NANOPLANT

INTERNATIONAL CONFERENCE ON PLANT NANOTECHNOLOGY

14-16 OCTOBER 2024, POZNAŃ, POLAND

BOOK OF ABSTRACTS

WWW.ICPN2024.PL

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement no 856961.

Organizing Committee

Core

Prof. Franklin Gregory
Dr. Dibyendu Mondal
Prof. Arkadiusz Kosmala
Prof. Paweł Krajewski

Other members of the Organizing Committee

Prof. Lidia Błaszczyk
MSc Joanna Dutkiewicz
Prof. Małgorzata Jędryczka
Dr. Leonard Kiirika
MSc Gabriela Majchrzak
Prof. Robert Malinowski
Dr. Pradeep Matam
Mr. Tadeusz Mirski
Dr. Lakshmipathy Muthukrishnan
Prof. Barbara Naganowska
Dr. Izabela Pawłowicz
Prof. Tomasz Pniewski
Dr. Megha Saxena

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement no 856961.

Honorary Patronage

Minister of Science
Republic of Poland

PAN
POLISH ACADEMY OF SCIENCES

Organizers

IGR PAN

NANO PLANT

Gold Sponsors

KENDROLAB

ANCHEM
SPEKTROMETRIA MAS I CHROMATOGRAFIA

Partner

PPNT
Poznan Science
and Technology Park

TABLE OF CONTENTS

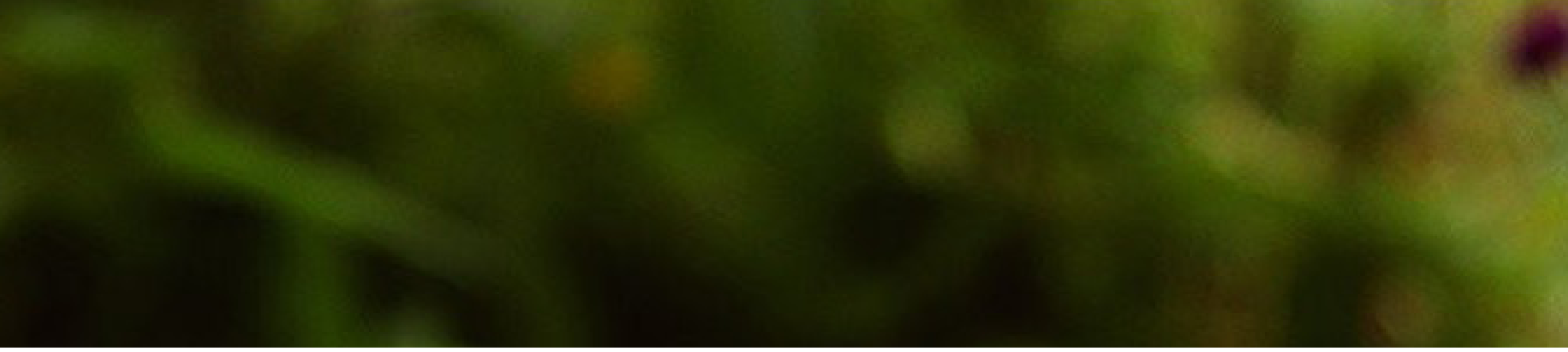
NANOPLANT

KEYNOTE LECTURES

INVITED LECTURES

ORAL COMMUNICATIONS

POSTER SESSION



Preface

Dear friends and colleagues,

It is a pleasure to welcome you all to Poznan for the International Conference on Plant Nanotechnology from 14 to 16 October 2024 (ICPN 2024). This conference is organized as a deliverable of NANOPLANT project funded by the European Union's Horizon 2020 research and innovation program.

ICPN 2024 aims to bring together experts, young scientists, students, industrial stakeholders and policy makers to explore the potential of plant nanotechnology to improve human well-being and environmental sustainability.

We are honored to welcome a consortium of distinguished scientists for keynote and guest lectures who will share their expertise on the current and future directions of plant nanotechnology. In addition, the conference will include a series of oral presentations, group discussions and poster sessions, providing the perfect opportunity for researchers, scientists and industry professionals from around the world to discuss and collaborate. We would like to thank all participants, sponsors and well-wishers.

The conference will address several important topics, including interfaces between plant biotechnology and nanotechnology, interactions between nanomaterials and microorganisms, nanomaterials for sustainable agriculture, green nanotechnologies, and nanotoxicity and food safety. The abstracts presented in this brochure highlight the cutting-edge research on these topics that is being conducted around the world - be it in the use of nanomaterials to combat biotic and abiotic stress to improve crop yields, or in their effects, such as toxicity to the environment. Fundamentally, these studies emphasize the interdisciplinary nature of plant nanotechnology and its ability to redefine agriculture and drive environmental sustainability, while highlighting the major threat posed by nanomaterials entering the environment as waste or as agrochemicals.

As you engage with the presentations and discussions over the next few days, we encourage you to take advantage of the networking opportunities and explore new avenues of collaboration that will advance the field of plant nanotechnology. Together, let us harness the power of nanotechnology to tackle the challenges of our time and create a sustainable future.

We wish you a productive and inspiring conference.

Best regards,
Franklin Gregory
Conference Chair, ICPN 2024

About the Nanoplant Project

The Nanoplant project (grant agreement No 856961), funded by the European Union's Horizon 2020 research and innovation program, is coordinated by the Institute of Plant Genetics, Polish Academy of Sciences (IPG PAS). This project has a duration of 6 years (2019-2025).

The main idea of Nanoplant is to promote cutting-edge research in the field of the interface between plant biology and nanotechnology and thus to promote interdisciplinary research expertise, scientific excellence, international cooperation and partnerships between science and industry at the IPG PAS by creating a Department of Plant Nanotechnology.

Plant nanotechnology is concerned with the potential application and effect of nanomaterials in plants. Due to their ability to improve plant growth and yield, their antimicrobial effect, etc., the incorporation of nanomaterials into agrochemicals (pesticides, fungicides, herbicides, fertilisers, etc.) is also expected to have great potential for nanotechnology-driven smart agriculture. On the other hand, plants are the most vulnerable to the presence of these new materials in the environment. The Nanoplant project is dedicated to scientific research in both these directions and will try to answer some of the unanswered questions of basic and applied research in plant nanotechnology in order to advance the state of the art.

Further information about the Nanoplant project
and its activities can be found on the following pages:

www.nano-plant.eu

facebook.com/nanoplanteu

linkedin.com/company/nanoplanteu

Conference Timetable

DAY-1: 14 October 2024	
3:00 pm to 5:00 pm	**Registration**
5:00 pm to 5:15 pm	**Opening Ceremony**
5:15 pm to 6:00 pm	**Inaugural Talk: Prof. Ajayan Vinu** *Title: Nanoporous carbon based materials for clean energy and environmental application*
6:00 pm to 7:00 pm	**Welcome Reception**

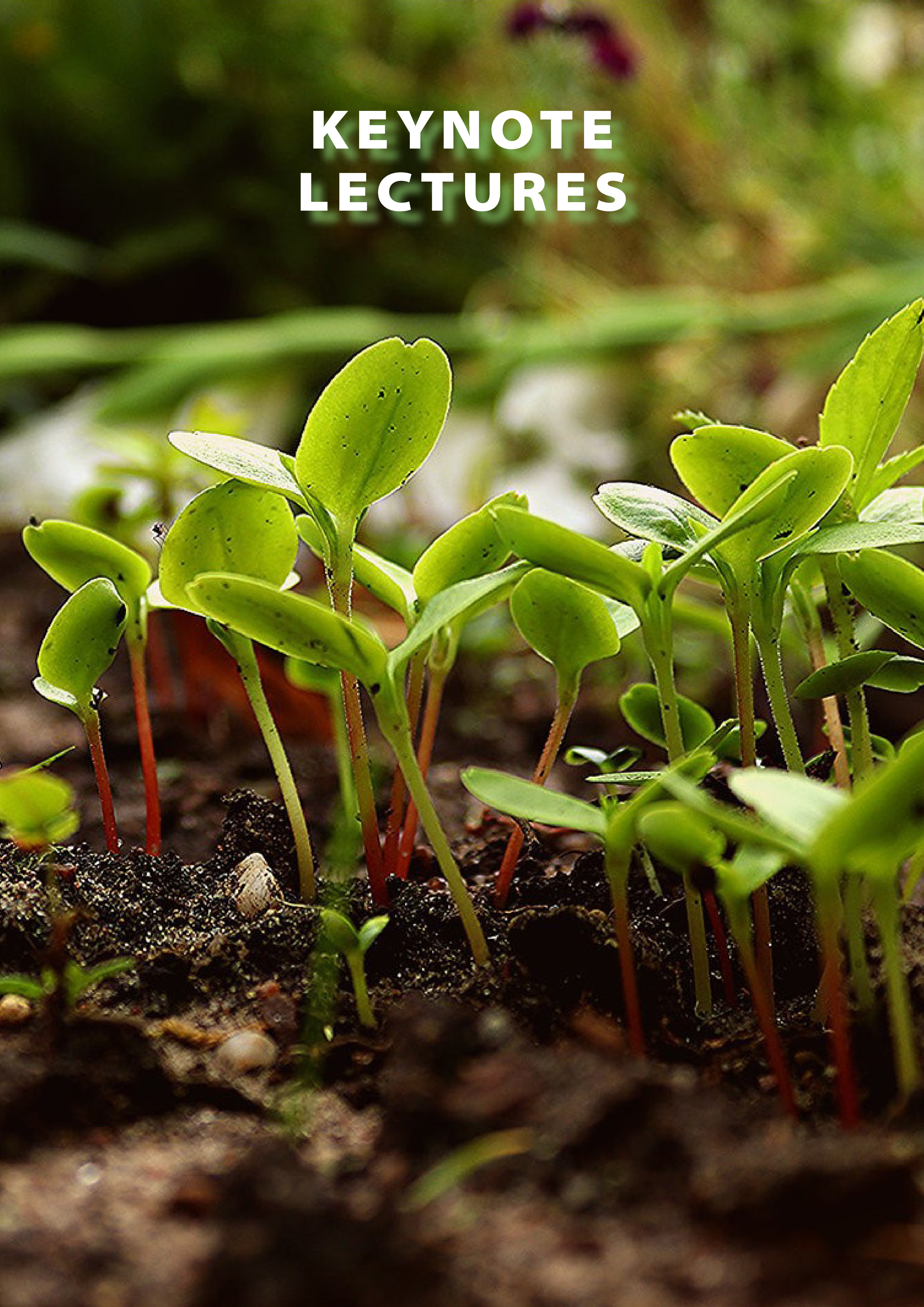
DAY-2: 15 October 2024	
SESSION 1: Plant biotechnology nanotechnology interfaces **Session Chair: Prof. Geetha Balakrishnan & Prof. Tomasz Pniewski**	
9:00 to 9:45 am	Keynote Address: Prof. Markita Landry *Title*: *Nanoscale approaches for nucleic acid and protein delivery to plants*
9:45 am to 10:15 am	Invited Talk: Prof. Zsuzsanna Kolbert *Title: Multiple scales and ways of plant-nanomaterial interactions*
10:15 am to 10:45 pm	Invited Talk: Prof. Søren Husted *Title: LA-ICP-MS and NANO-CT based bioimaging to study the fundamentals of mineral ion and nanoparticle interactions with plant tissue*
10:45 am to 11:10 am	**Tea Break and Group Photo**
11:10 am to 11:40 am	Invited Talk: Prof. Ewa Kurczyńska *Title: The influence of nanoparticles on selected aspects of plant development*
11:40 am to 11:55 am	Oral Talk: Dr. Dariusz Kulus *Title: Nanoparticles in plant cryopreservation: Effects on genetic stability, metabolic profiles, and structural integrity in bleeding heart cultivars*
11:55 am to 12:10 pm	Oral Talk: Dr. Alicja Tymoszuk *Title: Silver nanoparticles in chrysanthemum breeding*
12:10 pm to 12:25 pm	Oral Talk: Dr. Gloria B. Ramírez-Rodríguez *Title: Calcium phosphate nanoparticles for biofortification and protection of tomato plants*
12:25 am to 12:40 pm	Oral Talk: Vinnicius Henrique Cerqueira da Silva *Title: Feasibility of using SiO_2 NPs to mitigate mercury in transgenic soybeans grown in contaminated soils: respective effects and action of SiO_2 NPs*
12:40 pm to 12:55 pm	**Sponsor Talk by Kendrolab Sp. z o.o.**
12:55 pm to 1:55 pm	**Lunch Break**

SESSION 2: Nanotechnology for Sustainable Agriculture Session Chair: Prof. Susana Loureiro & Prof. Arkadiusz Kosmala	
1:55 pm to 2:40 pm	Keynote Address: Prof. Alejandro Pérez-de-Luque *Title: Nanotechnology reaches the field: Advances and challenges for agriculture*
2:40 pm to 3:10 pm	Invited Talk: Prof. Renata Szymańska *Title: Nanoparticles: A novel sustainable and eco-friendly approach for enhancing plant growth*
3:10 pm to 3:40 pm	Invited Talk: Prof. Gholamreza Gohari *Title: Next generation chemical priming: Utilizing nanocarrier technology for enhanced crop resilience*
3:40 pm to 4:10 pm	Invited Talk: Prof. Sebastian Kruss (online) *Title: Optical nanosensors to monitor plant health*
4:10 pm to 4:30 pm	**Tea Break**
4:30 pm to 4:45 pm	Oral Talk: Dr. Zoltan Molnar *Title: Impact of chitosan nanoparticles and cyanobacteria biomass supplementation under optimized nitrogen on maize (Zea mays L.) productivity in field condition*
4:45 pm to 5:00 pm	Oral Talk: Dr. Matej Baláž *Title: Plant-mediated solvent-free biomechanochemical synthesis of Ag nanoparticles and assessing the plant-As-Se interaction*
5:00 pm to 5:15 pm	Oral Talk: Nicola Carrara *Title: Enzyme-responsive chitosan-based phosphorus nanofertilizers*
5:15 pm to 5:30 pm	Oral Talk: Dr. Alessandra Azzali *Title: Bioinspired nanocarriers for controlled herbicide delivery: bettering performances and efficiency*
5:30 pm to 6:30 pm	**Poster Presentations**
7:00 pm to 10.00 pm	**Cultural Programme and Networking Dinner**

DAY-3: 16 October 2024	
SESSION 3: Green Nanotechnologies **Session Chair: Prof. Alejandro Pérez-de-Luque & Prof. Ajayan Vinu**	
9:15 am to 10:00 am	Key Note Address: Prof. João A. P. Coutinho *Title: Valorization of wastes through extraction and purification of plant metabolites using novel green solvents*
10:00 am to 10:30 am	Invited Talk: Prof. R Geetha Balakrishna *Title: From toxic lead perovskites to eco-friendly double perovskites: Innovations in foodstuff analysis and medical diagnostics*
10:30 am to 11:00 am	Invited Talk: Prof. Yogendra Kumar Mishra *Title: Tetrapods based Smart Materials for Advanced Technologies*
11:00 am to 11:20 am	**Tea Break**

11:20 am to 11:50 am	Invited Talk: Prof. Sanna K. Nataraj *Title: Sustainable Nanotechnology Practices in Environmental Applications*
11:50 am to 12:05 pm	Oral Talk: Martyna Anna Przewoźnik *Title: Production of chimeric virus-like particles in plant expression systems: a promising approach for veterinary vaccine development against Fasciola hepatica*
12:05 pm to 12:20 pm	**Sponsor Talk by Anchem Sp. z o.o.**
12:20 pm to 1:30 pm	**Lunch Break**
SESSION 4: Nanotoxicity and Nanomaterial-Microbial Interactions **Session Chair: Prof. Zsuzsanna Kolbert & Prof. Małgorzata Jędryczka**	
1:30 pm to 2:15 pm	Keynote Address: Prof. Susana Loureiro *Title: Nanomaterials and plants: Accumulation, toxicity, and agricultural applications*
2:15 pm to 2:45 pm	Invited Talk: Prof. Luiz R. G. Guilherme *Title: Nanotechnologies in agroecosystems and their effects on soil and food security*
2:45 pm to 3:00 pm	Oral Talk: Prof. Petra Peharec Štefanić *Title: Polystyrene particles impair photosynthesis in Chlorella vulgaris*
3:00 pm to 3:15 pm	Oral Talk: Dr. Adrian Augustyniak *Title: Stimulatory effect of nanomaterials on bacteria producing utile metabolites for agriculture*
3:15 pm to 3:30 pm	Oral Talk: Dr. Cristina De Miguel Rojas *Title: How nanotechnology would control fungal diseases*
3:30 pm to 3:45 pm	Oral Talk: Paweł Poznański *Title: Chitosan nanoparticles for plant protection against fungal pathogens*
3:45 pm to 4:05 pm	**Tea Break**
4:05 pm to 4:45 pm	**Panel Discussion**
4:45 pm to 5:15 pm	**Closing Ceremony**

KEYNOTE LECTURES



Nanoporous carbon based materials for clean energy and environmental application

Ajayan Vinu

The University of Newcastle, College of Engineering, Science and Environment, Callaghan 2308, Australia

E-mail: ajayan.vinu@newcastle.edu.au

Nanoporous Materials with ordered porous structures and functional elements offer excellent textural features and catalytic properties. Among the nanoporous materials, nanoporous carbon nitrides are quite unique as they possess highly stable semiconducting CN framework with tunable band gaps and basicity. These CN nanomaterials can deliver successful energy and environment solutions - such as converting waste or seawater into clean hydrogen with only sun-light and developing innovative devices for energy storage and conversion. In this talk, I will present the development, capabilities and current and future applications of multifunctional amorphous and crystalline nanoporous carbon nitride materials with different structures, band gaps, and nitrogen contents [1-9]. Especially, I will focus on the preparation of novel nanoporous amorphous and crystalline CN with different stoichiometries and their structural elucidation using different spectroscopic techniques. I will also demonstrate how the chemical composition, structure, porosity and the functionalization of these unique materials can be tuned [3, 10-11]. In the last part of the talk, I will present on the energy storage and photocatalytic performance of these unique nanoporous carbon nitrides and their hybrids on water splitting to produce clean hydrogen from seawater. I will also demonstrate the utilization of this technology on converting the seawater from different beaches along the coastal side of Australia.

