FIBOK 2026

7th National Conference of Young Biotechnologists

Programme and abstracts

February 5-6, 2026.

Szeged

7th National Conference of Young Biotechnologists

FIBOK 2026

Organised by the

Hungarian Academy of Sciences

Committee of Agricultural Biotechnology of the Section of Agricultural Sciences

and the

University of Szeged

Department of Plant Biology

together with the

Hungarian Society for Plant Biology

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the Scientific Committee of Agricultural Biotechnology of the Section of Agricultural Sciences of the Hungarian Academy of Sciences (MTA), and the

Department of Plant Biology of University of Szeged, together with the

Hungarian Society for Plant Biology

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Csaba VÁGVÖLGYI (SZTE, TTIK, Szeged)

Programme

Thursday (5 February)

9:30 Registration

10:45 – 11:00 Opening

Prof. Dr. István KRIZBAI (HUN-REN Biological Research Centre)
Prof. Dr. Csaba LANTOS (Cereal Research Non-Profit Ltd.)
Dr. Gábor FEIGL (University of Szeged)

11:00 – 12:00 Medical and pharmacological biotechnology

Chairperson: ***Prof. Dr. Mária DELI*** *(HUN-REN Biological Research Centre)*

11:00 – 11:30 Invited speaker: **Prof. Dr. László NAGY** (Johns Hopkins University)
HARNESSING THE HEALING POWER OF MACROPHAGES

11:30 – 11:45 **Máté Gergő HONVÁRI** (Budapest University of Technology and Economics)
BIOCATALYSIS FOR SYNTHESIS OF ENANTIOPURE SATURATED HETEROCYCLES AS POTENTIAL DRUG SCAFFOLDS

11:45 – 12:00 **Máté LAURINYECZ** (Budapest University of Technology and Economics)
NON-COVALENT ENZYME IMMOBILIZATION: DEVELOPMENT AND APPLICATION

12:00 – 12:10 **Áron NAGY**
INTRODUCTION OF THE HUNGARIAN BIOTECHNOLOGY STUDENTS ASSOCIATION

12:10 – 13:30 Lunch (own expense)

13:30 – 14:30 Animal biotechnology

Chairperson: ***Prof. Dr. Elen GÓCZA*** *(Hungarian University of Agriculture and Life Sciences)*

13:30 – 14:00 Invited speaker: **Dr. Bence SOMOSKŐI** (University of Veterinary Medicine)
IN VITRO CULTURE AND CRYOPRESERVATION OF PREANTRAL FOLLICLES IN VARIOUS SPECIES

14:00 – 14:15 **Dániel PÉTER** (Hungarian University of Agriculture and Life Sciences)
AFRICAN CATFISH SELECTION FOR BETTER HEAD AND FILLET SIZE

14:15 – 14:30 **Arnold TÓTH** (Hungarian University of Agriculture and Life Sciences)
HEAT TREATMENT EXPERIMENTS ON CHICKEN PRIMORDIA GERM CELLS: INVESTIGATING CHANGES IN THE CELL CYCLE AND FREEZING CAPABILITIES

14:30 – 15:00 Coffee break

15:00 – 16:00 Plant- and food biotechnology I.

Chairperson: ***Prof. Dr. Éva VÁRALLYAY*** *(Hungarian University of Agriculture and Life Sciences)*

15:00 – 15:30 Invited Speaker: **Prof. Dr. Miklós FÁRI** (University of Debrecen)
THE CHALLENGES OF GROWING PLANTS IN SPACE AND THE RESULTS OF THE HUNOR-VITAPRIC PROJECT

15:30 – 15:45 **Orsolya KEDVES** (University of Szeged)
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15:45 – 16:00 **Esther Ijeoma IDOGWU** (Hungarian University of Agriculture and Life Sciences)
EXPLORING THE GENETIC BACKGROUND AND POTENTIAL USEFULNESS OF A POTATO CHIMERA

16:30 – 17:30 Short poster presentations

Chairpersons: ***Prof. Dr. Elen GÓCZA*** *and* ***Dr. Ágnes GALLÉ*** *(University of Szeged)*

16:30 – 16:33 **Tamás BODOR** (University of Szeged)
SEED AND SEEDLING-STAGE RESPONSES TO NOVEL FORMULATIONS OF (NANO)ZINC-ENRICHED PLASMA-ACTIVATED WATER UNDER OSMOTIC STRESS IN ARABIDOPSIS
(PF#PE1)

16:33 – 16:36 **Gábor FEJES** (University of Szeged)
PLASMA-ACTIVATED LIQUIDS IMPROVE OSMOTIC STRESS TOLERANCE OF PEA PLANTS
(PF#PE2)

16:36 – 16:39 **Neda HESARI** (Hungarian University of Agriculture and Life Sciences)
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(PF#PE3)

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(PF#PE4)

16:42 – 16:45 **Diellza Dresha KASTRATI** (Hungarian University of Agriculture and Life Sciences)
RESISTANCE AGAINST TOMATO BROWN RUGOSE FRUIT VIRUS IN WILD SOLANUM SPECIES
(PF#PE5)

16:45 – 16:48 **Dóra KONDAK** (University of Szeged)
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(PF#PE6)

16:48 – 16:51 **Kamilla KOVÁCS** (University of Szeged)
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16:51 – 16:53 **Enikő MÉSZÁROS** (University of Szeged)
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17:09 – 17:12 **Nira PANDEY** (Hungarian University of Agriculture and Life Sciences)
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(MD#PE1)

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(MD#PE2)

17:15 – 17:18 **Chioma Lilian OZOADUCHE** (Hungarian University of Agriculture and Life Sciences)

ANTIBIOTIC RESISTANCE AND VIRULENCE DETERMINANTS OF *PSEUDOMONAS AERUGINOSA* ISOLATES CULTURED FROM HYDROCARBON-CONTAMINATED ENVIRONMENTAL SAMPLES

(MD#PE3)

17:18 – 17:21 **Raed A. ABURAWASH** (Hungarian University of Agriculture and Life Sciences)

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(AN#PE1)

17:21 – 17:24 **Gustavo DELGADO** (Hungarian University of Agriculture and Life Sciences)

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(AN#PE2)

17:30 – 19:00 Poster section (wine & cheese)

19:00 – 21:00 Dinner (Art Hotel)

Welcome and award ceremony: Prof. Dr. Elen GÓCZA and Dr. Péter POÓR
Béla FARKAS (violin)

Friday (6 February)

9:45 – 12:00 Plant- and food biotechnology II.

Chairperson: ***Prof. Dr. Attila FEHÉR*** *(University of Szeged)*

9:45 – 10:00 **Selahattin KONDAK** (University of Szeged)
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10:15 – 10:30 **Fruzsina NAGY** (HUN-REN Biological Research Centre)
CHARACTERIZATION OF THE BRASSICA GROWTH REGULATORY E2F-RBR PATHWAY

10:30 – 10:45 **Helga Fanni SCHUBERT** (ELTE Eötvös Loránd University)
POTENTIAL OF GREEN MICROALGAE TO ENHANCE PLANT SALT TOLERANCE

10:45 – 11:00 Short break

11:00 – 11:15 **Priyanka Pradeep PATIL** (HUN-REN Biological Research Centre)
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12:00 – 13:30 Lunch (own expense)

13:30 – 17:00 Bioinformatics and Microbial biotechnology I.

