data and subsequent biological network analysis studies, identified higher levels of oxidative phosphorylation, TCA cycle output and glycolysis in CON supplemented exercised muscle over only exercised muscle. This was further associated with significant increase in the mitochondrial respiratory capacity and muscle contraction, primarily due to higher levels of proteins involved in electron transport chain proteins like NDUFA9, SDHA, ATP5B and ATP5D, which were validated by real-time PCR and western blotting. Along with this, persistence of CON in muscle was evaluated with ICP-MS analysis, which revealed clearance of the particles after 90d, without exhibiting any inflammation or adverse effects on the health of the experimental animals. Thus, a higher physiological endurance of the CON supplemented exercised muscle' opens new avenues in skeletal muscle therapeutic, space and sports medicine.

Translation of Multiomics Data into Clinical Biomarkers

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Keywords: Biomarkers, high altitudes, proteomics, transcriptomics

Proteomics is typically labour-intensive, always biased toward peptides that are amenable to proteolytic digestion and assesses only a fraction of the expressed genome. Moreover, regulation of proteome is beyond transcriptome warranting integrative analyses that involve multiple data types. Currently, a multitude of omics-based technologies measuring mRNA dynamics, lipids and metabolite concentrations or completely orthogonal information on genomic variation across a population of samples are complementing proteomics investigations. These studies have allowed researchers to move toward a new field "integromics" concerned with mining information from the combination of omics-based techniques. This emerging form of proteomic analysis exploits large-scale measurements of concentrations, modifications and interactions of proteins with themselves and other molecules and being used to unravel new intracellular signalling pathways leading to new therapeutic targets that has helped in deciphering key pathway modulators and biomarkers. Since the use of clinical biomarkers is easier and less expensive than direct measurement of the final clinical endpoint, our group is using multi-omics

methods to identify potential biomarkers for high altitude maladies. These biomarkers have been translated into point-of-care devices for measuring blood and saliva metabolites at extreme environments.

Nitric Oxide Synthesis, Signaling and Nanoparticle Delivery for Stress Tolerance in Plants

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Key words: Nitric oxide, nanoparticles, germination, senescence, reactive oxygen species

Nitric oxide (NO) is a free radical signalling reactive nitrogen species produced in plants. In plants, NO is produced by multiple enzymes which are activated under several conditions. Nitrate reductase and mitochondrial electron transport chain are important sources which generate significant amount of NO. As a ubiquitous signalling molecule, NO is involved in multiple cellular processes. Our research revealed NO plays a major role ranging from germination to senescence, and NO is involved in flooding and salinity tolerance via regulation of reactive oxygen species and prevents peroxynitrite and concomitant tyrosine nitration of proteins. We demonstrated that form of nitrogen nutrition and nitrite play role in pathogen resistance. Control release of NO by nanoparticles is a key for enhancing plant resistance to stresses via priming. NO synthesis, its regulation and use of NO in the form of nanoparticles will be discussed in detail.

Exploration of Medicinal Plants using Metabolomics: An Emerging Analytical Approach

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Key words: Biomarkers, GC-MS, LC-MS, metabolomics, proteomics

Metabolites are well distributed in plant kingdom, their role in plant metabolism is of key interest among the scientific community. To understand the chemical processes novel analytical approaches, bring insightful information regarding the final product of cellular metabolism. Recently, metabolomics applications in medicinal plants have helped to identify numerous health promoting bioactive molecules and their interactive behaviour in disease control. Previously targeted metabolomics was able to identify and quantify only a few molecules which limits its application in the exploration of detailed biological actions. But non-targeted approach accelerates the identification of known and unknown metabolites using liquid and gas chromatography coupled with mass spectrometry (LC-MS, GC-MS). This analytical intervention helps to identify various classes and sub-classes of primary and secondary metabolites. Other advancements in MS is the hybrid utility with quadrupole-time-of-flight (QTOF) which permits to acquire MS/MS spectra, provides structural information and fragmentation patterns through supportive databases METLIN. Further, statistical tools and visualisation software (XC-MS, MPP, Metabo Analyst) help in understanding the correlation among the discriminate behaviour of the metabolites and pathway studies. Recently, Ion mobility mass spectrometry (IMS) was introduced in the area of metabolomics which is

helping to improve LC-MS and GC-MS based workflows. Future metabolomics investigation will also strengthen the other genre of sciences including medicinal and analytical chemistry. Future integration of metabolomics and proteomics will also greatly support the accurate identification of biomarkers in medicinal plants.