References

[1] Vinu et al. (2023). *Chemical Society Reviews*, 52 (21), 7602-7664
[2] Vinu et al. (2024). *Advanced Materials*, 36, 2306895.
[3] Vinu et al. (2023). *Progress in Materials Science,* 135, 101104
[4] Vinu et al. (2024). *Progress in Materials Science*, 101242
[5] Vinu et al. (2020). *Advanced Materials*, 32, 1904635.
[6] Vinu et al. (2020). *Chemical Society Reviews*, 49, 4360.
[7] Vinu et al. (2020). *Nano Energy*, 72, 104702.
[8] Vinu et al. (2018). *Angewandte Chemie*, 130 (52), 17381.
[9] Vinu et al. (2018). *Chemical Society Reviews*, 47, 2680.
[10] Vinu at al. (2021). *Nano Energy*, 82, 105733.
[11] Vinu et al. (2021). *Angewandte Chemie*, 60 (39), 21242.

New tools for delivery of nucleic acids and proteins to plants

Markita Landry

Department of Chemical and Biomolecular Engineering, University of California, Berkeley

DNA-free approaches to plant bioengineering offer many advantages over plant transgenics. For instance, RNA [1,2] or protein delivery [3] to plants offers many opportunities for gene editing, transcriptional regulation, and through direction of protein-protein interactions. However, the delivery of RNA and proteins to plants presents both practical and analytical challenges. We present a GFP bimolecular fluorescence complementation-based tool, delivered complementation in planta (DCIP) [3], which allows for unambiguous and quantitative measurement of protein delivery in leaves (**Figure 1**). Using DCIP, we demonstrate cell-penetrating peptide mediated cytosolic delivery of peptides and recombinant proteins in *Nicotiana benthamiana*. We show that DCIP enables measurement of delivery efficiency and enables functional screening of cell penetrating peptide efficacies for in-plant protein delivery. Finally, we demonstrate that DCIP detects cell penetrating peptide mediated delivery of recombinantly expressed proteins such as mCherry and Lifeact into intact leaves. We also demonstrate, for the first time, delivery of a recombinant plant transcription factor, WUSCHEL (AtWUS), in N. benthamiana. RT-qPCR analysis of AtWUS delivery in Arabidopsis seedlings also suggests delivered WUS can recapitulate AtWUS-overexpression transcriptional changes. In all, nanoscale delivery of DNA-free tools for plant bioengineering offers new approaches to control plant development and to engineer plant physiology.

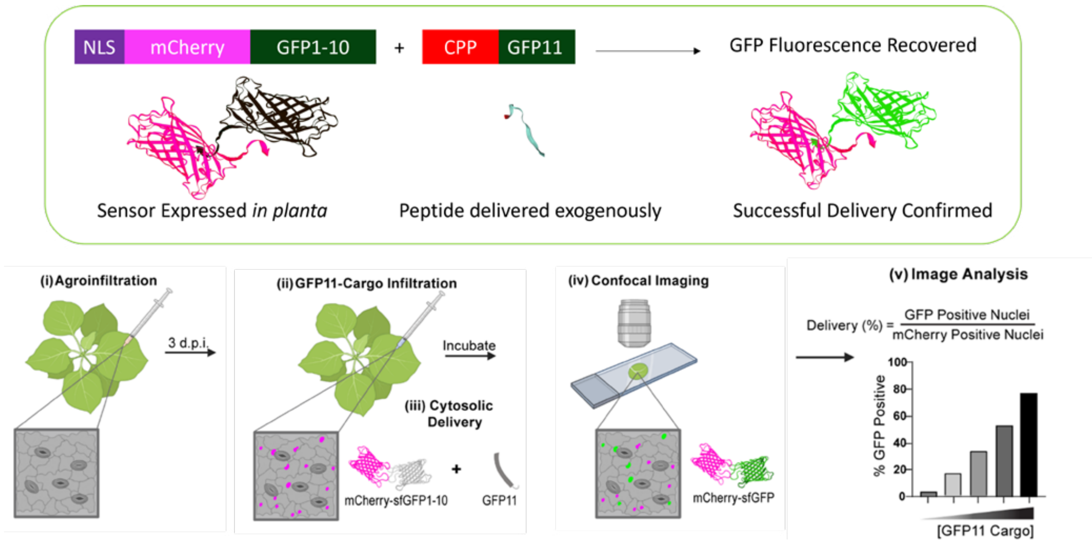


Figure 1. Expression of DCIP in plants allows expression of mCherry localized to the nucleus, and a dark GFP1-10 that will complement with a missing GFP11 fragment to reconstitute green fluorescence if and only if the missing GFP11 fragment is successfully delivered into the plant cell nucleus. GFP11 tagged with cell penetrating peptides enables their delivery into mature plant cells.

Acknowledgments

We acknowledge support from the USDA and an NSF CAREER award.

References

[1] Demirer, G.S., Zhang, H., Goh, et al. (2020). *Science Advances,* 6.

[2] Zhang, H., Demirer, G.S., Zhang, H., et al. (2019). *Proceedings of the National Academy of Science of the United States of America*, 116(15), 7543-7548.

[3] Wang, J., Squire, H.J., Goh, N.S., et al. (2023). *Nature Communications Biology,* 6, 840.

Nanotechnology Reaches the Field: Advances and Challenges for Agriculture

Alejandro Pérez-de-Luque

IFAPA, Centre Alameda del Obispo, Córdoba, Spain

In the context of climate change, agriculture faces significant challenges in increasing production and reducing environmental impact. Nanomaterial-based technologies, such as nanobiosensors and nanoformulations (NFs), offer promising solutions. Nanobiosensors can detect stresses in plants before they affect production, while NFs improve the efficiency and reduce the pollution of agrochemicals. These technologies facilitate the controlled application of inputs, optimising their use and reducing the necessary dosages, while precision agriculture can benefit from real-time data provided by nanobiosensors, aiding agricultural sustainability. An overview will be given of recent innovative uses of NFs and nanobiosensors in agriculture that can boost crop protection and production, as well as reduce the negative environmental impact of agricultural activities. However, to successfully implement these technologies, it is crucial to educate farmers and conduct field trials to ensure their effectiveness under real conditions.

Valorization of waste biomass through extraction of plant metabolites using green solvents

João A. P. Coutinho and Ana M. Ferreira Takahashi

CICECO-Aveiro Institute of Meterials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

The transformation of waste into valuable resources is a critical aspect of advancing a circular economy today. Numerous industrial processes discard substantial amounts of plant and animal biomass, resulting in the loss of valuable raw materials. To repurpose some of this recalcitrant biomass, new technologies based on novel solvents are essential, particularly for legnocellulosic materials that are not easily soluble in conventional solvents. This limitation can be turned into an advantage if intead of trying to solubilize the full biomass prior to fractionation an alternative approach of extracting particular fractions of interest is attempted. This approach has the advantage of requirinig the solubilization of smaller quantities of materials and also of allowing the use of aqueous based solvents instead of organic solvents.

In this lecture, we will explore how metabolites from three biomass sources can be extracted and applied. We will discuss the extraction of antioxidants from juçara fruits and their incorporation in soaps and skin care products [1], artemisinin form *Artemisia annua* L. And the evaluation of the extracts antimalarial activities [2] and levodopa from *Mucuna pruriens* and its use to tune the production of nanoparticles [3,4].

Acknowledgments

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 (DOI 10.54499/UIDB/50011/2020), UIDP/50011/2020 (DOI 10.54499/UIDP/50011/2020) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020) financed by national funds through the FCT/MCTES (PIDDAC).

References

[1] Soares, B.P., Ferreira, A.M., Justi, M., Rodrigues, L.G.G., Oliveira, J.V., Pinho, S.P., Coutinho, J.A.P. (2023). *Molecules,* 28, 1607.

[2] Ferreira, A.M., Sales, I., Santos, S.A.O., Santos, T., Nogueira, F., Mattedi, S., Pinho, S.P., Coutinho, J.A.P., Freire, M.G. (2024). *Chem & Bio Engineering,* 11, 44–52.

[3] Benfica, J., Miranda, J.S., Morais, E.S., Freire, M.G., Coutinho, J.A.P., de Sousa, R.C.S. (2020). *ACS Sustainable Chemistry and Engineering,* 8, 6682-6689.

[4] Benfica, J., Morais, E.S., Miranda, J.S., Freire, M.G., de Sousa, R.C.S., Coutinho, J.A.P. (2021). *Seperation and Purification Technology,* 274, 119084.

Nanomaterials and Plants: Accumulation, Toxicity and Agricultural Applications

Susana Loureiro[a], Zahra Khodaparast[b], Catarina Malheiro[a] and Rui Morgado[a]

[a]*CESAM- Centre for Environmental and Marine Studies & Department of Biology, University of Aveiro, Portugal*
[b]*CESAM- Centre for Environmental and Marine Studies & Department of Environment and Planning, University of Aveiro, Portugal*

Nanomaterials are increasingly present in agricultural fields, either intentionally through the application of nanopesticides and nanofertilizers or unintentionally through the use of sewage sludge as a soil amendment or fertilizer. Plants can serve as target organisms for these novel agrochemicals but may also be non-target recipients of other contaminants introduced through sludge or pesticide applications. Consequently, it is essential to investigate the toxicity and accumulation patterns of these novel materials and new sludge components, considering different plant life cycle stages and translocation mechanisms.

Laboratory-based toxicity and toxicokinetic studies, along with higher-tiered field trials, provide valuable insights into how these materials influence plant health, either positively or negatively, as target or non-target entities. Two case studies illustrate these approaches. The first focuses on the bioaccumulation and translocation of Ag_2S nanoparticles (NPs), a potential contaminant in sewage sludge, using the life cycle of a rapid-cycling turnip species (**Brassica rapa**) to assess toxicokinetics across different developmental stages. The second study explores the potential of Zn-Al-NO_3 layered double hydroxide (LDH) as a nanofertilizer, examining its impact on maize growth and soil biological and chemical properties compared to conventional zinc fertilizers. The Zn content in roots and its translocation were assessed, and soil enzyme activity was monitored, revealing improved multifunctionality indices and higher enzymatic activity at the end of the experiment.

These studies are critical for understanding the fate, toxicity, and beneficial traits of nanomaterials in agriculture, contributing to the development of safer and more efficient agrochemical products.

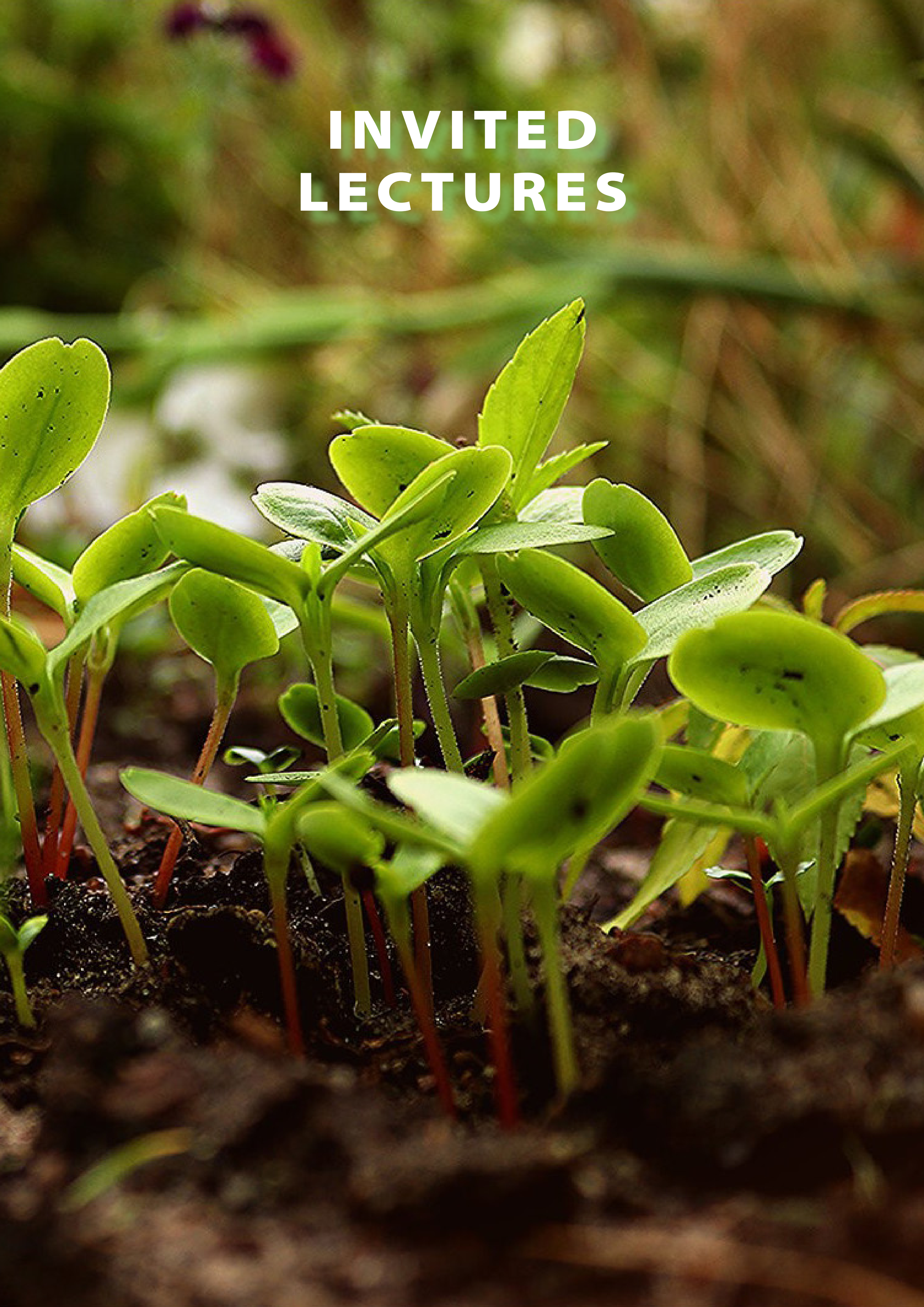
Acknowledgments

European Union's Horizon 2020 grant agreement No. 646002, NanoFASE - Nanomaterial Fate and Speciation in the Environment; ZK doctoral grant BD/UI88/7260/2015). FCT/MCTES for the financial support to CESAM (UIDP/50017/2020+UIDB/50017/
2020+LA/P/0094/2020) through national funds.