Chairperson: Dr. László GALGÓCZY (University of Szeged)

13:30 – 14:00 Invited speaker **Dr. Katalin GOMBOS** (University of Pécs)
DATA-TO-DIAGNOSIS: INTEGRATING GENOMICS, BIOINFORMATICS, AND CLINICAL WORKFLOWS

14:00 – 14:15 **Ádám HAJNAL** (University of Szeged)
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14:15 – 14:30 **Attila HLACS** (HUN-REN Biological Research Centre)
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SENSING PROTEIN DEVELOPMENT USING CLICK DISPLAY TECHNOLOGY

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15:30 – 17:00 Microbial biotechnology II.

Chairperson: Prof. Dr. Tünde PUSZTAHELYI (University of Debrecen)

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INTEGRATED ASSESSMENT OF EXTRACELLULAR ORGANIC MATTER-ENHANCED BIOREMEDIATION IN USED LUBRICANT OIL-CONTAMINATED SOILS: MICROBIAL RECOVERY, HYDROCARBON REMOVAL AND SPECIES-SPECIFIC TOXICITY RESPONSES

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16:30 – 16:45 **Valentina MADÁR** (Pázmány Péter Catholic University)
EVOLUTION IN MULTICELLULAR YEAST SYSTEMS

16:45 – 17:00 **Gergő TERNA** (University of Szeged)
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Prof. Dr. Elen GÓCZA, Dr. Péter POÓR

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Animal biotechnology

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Roy KIAMBI (Hungarian University of Agriculture and Life Sciences)
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Akhil KUMAR (Hungarian University of Agriculture and Life Sciences)
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Brian Josue LEMUS (Hungarian University of Agriculture and Life Sciences)
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Bánk PÁPAI (Hungarian University of Agriculture and Life Sciences)
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Sahilu Ahmad RABILU (HUN-REN Biological Research Centre)
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Ana RESTREPO (Hungarian University of Agriculture and Life Sciences)
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Kristóf UTASSY (Hungarian University of Agriculture and Life Sciences)
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Microbial biotechnology

Dániel HERCEGFALVI (University of Szeged)
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(MI#P1)

Henriett HUNKÁR (University of Szeged)
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(MI#P2)

Richárd MERBER (University of Szeged)
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Nomuun OYUNBAT (University of Szeged)
IDENTIFICATION OF NEW PEPTAIBIOTIC SEQUENCES FROM TRICHODERMA VELUTINUM ISOLATES
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Viktor SZENTPÉTERI (Hungarian University of Agriculture and Life Sciences)
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Bettina SZERENCSÉS (University of Szeged)
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Kitti TARI (University of Szeged)
ALTERATION OF THE FUNGAL ENDOPHYTE SECRETOME INDUCED BY SODIUM BUTYRATE
(MI#P7)

Abstracts

Medical and pharmacological biotechnology

MD#PLEN

HARNESSING THE HEALING POWER OF MACROPHAGES

Nagy, Laszlo[1,2,3]

[1] *Johns Hopkins University School of Medicine, Departments of Medicine, Pediatrics, Physiology, Pharmacology and Therapeutics and Biomedical Engineering, University, Institute, Department, Baltimore, MD, USA*
[2] *Johns Hopkins All Children's Hospital, Institute for Fundamental Biomedical Research, University, Institute, Department, St. Petersburg, FL, USA*
[3] *University of Debrecen, Faculty of Medicine, Department of Biochemistry and Molecular Biology, Debrecen, Hungary,*

Tissue regeneration is orchestrated by macrophages that clear damaged cells and promote regenerative inflammation. How macrophages spatially adapt and diversify their functions to support the architectural requirements of actively regenerating tissue remains unknown. In this study, we reconstructed the dynamic trajectories of myeloid cells isolated from acutely injured and early stage dystrophic muscles. We identified divergent subsets of monocytes/macrophages and DCs and validated markers (e.g., glycoprotein NMB [GPNMB]) and transcriptional regulators associated with defined functional states. In dystrophic muscle, specialized repair-associated subsets exhibited distinct macrophage diversity and reduced DC heterogeneity. Integrating spatial transcriptomics analyses with immunofluorescence uncovered the ordered distribution of subpopulations and multilayered regenerative inflammation zones (RIZs) where distinct macrophage subsets are organized in functional zones around damaged myofibers supporting all phases of regeneration. Importantly, intermittent glucocorticoid treatment disrupted the RIZs. Our findings suggest that macrophage subtypes mediated the development of the highly ordered architecture of regenerative tissues, unveiling the principles of the structured yet dynamic nature of regenerative inflammation supporting effective tissue repair.

MD#E1

BIOCATALYSIS FOR SYNTHESIS OF ENANTIOPURE SATURATED HETEROCYCLES AS POTENTIAL DRUG SCAFFOLDS

***Honvári, Máté Gergő*[1]; *Mócza, Levente András*[1]; *Kucsinka, Bence Attila*[1]; *Poppe, László*[1,2]; *Hornyánszky, Gábor*[1]**

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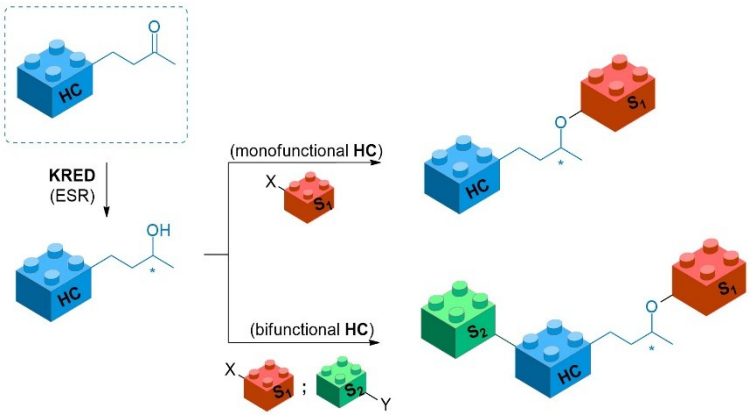

The objective of this study is to obtain enantiopure chiral "3D" fragments with asymmetric alcohol functionalities, that can be employed in fragment-based drug discovery. This was achieved by the enantiocomplementary bioreduction of ketones comprising flexible *N*-heterocycles using various biocatalysts with ketoreductase activity. Microbial whole-cell ketoreductases, such as wild-type yeast strains, including baker's yeast (*Saccharomyces cerevisiae*), and *Escherichia coli* cells expressing two enantiocomplementary recombinant alcohol dehydrogenases ((*S*)-selective ADH from *Rhodococcus aetherivorans* (*Ra*ADH) and (*R*)-selective ADH from *Lactobacillus kefir* (*Lk*ADH)) were screened for ketoreductase activity on numerous flexible *N*-heterocycles with prochiral carbonyl group in the *N*-(3-oxobutyl) substituent. Among the yeast strains tested, *Candida parapsilosis* (WY12) proved to be the most efficient biocatalyst, resulting in the corresponding enantiopure alcohols with good to excellent conversions (83–99%) and high enantiomeric excesses (ee > 99%). After screening as lyophilized whole cells, *C. parapsilosis* cells were immobilized in the form of calcium, zinc, nickel, and copper alginate beads. The whole-cell immobilization enabled recycling, with considerable residual activity of the biocatalysts over multiple cycles. The preparative-scale bioreductions showed comparable or even higher conversions than those observed in the small-scale screening reactions, resulting in virtually enantiopure (*S*)- and (*R*)-alcohols (ee > 99%), which are promising chiral fragments with a high degree of drug-likeness. Molecular docking of the substrates into the active site of the experimental structures of the ketoreductase enzymes rationalized their biocatalytic behavior and confirmed the assigned absolute configuration of the forming (*S*)- and (*R*)-alcohols. In summary, the present study broadens the range of applicability of wild yeast and recombinant alcohol dehydrogenase whole-cell preparations as biocatalysts to ketones involving flexible *N*-heterocycles and establishes a robust technology for producing chiral fragment library members with such 3D heterocyclic moieties.