Engineered Nanoparticles (ENPs) Dynamics in the Soil-Plant Continuum

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Keywords: Engineered nanoparticles, food chain, food safety, plant-soil system

Engineered nanoparticles (ENPs) are engineered materials having at least one dimension in the nanoscale (ca. 1-100 nm) dimension. ENPs can be grouped into four types namely carbon based, metal-based materials, dendrimers which are nano-sized polymers and composites which combine nanoparticles with other nanoparticles or with larger bulk-type materials and present different morphologies such as spheres, tubes, rods and prisms. ENPs are currently used in agriculture, aerogels, aerospace, automotive, catalysts, coatings, paints, pigments, composites, construction, cosmetics, electronics, optics, energy, environmental remediation, filtration, purification, food products, medical, packaging, paper and board, plastics; security, sensors and textiles. The inevitable release of ENPs into the environment including the soil-plant system is an increasing concern. These ENPs are generally produced with tailored elemental composition, particle size, shape, surface charge, and functionality. While the concentrations of most ENPs in the environments still remain unknown, exposure modelling suggests that soil could be a major sink of ENPs released into the environment. MNP concentrations in soil are higher than in water or air. Assessing the dynamics of these ENPs in the soil plant system requires an understanding of their mobility, reactivity, translocation, ecotoxicity and persistency. To date only few quantitative analytical techniques for measuring ENPs in natural systems are available, which results in a serious lack of information about their dynamics in the soil-plant system. Soil quality is defined as the capacity of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water as well as air quality, and support human health and habitation. Soil quality is the combination of soil physical, chemical and biological properties. How these properties affect the ENPs dynamics in soil-plant system need to be understood, vis-à-vis effect of ENPs on soil quality also need to be studied. Further, fates of ENPs in soil-plant system in their original or aggregated or modified form also needs to be analysed. These ENPs have also been reported to be biomagnified in the food chain. Interactions between plants and ENPs, such as uptake potential of different plant species, the effect of plant growth media condition, mechanisms of uptake and translocation, and the interactions between the ENPs and plant tissues at the cellular and molecular level, require further in-depth investigation. Such studies will help us understand the plant uptake of ENPs as a potential transport and exposure route and its role in bioaccumulation through the food chain. Further interactions amongst most highly used ENPs in agriculture; need to be examined in different environmental matrices to understand the behaviour and the risks associated with ENPs. There are many unknowns regarding the fate, transport, exposure, eco-toxicity, and lifecycle of ENPs in soil-plant system and therefore, we need strong research support for the development and implementation of environmentally benign nanotechnology for food safety and sustainable agriculture production.

Natural Product Based Nanoparticles to Target Protein Aggregation

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Keywords: Amyloid fibrils, gold nano particles, nano formulations, plant products, protein aggregates

Proteins are the most important biological macromolecules which are involved in almost every metabolic process in the living systems. Self-assembly of proteins into defined higher order structures is a fundamental process in biology which influences both structural and functional properties of many tissues. Formation of amyloid fibrils due to protein aggregation has been implicated in several complications such as the onset of various neurodegenerative diseases, complications during DNA-recombinant synthesis and formation of toxic aggregates during storage and formulation of protein therapeutic agents. Hence, it is necessary to find effective strategies against aggregation of proteins. Two important strategies, (1) Inhibition of protein aggregation, and (2) promotion of disassembly of preformed aggregates, are crucial for the development of efficacious antiaggregation formulations. This work explored the effect of selected surface-functionalized nanoparticles on amyloid fibril formation of proteins. We used plant-based natural products to design nano formulations against protein aggregation. Thermostable, uniform, and biocompatible gold nanoparticles coated with selected natural compounds/plant products have shown great potential to suppress the in vitro protein aggregation process and they show protective effect against amyloid induced toxicity under in vivo systems as well. Data generated from experimental and computational methods suggest a direct interaction between the nanoparticles and the aggregation-prone hydrophobic stretches of proteins. The results signify a unique approach to target protein aggregation through nanoparticle-based inhibitors.