References

[1] Khodaparast, Z., van Gestel, C. A. M., Verweij, R. A., Papadiamantis, A. G., Gonçalves, S. F., Lynch, I., & Loureiro, S. (2022). *Journal of Hazardous Materials*, 435, 128880. https://doi.org/10.1016/j.jhazmat.2022.128880.

INVITED LECTURES



Multiple scales and ways of plant-nanomaterial interactions

Zsuzsanna Kolbert[a,b]

[a]Department of Plant Biology, University of Szeged, Közép fasor 52., 6726 Szeged, Hungary
[b]MTA-SZTE "Lendület" MOMENTUM Plant NaNObiology Research Group, Közép fasor 52., 6726 Szeged, Hungary

Due to their unique properties, nanomaterials (NMs) behave peculiarly in biosystems. Understanding of the multilevel and bidirectional relationship between plants and NMs is of great relevance [1], since the development of nanotechnology opens up more and more possibilities for the application of nanoparticles (NPs) in plant cultivation. The interactions of NMs with plants can be interpreted on a spatial scale: from local interactions in cells to systemic effects on whole plants and on ecosystems. Interpreted on a time scale, the effects of NMs on plants may be immediate or subsequent. At the cellular level, the composition and structure of the cell wall and membranes are modified by NMs, promoting internalization. Our results obtained with different type of NMs (zinc oxide NPs, nickel oxide NPs, multi-walled carbon nanotubes, chitosan NPs) support the alterations in the levels of cell wall components including pectin, callose, lignin, suberine, cellulose. Additionally, we have evidenced the uptake of different nanomaterials in root cells/cell walls. The effects of nanomaterials on germination and seedling physiology and on the primary and secondary metabolism in the shoot are realized at organ and organism levels. Beyond the direct NM-effects on individual plants, NMs interact with the beneficial ecological partners of plants exerting indirect effects on plants. The bidirectional relationship between NMs and plants is supported by the *ex planta* transformation of NMs driven by root exudates and the possibility of *in planta* NM biotransformation [1]. Regarding the molecular processes of NM-induced plant responses, our research group extensively investigates nitro-oxidative signalling as the effect of metal-oxid NPs and carbon-based NMs including chitosan encapsulated nitric oxide donor.

This lecture will summarize our past and latest findings in association with plant-nanomaterial interactions.

Acknowledgments The nanomaterial-related works in the Kolbert lab is supported by the Hungarian Academy of Sciences (Momentum Programme, LP2023-14) and by the National Research, Development and Innovation Office (grant nos. K135303 and 146292). Thanks to all collaborating partners.

References

[1] Oliveira, H.C., Seabra, A.B., Kondak, S., Adedokun, O.P., Kolbert, Z.S. (2023). *Journal of Experimental Botany*, 74(12), 3406–3424.

LA-ICP-MS and Nano-Ct based bioimaging to study the fundamentals of mineral ion and nanoparticle interactions with plant tissue

Søren Husted, Maja Arsic, Morten Vestenaa, Francesco Minutello, Pauline Møs, Max Frank and Daniel Persson

University of Copenhagen, Department of Plant and Environmental Sciences, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

Conventional strategies for soil fertilization are remarkably inefficient, as only a fraction of most mineral elements added with fertilizers are taken up by plants, the rest are typically trapped in the soil or leached to the aqueous environment, causing eutrophication. Thus, one of the most important current challenges of agriculture is to improve the sustainability of food production via an improved fertilizer efficiency. However, fertilizers have practically not improved for decades and this calls for an acute rethinking of fertilization procedures.

We have proposed a novel approach that bypasses nutrient fixation and microbial immobilization in the soil. We take advantage of the most recent breakthroughs within bio-nanotechnology, which allow us to produce biocompatible fertilizers based on smart nanomaterials, tailored to more effectively deliver nutrients to agricultural crops, both when applied to the soil or via the foliage.

To study uptake and distribution of mineral ions and nanoparticles in plants we use a range of powerful multi-elemental imaging technologies, including laser ablation-ICP-MS (LA-ICP-MS), Nano-Computed Tomography (Nano-CT) and Confocal Laser Scanning Microscopy (CLSM).

I will demonstrate a range of recent cases where we have used elemental bioimaging techniques to study how essential mineral ions and nanoparticles interact with plant tissue. I will show how these techniques can be used to study the fundamental processes controlling uptake and assimilation at the single cell level. I will also show how we can extrapolate this information to produce a new generation of more efficient fertilizers for the benefit of farmers economy, environment and climate.

References

[1] Husted, S., Minutello, F., Pinna, A., Le Tougaard, S., Møs, P., Kopittke, P.M. (2023). *Trends in Plant Science*, 28, 90-105. DOI10.1016/j.tplants.2022.08.01.

[2] Kopittke, P.M., Lombi, E., van der Ent, A., Wang, P., Laird, J., Moore, K., Perrson, D., Husted, S. (2020). *Plant Physiology*, 182, 1869-1882 DOI: 10.1104/pp.19.01306

[3] Arsic, M., Persson, D.P., Schjoerring, J.K., Thygesen, L.G., Lombi, E., Doolette, C.L., Husted, S. (2022). *Physiologia Plantarum*, 174, 1-15 DOI10.1111/ppl.13761

[4] Egelund Szameitat, A., Sharma, A., Minutello, F., Pinna, A., Er-Rafik, M., Hansen, T.H., Persson, D.P., Andersen, B., Husted, S. (2021). *Environmental Science: Nano*, 8, 444-459. DOI10.1039/d0en00974a

The influence of nanoparticles on selected aspects of plant development

Ewa Kurczyńska

Plant Cell Biology Team, Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia, Katowice

The intensively developing nanotechnology raises many questions on the impact of nanomaterials on the development of living organisms, including plants. The questions that arise concern, among others, the influence of nanoparticles (NPs) on cell differentiation processes, including the chemical composition of cell walls and the exchange of information between cells on the example of symplasmic communication exposed to NPs.

Studies were conducted on: 1/*Hordeum vulgare* and *Arabidopsis thaliana* roots, 2/*Arabidopsis thaliana* protoplasts and 3/ the process of somatic embryogenesis of Arabidopsis. Analysis of gold nanoparticles (AuNPs) presence and distribution was performed using High Resolution Electron Microscope (HRTEM), chemical composition of cell walls was studied using antibodies directed against selected pectic and arabinogalactan proteins epitopes, while analysis of symplasmic communication was performed using symplasmic transport fluorochromes, the FRAP (Fluorescence Recovery After Photobleaching) method and confocal microscopy. AuNPs in diameter of 5 ± 2 nm with different surface charge were used during the studies. Conducted research showed that AuNPs do not enter roots of analysed plants but enter the Arabidopsis protoplasts [1, 2]. Moreover, AuNPs influence the root morphology, histology, chemical composition of root cell walls as well as symplastic communication which means that NPs modify the cell differentiation process. While during somatic embryogenesis, NPs completely blocked the changes in the direction of cell differentiation from somatic to embryogenic state, which resulted in blocking the process of somatic embryo formation [3].

Acknowledgments

This work was supported by the Ministry of Sciences and Higher Education of Poland as a part of the statutory activities of the Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia, Katowice

References

[1] Milewska-Hendel, A., Zubko, M., Karcz, J., Stroz, D., & Kurczynska, E. (2017). *Scientific Reports*, 7, 3.

[2] Milewska-Hendel, A., Zubko, M., Stróż, D., & Kurczyńska, E.U. (2019). *International Journal of Molecular Sciences*, 20, 1650.

[3] Godel-Jędrychowska, K., Milewska-Hendel, A., Sala, K., Barański, R., & Kurczyńska, E. (2023). *International Journal of Molecular Sciences*, 24, 10356.

Nanoparticles: A Novel Sustainable and Eco-Friendly Approach for Enhancing Plant Growth

Renata Szymańska, Aleksandra Orzechowska and Agnieszka Trela-Makowej

AGH University of Krakow, Faculty of Physics and Applied Computer Science, A. Mickiewicza 30, 30-059 Kraków

The increasing population, climate change, and shrinking arable land are prompting the exploration of new technologies in agriculture. In a sustainable approach, nanotechnology offers opportunities to support agricultural development on multiple levels. Our approach includes both controlled laboratory studies and field experiments. We investigated the impact of various concentrations of nano-TiO_2 on plant growth, cellular processes, photosynthetic activity, and antioxidant profiles. We found that high concentrations of nano-TiO_2 induce toxicity symptoms related to oxidative stress, while lower concentrations stimulate growth, developmental processes, and mitigate the effects of high light stress [1,2]. We conclude that nano-TiO_2 exhibits hormetic effects [3].

Recently, in collaboration with Wieliczka Vineyard, we studied the effects of nano-SiO_2 on grapevines in a field study. Our preliminary results show that nano-SiO_2 stimulates photosynthetic activity, transpiration rate, and photosynthetic pigment levels in these plants. Both oxides in nanoparticle form are used as fertilizers in both conventional and organic agriculture. Our findings suggest the potential benefits of nano-TiO_2 and nano-SiO_2 for sustainable agriculture. The mechanisms of nanoparticle action in plants remain an evolving area of research, and comprehensive studies are needed for the sustainable use of these nano-oxides in agriculture.

Acknowledgments

This project was supported by grant 2022/47/B/NZ9/00225 (OPUS 24) obtained from the National Science Centre (NCN) Poland.

References

[1] Szymańska, R., Kołodziej, K., Ślesak, I., Zimak-Piekarczyk, P., Orzechowska, A., Gabruk, M., Żądło, A., Habina, I., Knap, W., Burda, K., Kruk, J. (2016). *Environmental Pollution*, 213, 957-965. doi: 10.1016/j.envpol.2016.03.026.

[2] Orzechowska, A., Szymańska, R., Sarna, M., Żądło, A., Trtílek, M., Kruk, J. (2024). *Environmental Science and Pollution Research International*, 31(9), 13706-13721. doi: 10.1007/s11356-024-31970-6.

[3] Trela-Makowej, A., Orzechowska, A., Szymańska, R. (2024). *Science of the Total Environment*, 1, 910, 168669. doi: 10.1016/j.scitotenv.2023.168669.

Next generation chemical priming: Utilizing nanocarrier technology for enhanced crop resilience

Gholamreza Gohari[a,b] and Vasileios Fotopoulos[a]

[a]Cyprus University of Technology, Department of Agricultural Sciences Biotechnology and Food Science, Lemesos, Cyprus
[b]Department of Horticulture, Faculty of Horticulture, University of Maragheh, Maragheh, Iran

Climate change poses significant challenges to agricultural systems, subjecting plants to various stressors that result in substantial yield losses. In response, there is a critical need to develop innovative crop management tools. One promising approach is chemical priming, which enhances plant tolerance to stress. This technique involves the use of compounds such as phytohormones, reactive species, and synthetic chimeras, which have been identified as effective priming agents [1]. Recent advances in nanotechnology have opened up new possibilities for developing innovative delivery systems in agriculture. Chitosan (CTS), a biopolymer derived from chitin, has gained recognition as an effective nanocarrier for delivering various bioactive compounds in agriculture. Chitosan nanocarriers can encapsulate compounds like phytohormones and antioxidants, ensuring controlled, targeted release to plant tissues. This technology enhances the efficacy of these compounds while reducing chemical usage, promoting more efficient and eco-friendly crop management [1,2]. In this context, our research focused on using chitosan-based nanocarriers to deliver melatonin (Mel) as a chemical priming agent in spearmint plants under salinity stress. The results indicated that chitosan-melatonin nanoparticles (CTS-HPMC-Mel NPs) significantly mitigated the detrimental effects of salt stress. This was achieved through improved morphological traits, enhanced proline content, increased antioxidant enzyme activities, and a positive impact on the essential oil profile. In addition to foliar application, this technology was applied to seed priming, where chitosan was used to encapsulate both salicylic acid (SA) and melatonin. Seeds coated with CTS-SA and CTS-Mel NPs exhibited improved corn salad plants performance under salinity stress. Treatments with CTS-Mel NPs were particularly effective, enhancing fresh weight, dry weight, chlorophyll content, total phenolics, protein levels, and antioxidant enzyme activities (SOD and CAT). Furthermore, CTS-Mel NPs significantly increased the levels of chlorogenic acid, naringin, o-coumaric acid, and catechin hydrate under both control and salinity conditions. This nanotechnology-based priming system represents an eco-friendly, next-generation approach that improves the efficiency of chemical priming while reducing the overall use of chemicals in agriculture.

Acknowledgments
We would like to acknowledge financial support from Horizon 2020 (project 'RADIANT': 101,000,622)

References
[1] Gohari, G., Jiang, M., Manganaris, G. A., Zhou, J., & Fotopoulos, V. (2024). *Trends in Plant Science*, 29, 150-166.
[2] Ioannou, A., Gohari, G., Papaphilippou, P., Panahirad, S., Akbari, A., Dadpour, M. R., Fotopoulos, V. (2020). *Environmental and Experimental Botany*, 176, 104048.

Optical nanosensors to monitor plant health

Kruss Sebastian

Ruhr-University Bochum, Universitätsstrasse 150, 44801 Bochum, Germany

Nanoscale tools have the potential to enable smart sensors that communicate with plants and actuate devices. They can improve plant productivity, optimize and automate water and agrochemical allocation, and enable high-throughput plant chemical phenotyping. Reducing crop loss and minimizing the use of resources are major challenges in plant agriculture industries worldwide. For this purpose, new technologies are required to accurately monitor, in real time and with high spatial and temporal resolution, plant physiological responses to their microenvironment. Nanomaterials are allowing the translation of plant chemical signals into digital information that can be non-invasively monitored by standoff devices. Here, I will present our work on near infrared (NIR) fluorescent nanosensors that enable remote imaging of plant stress and pathogen-related responses. These optical sensors detect molecules that are typical for a certain physiological state. I will show examples of reactive oxygen species that are released in response to stress and polyphenols that are released in response to pathogen exposure. Our smart plant sensors are therefore promising tools for next-generation precision agriculture.

From Toxic Lead Perovskites to Eco-Friendly Double Perovskites: Innovations in Foodstuff Analysis and Medical Diagnostics

Kiran Hiremath, Jomy Jose Philip and R Geetha Balakrishna

Centre for Nano and Material Sciences, Jain Global Campus, JAIN (deemed-to-be-University), Jakkasandra Post, Ramanagaram, Bangalore

Email: br.geetha@jainuniversity.ac.in

Perovskite quantum dots (PQDs) have shown good promises as fluorescent probes over the conventional quantum dots (CQDs) like CdSe, CdTe, carbon dots etc., for sensing analytes, mainly due to their fascinating properties like tunable emission, high quantum yield and high absorption coefficient that renders high sensitivity [1]. Despite rapid advances in their development, the low stability and intrinsic toxicity of lead ions have hindered their commercial application. Lead-free halide double perovskites are explored as an alternative and have shown good stability, non-toxicity, and environmental friendliness [2]. In particular, CsM+M3+Cl6 type double perovskites have emerged at the forefront with excellent stability and interesting optoelectronic properties. A series of such doped and co-doped double perovskite nanocrystals (DPNCs), wherein dopants act as energy channels, thus showing wavelength-tunable NIR light emission (800–1600 nm), have been explored [3]. This series of NIR emission wavelengths of doped DPNCs include not only shorter-wavelength NIR light (≤900 nm) but also longer-wavelength NIR light (>900 nm), which are more appropriate for foodstuff analysis and medical diagnosis applications [4]. On appropriate tuning, both white light and broad near-infrared (NIR) radiation can be achieved simultaneously, to provide visual inspection and early signs of rotting of food products. The broad NIR emission is absorbed by the vibrational overtones of water molecules present in food items, providing the non-invasive image contrast to assess the food freshness. This detection technique if developed further can become a easy diagnostic tool to complement the existing, complex but high throughput and high-sensitivity approaches.

References

[1] Kovalenko, M.V., Protesescu, L., & Bodnarchuk, M.I. (2017). *Science,* 358(6364), 745-750.
[2] Wu, Y., Li, X., Zeng, H. (2021). *Small Structures,* 2(3), 2000071.
[3] Cao, L., Jia, X., Gan, W., Ma, C.G., Zhang, J., Lou, B., & Wang, J. (2023). *Advanced Functional Materials,* 33(13), 2212135.
[4] Wang, H., Yao, J., & Zeng, R.J.N. (2024). *Nanoscale,* 16, 6837-6852.