Keywords: *Alcohol dehydrogenase, Ketoreductase, Whole-cell bioreduction*

Acknowledgement: *TKP2021-EGA-02; RRF-2.3.1–21-2022–00015; SNN-146370*
The authors acknowledge Euroapi Hungary Ltd. for the PhD fellowship of MGH via the Varga Jozsef Foundation. The project was also supported by the Doctoral Excellence Fellowship Programme.

MD#E2

NON-COVALENT ENZYME IMMOBILIZATION: DEVELOPMENT AND APPLICATION

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The immobilization of enzymes can be used to overcome several drawbacks, including low enzyme stability and limited reusability of the native biocatalyst. Among the various methods of enzyme immobilization, each of which possesses its own set of benefits and drawbacks, the selective enzyme complexation technique based on immobilized metal ion affinity chromatography (IMAC) was utilized in this study.
Phenylalanine ammonia-lyase from parsley (*Pc*PAL) and from *Pseudozyma antarctica* (*Pza*PAL), both with a His_{10}-tag, were selected as model enzymes due to the ease of measuring their reaction products. Metal ion chelating groups on the surface of enzyme supports were created from the alkylamino moieties by treatment with ethylenediaminetetraacetic dianhydride and subsequent complexation with cobalt(II) ions.
Three porous polymer beads, two silica-based supports and a silica-coated magnetic nanoparticle (MNP) were investigated as potential supports. The most effective *Pc*PAL-based biocatalysts were tested in kinetic resolution and ammonia addition reactions with substrates containing phenyl and thiophen-2-yl rings. In the selective ammonia addition reaction onto the (hetero)arylacrylates, requiring a harsh medium of a 6 M ammonia solution, the biocatalysts exhibited excellent stability, resulting in the production of L-amino acids in high yield and excellent enantiomeric excess.
Following the inactivation of the biocatalyst, the rechargeability of the costly supports is of major importance. Consequently, in the subsequent phase of our study, the rechargeability of the MNP-based, EDTA-Co^{2+}-type support was investigated. After screening for eluents, five subsequent reaction cycles were carried out. These cycles involved elution with 5% diethylenetriamine, followed by reloading with fresh *Pc*PAL or *Vibrio fluvialis* transaminase. During the third cycle, a retention rate of over 80% of the relative activity was observed in both cases. Further optimization involving various eluents and detergents resulted in a significant improvement when 1% citric acid and Triton X-100 were used at 90 °C. Utilizing *Pza*PAL-MNP, 88 ± 11% relative activity was observed at the 8th cycle.

Keywords: *metal ion affinity binding, enzyme immobilization, phenylalanine ammonia-lyase, magnetic nanoparticle*

Acknowledgement: *TKP2021-EGA-02; RRF-2.3.1-21-2022-00015; Renewable Enzyme Immobilization - RENZI; József Varga Foundation; This research was supported by the National Research, Development and Innovation Fund of Hungary and the ICGEB Research Grants Programme 2023.*

MD#PE1

HDR-MEDIATED INTRODUCTION OF A PATHOGENIC SCN5A VARIANT INTO A RABBIT MODEL OF LQT3

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Sudden cardiac death (SCD) in individuals is often caused by inherited channelopathies such as Long QT Syndrome type 3 (LQT3), linked to gain-of-function mutations in the SCN5A gene. The missense N1324S (Asn1324Ser) variant—resulting from an A>G transition —has been classified as pathogenic for congenital LQTS. Located within a functionally critical and evolutionarily conserved region of Nav1.5 channel, it is implicated in impaired sodium channel inactivation and QT prolongation.
Leveraging the high sequence homology between rabbit and human SCN5A around codon 1324, we designed a HDR-based CRISPR/Cas9 approach to introduce the N1324S mutation into the endogenous rabbit gene. Following in silico guide RNA and donor template design, we performed microinjection of the gRNA–Cas9 RNA alongside donor DNA into rabbit zygotes. Preliminary genotyping of blastocysts is underway to assess editing efficiency and on-target integration.
The resulting humanized N1324S rabbit model is expected to serve as a superior translational platform for electrophysiological characterization and proarrhythmic drug assessment—addressing a key gap left by murine models. This approach aims to enhance mechanistic insight and therapeutic evaluation for LQT3.

Keywords: *genome editing; rabbit model; LQT3, CRISPR/Cas9*

Acknowledgement: *This work was supported by the National Research, Development and Innovation Office (NKFIH), Hungary, grant number K147212 and Agribiotechnology and Precision Breeding for Food Security National Laboratory RRF-2.3.1-21-2022-00007.*

MD#PE2

EFFECT OF PLASTIC NANOPARTICLES ON THE AGGREGATION, STRUCTURE AND CYTOTOXICITY OF AMYLOIDOGENIC PROTEINS.

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Nanoparticles have attracted increasing scientific attention due to both their potential applications and significant associated environmental risks. Among them, micro- and nanoplastics are omnipresent contaminants that have been detected in almost every biological system, including consumer products intended for human use, as well as in animal and human tissues and organs. Recent evidences demonstrate that micro- and nanoplastics can be a source of immune activation, inhibition of cellular proliferation, and induction of oxidative stress. The studies of the molecular-level effects, for example, conformational changes and protein denaturation, are also increasing, and in this way, we get a much more complex picture about them. In this study, we investigated the influence of polystyrene plastic nanoparticles on the amyloidogenic protein amyloid-β. In our study we employed a wide range of tools, such as thioflavin-T fluorescence, circular dichroism spectroscopy (CD), electron microscopy, isothermal titration calorimetry, mass spectrometry (MS), and cytotoxicity assays. Our results show that even at low concentrations, polystyrene nanoparticles alter the aggregation behavior of the peptide and have a strong impact on the kinetics. Notably, these nanoparticles display high-affinity binding to monomeric amyloid-β. MS analysis revealed specific interaction sites of the peptide. From CD spectroscopy results, we obtained information about the secondary structure change of the amyloid fibrils. Furthermore, in cellular models we established the correlation between the particle size and their toxicity, as well as the fact that they appeared to reduce the cytotoxic effects of amyloid-β in cellular models. These results underscore the possible involvement of nanoplastics in influencing protein aggregation processes and stress the importance of continued investigation into their wider biological implications.