Green Synthesis of Nanoparticles -- An Alternative Way for Environmental Protection

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Key words: Anti-cancer drug, green synthesis, metal nano-particles, nano-fibres, nanotechnology

As of now, the nanotechnology has drawn much exploration consideration because of its multidisciplinary nature in different parts of science and designing. The NPs are around one hundred to multiple times less than human cells, can offer remarkable collaborations with biomolecules both on a superficial level and inside the cells. In this way, offering numerous improvements in different spaces of advances like biotechnology, biomedicine, bio-sensing and environmental technology, ceramics, nanofiber and others. Metal nanoparticles has various properties but applications are limited due to their synthesis strategies which are time consuming, produce hazardous by products, use toxic chemicals, stability and generation of large sized and aggregated particles. Hence, an alternative strategy is required, which will be harmless to the ecosystem contrast with customary and compound innovations. Therefore, the green synthesis of nanoparticles has been shown by the utilization of organic specialists like microorganisms and plants extracts. In this regards we have synthesized various organic and metallic nanoparticles by green technology and utilized them in various applications as anticancer drug, antibacterial agent, waste water treatment, environmental pollutant degradation (Dye degradation).

Novel Plant Haemoglobin Proteome from *Arabidopsis thaliana*: What do their Structure and Fold Unfold?

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Key words:crystal structure, globins, haemoglobin, myoglobin, non-symbiotic

Globins are ubiquitous proteins diversely present in all organisms ranging from bacteria to higher eukaryotes. Based on the globin fold architecture, globins are broadly classified into two distinct classes: classical globins with 3-on-3 α-helical globin fold, as in myoglobin and human haemoglobin, and more recently discovered 2-on-2 fold, as seen in bacterial globins. Plant genomes encode multiple globin genes which differ in their sequence, ligand binding kinetics and structural fingerprint. In contrast to symbiotic haemoglobins present in root nodules of leguminous plants, nonsymbiotic haemoglobins have ubiquitous expression profile and are phylogenetically classified into three distinct classes: class I and class II nonsymbiotic haemoglobins and recently discovered bacteria like plant truncated haemoglobins (class III). Though these plant haemoglobins have been implied in a number of distinct physiological functions, most importantly NO detoxification, the functions may differ among different globin types and possibly between different plant species. The model plant Arabidopsis thaliana presents a perfect system for investigating both function and structural characteristics of globins since it contains three globins that are distinctly different. We have solved the crystal structures of two of these globins - AHb1 and AHb3. The structural characteristics of these two globins provide remarkable insights into their potential function, evolution, and ligand binding properties. AHb1, with the classical globin fold, surprisingly displayed an internal tunnel connecting the two haem prosthetic groups, previously not described in any globins. AHb3, on the other hand, revealed a 2-on-2 globin fold observed for the first time in the crystal structure of a plant haemoglobin. These and other such novel properties will be discussed in light of globin function and evolution.

Development of Nano-Zinc based Biofertilizer Formulation and its Effect on Growth and Yield of Wheat

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Key words: Bioinspired fabrication, bioslurry, nano-biofertilizers, sustainable agriculture, ZnO NPs

Zinc (Zn) is an important micronutrient for crop plants and plays an important role in plant and animal metabolism. However, as per the available literature, it is one of the yield-limiting nutrients and over 50% of Indian soils are predicted to be Zn deficient in the coming years. The Zn requirement in deficient soils is commonly met through the inorganic fertilizers, which upon long-term applications and over-use can affect soil physio-chemical properties, soil-microbiota and organic carbon pool. Considering such consequences, technological interventions leading to the development of eco-friendly Zn-based nutrient inputs are imperative. The present study aimed to develop the Nano Zn-based biofertilizer formulations (NBFs) followed by testing their effects on the growth and yield of Wheat. The bio fabricated ZnO-NPs (synthesized using aqueous bioslurry extract as a potential source of capping agent under optimized reaction conditions) were monodispersed, with negative zeta-potential and having hexagonal wurtzite geometry. The resultant ZnO-NPs followed by Phyto- and Microbial toxicity testing were incorporated with the microbially enriched organic manures and the resultant NBFs were applied for soil fertigation in pot studies on wheat. The findings pertaining to the concentration-dependent effects of