Tetrapods based Smart Materials for Advanced Technologies

Yogendra Kumar Mishra

Mads Clausen Institute, NanoSYD, Smart Materia, University of Southern Denmark, Alsion 2, 6400, Sønderborg, DENMARK

Email: mishra@mci.sdu.dk

Considering the size dependent utilization complexities of nanoscopic dimensions in real technologies, the focus of nanomaterials community is converging to three-dimensional (3D) nanomaterials which are built out of interconnected nanostructures building blocks. This talk will briefly introduce the importance of tetrapod nanostructures towards smart 3D nanostructuring via a simple and single step flame-based approach for synthesis of zinc oxide tetrapods. These tetrapods have already demonstrated their potential roles in many different technologies. These zinc oxide tetrapods can be used as solid backbone or sacrificial templates to design hybrid or new tetrapods as smart materials. These smart 3D nanomaterials offer many applications in engineering and advanced technologies. Application examples of 3D tetrapods in nanosensing, composite engineering, antiviral candidates, water purification, piezotronics, agriculture, and in several other applications will be demonstrated. The integration of tetrapods in electrospun fibers offer many advantages in biomedical engineering and few examples about nano-engineered electrospun fibers will be presented as recent developments.

Sustainable Nanotechnology Practices in Environmental Applications

Nataraj Sanna Kotrappanavar

Centre for Nano & Material Sciences, JAIN University, Jain Global Campus, Bangalore 562112, India

Email: sk.nataraj@jainuniversity.ac.in; sknata@gmail.com

In recent years, nanotechnology practices have enabled efficient utilization of natural resources to their maximum capabilities in various applications. On the other hand, finding new class of materials and sustainable technologies to overcome limitation in conventional materials stock and practices is a challenging task. Even in presence of advanced technologies world will continue to experience newer challenges which will directly affect the environmental sustainability at large. These challenges include (a) increasing water scarcity, (b) vulnerability to pollution and contamination, (c) degrading ecosystem, (d) deforestation, (e) continuing urbanization, (f) excessive use of agricultural chemicals, etc. Nevertheless, collective efforts and innovative strategies can provide futuristic solution to achieve water and environmental sustainability. Some of these may include (a) Integrated Water Resource Management, (b) Utilization of natural resources as material stocks, (c) Adopting new methodologies, (d) Promotion of Green Infrastructure, and so on. This talk will discuss about the recent advances in the utilization of sustainable materials through nanotechnology practices and developing greener process designs aiming water treatment, wastewater treatment and energy applications. These practices are crucial for conserving natural water resources, safeguarding groundwater, minimizing water consumption, recycling used water, discharge of wastewater and environmental sustainability which promotes overall sustainable living practices.

Acknowledgments

SKN expresses sincere gratitude to Jain University for the Management Research Project Grant (JU/MRP/CNMS/18/2022), and Department of Science and Technology (DST) for NANOMISSION GRANT (SR/NM/NT-1073/2016), the INSPIRE faculty grant (IFA12-CH-84) and DST-Technology Mission Division (DST/TMD/HFC/2K18/124G).

References

[1] Santhosh, K.N., Anita Samage, Mahadevaprasad, K.N., Aditya, D.S., Jayapandi, S., Hyeonseok Yoon, & Nataraj, S.K. (2024). *Chemical Engineering Journal,* 484, 149747.

[2] Aditya, D.S., Mahadevaprasad, K.N., Santhosh, K.N., Hemavathi, A.B., Mahaveer Halakarni, Hyeonseok Yoon, Nataraj, S.K. (2024). *Chemical Engineering Journal*, 488, 150910.

Nanotechnologies in agroecosystems and their effects on Soil and Food Security

Luiz Roberto Guimarães Guilherme

Professor of Soil Science, Federal University of Lavras (UFLA), School of Agriculture (ESAL), Soil Science Department (DCS)
Research Fellow (CNPq), Member of the Brazilian Academy of Sciences (ABC)
Coordinator of the National Institute of Science and Technology INCT - Soil and Food Security, Lavras (Minas Gerais), Brazil

Concerns about Food Security and how to address problems relevant to this global challenge are recurrent and had a worldwide landmark during *The World Food Summit*, in 1996. Meanwhile, Soil Security is a new concept treated as crucial to guaranteeing Food Security only after the declaration of the *International Year of Soils*, in 2015. The assessment and proposition of actions to ensure global Food Security require continued studies that could develop better indicators to more assertively represent and solve problems related to the five dimensions of Food Security: 1) Quantity, 2) Quality, 3) Acceptability, 4) Safety and 5) Certainty/Stability. Similarly, the concept of Soil Security is also multidimensional and recognizes the importance of appropriately tackling the following dimensions: 1) Capacity, 2) Condition, 3) Capital, 4) Connectivity and 5) Codification. Concerning Food Security, nanotechnologies could help us solve many aspects of food quantity / quality. Yet, a better understanding of the food safety aspects of using such technologies is still a significant gap. Considering Soil Security, relevant information regarding capacity (soil functions) and condition (change in capacity) can also be obtained via resources and capabilities provided by nanotechnologies. Our talk will discuss the pros and cons, as well as the major gaps of nanotechnologies for addressing issues concerning Food Security - namely food quality and safety - while also relating these aspects with relevant topics of Soil Security that are essential to assuring the sustainability of agroecosystems.

ORAL COMMUNICATIONS

Nanoparticles in plant cryopreservation: Effects on genetic stability, metabolic profiles, and structural integrity in bleeding heart cultivars

Dariusz Kulus[a], Alicja Kulpińska[a], Alicja Tymoszuk[a], Agata Michalska[b], Julita Nowakowska[c] and Dorota Wichrowska[d]

[a]Laboratory of Horticulture, Department of Biotechnology, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, Bernardyńska 6, 85-029 Bydgoszcz, Poland. Email: dariusz.kulus@pbs.edu.pl
[b]Department of Biogeochemistry and Soil Science, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, Bernardyńska 6, 85-029 Bydgoszcz, Poland
[c]Imaging Laboratory, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland
[d]Department of Microbiology and Food Technology, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, Kaliskiego 7, 85-796 Bydgoszcz, Poland

This research focused on evaluating the impact of gold (AuNPs), silver (AgNPs), and zinc oxide (ZnONPs) nanoparticles on the structural integrity, genetic stability, and metabolic activity of cryopreserved plant material with medicinal properties. The study involved cryopreserving shoot tips from two cultivars of bleeding heart (*Lamprocapnos spectabilis* (L.) Fukuhara), i.e. 'Gold Heart' and 'Valentine', using the encapsulation-vitrification technique. Nanoparticles were incorporated at concentrations of 5 or 15 ppm into either the preculture medium (the first step of the cryo protocol) or the alginate bead matrix (the second step). Following *in vitro* recovery and acclimatization in a greenhouse, the plants underwent histological, molecular, and biochemical assessments. Electron microscopy analysis (TEM) of the LN-derived plant material revealed that the cells maintained micro-morphological stability. It was observed that nanoparticles could penetrate the cells and accumulate in various compartments, including the nucleus. Genetic analysis using SCoT markers showed polymorphisms in 11.5% of 'Gold Heart' plants, while RAPD markers identified mutations in 1.9% of 'Valentine' specimens. Analysis of Molecular Variance (AMOVA) indicated that all genetic variation in 'Valentine' was sourced within populations and was not significantly influenced by nanoparticle treatments. In contrast, for 'Gold Heart', 94% of genetic variation was found within populations, while 6% was linked to nanoparticle treatments, predominantly from the application of 15 ppm ZnONPs. The introduction of NPs significantly affected the metabolic profile of bleeding heart plants, particularly influencing the synthesis of phenolic acids and aldehydes, as well as antioxidant mechanisms in both cultivars. In 'Gold Heart', there were notable changes in protein content and plant pigments (anthocyanins, carotenoids, and chlorophylls), whereas 'Valentine' showed no such alterations. The results indicate that different types and concentrations of nanoparticles exert distinct effects on the production of specific metabolites. This variability could be leveraged to enhance plant secondary metabolism for targeted pharmacological benefits.

Acknowledgments
This research was funded in part by the National Science Centre, Poland (Grant number: 2020/39/D/NZ9/01592).

Silver nanoparticles in chrysanthemum breeding

Alicja Tymoszuk and Dariusz Kulus

Laboratory of Horticulture, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, Bernardyńska 6, 85-029 Bydgoszcz, Poland

Chrysanthemum × *morifolium* (Ramat.) Hemsl. is one of the most popular and economically important ornamental plant species worldwide. This popularity is due to various-shaped and multi-colored inflorescences, long and uniform flowering period, habit diversity, as well as broad use in horticulture and medicine. The market demand for chrysanthemum cultivars with new inflorescence characteristics, improved stress tolerance, or quality attributes is increasing annually, being a great challenge for chrysanthemum breeders, in Poland and abroad.

Nanoparticles (NPs) can easily interact with plant cells and influence plant growth and development. Despite many perspectives and benefits arising from the great progress in nanotechnology development, nanoparticles may also have adverse effects that have not been sufficiently explored. A good understanding of NPs influence on plants is of paramount importance for assessing NPs toxicity, especially the genotoxic effects, which are difficult to predict, and need deeper study.

The most frequently used chrysanthemum breeding methods are generative crossing and mutagenesis. Mutation induction with X- or gamma rays, unfortunately, is associated with the use of specialized devices located in medical centers or scientific institutions. The application of nanoparticles, as a new chemical mutagen added into the micropropagation medium, could make chrysanthemum breeding relatively easy, and could be routinely performed in *in vitro* plant laboratories, without the need for sophisticated equipment used in the new breeding technologies.

This study aimed to analyze the biochemical activity of leaf and internode explants during *in vitro* culture; evaluate the effectiveness of *in vitro* adventitious shoots regeneration; identify genetic and phenotypic variation among *ex vitro*-cultivated plants in *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. 'Lilac Wonder' and 'Richmond' as a result of *in vitro* application of Ag NPs at the concentration of 5-100 $mg \cdot L^{-1}$; 20 nm in size. We proved that *in vitro* treatment of explants with Ag NPs can be used as a novel breeding technique in chrysanthemum. However, the individual cultivars may differ in the biochemical response, regeneration efficiency, genetic variation, and frequency of induced mutations in flowering plants. The results significantly broaden the understanding of the impact of Ag NPs on plants at the biochemical, physiological, molecular and phenotypic levels, and are of importance for both, nanotechnology and plant biotechnology. This is an innovative research in the area of plant breeding.

Acknowledgments

This research was funded by The National Science Centre (NCN) in Poland, founding MINIATURA 4 "Studies on the use of silver nanoparticles in chrysanthemum breeding" (DEC-2020/04/X/NZ9/01667).

Calcium Phosphate Nanoparticles for Biofortification and Protection of Tomato Plants

Gloria B. Ramírez-Rodríguez [a], Belén Parra-Torrejón [a], Manuel J. Sánchez del Castillo[b], Andrés Cáceres[c], and José M. Delgado-López[a]

[a]Department of Inorganic Chemistry, University of Granada (Granada, Spain)
[b]Donostia International Physics Center (DIPC, Spain)
[c]Nanointec S.L. (Almería, Spain)

Nanotechnology offers new opportunities to enhance crop production and improve crop resilience against diseases, pests, and weeds, while also reducing the environmental impact of conventional agrochemicals.[1] Among the promising nanomaterials for agriculture are calcium phosphate nanoparticles (CaP NPs), which mimic the mineral composition of bone. These nanoparticles stand out due to its composition (two relevant plant nutrients), inherent biocompatibility and biodegradability and non-toxicity. [2] Additionally, CaP NPs exhibit excellent adsorption capacity for organic molecules (*e.g.* urea, elicitors) and can accommodate a wide range of ionic substitutions (*e.g.* K^+, Zn^{2+}, NO_3^-, etc.).[2] CaP NPs, predominantly in the forms of apatite (Ap) and amorphous calcium phosphate (ACP), have been explored in precision agriculture for the targeted delivery of macronutrients, elicitors, and biostimulants to plants.[2]

In this study, we developed a multifunctional nanomaterial for biofortification and plant protection by doping ACP NPs with zinc ions (nanoZn).[3] The resulting nanoparticles were spherical, with an average diameter of 25 ± 2.6 nm, and consisted primarily of 20.3 wt % Ca, 14.8 wt % P, and 13.4 wt % Zn. Although nanoZn remained stable in aqueous solutions at neutral pH, it dissolved in citric acid at pH 4.5, enabling a pH-responsive delivery of its constituents. Foliar application of nanoZn on tomato plants led to significantly higher concentrations of Zn, Ca, and P in the fruit, as well as an increased yield compared to conventional treatments. Furthermore, nanoZn inhibited the growth of *Pseudomonas syringae* (*Ps*), the main cause of bacterial speck, and significantly reduced *Ps* incidence and mortality in infected tomato seeds.

Acknowledgments
This research was funded by FEDER/MCIN/AEI/10.13039/501100011033 and "European Union NextGenerationEU/PRTR" through the projects nanoFERTI2 (CPP2021-008801), nanoBip (TED2021-132151A-I00) and RYC2021-032734-I.

References
[1] Lowry, G.V., et al. (2024). *Nature Nanotechnology*, 2024, 1-15.
[2] Ramírez-Rodríguez, G.B. et al. (2020). *ACS Applied Bio Materials,* 3, 1344–1353.
[3] Parra-Torrejón, B. et al. (2023). *Environmental Science and Technology,* 57, 14950−14960.

Feasibility of using SiO_2 NPs to mitigate mercury in transgenic soybeans grown in contaminated soils: respective effects and action of SiO_2 NPs

Vinnicius H. C. Silva[a,b], Marco A. Zezzi Arruda[a] and Jörg Feldmann[b]

[a]*Spectrometry Sample Preparation and Mechanization Group, Institute of Chemistry, State University of Campinas – UNICAMP, Campinas - SP, Brazil*
[b]*Trace Element Speciation Laboratory, Institute of Chemistry, University of Graz, Universitätsplatz, Graz, Austria*

Mercury (Hg) is recognized as a global pollutant, standing out for being present at various trophic levels. It is considered a bio accumulative trace element, with the food pathway being the primary route of contamination in humans. The application of silica nanoparticles (SiO_2 NPs) as a mitigating agent on heavy metal contamination in plant crops, thereby reducing the abiotic stress induced by these contaminants, has shown promising results [1,2]. Therefore, the aim of this study was to investigate the potential of SiO_2 NPs to play an effective role against the absorption, accumulation and toxicity of Hg in soybean plants, through chemical speciation and total determination of Hg. The protective role of SiO_2 NPs was assessed taking into account morphological characteristics of soybean plants, macro and micronutrients and metabolites. Treatment with SiO_2NPs showed a significant decrease in Hg concentration in the roots of both transgenics, 17% and 29% for RR and INTACTA, respectively. With the presence of Na_2SiO_3, the reduction was 15% and 37%. In the leaves, this decrease was 25% and 22% for the RR transgenic under treatments with SiO_2 NPs and Na_2SiO_3 , while for the INTACTA transgenic, this decrease was 14% and 34%. Through this study of chemical speciation in different soybean plant compartments, only the species Hg (II) was found among the investigated groups (Hg; SiO_2 NPs + Hg; and Na_2SiO_3 + Hg), which indicates an absence of Hg methylation in the soil by the soil microbiome and in plant was possible to observe that the presence of Hg in the medium, either alone or in the presence of Si (SiO_2NPs or Na_2SiO_3), interferes with plant metabolism, causing alterations in the absorption of macro and micronutrients by the plant [3]. Furthermore, morphological analyses of the plant demonstrate a mitigating effect on Hg contamination at the cellular level by applying Na_2SiO_3 and SiO_2NPs. The mechanism of action of silica nanoparticles was evaluated, aiming to investigate a possible adsorption of Hg by the nanoparticles. No SiO_2 NPs could be detected in the plant roots allowing us to infer that there is no translocation of the nanoparticles into the soybean plant.

Acknowledgments
The authors thank the Fundação de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP, Grants 2021/11685-0, 2018/125207-0, 2019/24445-8 and 2023/14635-0).