Keywords: *amyloid, plastic nanoparticles, polystyrene, interaction, protein aggregation, cytotoxicity, neurodegenerative disease*

Acknowledgement: *This work was supported by the National Research, Development, and Innovation Fund of Hungary (K138937, DKOP-23), the Hungarian Academy of Sciences (NAP3.0 Program NAP2022-I-3/2022) the Eötvös Loránd University Excellence Fund (EKA 2022/045-P278-1).*

MD#PE3

ANTIBIOTIC RESISTANCE AND VIRULENCE DETERMINANTS OF *PSEUDOMONAS AERUGINOSA* ISOLATES CULTURED FROM HYDROCARBON-CONTAMINATED ENVIRONMENTAL SAMPLES

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Crude oil and its derivates are among the most important environmental pollutants, where *P. aeruginosa* strains producing AlkB1 and AlkB2 alkane hydroxylases are often involved in their biodegradation. Looking at the environment from a One Health perspective, crude oil production contributes at about 6% to the GDP of Nigeria and at over 82% to its commercial exports, and Ogoniland has been a major oil-producing region in Nigeria since 1957. Our study aimed to characterize the antibiotic resistance and virulence determinants of a *P. aeruginosa* isolate cultured from crude oil-impacted soil in Ogoniland, Nigeria, and to compare its antibiotic resistance and virulence determinants with those of *P. aeruginosa* strains obtained worldwide from hydrocarbon contaminated environments and from clinical settings. ResFinder analysis revealed the consistent presence of the *catB7* chloramphenicol acetyltransferase gene, an *ampC*-type PDC β-lactamase gene, and an OXA-50 family β-lactamase gene across all strains examined. *P. aeruginosa* possesses several efflux pumps capable of removing both organic solvents and antibiotics from the cell, with MexAB-OprM being the most significant from this respect. Thereby, antibiotic resistance in *P. aeruginosa* inhabiting hydrocarbon-contaminated sites may also emerge as a result of acquiring tolerance to *n*-alkanes and other hydrocarbons. Accordingly, several of the *P. aeruginosa* isolates analysed in our study harboured a loss-of-function mutation or deletion in the *mexR, nalC,* or *nalD* regulatory genes of *mexAB-oprM,* suggestive of efflux-mediated acquired resistance mechanisms. Furthermore, several *P. aeruginosa* sequence types (STs) identified in oil-contaminated environments have also been reported among *P. aeruginosa* clinical isolates globally. These findings underscore the potential of environmental *P. aeruginosa* strains to cause human infections, and highlights the need for a One Health–oriented surveillance and careful evaluation of the public health risks that may be associated with the *in situ* bioremediation of hydrocarbon-polluted ecosystems.

Keywords: *P. aeruginosa, Hydrocarbon pollution, Antimicrobial resistance, Efflux pump*

Acknowledgement: *We gratefully acknowledge the support provided by the National Laboratory Grant RRF-2.3.1-21-2022-00007 and by the Tempus Public Foundation/ Stipendium Hungaricum programme.*

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Animal biotechnology

AN#PLEN

IN VITRO CULTURE AND CRYOPRESERVATION OF PREANTRAL FOLLICLES IN VARIOUS SPECIES

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In the field of assisted reproduction and conservation biology, there is an increased need to develop effective methods to store and maintain the reproductive potential of species that are either endangered or possess significant genetic value. The cryopreservation of preantral follicles (PAFs) has emerged as a promising fertility preservation approach. These immature follicles contain oocytes at an early developmental stage. Therefore, they are more resistant to freezing-induced damage compared to fully grown mature oocytes. Since mammalian ovaries contain large number of preantral follicles, culturing frozen/thawed follicles could be a viable method to obtain a considerable quantity of oocytes. These oocytes can undergo maturation and fertilization in vitro, with the resulting embryos being suitable for either immediate transfer or cryopreservation for future use.
Preantral follicles can be cryopreserved together with the ovarian tissue (ovarian tissue cryopreservation - OTC) or after isolation from the ovarian cortex. OTC is mainly applied in the human healthcare as a tool for fertility preservation before anti-cancer therapy which is harmful for the ovary and follicles. In the animal breeding/veterinary field, the storage of isolated preantral follicles could be a more feasible approach. For the isolation different procedures can be used such as: mechanical technique, enzymatic method, or combination of them. Cryopreservation can be performed by two main methods: slow (conventional) freezing and vitrification.
Despite some promising results achieved on this field, cryopreservation and in vitro culture of isolated preantral follicles are still a big challenge and protocols have not been standardized. Therefore, the procedure is far from practical application. Over the past few years, our research team (the Assisted Reproduction Research Group) has conducted studies on PAF IVC/CRYO of mouse, dog, pig and ruminants with tha aim to establish repeatable, general protocols for various species. Our specific aims were: (1) to compare the effect of different CRYO (slow freezing VS vitrification) on PAF (2) to find the most effective *multispecies* IVC system which provides satisfactory survival rate of PAF and oocytes.
In our presentation, we summarize the main results, challenges, and future prospects of our research.

Keywords: *ovarian follicle, cryopreservation, vitrification*

Acknowledgement: *Project no. 134887 has been implemented with the support provided by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund, financed under the FK_20 funding scheme; Project no. TKP2020-NKA-01 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the Tématerületi Kiválósági Program 2020 (2020-4.1.1-TKP2020) funding scheme. This study was supported by the strategic research fund of the University of Veterinary Medicine Budapest (Grant No. SRF-001; Grant No. SRF-002). This study was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.*

AN#E1

AFRICAN CATFISH SELECTION FOR BETTER HEAD AND FILLET SIZE

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Hungary is the most significant African catfish (*Clarias gariepinus*) producer in the European Union, with more than 5,000 tonnes of live fish produced annually. To ensure long-term sustainability, new lines that generate less waste should be developed.
We created two fish lines: one selected for a smaller head-to-body size ratio and the other selected for a larger fillet-to-body size ratio. These two lines served as controls for each other. After four generations of selection, we collected brain, muscle, and liver samples for further genetic analyses.
In the group selected for smaller head sizes, there was a 40% higher proportion of individuals whose head size was approximately 2% smaller relative to the body length. During the measurements, we did not differentiate between sexes. In total, 500 fish were measured in each group.
Compared to the control males, the males selected for increased body circumference showed a 27% higher proportion of individuals with approximately 3% larger circumferences. A similar trend was observed in females: relative to the control females, the circumference-selected group contained 16% more individuals with roughly 3% larger circumference. Additionally, within the circumference-selected group, females displayed on average 9% greater circumference than males.
For genetic analysis we used total RNA sequencing. We identified 10 genes (*acp5a, lrp13, megf6a, wnt9a, cavin4b, fkrp, six1b, jarid2a, kdm66b, cfap251*) which connected head/muscle growth and were significant between the groups. To verify the sequencing results, we will check gene expression using the qPCR method.
We aimed to identify the genetic background of the two groups' phenotypic differences using gene expression pattern analysis.

Keywords: *African catfish, head size, fillet size*

Acknowledgement: *Supported by the EKÖP-MATE/2024/25/D university research scholarship Programme of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund and the 2.1.1.-21-2022-00100 identification number GINOP Plus. This research was also supported by the Research Excellence Programme of the Hungarian University of Agriculture and Life Sciences.*

AN#E2

HEAT TREATMENT EXPERIMENTS ON CHICKEN PRIMORDIA GERM CELLS: INVESTIGATING CHANGES IN THE CELL CYCLE AND FREEZING CAPABILITIES