the NBFs on plant growth, seed yield, and anti-oxidant defence system in soil cultivated wheat were recorded, analysed, and will be presented at the conference. Such integrated studies have the potential to meet the Zn requirements in deficient soils and can also act as a conceivable approach for valorisation and value-addition of organic manures such as bioslurry.

Environmental Nanotechnology - Perks, Challenges and Risk Assessment

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Key words: Engineered nanomaterials environment, nanotechnology, nanotoxicology, remediation, risk assessment

Understanding of the environmental effects and risk associated with nanotechnology is very limited and contradictory. There is no sufficiently reliable information concerning potential environmental risks and harmful effects associated with advanced nanotechnology applications. Engineered nanomaterial production requires a lot of water and energy and the used chemicals are often highly toxic. Though a large volume of studies on engineered nanomaterials have been conducted however, responsible and safe synthesis as well as applicability of desirable nanomaterials is not practiced. Basic understanding, documentation and management of the nano-bio interfaces are the major challenges identified in environmental nanotechnology. Rapid growth in this exhilarating area of nanotechnology demands identifying the potential hazards and risk assessment of nanoscale domain to human health and environmental effects. Life Cycle Assessment (LCA) is an interesting approach designed for improving the understanding of the impending impacts of nanotechnology. This comprehensive analysis tool can be used for the estimation of the effect of a product or material on the life cycle an organism in ecosystems and human health. With development of different aspects of nanotechnology, the broader environmental impacts needs to be discussed, including determination of potential benefits of reduction or prevention of pollutants from industrial sources, but also monitoring or controlling unintended negative effects by proper and comprehensive risk assessment and life cycle analysis.

Effects of Abiotic Stress (Temperature, Heavy Metal and Salinity) *Vis-À-Vis* Nitric Oxide (NO) in Moth Bean

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Key words: Abiotic stress, climate change, moth bean, nitric oxide, reactive oxygen species

Climate change, heavy metal (HMs) contamination of soil, and salinity are emerging challenges for crops and biodiversity in present scenario and their adverse effects are visible on plants as temperature stress, salinity and heavy metals stress. Unfavourable temperature, HMs and salinity becomes lethal when threshold levels are crossed. As and Cd are two hazardous heavy metals, that are toxic to our ecosystem even at low concentrations. These changes affect various parameters of plants like root-shoot inhibition, senescence, abscission, plasma membrane damage, reproductive structure, increased respiration, decreased chlorophyll and photosynthesis, cell dehydration, increased protein denaturation, generation of reactive oxygen (ROS) and reactive nitrogen species (RNS) resulting in oxidative stress, altered ion balance, change in cytosolic pH and overall imbalance in metabolism leading to decline in plant productivity. In response to these stresses plants induce synthesis of

compatible solutes, phytochelatins and antioxidants including generation of NO. It is well reported that NO is involved in cold acclimation, HMs and salinity stress, and tolerance in legume through ROS scavenging, s-nitrosylation and involving activation of Ca²⁺ signalling. S-nitrosylation mediated by NO has also been found to greatly influence plant growth and development, as well as responses to environmental changes, and cold-induced modifications of S-nitrosylation proteins have been identified in various plant species, such as *Brassica juncea* and *Arabidopsis*. Moth bean (*Vigna acontifolia* (Jacq.) Marechal) is a legume crop which belongs to Fabaceae. The crop is mainly grown in hot and dry regions of India because of its hot and dry condition tolerance and adaptability. The moth bean performs well in the range of 25-37 °C, 250-500 mm rainfall and proper drainage. In this light, we are interested to study the effect of temperature stress (both low and high temperature), HMs like As and Cd and salinity on morpho-anatomical, bio-physiochemical effect *vis-à-vis* NO with its biochemical presence, enzymatic generation and proteomics analysis.