References
[1] Li, Y. et al. (2020). *Environmental Science: Nano*, 7. https://doi.org/10.1039/D0EN00091D.
[2] Carneiro, J.M.T. et al. (2017). *Journal of Trace Elements in Medicine and Biology,* 44. http://dx.doi.org/10.1016/j.jtemb.2017.05.010.
[3] Yadav, M.et al. (2023). *Environmental Pollution*, 333. https://doi.org/10.1016/j.envpol.2023.122112.

Impact of Chitosan Nanoparticles and Cyanobacteria Biomass Supplementation Under Optimized Nitrogen on Maize (*Zea mays* L.) Productivity in Field Condition

Zoltan Molnar and Wogene Solomon

Albert Kázmér Faculty of Agricultural and Food Sciences, Department of Plant Sciences, Széchenyi István University, Győr - Mosonmagyaróvár, Hungary

The integration of agriculture with other scientific fields, such as using chitosan and cyanobacteria can significantly optimize fertilizer efficiency and improve soil health. Chitosan's nutrient delivery and metabolic enhancement, coupled with cyanobacteria's nitrogen-fixing ability within the plant, offer a comprehensive approach to boosting crop growth and promoting sustainable soil management. The exploration of chitosan nanoparticles and cyanobacteria biomass in agriculture is gaining increasing attention for its potential to enhance sustainability and crop performance [1]. However, the combined application of chitosan nanoparticles and cyanobacteria biomass remains largely unexplored, necessitating further investigation into their synergistic potential alongside nitrogen fertilizers. This research seeks to investigate the effects of integrating chitosan nanoparticles and cyanobacteria biomass – either independently or in combination – with recommended and reduced nitrogen fertilizer on maize crop productivity in field conditions. The results reveal that the integration of chitosan nanoparticles and cyanobacteria biomass significantly improves crop yield and chlorophyll content, while also positively influencing a range of plant growth parameters. This combined application not only boosts overall plant health and productivity but also enhances physiological traits that contribute to more robust and efficient crop development. The strategic integration of agricultural practices with advanced scientific approaches, such as combining chitosan nanoparticles and cyanobacteria biomass, can effectively extend the benefits of fertilizers while minimizing their limitations. This multidisciplinary approach not only optimizes nutrient use but also enhances plant growth and productivity, paving the way for more sustainable and efficient agricultural systems.

Reference

[1] Molnár, Z., Lamnganbi, M., Solomon, W., & Janda, T. (2023). *Agrosystems, Geosciences & Environment*, 6, e20428. https://doi.org/10.1002/agg2.20428

Plant-mediated solvent-free biomechanochemical synthesis of Ag nanoparticles and assessing plant-As-Se interaction

Matej Baláž and Zdenka Lukáčová Bujňáková

Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 04001 Košice, Slovakia

The interaction between nanomaterials and plants is an important issue nowadays, due to environmental pollution on one hand, but also due to phyto-remediation on the other one. However, many plants possess a unique option to participate in the nanomaterials synthesis by utilizing reducing species, such as phenols and flavonoids, which are naturally present in their structures to reduce metallic ions and thus yielding the nanoscale metal in its elemental form [1]. This synthesis methodology, known as green synthesis is particularly well-known for the preparation of silver nanoaprticles with antibacterial activity. However, this methodology is a multi-step process, utilizes liquids and external heating. Our research group has developed a one-step solid-state alternative by grinding the plant material and silver precrusor in a ball mill, entitled bio-mechanochemical synthesis [2]. So far, it has been successfully used to obtain Ag NPs using common plants such as oregano, wild thyme, lavender, and even lichens [3,4]. Currently, we are also interested in the plant interactions with arsenic and selenium, since we are synthesizing such nanomaterials in our lab [7] and we are aware of their toxicity. It is known that these two elements play an important role in the eosystem [5] and that plants are capable of biomineralizing As and producing Se nanoparticles [6]. Thus, we are interested in potential phyto-remediation of the soils contaminated by these two elements. This lecture will provide a short overview on the field of mechanochemistry [8] and its potential to prepare plant-based nanomaterials.

Acknowledgments

This work is supported by Slovak Research Development Agency, APVV (SK-PL-23-0002) and by The Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic (project 2/0112/22).

References

[1] Ahmad, S. et al. (2019). *International Journal of Nanomedicine*, 14, 5087-5107.
[2] Baláž, M. et al. (2017). *Advanced Powder Technology*, 28, 3307-3312.
[3] Baláž, M. (2021). *Nanomaterials,* 11, 1139.
[4] Baláž, M. et al. (2020). *ACS Sustainable Chemistry and Engineering*, 8, 13945-13955.
[5] Guan, D. X. (2022). *Critical Reviews in Environmental Science and Technology*, 52, 3567-3572
[6] Bravo, F. et al. (2024). *Environmental Technology and Innovation*, 34, 103575.
[7] Shpotyuk, O. et al. (2024). *Molecules,* 29, 3245.
[8] Baláž, M. (2021). Environmental Mechanochemistry: Recycling Waste into Materials Using High-Energy Ball Milling. Springer, pp.619.

Enzyme-responsive Chitosan-based Phosphorus Nanofertilizers

Nicola Carrara[a], Alice Boarino[a], Joaquin Clua[b], Maria Victoria Aparicio Chacon[b], Yves Poirier[b] and Harm-Anton Klok[a]

[a]*Institut des Matériaux et Institut des Science et Ingénierie Chimiques, École Polytéchnique Fédérale de Lausanne (EPFL), Rte Cantonale, 1015 Lausanne, Switzerland*
[b]*Department of Plant Molecular Biology, University of Lausanne, Quartier Centre, 1015 Lausanne, Switzerland*

Hunger is a persistent problem that is heightened by an ever-increasing global population. Providing food to the growing world population will require a further increase in agricultural productivity. However, the current agricultural practices are inefficient and involve an overuse of agrochemicals. The loss of fertilizers due to run-off and volatilization among other processes, is having detrimental effects on the environment [1]. In this project, new strategies are sought to improve the delivery of plant nutrients. The goal is to develop nanoparticle-based fertilizers that allow for an enzyme-triggered release of active ingredients, specifically harnessing acid phosphatase (AcP) activity [2]. Chitosan-based particles are highly attractive not only because they are obtained from a waste product but also because of the biocompatibility, biodegradability and non-toxicity of this biopolymer. Moreover, chitosan is known to have plant-growth promoting and antimicrobial properties. For these reasons and the simple and cost-effective production of nanoparticles through the ionotropic gelation method, tripolyphosphate-crosslinked chitosan nanoparticles have been extensively studied as next-generation agrochemicals [3].

In this study, polyphosphate-crosslinked chitosan nanofertilizers were synthesized by varying the polyphosphate and chitosan average molecular weight as well modifying the nanoparticles with the coupling agent 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). The response of the nanomaterial to AcP was tested *in vitro* in an aqueous environment, showing that the nutrient (phosphorus) release kinetic was mainly influenced by the polyphosphate average chain length. The effect of the nanoparticles on *Arabidopsis thaliana* grown in an agar medium under phosphorus starvation was investigated in terms of shoot weight and phosphorus use efficiency (PUE). Samples synthesized with the higher molecular weight chitosan and chemically modified with EDC performed comparable to the positive control in terms of shoot weight, displaying at the same time a higher PUE.

Acknowledgments
This work was financially supported by the Swiss National Science Foundation (SNSF).

References
[1] Lowry, G.V., Avellan, A., & Gilbertson, L.M. (2019). *Nature Nanotechnology,* 14, 517–522.
[2] Kuzyakov, Y., & Razavi, B. S. (2019). *Soil Biology and Biochemistry,* 135, 343–360.
[3] Riseh, R. S., et al. (2024). *Internatinal Journal of Biolgical Macromolecules,* 260, 129522.

NANOPLANT

Production of chimeric Virus-Like Particles in plant expression systems: a promising approach for veterinary vaccine development against *Fasciola hepatica*

Martyna A. Przewoźnik[a] and Kacper Karczmarzyk[b], Estera Wojtkowiak[a], Hanna Pudelska[a], Joanna Bernard[c], Jerzy Kozłowski[c], Emilia Mroczko[d], Maja Renicka[e], Roman Marecik[e], Wojciech Białas[e], Małgorzata Kęsik-Brodacka[b] and Tomasz Pniewski[a]

[a]*Institute of Plant Genetics of Polish Academy of Sciences, Poznań, Poland*
[b]*National Medicine Institute, Warsaw, Poland*
[c]*Adam Mickiewicz University in Poznań, Poland*
[d]*University of Wrocław, Poland*
[e]*Poznań University of Life Sciences in Poznań, Poland*

E-mail: mprz@igr.poznan.pl; k.karczmarzyk@nil.gov.pl; tpni@igr.poznan.pl

Fasciola hepatica, commonly known as the liver fluke, is a significant parasite that affects wild and domestic animals, mostly ruminants, worldwide. This flatworm parasite primarily targets the liver of ruminants, causing a disease known as fascioliasis. Sheep and cattle become infected with *Fasciola hepatica* by ingesting contaminated water or vegetation containing the parasite's larvae. Once inside the ruminant's body, the larvae migrate to the liver where they mature into adult flukes and begin to feed on the liver tissue, causing damage and leading to various health issues.

Chimeric virus-like particles (VLPs) have emerged as an attractive candidate for vaccine development due to ability to elicit robust immune responses. Plant expression systems offer a versatile platform for the production of chimeric VLPs, presenting advantages such as scalability, cost-effectiveness, and proper post-translational modifications.

Production of subunit vaccines in edible plants, such as lettuce, represents a groundbreaking approach to modern vaccine development. By leveraging the genetic engineering capabilities of plants researchers can introduce specific genes encoding vaccine antigens into the plant's genome. This strategy allows toproduce edible plant tissues containing the desired vaccine antigens, which can be easily converted via lyophilisation into an oral form. Not only does this method offer a cost-effective and scalable alternative to traditional vaccine production systems, but it also eliminates the need for complex purification processing, making it particularly appealing for veterinarian vaccination. The utilization of edible plants like lettuce as bioreactors for subunit oral vaccine production holds great promise in revolutionizing the field of immunization and expanding access to safe and effective vaccines targeted to domestic animals.

Acknowledgments
This study was performed within NCN project No. 2019/35/B/NZ6/04002.

Polystyrene particles impair photosynthesis in *Chlorella vulgaris*

Petra Peharec Štefanić[a], Bruno Komazec[a], Nino Dimitrov[b] and Biljana Balen[a]

[a]*University of Zagreb, Faculty of Science, Department of Biology, Horvatovac 102a, HR-10000 Zagreb, Croatia*
[b]*Croatian Institute for Public Health, Rockefellerova 7, HR-10000 Zagreb, Croatia*

The increasing use of polystyrene (PS) in industrial and commercial products leads to a significant accumulation of plastic waste in the environment [1]. Due to its low mass and density, PS waste often accumulates on the surface of aquatic ecosystems, where their impact has not yet been fully investigated. To examine the effects of PS particles (PS-P) on the freshwater alga *Chlorella vulgaris*, cells were treated with 40 mg L^{-1} concentration, which is considered the upper limit for human exposure to styrene monomers. Their uptake in the cells and on the extracellular polymeric substances (EPS) layer was analyzed by pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). Visualization of the cells under native conditions and ultrastructural analysis were performed by transmission electron microscopy (TEM). The EPS layer was visualized by confocal microscopy using the fluorescently labeled lectin ConA (Fig. 1), while its quantification was performed by a microplate reader. To investigate the effect of PS-P on photosynthesis, we measured the oxygen evolution, maximum quantum yield (F_v/F_m) and efficiency (PI_{abs}) of photosystem II using a Clark electrode and an AquaPen, while the expression of Rubisco and D1 protein was analyzed by immunoblotting. Py-GC-MS detected the PS-P accumulation in the algal cells and the EPS layer, while TEM confirmed their retention in the EPS layer. PS-P induced cell plasmolysis and decreased the number of thylakoids and starch compared to the control, indicating destabilization of the thylakoid system, while confocal microscopy showed an increase in the EPS layer. In addition, PS-P led to a decrease in photosynthetic rate and an increase in PI_{abs}, but had no effect on F_v/F_m. They also slightly increased Rubisco and decreased D1 protein expression. The results suggest that the primary mechanism of PS-P toxicity is binding to cell surfaces, impairing gas and nutrient exchange and reducing light available for photosynthesis.

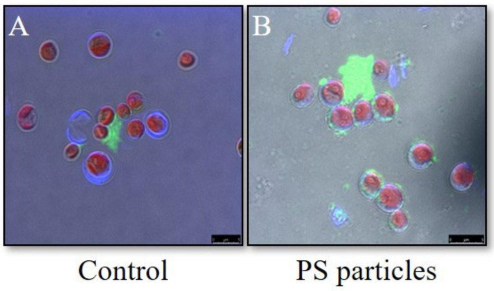


Figure 1. EPS layer around control *C. vulgaris* cells (A) and after PS-P treatment (B) visualized by confocal microscopy using the fluorescently labeled lectin ConA (green).

Acknowledgments
This research has received funding from the Croatian Science Foundation under grant number IP-2022-10-3824, from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 823717-ESTEEM3 and University of Zagreb, Croatia.

References
[1] Gewert, et al. (2015). *Environmental Science: Processes and Impacts,* 17, 1513 – 1521. https://doi.org/10.1039/C5EM00207A

Stimulatory effect of nanomaterials on bacteria producing utile metabolites for agriculture

Adrian Augustyniak[a, c], Natalia Gurgacz[b], Karolina Widoniak[b], Kamila Dubrowska[a], Joanna Honselmann gennant Humme[a] and Rafał Rakoczy[a,c]

[a]*Faculty of Chemical Technology and Engineering, West Pomeranian University of Technology in Szczecin, Piastów Avenue 42, 71-065 Szczecin, Poland*
[b]*Institute of Biology, University of Szczecin, Wąska Str. 13, 71-415 Szczecin, Poland*
[c]*Center for Advanced Materials and Manufacturing Process Engineering (CAMMPE), Piastow Avenue 42, 71-065 Szczecin, Poland*

Nanomaterials are often used to inhibit the growth of microorganisms. However, in sublethal concentrations and variable environmental conditions, these substances may stimulate primary and secondary metabolism [1]. Therefore, this study aimed to evaluate the effects of metal oxide nanoparticles and carbon nanotubes on reference bacteria.

All experiments were conducted on *P. aeruginosa* ATCC 27853. Selected nanomaterials included zinc oxide nanoparticles (ZnO NPs) and multi-walled carbon nanotubes (MWCNT). Physiological properties of cells, including their viability, metabolic activity (respiration), production of pyocyanin, biofilm formation, and oxidative stress, were measured with spectrophotometry, flow cytometry, confocal microscopy, and quantitative PCR.

Studied nanomaterials can have stimulatory properties on *P. aeruginosa* if used in specific concentrations. Stimulated properties included pyocyanin production, which indicated the strain's increased virulence. The biomass was actively interacting with tested nanomaterials and forming agglomerates. This effect was strengthened by the increased concentration of nanomaterials. The interaction with nanomaterials caused changes in viability and gene expression, including upregulation of efflux pumps responsible for zinc removal [2].

Obtained data have shown that nanomaterials may stimulate virulence factors in *P. aeruginosa*, which imposes caution on using nanoparticles in medical devices that have direct contact with patients. On the other hand, upregulated pyocyanin production could be used in biotechnology, as this phenazine may be a potential biopesticide for agriculture.

Acknowledgments

The study was funded by The National Science Centre Poland within PRELUDIUM 20 (2021/41/N/ST8/01094) and by The National Centre for Research and Development within LIDER XIV (LIDER14/0169/2023) programmes.

References

[1] Jabłońska, et al. (2023). *World Journal of Microbiology and Biotechnology*, 39, 103.
[2] Honselmann, et al. (2024). *Microbial Cell Factories*, 23(1), 1–15.