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In case of avian species, a modern *in vitro* method for gene preservation is cryopreservation and storage primordial germ cells (PGCs) in gene banks. PGC migration takes place in the extra-embryonic blood vessels, which makes their isolation from embryonic blood possible.
The goal of our project was to deepen our understanding of the molecular processes involved in germ cell cryopreservation, and to test a new heat treatment method to prepare the cells for deep cooling. We applied a preliminary heat treatment to PGCs with the intention of activating heat-shock proteins.
In this study, we conducted two experiments. In the first experiment, we were using transgenic FUCCI PGC lines and we monitored changes in the cell cycle.
The heat treatment was carried out for 3 hours at 40-41-42-43°C in 1.5ml Eppendorf tubes, 6x100.000 cells/group, in 500 µl medium each tube. An Eppendorf Thermomixer device was used for the heat treatment at 350 rpm to prevent cell settling. After treatment, we used the ImageXpress Pico Automated Cell Imaging System for 48h to monitor the cell-cycle changes. Based on the results the 42 °C heat treatment had significant effect on the cell cycle that differed from the other groups.
In the second experiment we used newly established PGC lines that had not been frozen before, and we applied the 42 °C heat treatment using the same protocol. PGCs were collected immediately after treatment, for RNA analysis. A necrosis test was performed after 48 hours. From all experimental groups the cells were frozen after the treatment and stored at -150°C.
After the thawing, cell lines were cultured, and RNA was isolated. Gene expressions were measured by qPCR method. We monitored genes associated with heat stress (HSP70, HSP90, HSF1), stem cell specific markers (CVH, DAZL) and miRNAs (miR-92, miR-138).
Preliminary results indicate that the heat treatment influenced the expression of genes related to heat stress. The HSP70 significantly over-expressed after the treatment but detected at normal levels after 48 hours.

Keywords: *Heat treatment, Cryopreservation, PGC, FUCCI*

Acknowledgement: *This study was supported by grants RRF-2.3.1-21-2022-00007 and ÚNKP-23-3-I - New national excellence program of the ministry for culture and innovation from the source of the national research, development and innovation fund.*

AN#PE1

ESTABLISHMENT OF A PEROXIDASIN-LIKE KNOCKOUT NON-RODENT MODEL

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The peroxidasin-like protein (PXDNL) is a non-enzymatic peroxidase homologue that is expressed almost exclusively in cardiomyocytes and implicated in dilated cardiomyopathy, yet its in vivo function remains poorly understood. As rodents lack the Pxdnl gene, a rabbit model is particularly suitable because rabbit cardiac physiology, anatomy and early embryonic development more closely resemble those of humans, and PXDNL is robustly expressed in rabbit heart.
The aim of this work was to generate a PXDNL knockout (KO) rabbit line to investigate the consequences of PXDNL loss of function and to clarify its potential role in cardiac function. Single guide RNAs were designed to target the first coding exon of the rabbit PXDNL gene, and a mixture of CRISPR/Cas9-guide RNA was microinjected into New Zealand White rabbit zygotes to introduce disruptive mutations by error-prone non-homologous end joining.
Founder animals were identified, and following initial characterization of the induced mutations, a single founder carrying a PXDNL allele predicted to have an early stop codon was selected for breeding to establish a mutant line.
This newly established PXDNL KO rabbit line provides the first non-rodent in vivo model to study PXDNL function and its contribution to cardiac remodeling. CRISPR/Cas9 off-target analysis revealed no detectable off-target mutations. Ongoing work includes the generation of a homozygous knockout population for downstream experiments and the development of rabbit-specific PXDNL antibodies. The main objective is comprehensive cardiovascular phenotyping to relate molecular alterations to functional cardiac outcomes.

***Keywords**: peroxidasin-like protein; CRISPR/Cas9; rabbit model; genome editing*

***Acknowledgement**: K143509, RRF-2.3.1-21-2022-00007 and NVKP16-1-2016-0039.*

AN#PE2

EFFECT OF THE IN VITRO MATURATION CONDITIONS ON OOCYTE COMPETENCE

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In vitro embryo production (IVEP) is a key tool for genetic improvement in cattle. Multiple intrinsic and extrinsic factors influence embryo development *in vitro* conditions. Among the extrinsic factors, ovary storage temperature and maturation media can significantly affect embryonic development. A crucial area for optimization in IVEP systems is the identification of predictive indicators of developmental competence, such as oocyte morphology, particularly polar body (PB) extrusion. Random *Bos taurus* ovaries from the slaughterhouse of TENDON Kft. In Gyöngyös, Hungary was used for the IVEP programs. Five IVM protocols were tested: A0 (22 h in BO-IVM), A1 (22 h in BO-IVM + 2 h in BO-IVF), A2 (24 h in BO-IVM), A3 (22 h in BO-IVM + 4 h in BO-IVF), and A4 (26 h in BO-IVM). After maturation, the oocytes were stripped of their cumulus oophorum in a denudation process by vortexing and pipetting, these oocytes were denominated as DOs, after it, were classified according to PB status (positive or negative), the classification was made by visualization under stereo microscope, then fertilized and cultured to the blastocyst stage. IVF was performed in 80-µl BO-IVF droplets, followed by embryo culture in 30-µl BO-IVC droplets. Oocytes collected from ovaries stored overnight at 4 °C were used only to assess PB extrusion after IVM, while those from non-stored ovaries were evaluated for cleavage rate, blastocyst formation, and measurement of the size of the oocyte area. The oocyte size measurements were performed using digital pictures taken by S9 Series Greenough Stereo Microscope, where the whole area of the embryo it was measured by the use of a free software tool ImageJ (https://imagej.net/ij/). Statistical analyses included the ART (Align-Rank-Transform) test, Spearman correlation, and post hoc procedures (Duncan's Multiple Range Test, ART procedure, and Bonferroni-adjusted contrasts) were done using R 4.3.1 software, with significance set at $p<0.05$.

Results showed that DOs IVEP protocols achieved similar blastocyst formation compared to routine IVEP programs. Cleavage rates were higher in oocytes with a visible polar body (65.02%), than in those without, and their ability to reach the blastocyst stage remained robust (29.37%). Oocyte size analysis revealed that cleaved embryos had a larger oocyte area (61,100µm^2) than non-cleaved embryos. Ovary storage significantly reduced the number of PB-positive oocytes, indicating impaired maturation. However, PB extrusion was still observed in some stored oocytes, suggesting that their developmental capacity was diminished but not entirely lost.

This study showed polar body extrusion as a reliable predictor of oocyte competence, PB-positive oocytes demonstrating improved cleavage and blastocyst formation. Maturation conditions influenced developmental performance when combined with PB assessment. Although ovarian storage negatively impacted oocyte maturation, however, some degree of developmental capacity was retained.

Keywords: *IVEP, Embryo, Competence, Denudated Oocytes*
Acknowledgment: *This study was supported by 2020-1.1.2-PIACI-KFI-2021-00332 and Stipendium Hungaricum Scholarship.*

AN#P1

THE DIFFERENCE IN AMINO ACID COMPOSITION OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) FED BLACK SOLDIER FLY LARVAE

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In aquaculture feeds, black soldier fly larvae (BSFL; Hermetia illucens) have become a viable substitute protein source, especially for African catfish (Clarias gariepinus). The current understanding of how dietary inclusion of BSFL influences the amino acid composition of African catfish muscle tissue is summarized in this abstract. Essential amino acid (EAA) levels in the fillet are typically maintained when fishmeal is partially or completely replaced with BSFL, though certain changes may take place. Due to their abundance in BSFL, branched-chain amino acids (leucine, isoleucine, and valine) tend to remain stable or increase, while muscle lysine and methionine concentrations, which are frequently limiting in insect meals, may show slight reductions depending on inclusion level. This study aimed to assess differences in the amino acid composition of African catfish (*Clarias gariepinus*) fed diets in which fishmeal was replaced with black soldier fly larvae. Juvenile African catfish were randomly assigned to dietary treatments in a Recirculating Aquaculture System (RAS). There were three test groups: control (Haltáp Ltd. catfish breeding feed), 33% (33% black soldier fly meal + 67% catfish feed), 50% (50% black soldier fly meal + 50% catfish feed) At the end of the experiment, muscle samples were collected, homogenized, and analyzed for amino acid content using standard chromatographic techniques (such as high-performance liquid chromatography). Proximate composition and growth performance were also monitored to support interpretation of amino acid results. Inclusion of black soldier fly larvae at 33% and 50% produced only minor decreases in measured amino-acid concentration (2.1% and 2.8% lower than the control, respectively). These small reductions are unlikely to be biologically important, but formal statistical testing and analyses of individual essential amino acids and digestibility are needed to confirm equivalence.
Therefore, the minimal decrease in amino-acid concentration at 33–50% BSFL inclusion likely reflects the chitin-rich fraction of the larvae which does not contribute to amino-acid yield.