Development, Characterization and Efficacy Evaluation of Nanoparticle-protein Conjugates and Microemulsion-based Formulations Containing Insecticidal Protein

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Key words: Insect pests, microemulsion, nanoparticles, nanoparticle protein conjugates, plant defence proteins.

Plants have evolved an array of proteins and peptides that are involved in defence against pests and pathogens. Several different proteinase inhibitors and defence peptides occur in high abundance in aerial plant tissues like leaves and flowers of individual plants where their natural function is thought to be in host defence as an insecticidal agent. In this work, we are trying to develop an efficient surface delivery mechanism for defence proteins/peptides to susceptible crop plant species for the control of insect pests.

Development of a formulation containing protein/peptide or active ingredients (in a plant extract) is an advanced thrust area and has been explored to a lesser extent. A microemulsion formulation containing water, vegetable oil (lipid), soybean lecithin (emulsifier) and protein (STI/Kb) was optimized. We have characterized the formulation for peptide solubility and stability, homogeneity, particle size, contact-angle measurement with leaf surface. The formulation is being used for application on leaves/fruits of test plants (Tomato and Chickpea – host plants of lepidopteran insect pests). The evaluation of the formulation for leaf permeation and efficacy against insect pests is underway. In another approach, we are using Silica and Gold nanoparticles as mediators for delivery of defence proteins in tomato plants. Functionalized Silica and gold nanoparticles were conjugated with plant protein (STI/Kb) and administered to the growing plants for evaluating the effects of Np on growth as well as the uptake of the Np-protein conjugates by the plant through the vascular system. Development of a plant protein-based product for control of insect pests is a novel approach and can be a sustainable advance towards integrated pest management.

Development of Zinc Based Nanofertiliser for Alkali Soils: A Green Synthesis Approach

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Key words: Nanotechnology, soil organic content, sugar press mud (SPM), zinc deficiency

The focus of the current plant nutrition research is increasing use efficiency, as most of conventional chemical fertilizers score very low (< 45-50%) in this. In addition to this, efficient use of mineral resources, demand for high productivity and maintenance of soil health all in a sustainable manner, are some big challenges in practicing agriculture. The majority of soils across the world are suffering from multi nutrient deficiencies along with alarming levels of soil organic carbon status. Among these, the deficiency of zinc is very common, at around 67% of the soil around the world being deficient in plant available zinc— the underlying reason for hidden hunger in masses. Scientists around the world are dealing with this problem in two ways— one is the attempt to change the whole bulk regime of conventional chemical fertilizer to the new and efficient nano regime using nanotechnology, and the other is the genetic modification of the plants to develop nutrient efficient cultivars. We, in our research, are trying to develop a nano zinc oxide-based fertilizer to solve the problem of zinc deficiency, especially in alkali soils where it is very common. In an attempt to do this, we have synthesized highly stable, monodisperse zinc oxide nanoparticle of the size, ranging from 18 to 25 nm using sugar press mud water extract as stabilizing agent by sol-gel method. The process is environmentally benign and utilizes waste. We have done pot level study in wheat crop in order to establish its efficiency. The result of these experiments will be presented at the conference.

Mapping the *Picrorhiza kurroa* Proteome and Understanding its Adaptive Strategies to an Altitudinal Gradient in Western Himalaya

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Keywords: Medicinal herb, metabolome, proteome, high altitude, stress