How nanotechnology would control fungal diseases

C. Miguel-Rojas, A. Perez-de-Luque and J. Sillero

Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA) Alameda del Obispo, Córdoba, Spain

Fungal diseases are major threats to the most important crops upon which humanity depends. Essential crops such as wheat, maize and rice are subject to infection by fungal pathogens, threatening global food security [1]. There is also concern about global warming contributing to the movement of disease-causing agents into new territories [2]. In this context, reducing crop losses caused by pests and diseases is a priority of the European Union. However, application of pesticides has increased yields but at a high environmental cost such as climate change, and soil degradation [3]. Within this framework, the application of the safest nanotechnology in agriculture could play a key role to enlarge the range of tools for integrated and sustainable pest/disease management.

Lately, RNA interference-based fungicides are emerging for their potential uses in crop protection [4], opening new avenues for agrochemicals design. However, the rapid degradation of naked dsRNA limits its potential to trigger its action, and therefore nanoparticles may be used as carriers to minimize its degradation and increasing the cellular uptake of intact dsRNA.

This study assessed the potential of this innovative approach targeting a critical gene involved in the pathogenicity process of two wheat fungal pathogens. We tested the effect of topical applications of dsRNA constructs in vitro, by a fungal growth assay in microtiters plates in *Zymoseptoria tritici*, and in vivo on wheat leaves artificially inoculated, in *Puccinia triticina*. Topical application of dsRNA led to a delay in conidia germination, and growth retardation in *Z. tritici*, and a strong reduction in appressorium formation in *P. triticina.* Given the outstanding results of this new approach to controlling fungal diseases, nanotechnology could help us advance further and transform the system into a practical field application. Therefore, we aim to load functional dsRNA into nano-hydroxyapatites (NHA) to test the effects *in planta* assays and investigate the interactions of dsRNA-NHA.

Acknowledgments

This research was financially supported by the Project PID2020-118650RR-C32.

References

[1] Flood, J. (2010). *Food Security*, 2, 215-231.
[2] Bebber, D.P., Ramotowski, M.A.T., & Gurr, S.J. (2013). *Nature Climate Change,* 3, 985-988.
[3] Simon-Delso, Martin, G.S., Bruneau, E., & Hautier, L. (2018). *Scientific Reports,* 8(1), 7241.
[4] Koch, A., Kumar, N., Weber, L., Keller, H., Imani, J., & Kogel, K-H. (2013) *Proceedings of the National Academy of Sciences of the United States of America*, 110, 19324–19329.

Chitosan nanoparticles for plant protection against fungal pathogens

Paweł Poznański, Abdullah Shalmani and Wacław Orczyk

Plant Breeding and Acclimatization Institute—National Research Institute, Radzików, 05-870 Błonie, Poland

Chitosan (CS) is a linear polysaccharide obtained through the deacetylation of chitin. It is biodegradable, non-toxic, and exhibits a wide spectrum of biological activities. In plant protection, CS functions as an antifungal agent, a plant immunity elicitor, and a growth stimulator. Most reports on CS-derived nanoparticles refer to chitosan molecules crosslinked with sodium tripolyphosphate (TPP). However, we found that in mildly acidic solutions (pH 5.6), chitosan molecules form nanoparticle-like structures. These structures can be fully characterized, like other nanoparticles, by their hydrodynamic diameter and surface zeta potential.

The main objective of this study was to characterize the physicochemical and biological properties of these self-formed CS nanoparticles. During testing these particles, we found that their characteristics (i.e., hydrodynamic diameter and zeta potential) depend on the protonation rate (pH of the solution) as well as the molecular weight, degree of deacetylation, and variability of these parameters in a given CS batch. By testing antifungal actvity of the self-formed CS nanoparticles we found that they outperformed the antifungal activity of CS nanoparticles formed via the conventional TPP ionic gelation method. From a collection of thirteen CS samples with different parameters, the sample CS_10 with the highest antifungal activity was selected. Investigating biological effects of self-formed CS_10 nanoparticles we used barley-*Fg* and barley-*Puccinia hordei* (*Ph*) pathosystems. Both pathogens were employed to test the impact of the CS on host immune response. In barley, the CS_10 sample stimulated biomass growth and immunity-related processes. CS-induced immunity in barley was detected as reduced symptoms of *Fg* infection and elevated micronecrotic reactions against *Ph*. Detailed comparative transcriptome analysis revealed that the gene regulation patterns in CS-sprayed barley overlaps with barley response to *Fg* infection.

Salicylic acid (SA) is a plant regulator of immune reactions and growth regulation. The content of SA in CS-treated and *Fg*-inoculated barely was found to be elevated. CS_10 application was found to increase biomass growth of barley plants. The effect was similar to SA treatment. We propose that CS-induced immune response and biomass growth stimulation is mediated by SA-dependent regulation.

The cumulative results contribute to a better understanding of the interplay between chitosan's physicochemical parameters and its biological activity, highlighting its potential practical uses in agricultural plant protection.

Acknowledgments

Research financed by National Science Centre, Grant no 2019/35/B/NZ9/00323 (WO)


POSTER
SESSION

Next Generation Chitosan Nanofertilizer: The Future of Sustainable Agriculture

Damyanti Prajapati, Kinjal Mondal and Vinod Saharan

Nano Research Facility Laboratory, Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan 313 001, India

The low nutrient use efficiency of conventional fertilizers significantly jeopardizes the global food security and causes hazardous environmental impacts. Chitosan nanofertilizers have emerged as potential solution to overcome these challenges and meet the rising food demand. Chitosan nanofertilizers are of great interest due to their biodegradability, diverse biological activities and sustained release of active ingredients [1]. The functionalized chitosan nanofertilizers can synchronize the crop nutrient demand by prolonged nutrient availability through slow, controlled or targeted delivery of macro and micro nutrients. The strategic nutrient release not only enhances the nutrient use efficiency but also maintains cellular homeostsais improving source activity and sink strength of the plant [2]. This in turn, reduces conventional fertilizers requirement, thereby lowers the input cost as well as the ecological footprint. Therefore, chitosan nanofertilizers can be envisioned as a sustainable, eco-friendly and cost-effective solution for targeted and precise delivery of different nutrient payloads for higher crop productivity.

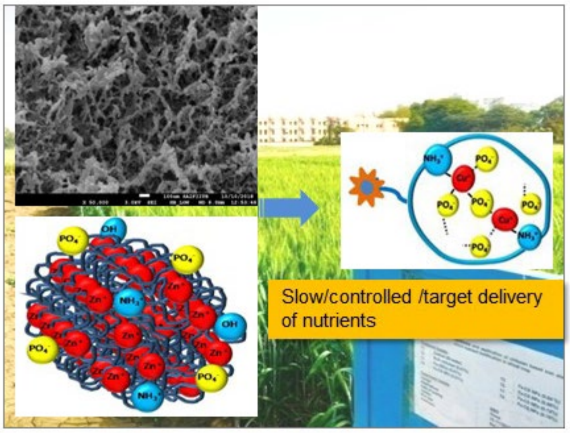


Figure 1. Chitosan Nanofertilizer

References

[1] Prajapati, D., Pal, A., Dimkpa, C., Singh, U., Devi, K.A., Choudhary, J.L., & Saharan, V. (2022). *Carbohydrate Polymers,* 119356.

[2] Kumar, A., Prajapati, D., Devi, K. A., Pal, A., Choudhary, U., Dashora, A., Choudhary, J., Joshi, A., & Saharan, V. (2021). *Plant Physiology and Biochemistry,* 168, 272–281.

Differential induction of plant promoter by metal nanoparticles

Rakesh Sinha and Gregory Franklin

Institute of Plant Genetics of the Polish Academy of Sciences (IPG-PAS), Strzeszyńska 34, 60-479, Poznań, Poland

Although positive and negative effects of metal nanoparticles on plant metabolism have been reported, how plants recognise these nanoparticles is not well understood. Here we report the ability of a plant promoter to discriminate between different types of metal nanoparticles. Briefly, transgenic tobacco seedlings expressing the GUS gene under the control of the HyPRO promoter were treated with Au, Ag, Zn and Fe nanoparticles and the corresponding ions. GUS expression in the treated seedlings was analysed by both quantitative real-time PCR and a histochemical GUS assay. Our results show that Au and Zn nanoparticles significantly increase GUS expression compared to their ionic form and the untreated control. While no significant difference in GUS expression was observed after treatment with the ionic and nano form of Fe, both the nano and ionic forms of Ag reduced GUS expression compared to the control seedlings. The differential induction of HyPRO in response to different types of nanoparticles suggests that this promoter could be useful for nanosensing applications in the future.

Acknowledgements

This work was supported by the National Science Centre (NCN), OPUS project no Reg. No UMO-2022/45/B/NZ9/02135 and the NANOPLANT project, which received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 856961.

Barley *MIR444c* gene encodes PEP444c: a regulator of microRNA444c biogenesis

Aleksandra Chojnacka[a,b], Dawid Bielewicz[a,c], Andrzej Pacak[a], Goetz Hensel[d,e], Jochen Kumlehn[d], Artur Jarmołowski[a] and Zofia Szweykowska-Kulińska[a]

[a]*Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland*
[b]*Present address: Department of Plant Microbiomics, Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland.*
[c]*Centre for Advanced Technologies, Adam Mickiewicz University, Poznan, Poland*
[d]*Plant Reproductive Biology, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, Germany*
[e]*Centre for Plant Genome Engineering, Institute of Plant Biochemistry, Heinrich-Heine-University Dusseldorf, Dusseldorf, Germany*

Email: acho@igr.poznan.pl

MicroRNAs (miRNAs) are small single-stranded RNAs that regulate gene expression. There are 72 barley microRNAs and 69 pre-miRNAs deposited in miRBase (release 22.1). However, the regulation of barley *MIR* 444 – derived miRNA accumulation remains poorly understood. Our studies reveal the complex exon-intron structures of three barley *MIR444* genes. Their transcripts, pri-miRNAs444, undergo alternative splicing to produce various isoforms, classified as functional (producing miRNA) or non-functional (not producing miRNA). Bioinformatic analysis and polysome profiling techniques revealed numerous open reading frames (ORFs) within barley pri-miRNAs444, encoding peptides of 119 (PEP444a), 51 (miPEP444b), and 168 (PEP444c) amino acids. Using specific antibodies directed against these peptides, we confirmed the presence of PEP444a in barley shoots under nitrogen excess stress and PEP444c in both shoots and roots under control and nitrogen excess conditions. Additionally, CRISPR-associated endonuclease 9 (Cas9)-mediated mutagenesis of the PEP444c-encoding sequence led to a decrease in PEP444c transcript levels in barley shoots and roots, and a five-fold reduction in microRNA444c levels in roots. Our findings strongly suggest that PEP444c, encoded by the *MIR444c* gene, plays a crucial role in the biogenesis of microRNA444c in barley.

Biosynthesized metal nanoparticles for pre-sowing seed treatment and growth stimulation of *Zea mays*

Joanna Trzcińska-Wencel[a], Natalia Mucha[b], Jarosław Tyburski[b] and Patrycja Golińska[a]

[a]*Department of Microbiology, Nicolaus Copernicus University in Toruń, Lwowska 1, 87-100 Torun, Poland*
[b]*Department of Plant Physiology and Biotechnology, Nicolaus Copernicus University, Lwowska 1, 87-100 Torun, Poland*

The use of nanomaterials to improve crop plants growth requires understanding of the interaction between the nanoparticles (NPs) and plants is essential to identify their potential to growth stimulation, while also determining the risk of toxicity [1]. Therefore effect of biologically synthesized zinc oxide (ZnONPs) and silver (AgNPs) nanoparticles on *Zea mays* seeds germination, seedlings growth and condition was evaluated.

The *Fusarium solani* IOR 825 strain was used to synthesize ZnONPs and AgNPs and the physicochemical properties of biofabricated NPs were determined by using Transmission Electron Microscopy (TEM), X-ray Powder Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Nanoparticle Tracking Analysis (NTA). The maize seeds were treated with NPs solutions at concentrations of 32, 128 and 512 µg mL^{-1} and cultivated at ± 22°C. The germination parameters and seedling vigour were evaluated after 14 days. The seedling condition was estimated by determination the chlorophyll content in bio-NP-treated seedlings.

The AgNPs at all tested concentrations revealed sterilization effect on maize seeds. Results showed no effect on seed germination and dose-dependent effects of bio-NPs on maize seedlings growth. The increase of fresh and dry biomass of seedling shoot and root, as well as higher values of vigour index I and II by 8-12% and 15-29%, were noted after the treatment with both bio-NPs at the concentration of 32 and 128 µg mL^{-1} (ZnONPs) and 512 µg mL^{-1} (AgNPs), respectively. The reduced chlorophyll content by 12-13% was observed in seedlings treated with ZnONPs and AgNPs at concentration of 512 µg mL^{-1}.

The pre-sowing treatment of maize seeds with biosynthesized AgNPs and ZnONPs results in improved maize biomass production. In addition, AgNPs showed efficacy as an antimicrobial agent and inhibited pathogen growth on the seed surface. The application of low concentrations of bio-NPs revealed no adverse effect on seeds germination, seedling condition or chlorophyll content, indicates their potential for use in agriculture as nano-agrochemicals to provide nutrients and protection against microbial pathogens.

Acknowledgements
This research was funded by grant No. 2022/45/N/NZ9/01483 from National Science Centre, Poland.

References:
[1] Singh, S.P., Prasad, S., Misra, M., Rajput, R., & Ranjan, V.D.R. (2023). *Hybrid Advances*, 100097.

Application of FTIR-ATR spectroscopy to assess the vigour of crop seedlings after foliar biostimulation

Paulina Pipiak, Dorota Gendaszewska, Dorota Wieczorek and Katarzyna Ławińska

Łukasiewicz Research Network—Lodz Institute of Technology, 73 Zgierska Str., 91-463 Lodz, Poland

In recent years, Fourier Transform Total Reflection Infrared Spectroscopy (ATR-FTIR) has become an increasingly popular technique for the analysis of plant biological material. FTIR analysis offers a rapid, cost-effective and relatively non-invasive approach to the chemical characterisation of biological samples [1,2]. The technique allows for the identification of chemical bonds and functional groups present in the material under examination. In the context of plant samples, FTIR enables the alteration of the proportions of the principal organic compounds present in the analysed plant material, both between different plant species and within the same species. In addition, it makes it possible to assess the variability of the chemical profile of plants of the same species grown under different conditions or using various cultivation methods [3]. The aim of the study was to assess the efficacy of FTIR spectroscopy in evaluating the impact of foliar biostimulants on crop productivity. As part of the ongoing project, an innovative foliar preparation was developed based on collagen and keratin hydrolysates derived from tannery waste and enriched with bioactive substances (salicylic acid derivatives and/or organic titanium salts) was developed and used in conjunction with the fungicide azoxystrobin. The efficacy of the formulation was evaluated through a series of experiments, including a trial conducted during the cultivation of cabbage (*Brassica oleracea* L. *var. capitata* L.). FTIR analyses of leaves of head cabbage treated with foliar biostimulants were carried out as part of the study. In the recorded spectra, particular attention was paid to the position of the bands recorded in the region characteristic of the functional groups found in proteins. Based on preliminary studies, it can be concluded that the FTIR technique used has great application potential in the study of foliar preparations.

Acknowledgments

This study was carried out as part of the EUREKA DUO_PLANT_Protect research project funded by the National Center for Research and Development, Poland, EUREKA/DUO_PLANT_Protect/5/2021, grant title "Multifunctional foliar preparations for protect and stimulate plant growth".

References

[1] da Costa, R.M., Barrett, W., Carli, J., & Allison, G.G. (2020). *The plant cell wall: methods and protocols*, 297-313.
[2] Durak, T., & Depciuch, J. (2020). *Environmental and Experimental Botany*, 169, 103915.
[3] Talari, A.C.S., Martinez, M.A.G., Movasaghi, Z., Rehman, S., & Rehman, I.U. (2017). *Applied Spectroscopy Reviews*, 52(5), 456-506.