Keywords: *African catfish, Black Soldier Fly Larvae, Amino Acid Composition*

Acknowledgement: *The research is supported by Stipendium.*

Plant- and food biotechnology

PF#PLEN

THE CHALLENGES OF GROWING PLANTS IN SPACE AND THE RESULTS OF THE HUNOR-VITAPRIC PROJECT

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The cultivation of plants beyond Earth faces a constellation of interdependent environmental, biological, and engineering challenges. These include limited solar radiation, elevated ionizing radiation, large thermal variability, non-Earth atmospheric pressures, reduced gravity, regolith substrates with low nutrient-holding capacity, high-CO_2/low-O_2 atmospheres, pervasive dust, constrained water and nutrient availability, altered plant physiology, as well as overarching need for closed-loop, resource-efficient systems. Therefore, the establishment of a special Controlled Environment Agriculture (CEA) is foundational technology for space plant research, enabling the growth of crops in environments where natural survival is impossible. By precisely manipulating light, temperature, humidity, and nutrients, researchers create artificial ecosystems that act as Bioregenerative Life Support Systems (BLSS) to sustain astronauts on long-duration missions. In Hungary, the VITAPRIC project is one of the newest and most successful development directions of space plant research, which began in 2019 and is being implemented with the participation of twenty researchers. In a narrower sense, the Vitapric program is considered the very first space plant biological experiment designed exclusively by Hungarian scientists. In a broader sense, this is the first Hungarian space agricultural research activity. In parallel with the AXIOM-4 space mission, the Vitapric ground experiments were also tooke place at the Biodrome research greenhouse of the University of Debrecen where the Vitapric ground simulation space chamber has been set up for this purpose, including, i.e. a novel Multifunctional clinostat system with 12-20 workstations that partially simulates microgravity. After the mission, these samples will be subjected to detailed analytical, biochemical and molecular biological tests which may begin in the coming weeks. It is also interesting to mention that in memory of the Vitapric program, the University of Debrecen has placed a 50-year-old TIME CAPSULE (2025-2075). In addition to the samples of experimental payloads, plant seeds and the researchers' vision for the future were placed into two time capsules through which we sent messages to the researchers of the future generation. The HUNOR program was strategically coordinated in every detail and has been implemented over the past years in close cooperation with AXIOM on the one hand and NASA experts on the other. The Vitapric program can draw attention to the more health-conscious nutrition of the, i.e. European population, especially the youth, including the demand for natural substances with higher biological value. Finally, the tools and methods developed during the Vitapric program and their improved versions can spread in international and domestic biological education sistem, from primary schools to universities as well as in the research sectors.

Keywords: *Space plants, Hungary, Hunor project*
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PF#E1

DEVELOPMENT OF MULTIFUNCTIONAL SOIL MICROBIAL CONSORTIA TO ENHANCE CROP PERFORMANCE UNDER CLIMATIC STRESS

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The intensifying impacts of climate change, manifesting as elevated average temperatures, erratic precipitation regimes, and the emergence of novel plant pathogens pose critical challenges to agricultural productivity in temperate regions. Simultaneously, the extensive use of synthetic fertilizers and pesticides has led to widespread ecological disturbances, including nutrient imbalances, groundwater contamination, and soil health degradation. In light of these concerns, there is a pressing demand for sustainable soil management strategies that leverage the functional capabilities of beneficial soil microbiota.
This study details the development and open-field validation of functionally diverse microbial consortia to enhance the resilience of key crops—maize, soybean, sunflower, and walnut—under climate-induced stress. The inoculants, composed of synergistically interacting microbial strains, were selected for their abilities to improve drought tolerance, mobilize nutrients, suppress soilborne pathogens, and promote overall plant vigor. A central objective was to overcome the variability in field efficacy commonly associated with microbial soil amendments through the rational design of multi-strain consortia tailored to specific agroecological contexts. Most notably, the performance of these prototype formulations was rigorously assessed in large-scale field trials under variable climatic conditions. The results consistently demonstrated improved plant growth, enhanced drought resilience, and reduced dependence on chemical fertilizers, alongside measurable improvements in soil structure and fertility. These outcomes provide empirical validation for the field applicability of consortium-based microbial inoculants and highlight their potential as a scalable, nature-based solution for sustainable crop production in temperate agroecosystems facing increasing climatic uncertainty.

Keywords: *microbial inoculants, microbial consortia, biofertilizers, sustainable crop production*

Acknowledgement: *This study was supported by the National Research, Development and Innovation Office (2022-1.2.6-TÉT-IPARI-TR-2022-00009) and the Interreg-IPA Cross-border Cooperation Programme Hungary-Serbia (PLANTSVITA project, HUSRB/1602/41/0031).*

PF#E2

EXPLORING THE GENETIC BACKGROUND AND POTENTIAL USEFULNESS OF A POTATO CHIMERA

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Plant chimerism is the concurrence of genotypically different tissues within a single plant organism (Balkema,1971), it can be a conglomerate of cells originating from different zygotes (Rossant and Spence, 1998). In essence, plant chimerism occurs when a plant is composed of genetically distinct cell lines that coexist within the same plant (Frank and Chitwood, 2016). These genetically distinct cell lines can confer different phenotypes which are much easier to detect, like the change in colour of tubers (Howard, 1969), loss of pigmentation, or cytological changes that are less visible to detect, like changes in chromosome number or arrangement. It can be classified as: mericlinal, periclinal, and sectoral chimerism. It was first described in the seventeenth century and, with advances in science, now serves as a tool for understanding plant cellular, tissue, and organismal development.
Using flow cytometry, we discovered a spontaneously evolved, not artificially induced, chimeric line of a potato cultivar. These results were further reiterated via a haploid chromosome-resolved WGS from short (150 bp Illumina) and long (~8,000 bp PacBio) reads, which showed a genome size of about 2 Gbp (no other organism detected when compared with the *Solanum tuberosum* Group *Phureja*) instead of 850 Mbp with abundant repeats and difficulty reconstructing the contigs.
The chimeric plants found in this study were *in vitro* plants cultured on MS 100 and fieldpropagated plants and have maintained stable chimeric states via vegetative propagation after 20+ passes every 6 weeks of the *in vitro* plants and a couple of generations of the fieldpropagated plants. Regeneration using direct and indirect somatic embryogenesis with auxins and cytokines from the internodal segments and leaves also yielded chimeric plants. Analysis of the chromosomes in the tetraploid state showed extra copy numbers in some chromosomes. Results will be presented at the conference.