Picrorhiza kurroa Royle ex Benth. is a promising medicinal herb growing in higher altitudes of Himalaya. The medicinal potential of the plant is ascribed to picrosides, which are synthesized in organ-specific manner through highly complex pathways. As an initial step, we assembled the first proteome map of P. kurroa using 1D-GE coupled with LC-MS/MS covering all four major organs at two developmental stages. The identification of 5186 protein accessions provided deep coverage, spanning around six orders of magnitude. Most of the proteins are linked with metabolic processes, response to abiotic stimuli and cellular processes that provide medicinal and adaptive significance to P. kurroa. Organ-specific sub proteomes reveal organ specialized functions, while vegetative phase is found to be enriched with growth processes, whereas the reproductive phase harvests more energy in secondary metabolism. To test the applicability of proteome map, phytochemical analysis reveals the accumulation of marker compounds in rhizome and overall, during the late stage of development. This study presents the first extensive proteomic description of P. kurroa and identifies several candidates having a role

in providing tolerance to multiple stress conditions. Next, we studied the effect of altitude on metabolome variation of *P. kurroa* in organ specific manner. Environmental stress gradients are the main drivers of plant variation and stress gradient hypothesis assumes higher altitudes to be more stressful for plants. Differential metabolites accumulation reveals source-sink partitioning, modulation of sugar and amino acid metabolism, ascorbate metabolism, partial TCA cycle, purine catabolism and salvage, pyrimidine synthesis, lipid alteration besides gibberellins and cytokinin inhibition. This metabolome reshuffling along with other omics tools might expand avenues for metabolic engineering in future.

Preparation and Evaluation of a Protein Fraction, Isolated from a Medicinal Plant, for Biological Activities

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Key words: Anticancer activity, antifungal activity, antioxidant activity, medicinal plant, proteins

Continuous use of conventional drugs and therapies in addition to imparting deleterious effects on normal cells and tissues pose a threat. Thus, constraining their use and making it compulsory for researchers to search for drugs/therapies which can overcome these shortcomings. In this regard, plant-based drugs have been used since antiquity to treat infections and deadly diseases. The medicinal importance of plants lies in their active constituents which mainly include secondary metabolites and proteins/peptides. However, drugs derived from secondary metabolites of plants in spite of their huge success in therapeutics do have some drawbacks such as less bioavailability, non-specificity and thus toxicity. This makes a way for proteins/peptides to be used in therapeutics although some are clinically approved and some are in clinical trials. The relevance of plant-based proteins/ peptides is justified due to their higher specificity, lower toxicity and their higher efficiency. In this context, we tried to explore proteome of a medicinal herb of Kashmir Himalayas and evaluate its biological activity. So far, extraction of this from this plant has been carried out and SDS-PAGE confirmed presence of one prominent band and four lighter bands. The crude protein fraction of this plant has been evaluated for various biological activities and its showing anti-cancer, antioxidant and antifungal activity. Further studies to the actual protein responsible for these biological activities are under way.

Reduction Mechanism during Nanoparticles Synthesis Using Algal Extract

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Key words: AgNPs, algae, bioactivity, capping, reduction mechanism

Nanotechnology is a growing scientific field with many beneficial applications for humans and the environment. Nanoparticles (NPs) have unique physico-chemical properties like high electrical and thermal conductivity, chemical stability, catalytic and biological activity, which make them potentially useful in industrial and biomedical applications. Physical, chemical, and biological methods are used to synthesize NPs. The physical approach, high energy inputs in the form of pressure and temperature are mostly required. Chemical methods mainly involve using various reagents for reduction/oxidation through sol-gel processes, atomic or molecular condensation, chemical etching, sputtering, and spray pyrolysis. In recent years, researchers have focused their efforts on creating ways for making safe and low-cost NPs. Bio-nanofabrication, a biological method, is used extensively

to create safe, biocompatible, and cost-effective NPs. For the fabrication of NPs, certain biogenic compounds found in algae serve as good reducing and capping agents. In comparison to plants and other microorganisms, algae are easy to maintain and mass multiply. Polysaccharides, proteins, and lipids are present, which act as reducing agents. During the green synthesis of NPs, a cocktail of biomolecules that initially serve as reducing agents can also be used as a capping agent. In contrast, during chemical synthesis, separate reducing agents and capping agents are employed to synthesize NPs. Because of the extensive and diversified chemical ecology of algae, significant research efforts are required to understand the specific mechanism by which algae are involved in the more efficient synthesis of metallic NPs, giving advantages over other organisms. In this presentation, the information regarding the reduction mechanism for nanoparticles synthesis by algae is shared.