Monitoring of quality and environmental parameters of water and associated ecosystems accompanying

Dorota Gendaszewska, Dorota Wieczorek, Katarzyna Ławińska and Renata Żyłła

Lukasiewicz Research Network - Lodz Institute of Technology, Street Marii Skłodowskiej-Curie 19/27, 90-570 Lodz, Poland

Chemical methods used to assess the impact of water and soil environments on plant growth are not always quick and cost-effective. Therefore, environmental monitoring practices increasingly employ biological methods such as biotests, which often meet these criteria [1]. Compared to chemical tests, phytotests not only measure results but also demonstrate the dynamic biotic response to toxicants [2]. This allows for a more comprehensive understanding of the ecological impact of various pollutants. Additionally, phytotests are often easier to implement in field conditions, making them a practical choice for ongoing monitoring efforts. Contamination assessment includes evaluating seed germination, seedling growth, and root inhibition in seed plants [3]. Phytotests can also investigate the effect of different constituents intended to support seed growth [4]. These methods provide valuable data that can inform strategies for environmental protection and sustainable agricultural practices. This work presents the results of toxicological research on the impact of wastewater from the process of washing fibers on the growth of reference plants (*Sorghum saccharatum, Lepidium sativum, Sinapis alba*) using effective phytotests (PHYTOTOXKIT). The determination is carried out in special transparent containers that allow direct observation and measurement of the length by image analysis at the end of the test. No significant acute phytotoxicity was observed in two sewage samples tested at three different concentrations: 10%, 50%, and 100%. The studies on the impact of wastewater on green plants ruled out toxicity, indicating the potential for developing methods to treat and recirculate the treated water, and even manage the filtrate. These studies are a key component of the system for monitoring the environmental parameters of water and wastewater.

Acknowledgments

The work is co-financed from the state budget under the program of the Minister of Education and Science called "Science for Society II" project number NdS-II/SN/0269/2023/01, funding amount PLN 994,800.00, total project value PLN 994,800.00.

References

[1] Jakubus, M. (2012). *Fresenius Environmental Bulletin,* 21(11a), 3464–3472.
[2] Bengtson Nash, S.M., Quayle, P.A., Schreiber, U., & Müller, J.F. (2005). *Aquatic Toxicology*, 72(4), 315-326. https://doi.org/10.1016/j.aquatox.2005.02.004.
[3] ASTM 1997. Standard Practice for Conducting Early Seedling Growth Tests. ASTM E 1598–94.
[4] Gendaszewska, D., & Wieczorek, D. (2022). *Technologia i Jakość Wyrobów*, 67, 141-154. https://doi.org/10.57636/67.2022.1.11

NANOPLANT

POSTERS SESSION

P 8

The influence of selected fungi on the mass decomposition of processed wood raw material made of sapwood and heartwood *Pinus sylvestris* L.

Małgorzata Osmenda[a] and Katarzyna Nawrot-Chorabik[b]

[a]*Olkusz Forest District, The State Forests National Forest Holding, Łukasińskiego 3, 32–300 Olkusz, Poland. Email: malgorzata.osmenda@katowice.lasy.gov.pl*
[b]*Department of Forest Ecosystems Protection, Faculty of Forestry, University of Agriculture in Kraków, 29 Listopada Ave. 46, 31-425 Kraków, Poland*

Email: katarzyna.nawrot-chorabik@urk.edu.pl

Wood of coniferous tree species, including Scots pine (*Pinus sylvestris* L.), is a renewable raw material used in a strategically important economic sector, such as energy industry. Currently, large amounts of mechanically processed wood material in the form of chips and sawdust are used for heating purposes, which enables the production of high-quality pellets. The most important advantages of processed wood materials, such as chips and pellets made of sawdust, include low emission of carbon dioxide (CO_2) released from processed wood biomass into the atmosphere. Other advantages of wood biomass include high energy efficiency and alternative uses of the raw material compared to non-renewable fossil fuels, including: natural gas and hard coal [1]. Unfortunately, the quality and quantity of wood sawdust mass often change due to the negative and expansive impact of many fungi species. Fungal organisms, through their colonization and then the development and intensive growth of mycelium, lead to the wood substrate decomposition and the reduction in the energy efficiency of the processed wood mass [2]. Visible changes in the amount of wood substrate mass are the result of the enzymes secretion by fungal hyphae, i.e. lignolytic and cellulolytic enzymes, which are responsible for the decomposition of the basic building blocks of wood, including: cellulose and lignin [3].

For this reason, in order to determine the impact of the intensity of decomposition of pine sawdust through the development of cultures of selected fungi with diverse nutritional adaptations, i.e. *Heterobasidion annosum* (pathogen), *Ophiostoma minus* (saprotroph) and *Serpula himantioides* (saprotroph), laboratory tests were carried out under *in vitro* conditions [1, 3]. *In vitro* tests help determine the rate of colonization and decomposition of sawdust by selected fungi species, which enables the identification of the fungus causing the greatest weight loss of the processed wood raw material. The experiments carried out consisted in determining the percentage loss in the mass of individual fractions of sawdust made of solid sapwood and heartwood of pine caused by the growth of fungi. Based on the experiments carried out, it was found that the processed energy substrate, i.e. sapwood sawdust, undergoes colonization and decomposition to a greater extent than heartwood sawdust. This phenomenon is caused by the presence of larger amounts of fungicidal phenolic compounds in heartwood than in sapwood [4]. It was also found that fungi with balanced life strategies colonize and reduce the amount of wood mass to varying degrees. The research showed that the fungus species that caused the greatest weight loss in processed wood was *S. himantioides*. This species caused increased decomposition of sapwood sawdust by 14,3% compared to *H. annosum* and by 17,1% compared to the development of *O. minus*. *S. himantioides* also contributed to the highest observed reduction of heartwood sawdust compared to the other species examined. The reduction of heartwood sawdust caused by *S. himantioides* was 8,7% higher compared to the impact of *H. annosum* and 11,1% higher compared to *O. minus.*

The obtained results of *in vitro* tests using processed plant material and fungi cultures constitute the basis for developing a modern nomenclature for drying and impregnating with fungicides sawdust of coniferous trees exposed to the harmful effects of fungi that reduce both the quality and calorific value of the wood energy raw material.

Acknowledgments
The authors thank Ph.D. Czesław Bartnik and M.Sc. Dominika Ledwich for identification and analyzing the morphology and genetics of the fungi used in this work.

References
[1] Nawrot-Chorabik, K., Osmenda, M., Bartnik, C. (2024). *Drewno. Prace naukowe. Doniesienia. Komunikaty,* 67(213), 00024. DOI: https://doi. org/10.53502/wood-191794.
[2] Goodell, B., Qian, Y., Jellison, J. (2008). *Development of Commercial Wood Preservatives*, 2, 9-31. DOI: 10.1021/bk-2008-0982.ch002.
[3] Osmenda, M., Nawrot-Chorabik, K. (2024). *Sylwan*, 168(2), 127–145. DOI: https://doi.org/10.26202/sylwan.2023107.
[4] Bartnik, C., Nawrot-Chorabik. K., Woodward, S. (2020). *Forest Pathology*, 50, e12652. DOI: https://doi. org/10.1111/efp.12652.

The effect of silver nanoparticles and cross-talk of silver nanoparticles and *Fusarium oxysporum* f.sp. *lupini* on ultrastructure of root cap cells of *Lupinus luteus* L.

Anielkis Batista[a,b], Elżbieta Gabała[c], Sławomir Samardakiewicz[d], Katarzyna Sadowska[e], Zbigniew Karolewski[f] and Iwona Morkunas[a]

[a]*Department of Plant Physiology, Faculty of Agriculture, Horticulture and Biotechnology, Poznań University of Life Sciences, Wołyńska 35, 60-637 Poznań, Poland*
[b]*Polytechnic Institute of Huila, Universidade Mandume ya Ndemufayo, 3FJP+27X, Lubango, Angola*
[c]*The Research Centre of Quarantine, Invasive and Genetically Modified Organisms, Institute of Plant Protection – National Research Institute, Władysława Węgorka 20; 60-318 Poznań, Poland*
[d]*Laboratory of Electron and Confocal Microscopy, Faculty of Biology, Adam Mickiewicz University, Uniwersytetu Poznańskiego 6, 61-614 Poznan, Poland*
[e]*Laboratory of the Plant Diseases Clinic and Pathogen Bank, Institute of Plant Protection - National Research Institute, Władysława Węgorka 20; 60-318 Poznań, Poland*
[f]*Department of Phytopathology, Seed Science and Technology, Faculty of Agriculture, Horticulture and Biotechnology, Poznań University of Life Sciences, Dąbrowskiego 159, 60-594 Poznań, Poland*

In the present study, we have attempted to elucidate the alterations in cell ultrastructure and some metabolic reactions in root cells of yellow lupine (*Lupinus luteus* L.) triggered by silver nanoparticles (AgNPs) during the seed germination stage and development of the seedlings, as well as the role of AgNPs in defense mechanisms to hemibiotrofic patogen *Fusarium oxysporum* f.sp. *lupini*. Ultrastructural analysis of root cap cells of yellow lupine (*Lupinus luteus* L. cv. Diament and *Lupinus luteus* L. cv. Mister varieties) in transmission electron microscopy (TEM) were examined with the Hitachi HT7700 (Hitachi, Tokyo, Japan). The experimental protocol used in this study was based on a model system: the germinating seeds pre-treated with AgNPs or non-treated, were inoculated with a *F. oxysporum* f. sp. *lupini* spore suspension or non-inoculated and were transferred to hydroponic grow boxes containing Hoagland medium. While analyzing changes in cell ultrastructure of root cells of yellow lupine after pretreated with AgNPs or pretreated with AgNPs and inoculated with *F. oxysporum*, we found distinct alterations. The obtained research results show that AgNPs pretreatment causes local, clearly visible cell wall thickenings. Particular attention is drawn to the large number of dictyosomes characterized by a highly developed cisternae system (in cells from AgNPs variant and AgNPs+*F. oysporum*) compared to the control. Electron micrographs revealed increase in the endocytosis and exocytosis associated with transport of material enclosed or inserted in membranes vesicles. Yellow lupine inoculation with *F. oxysporum* causes strong autophagic activity, the occurrence of multivesicular bodies, which is associated with transport processes and membrane flow.

Acknowledgments
The authors would like to thank Prof. Krzysztof Szoszkiewicz, Rector, Poznań University of Life Sciences and Prof. Natasza Borodynko-Filas, Institute of Plant Protection - National Research Institute, Poznań, Poland, for financial support of the research and supporting international cooperation. The authors would like to mention Prof. Natasza Borodynko-Filas.

Silver nanoparticles produced by various plant extracts and their antioxidant and antimicrobial properties

Zdenka Bedlovičová[a], Matej Baláž[b], Nina Daneu[c] and Ľudmila Tkáčiková[d]

[a]Department of Chemistry, Biochemistry and Biophysics, The University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice, Slovakia
[b]Department of Mechanochemistry, Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 04001 Košice, Slovakia
[c]Advanced Materials Department, Jozef Stefan Institute, Jamova cesta 39, SI-1000 Ljubljana, Slovenia
[d]Department of Microbiology and Immunology, The University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice, Slovakia

Silver and its compounds is well-known as antimicrobial agent. Silver nanoparticles (AgNPs) dispose broad possibilities of use in medicinal applications as well as in other scientific or industrial fields. In medicine, AgNPs are being studied for drug transport, screening of various diseases, and cancer therapy [1,2]. Due to increasing resistence of pathogens to conventional antibiotics, AgNPs are also intensively studied as antimicrobial agents [3]. These facts lead to study the silver nanoparticles as inhibitors of resistant bacterial strains by multiple mechanisms of action, including oxidative stress, DNA replication inhibition, or interaction with proteins and enzymes [4].

Our study is focused on the extraction of various medicinal plants and the green synthesis of AgNPs using these aqueous extracts. The antioxidant properties of extracts and AgNPs were investigated. The antibacterial activities of selected AgNPs against *S. aureus*, and *E. coli* bacterial strains were also studied. A set of water extracts of various plants, namely *Alchemilla vulgaris* and *Trifolia pratens* leaves and flowers, *Stachys recta* leaves, *Fallopia japonica* leaves and *Berberis vulgaris* L. fruit.

Acknowledgments

The study was supported by project of the Grant Agency of the Ministry of Education, Research, Development and Youth of the Slovak Republic (2/0112/22).

References

[1] Justin Packia Jacob, S., Finub, J.S., & Narayanan, A. (2012). *Colloids Surfaces B Biointerfaces,* 91, 212 – 214. doi.org/10.1016/j.colsurfb.2011.11.001
[2] Nazem, A., & Mansoori, G.A. (2008). *Journal of Alzheimer's Disease*, 13, 199–223
[3] Abbasi, E., Milani, M., Fekri Aval, S. et al. (2014). *Critical Reviews in Microbiology,* 7828, 1–8. https://doi.org/10.3109/1040841X.2014.912200
[4] Tian, X., Jiang, X., Welch, C. et al. (2018). *ACS Applied Materials and Interfaces,* 10, 443–8450. https://doi.org/10.1021/acsami.7b17274

Zinc oxide submicron particles and zinc oxide nanoparticles effects on *Alternatia alternata*, *Botrytis cinerea* and *Fusarium oxysporum* mycelium growth

Jolanta Kowalska[a], Małgorzata Antkowiak[a], Joanna Krzymińska[a], Urszula Szałaj[b], Jacek Wojnarowicz[b], Alicja Tymoszuk[c], Małgorzata Jeske[d], Aleksander Łukanowski[d] and Pańka Dariusz[d]

[a]*Department of Organic Agriculture and Environmental Protection, Institute of Plant Protection – National Research Institute, Władysława Węgorka 20, 60-318 Poznan, Poland*

[b]*Laboratory of Nanostructures, Institute of High Pressure Physics, Polish Academy of Science, Sokolowska 29/37, 01-142 Warsaw, Poland*

[c]*Laboratory of Horticulture, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, Bernardynska 6, 85-029 Bydgoszcz, Poland*

[d]*Department of Biology and Plant Protection, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, Kaliskiego 7, 85-796 Bydgoszcz, Poland*

Zinc oxide exhibit antifungal properties and could be applied in plant production against plant pathogens. The aim of this study was verify and compare the effects of ZnO submicron particles (ZnO SMPs) and ZnO nanoparticles (ZnO NPs) on the *in vitro* and *in vivo* response of *Alternaria alternata*, *Botrytis cinerea* and *Fusarium oxysporum*. In the *in vitro* experiment, all pathogens were grown on the PDA medium supplemented with 0, 100, 200, 500, 1000 and 2000 mg·L^{-1} ZnO SMPs or ZnO NPs. Mycelium discs were cut from seven-day-old pathogen cultures and placed in the center of each Petri dish with PDA medium. Cultures were then incubated at 23°C. The mycelium diameter (mm) was measured every 24 hours until it reached the edge of the dish in one of the tested treatments (for each pathogen separately). In the *in vivo* experiment conducted in growth chamber, potted tomato (*Solanum lycopersicum* L. 'Bawole Serce') plants were infected with the tested pathogens and sprayed with 500 mg·L^{-1}ZnO SMPs or ZnO NPs. Experimental objects included also non-infected control plants, as well as infected and ZnO SMPs/NPs non-treated control plants. It was found that the addition of ZnO NPs and ZnO SMPs to the medium significantly inhibited the growth of mycelium of all tested pathogens. However, regardless of the concentration used, the advantage of ZnO NPs over ZnO SMPs in inhibiting the growth of mycelium has not been demonstrated. Interestingly, the lowest tested ZnO SMPs/NPs concentration (100 mg·L^{-1}) caused a significant reduction of mycelium growth of *A. alternata* and *F. oxysporum*. However, in *B. cinerea*, the concentration of 500 mg·L^{-1} and higher concentrations of tested material samples limited the growth of its mycelium. *In vivo* experiment on tomato plants did not confirm the effectiveness of ZnO SMPs in reduction of *B. cinerea* and *A. alternata* infection, in contrast to ZnO NPs, which significantly limited the growth of all tested pathogens.

Enhancing the performance of chrysanthemum synthetic seeds through auxin and iron oxide nanoparticles supplementation

Dariusz Kulus[a], Alicja Tymoszuk[a], Magdalena Osial[b] and Daria Groń[a]

[a]Laboratory of Horticulture, Department of Biotechnology, Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology, Bernardyńska 6, 85-029 Bydgoszcz, Poland. Email: dariusz.kulus@pbs.edu.pl
[b]Institute of Fundamental Technological Research, Polish Academy of Sciences, Pawińskiego 5B, 02-106 Warsaw, Poland

Chrysanthemum holds significant economic importance as a popular ornamental plant. Developing synthetic seeds may become necessary, for ensuring efficient propagation and genetic preservation of elite cultivars. Nanoparticles can be a valuable additive in the production of manufactured seeds as carriers of growth regulators. The aim of this study was to investigate the effect of iron oxide nanoparticles (Fe_3O_4NPs) in combination with auxin indole-3-acetic acid (IAA) on the growth and development of chrysanthemum synthetic seeds.