Keywords: *chimera, chromosomes, somatic embryogenesis*

Acknowledgement: *This study was supported by the Stipendium Hungaricum scholarship of the Tempus Foundation, Hungary.*

PF#E3

IMPROVING EFFECTS OF SEED PRIMING WITH DIFFERENT SIZED ZNO NANOPARTICLES IN ZINC-DEFICIENT *SOLANUM LYCOPERSICUM* L. CV. MANO

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Zinc (Zn), the second most abundant transition metal in biological systems, plays a crucial role in sustaining life, yet Zn-deficient soils remain widespread worldwide, including in Hungary. In this study, we examined the impact of zinc oxide nanoparticles (nZnOs) of two sizes (~8 nm and ~45 nm) applied as seed priming agents (100 mg/L) on *Solanum lycopersicum* L. cv. Mano grown under Zn-deficient nutrient conditions. Tomato seeds were primed for 24 hours and subsequently germinated for three weeks in nutrient solutions either supplemented with $ZnSO_4$ (Zn +) or lacking $ZnSO_4$ (Zn -). Plant responses to Zn availability were assessed through Zn concentration and spatial distribution analyses, growth-related parameters (fresh and dry biomass of shoots and roots, shoot/root length, and leaf area), and the evaluation of reactive oxygen and nitrogen species. Antioxidant enzyme activities (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD)) and metabolites (amino acids, organic acids, sugars and polyamine) were also measured from both organs (leaf and root). Fluorescent probes were employed to monitor nitric oxide (NO), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), superoxide ($O_2^{\cdot -}$), Zinc levels, and cell viability in root tissues. The three-week treatments effectively induced Zn deficiency in tomato, as reflected by reduced biomass accumulation. In Zn-sufficient plants, both nanoparticle sizes enhanced Zn accumulation, with the larger particles producing a more pronounced increase. Zn deficiency markedly lowered Zn content in the root tip, while both nZnO treatments elevated Zn levels relative to hydroprimed (HP) control plants under Zn-deficient conditions. Concerning reactive molecules, Zn supply resulted in decreased NO, H_2O_2, and $O_2^{\cdot -}$ levels but elevated $ONOO^-$ in roots. Seed treatments with 8 nm and 45 nm nZnO also improved leaf area and both shoot and root biomass compared to HP controls. Distinct antioxidant and metabolic responses were detected in both organs. In the leaves, SOD and APX activities declined, whereas CAT activity increased. In the roots, SOD activity increased while APX activity decreased under both nZnO treatments in the Zn-deficient (Zn -) condition. Overall, nZnO-based seed priming represents a promising strategy to enhance tomato biomass production under Zn-limited environments.

Keywords*: Solanum lycopersicum, Zn deficiency, Nanoparticle, seed priming*

Acknowledgement: *This work was supported by the National Research, Development and Innovation Office of Hungary under grant No. K 135303 and the 'Lendület' MOMENTUM project of the Hungarian Academy of Sciences (LP2023-14/2023).*

PF#E4

THE ROLE OF RNA-DIRECTED DNA METHYLATION IN BARLEY

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Plants regulate their gene expression at various developmental stages, and in response to biotic and abiotic stresses. RNA Directed DNA Methylation (RdDM) is one of such mechanisms through which genome stability is maintained and the expression of stress related genes is regulated at different phases in their life cycle. RdDM involves the production of small interfering RNAs of 24 nucleotide length via the action of DICER-LIKE (DCL) RNase-III enzymes that cleaves double stranded RNA precursors. Small RNAs are then loaded into an effector protein called Argonautes to form the RNA induced silencing complex (RISC) which mediates sequence specific DNA methylation leading to transcriptional repression. The aim of our work is to characterize the RdDM pathway genes in Barley with the goal of revealing their role during stress response and development. We identified some of the pathway genes to be important for normal reproductive development, some required for reproductive development only under heat stress conditions. Overall, while this pathway is reported to be of less significance in Arabidopsis, Mutant barley lacking genes involved showed abnormality.

Keywords: *RdDM, Argonautes, Barley*

PF#E5

CHARACTERIZATION OF THE BRASSICA GROWTH REGULATORY E2F-RBR PATHWAY

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Oilseed rape (*Brassica napus*), a crop plant closely related to *Arabidopsis thaliana*, the model plant for molecular studies, originated approximately 7,500 years ago through the hybridization of *B. rapa* (field mustard) and *B. oleracea* (wild cabbage), followed by chromosome doubling, a process known as allopolyploidy. Consequently, the *B. napus* genome encompasses a multitude of gene alleles, including six RETINOBLASTOMA-RELATED sequences and four homologues of the *Arabidopsis* E2FB transcription factor, complicating the study of their functions in the rapeseed. To address this complexity, a homologue of the *Arabidopsis* E2FB gene in *B. napus* was identified and employed to complement the growth-related phenotype of the *e2fb*-2 mutant *Arabidopsis* line. Consistent with this, the BnE2FB protein exhibits a similar expression pattern to its *Arabidopsis* counterpart in the *Arabidopsis* root apical meristem and interacts with the components of the multi-protein complexes known as DIMERIZATION PARTNER-RB-E2F-and MYB-INTERACTING (DREAM). Furthermore, we demonstrated that RBR proteins undergo multiple phosphorylation events in proliferating *B. napus* leaves, thereby inhibiting their interaction with E2Fs. Surprisingly, phosphorylated RBR forms were identified in complexes with proteins involved in ribosome biogenesis and translation. This suggests that the phosphorylation of RBR may alter its function from transcriptional to post-transcriptional regulation in proliferating plant cells.

Keywords: *RBR, E2FB, phosphorylation, Brassica*

Acknowledgement: *This work was supported by the Biological Research Centre, and by the National Research, Development and Innovation Office (OTKA-K 139202, OTKA-K 146386). Fruzsina Nagy was supported by a PhD fellowship of the University of Szeged.*

PF#E6

POTENTIAL OF GREEN MICROALGAE TO ENHANCE PLANT SALT TOLERANCE

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High soil salinity and the excessive application of chemical fertilizers is causing problems in agricultural regions worldwide, leading to the progressive degradation of soil quality. For the sustainable crop production, algae- or bacteria-based soil inoculants can offer a natural and environmentally friendly alternative. In this study, we investigated the effects of two microalgal cultures – *Tetradesmus obliquus* (MACC 34, KH/1, isolated from Lake Balaton) and *Chlorella variabilis* (KH/2, isolated from Széchenyi thermal baths) – on salt stressed plants. Our previous experiments revealed that the photosynthetic activity of plants was significantly reduced after very short term (30 min) treatment – using 200 mM and 300 mM NaCl solution. In this study, *Arabidopsis thaliana* L. (ecotype Columbia-0) plants were grown under control conditions for 28 days, and then exposed to salt stress (100 mM or 200 mM NaCl), liquid living algae culture, or lyophilized algae. Combined treatments were also applied: plants received both salt stress and algae culture or salt stress and lyophilized algae. Treatments were administered by spraying on day 1 and again on day 4 or 5. Following the treatment, non-invasive photosynthetic activity measurements were performed on the plants almost daily for three weeks. Our results revealed a significant decrease in effective and maximal quantum yield. The effect of salt treatment became detectable from day 10. By day 25, however, a difference emerged between the PSII quantum efficiency values of salt-treated plants and those that received both salt and algae treatments. In the case of KH/1, algae application slightly increased the effective quantum yield. Regarding KH/2 algae, a significant positive effect was observed in the treatment combining lyophilized algae with 200 mM NaCl, when compared to salt treatment alone. At 100 mM NaCl, the positive effect of lyophilized algae was smaller, but photosynthetic activity still showed improvement compared to salt treatment. *Tetradesmus obliquus* and *Chlorella variabilis* can enhance plant tolerance to salt stress, especially with lyophilized algae treatments. These results highlight the potential of microalgae-based biofertilizers as sustainable tools to improve crop resilience under saline conditions.