In vitro-derived shoot tips of *Chrysanthemum* × *morifolium* (Ramat.) Hemsl. 'Richmond' were encapsulated in MS-based calcium alginate beads either with an addition of Fe_3O_4NPs alone or IAA or both the auxin and NPs. A control without Fe_3O_4NPs or IAA was included. Next, the synthetic seeds were inoculated on a water agar medium for eight weeks and, then, sown in a greenhouse in a mixture of peat and perlite (2:1).

It was found that the supplementation of alginate beads with Fe_3O_4NPs or IAA (alone or in combination) increased the germination efficiency (76 – 83%) compared with the control (57%). Even though the addition of nanoparticles did not enhance rhizogensis, synthetic seeds supplemented with Fe_3O_4NPs produced longer shoots that survived acclimatization better than the plant from the other treatments. The reason for these results is that nanoparticles can ensure better nutrient and moisture retention. No polymorphisms were detected within the studied plants according to the start codon targeted (SCoT) marker system, confirming the genetic homogeneity of the plant material. Upcoming studies will focus on the observation of inflorescence colour and parameters. The utilization of nanoparticles in the production of synthetic seeds for chrysanthemum is a cutting-edge technique that can improve their viability and performance.

Phytochemicals mediated fabrication of zinc oxide (ZnO) and cellulose bio-nanocomposite for sustained nutrient use efficiency

Lakshmipathy Muthukrishnan, Dibyendu Mondal and Gregory Franklin

Institute of Plant Genetics of the Polish Academy of Sciences (IPG-PAS), Strzeszyńska 34, 60-479, Poznań, Poland

Nutrient use efficiency remains one of the cornerstones of sustainable agriculture aimed at integrating nanomaterials to improve nutrient supply and augment productivity with reduced resource input and environmental impact [1]. A green synthesis approach in nanocrafting bionanocomposite, incorporating zinc oxide nanoparticles woven onto nanocellulose (ZnO_NC) has been reported. Bioactive phytochemicals were extracted from *Hypericum perforatum* L. and used for the synthesis of ZnO NPs. The phytochemicals involved in the nanocapping were determined using UPLC-PDA [2]. The residual biomass was subjected to alkaline treatments for the extraction of cellulose and fine-tuned to nanocellulose. The interweaving of nanoparticles with nanocellulose was achieved and characterized by spectroscopy and electron microscopy. UV-vis spectroscopic analyses revealed the typical surface plasmon resonance (SPR) of zinc with λ_{max} at 365 nm whereas the ZnO_NC showed slight shift in the absorbance maxima. The hydrodynamic diameter of ZnO NPs, nanocellulose and ZnO_NC was found to be around ≤ 200 nm with polydispersity indices of 15% - 25% and negative surface charges as determined by dynamic light scattering (DLS). Electron micrographs revealed spherical particles, cellulose fibrils and nanoparticles interwoven fibrils of size ≤ 100 nm indicating effective assembly. Thus, the holistic approach in bionanocomposite assembly with essential element might explore the possibilities to stabilize the nutrient uptake by the plants and managing soil health, contributing towards sustainability.

Acknowledgments

This work was supported by the National Science Centre (NCN), OPUS project Reg. No UMO-2022/45/B/NZ9/02135 and the NANOPLANT project, which received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No.856961.

References

[1] Lowry, G.V., Avellan, A., & Gilbertson, L.M. (2019). *Nature Nanotechnology,* 14, 517 – 522.

[2] Pradeep, M., Kruszka, D., Kachlicki, P., Mondal D., & Franklin G. (2022). *ACS Sustainable Chemistry & Engineering*, 10(1), 562–57.

Impact of ionic liquid-based nanoformulations on the bacterial and plant cell viability

Diksha Dhiman, Rakesh Sinha, Gregory Franklin and Dibyendu Mondal

Institute of Plant Genetics of the Polish Academy of Sciences (IPG-PAS), Strzeszyńska 34, 60-479, Poznań, Poland

E-mail ddhi@igr.poznan.pl

Integrating ionic liquid (IL)-based nanoformulations into agriculture offers inventive solutions for sustained nutrient delivery and promoting sustainable agriculture.[1] Understanding the impacts of such nanoformulations on both plants and phytopathogens is essential for advancing agricultural biotechnology. In this study, we developed IL-based nanoformulations containing CeO_2 nanoparticles (NPs) and assessed their effects on the viability of *Agrobacterium tumefaciens* and *Hypericum perforatum* cells. Our findings demonstrate that among the various concentrations, 50 mM IL had a significant inhibitory effect on agrobacterial growth at all the time period. However, 25 mM IL shown partial inhibition of agrobacterium growth. Additionally, the stimulatory effect of CeO_2 NPs on agrobacterial growth have been observed at lower concentration while, significantly inhibit bacterial growth at higher concentration. IL when combined with CeO_2 NPs, showed a better agrobacterium growth inhibition compared to alone IL or CeO_2 NPs. Further, our results showed that the IL, NPs and IL+NPs behaved similar to the control system for *H. perforatum* cells viability, thus highlighting their potential as plant growth stimulant against biotic stress. Overall, this study can systematically contribute to the development of sustainable strategies for crop protection and enhancement, with implications for agricultural productivity and environmental sustainability.

Acknowledgments

This work was supported by the National Science Centre (NCN), OPUS project no Reg. No 2017/25/B/NZ9/00720 and the NANOPLANT project, which received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 856961

References

[1] Martins et al. (2024). *ACS Sustainable Resource Management,* 1(6), 1291–1301.

Nanoadsorption of low-polar secondary metabolites for their purification from the complex extract of *Hypericum perforatum* L.

Pradeep Matam, Dibyendu Mondal and Gregory Franklin

Institute of Plant Genetics of the Polish Academy of Sciences (IPG-PAS), Strzeszyńska 34, 60-479, Poznań, Poland

The purification of low-polar secondary metabolites from complex plant extract presents significant challenges due to the matrix of compounds present in the source material. *Hypericum perforatum L*., which is commonly known as St. John's wort, is known for having many bioactive secondary metabolites that are useful for therapeutic purposes. These include phenolic acids, flavonoids, xanthones, phloroglucinols, and naphthodianthrones [1]. Traditional purification methods often fall short in terms of efficiency and selectivity, necessitating the exploration of innovative techniques. Previously, the phytochemical basis and mechanism of *H. perforatum* extract mediated sustainable synthesis of nanoparticles (NPs) were shown to be that phenolic acids and flavonoids are employed in the reduction, whereas xanthones and phloroglucinols act as capping agents, and naphthodianthrones are involved in both steps [2]. In this study, we investigate the use of *H. perforatum* extract in combination with NPs for the separation and recovery of capping agents. These agents were then analyzed by ultra-performance liquid chromatography coupled with a photodiode array (UPLC-PDA) to quantify changes in the phytochemical profile. The results revealed an extraction efficiency of 35% for xanthones and 50% for phloroglucinols. Nanoadsorption emerges as a promosing approach for the selection extraction and purification of low-polar compounds from *H. perforatum,* providing a potential solution to the challenges posed by traditional purification methods.

Acknowledgments

This work was supported by the National Science Centre (NCN), OPUS project Reg. No UMO-2022/45/B/NZ9/02135 and the NANOPLANT project, which received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 856961.

References

[1] Dsouza, A., Dixon, M., Shukla, M., & Graham, T. (2024). *Journal of Experimental Botany*, erae248.

[2] Pradeep, M., Kruszka, D., Kachlicki, P., Mondal D., & Franklin G. (2022). *ACS Sustainable Chemistry & Engineering*, 10(1), 562–57.

Highly efficient green synthesis of copper-based bionanocomposites and their potential applications in advancing sustainable agriculture

Megha Saxena, Lakshmipathy Muthukrishnan, Gregory Franklin and Dibyendu Mondal

Institute of Plant Genetics of the Polish Academy of Sciences (IPG-PAS), Strzeszyńska 34, 60-479, Poznań, Poland

Nanofertilizers exhibit improved plant absorption due to their high surface area-to-volume ratio, enabling controlled and sustainable nutrient delivery while minimizing environmental impact. These fertilizers also effectively mitigate biotic and abiotic stress in plants. Copper, an essential micronutrient, plays a vital role in various physiological and biochemical processes. At low concentration Cu nanoparticles (NPs) act as nanofertilizers whereas, at high concentration it acts as nanopesticide and showed retarded plant growth. Therefore, slow and controlled release of Cu ion from Cu-based NPs is crucial to maximize benefits and minimize ecological harm [1-2]. Hence, the objective of this work is to synthesize nanocomposites (NCs) of copper with functional biopolymers. For this, *Hypericum perforatum L,* plant was used which served dual purposes, its phytochemicals extracted in methanol were used for the synthesis of CuO NPs and the remaining biomass was used for extraction of two important biopolymers, lignin and cellulose. These extracted biopolymers were further converted into cellulose NPs and lignin NPs and then combined with CuO NPs to make the CuO/lignin NC and CuO/cellulose NC. To evaluate the slow-release profile of copper from these nanocomposites a dialysis method was used and the concentration of copper was determined by total reflectance X-ray fluorescence spectroscopy (TXRF). The experiment was performed for 15 days. The results obtained indicated that CuO/lignin NC showed slow releases of Cu in comparison to control (CuO NPs) while, CuO/cellulose NC showed marginally higher Cu ion release than control at after one week. Hence, it can be concluded that the designed bionanocomposites helps in the slow and controlled release of copper which is crucial for sustainable agriculture.

Acknowledgments

This work was supported by the National Science Centre (NCN), OPUS project Reg. No UMO-2022/45/B/NZ9/02135 and the NANOPLANT project, which received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 856961.

References

[1] Santás-Miguel, V., Arias-Estévez, M., Rodríguez-Seijo, A., & Arenas-Lagoet, D. (2023). *Environmental Pollution*, 334, 122222.
[2] Kalwani, M., Chakdar, H., Srivastava, A., Pabbi, S., & Shukla, P. (2022), *Chemosphere*, 287, 132107.

Gold and silver nanoparticles affect the expression of transcripts implicated in various biological processes in plants

Rajendran K. Selvakesavan, Maria Nuc, Paweł Krajewski and Gregory Franklin

Institute of Plant Genetics of the Polish Academy of Sciences (IPG-PAS), Strzeszyńska 34, 60-479, Poznań, Poland

The impact of metal nanoparticles on the environment, either as waste products or applied directly to plants in the form of nanoformulations (e.g. smart fertilizers, pesticides, fungicides, growth promoters), remains poorly understood [1]. In this study, we investigated the effects of silver (Ag) and gold (Au) nanoparticles (NPs) on cell suspension cultures of the pharmaceutically important plant *Hypericum perforatum*. Exposure to 25 ppm AgNPs significantly reduced cell viability, while 25 ppm AuNPs had no effect [2]. Transcriptomic analysis revealed a greater number of differentially expressed genes after treatment with AgNP compared to AuNP. After 30 minutes of exposure, 27 genes were upregulated in response to AuNP, while 46 genes were upregulated in response to AgNP. After 4 hours, 254 genes were upregulated in the AuNP treatment compared to 1109 in the AgNP treatment. After 12 hours, 308 genes were upregulated with AuNP and 1946 with AgNP. Genes related to reactive oxygen species production, secondary metabolism and plant defence were significantly differentially expressed in response to nanoparticle-induced stress. Both nanoparticles induced the expression of several key genes, including polygalacturonase-inhibiting protein, pathogen-related protein (PR10), phenolic oxidative coupling protein, benzophenone synthase and 1,3,7-trihydroxyxanthone synthase. In particular, the alternative oxidase was upregulated after 4 and 12 hours of AgNP treatment, while AuNP had no effect on its expression. Our results show that AgNP induces more transcriptomic changes in plant cells than AuNP at the same concentration and that the former is more toxic compared to the latter.

Acknowledgments

This work was supported by the National Science Centre (NCN), OPUS project no Reg. No UMO-2022/45/B/NZ9/02135 and the NANOPLANT project, which received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 856961.

References

[1] Adhikari, T., & Dharmarajan, R. (2022). *International Journal of Environmental Science and Technology*, 19, 9129–9148

[2] Selvakesavan, R.K., & Franklin, G. (2020). *Nanotechnology, Science and Applications*, 13, 77-88.

Nanomaterials and Ionic Liquids Synergistically Enhance Tobacco Growth and Yield Under Controlled Greenhouse Conditions

Leonard Kiirika, Dawid Perlikowski, Dibyendu Mondal and Gregory Franklin

Institute of Plant Genetics of the Polish Academy of Sciences (IPG-PAS), Strzeszyńska 34, 60-479, Poznań, Poland

As the world faces increasing agricultural demands and unpredictable environmental challenges, there is a pressing need to improve crop productivity. The pioneering role of nanomaterials (NMs) and ionic liquids (ILs) in promoting plant growth performance has previously been demonstrated [1], showing alterations in physiological and morphological traits using tobacco plants, culminating to alleviation of plant stress and increased resilient. The unique physical and chemical properties of NMs combined with ILs exhibit positive synergistic potential to enhance efficient nutrient delivery and utilization, resulting in overall increased crop production. The findings in [1] prompted further investigation into the connection between these alterations and their influence on plant growth vigor and yield traits. We conducted a study to test the effects of NMs combined with ILs on the development, vigor, and yield performance of tobacco (*Nicotiana tabacum*) plants grown under controlled greenhouse conditions. Commercially acquired NMs were characterized for their physico-chemical properties and dissolved in sterile deionized water together with the ILs. 19-day-old plants were foliar-applied with the formulation as previously described [1], and the response of the plants was monitored regularly every 5 days. Growth parameters including physiological status, growth vigor, biomass weight, and yield were assessed.

Compared to the control, plants treated with NMs and ILs showed significant increases in height (62.1%), growth rate (67.7%), biomass yield (70.6%), intercellular CO_2 concentration (11.7%) and stomatal conductance (50%). Considering the overall yield, we observed increased leaf weight (76%), pod size (58.8%), pod dry weight (37.5%), and the number of seeds per pod (44.2%) compared to control plants (mock-treated with water.

Our results demonstrate the synergistic potential of NMs and ILs in promoting plant performance and over all yield, towards establishment of nanofertilizers for improving crop productivity.

Acknowledgments

This work was supported by the NANOPLANT project, which received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 856961.

References

[1] Martins et al. (2024). ACS Sustainable Resource Management, 1(6), 1291–1301.

Field Evaluation of Liquid Nano fertilizer (Liquina®) on the growth of okra (*Abelmoschus esculentus* L.) under tropical conditions

Shyni Nixon[a], Dibyendu Mondal[b], Gregory Franklin[b] and Prakash Vincent[a]

[a]*Centre for Marine Science and Technology (CMST), Manonmaniam Sundaranar University, India*
[b]*Institute of Plant Genetics of the Polish Academy of Sciences (IPG-PAS), Strzeszyńska 34, 60-479, Poznań, Poland*

A liquid nano fertilizer formulation (Liquina) developed by the Nanoplant team has been field tested at the Centre for Marine Science and Technology (CMST) in India to evaluate its effectiveness on the tropical vegetable okra (*Abelmoschus esculentus* L. Moench). The seeds were procured and germinated under field conditions. When the plants had reached a height of 9 to 10 cm, they were divided into experimental (n=3) and control (n=3) groups. The experimental plants were sprayed weekly with 400 µl of liquid nano-fertilizer, while the control plants were sprayed with distilled water. The comparison of the data from the first day with those from the 21st day showed a significantly higher growth rate of the treated plants (25-30%) compared to the control plants. This preliminary field study shows the promising potential of the newly developed nano-fertilizer in agriculture.

Acknowledgments
This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No.856961.

Editorial Office

Poznan Science and Technology Park
Professional Congress Organizer
Rubież 46, 61-612 Poznań, Poland
e-mail: bok@ppnt.poznan.pl

DTP/Technical Editor

Reginaldo Cammarano

This volume has been compiled from files supplied by the Authors

This project has received funding
from the European Union's Horizon 2020
research and innovation programme
under Grant Agreement no 856961.