Keywords: *microalgae, salt stress, photosynthesis*

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PF#E7

INVESTIGATION OF THE EFFECT OF SALT STRESS ON PHOTOSYNTHETIC ELECTRON TRANSPORT PATHWAYS IN EUKARYOTIC MICROALGAE

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Salt stress caused by elevated NaCl concentrations represents a major environmental factor affecting microalgae, where it significantly impairs photosynthetic performance. Although the physiological effects of salt stress have been investigated for several decades, it is not known exactly which steps of the photosynthetic electron transport chain are inhibited, especially regarding the functioning of the Calvin-Benson-Bassham cycle. The impact of salt stress on the photosynthetic processes was investigated in the microalga *Chlorella sorokiniana* to resolve long-standing contradictions in the literature regarding the sites of NaCl-induced inhibition at the donor and acceptor sides of PSII, and of PSI, as well as to explore the inhibitory effects of salt stress downstream of PSI. Our data show that NaCl (200-1000 mM) has multiple inhibitory sites in the photosynthetic electron transport chain, ranging from the water-oxidizing complex to the Calvin-Benson-Bassham (CBB) cycle, which serve as the ultimate electron donor and final electron acceptor, respectively. We have demonstrated that NaCl in PSII affects both the donor and acceptor site processes by inhibiting the water-oxidizing complex and electron transfer between the Q_A and Q_B quinone electron acceptors, respectively. Importantly, our data demonstrated that the main inhibitory sites of salt stress, which have the largest impact on the overall photosynthetic efficiency, are located downstream of PSI. The direct NaCl-induced inhibition of specific steps of the CBB cycle have an important potential to identify targets for biotechnological improvement of salt-stress tolerance. Furthermore, the insights gained from this study will provide deeper understanding of the salinity responses of other eukaryotic microalgae as well, including the symbiotic dinoflagellates (Symbiodiniaceae) involved in coral symbiosis.

Keywords: *Salt stress, Microalgae, Electron transport chain, Chlorophyll fluorescence, NADPH fluorescence, P700, Calvin-Benson-Bassham cycle, PSII inhibition, Symbiodiniaceae*

Acknowledgement: *This research was partly supported by the National Research, Development, and Innovation Office, grant number FK-146298.*

PF#E8

INCREASED EXPRESSION OF ATSPQ IMPROVES DROUGHT TOLERANCE IN *BRASSICA NAPUS*

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Brassica napus (rapeseed) is an important oilseed crop cultivated globally for edible oil and biofuel production, but its productivity is severely impacted by drought stress. Drought restricts leaf expansion, induces stomatal closure, and disrupts key physiological processes like photosynthesis, respiration, and phytohormonal balance. Additionally, it leads to excessive accumulation of reactive oxygen species (ROS), which trigger antioxidant defence responses, phytohormone synthesis, and osmotic adjustments through organic solutes. These adaptive mechanisms help maintain cellular homeostasis and sustain photosynthetic activity, contributing to improved drought tolerance in plants. Previously, overexpression of the Small Paraquat resistant (SPQ) gene from *Arabidopsis thaliana* and *Lepidium crassifolium* was shown to improve drought tolerance in Arabidopsis by increasing viability, reducing oxidative damage, maintaining photosynthetic activity and increasing resistance to paraquat. To test the effect of SPQ on the drought responses of a crop plant, rapeseed (Westar) was transformed with AtSPQ to overexpress it under the control of the constitutive CaMV35S promoter. Transgenic plants were subjected to water stress by suspending watering in controlled conditions. Several parameters, like physiological, biochemical and stress-induced gene expression were analysed in stressed and control plants. Preliminary results indicate that the AtSPQ-overexpressing lines exhibit enhanced viability, better stomatal conductance, and lower accumulation of osmo-protectants, suggesting that the *SPQ* overexpression can reduce the deleterious effects of water deprivation. Our results suggest that SPQ overexpression in crop plants has potential for crop improvement to mitigate drought damage. Further studies are underway to investigate the underlying mechanisms in more detail.

Keywords: *rapeseed, drought, AtSPQ overexpression lines*

Acknowledgement: *The research was supported by NKFI FK128920, K128728, K143620, FK142852, NKFI-Advanced-151222.*

PF#E9

PHYSIOLOGICAL EFFECTS OF UVR8 PHOTORECEPTOR PHOSPHORYLATION IN *ARABIDOPSIS THALIANA* SEEDLINGS

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Ultraviolet B (UV-B) radiation (280–315 nm) is a high-energy component of sunlight that can be absorbed by many biological macromolecules, thereby damaging them and influencing the growth and development of living organisms. Because plants are sessile organisms, mitigating the harmful effects of UV-B radiation poses a particular challenge for them. The UV RESISTANCE LOCUS 8 (UVR8) photoreceptor senses UV-B radiation and regulates plant responses through its control of downstream signaling pathways. Understanding the function of UVR8 and its signaling networks can provide us a deeper insight into how plants respond to UV-B radiation.
Our preliminary results have shown that UVR8 can undergo phosphorylation, and we hypothesize that this post-translational modification affects UVR8 function by influencing its interactions with other proteins and modulating adaptive responses to UV-B. To investigate this, we expressed modified UVR8 proteins in uvr8-deficient plants, in which phosphorylated amino acids were replaced either with non-phosphorylatable residues or residues that mimic constitutive phosphorylation. These transgenic plants were analyzed using photobiological and biochemical approaches to elucidate the physiological role of UVR8 phosphorylation.
We found that phosphorylation of UVR8 reduces its activity and thereby influences the UV-B-specific growth responses it regulates. Our findings may reveal connections between UVR8-dependent signaling pathways and plant UV-B responses, highlighting a possible fine-tuning mechanism within UVR8 signaling. Such regulatory mechanisms could potentially be applied in agriculture and environmental protection. Manipulation of UVR8-dependent signaling pathways may enhance plant stress tolerance and yield, contributing to the development of sustainable agriculture.

Keywords: *UVR8, UV-B, signaling, phosphorylation*

Acknowledgement: *The work was supported by the National Research Development and Innovation Fund OTKA K-138022 grant.*

PF#E10

PROBIOTIC COCOA POWDER FORMULATION PREPARED BY CONTINUOUS WET GRANULATION-BASED POWDER-TO-GRANULE LINE

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Functional foods, particularly probiotic formulations, are gaining increasing attention today, as maintaining the balance of the gut microbiota can prevent numerous diseases. These formulations often contain various *Lactobacillus* strains. Notable examples include *Lactobacillus acidophilus*, which is frequently used in antidiarrheal treatments, and *Lactobacillus paracasei*, which could be suitable to support oral hygiene and reduce the risk of dental caries in children. The production of probiotic formulations presents a complex challenge, as the viability of these microorganisms is significantly affected by various environmental stress factors during both processing and storage. Therefore, the development of a manufacturing process that ensures gentle processing parameters is essential for producing effective probiotic products. Continuous twin-screw wet granulation technology offers a potential solution, providing a gentle processing method for the formulation of heat-sensitive biological active ingredients. The primary objective of this research was to develop a continuous manufacturing process to produce probiotic functional food formulations. Cocoa powder was selected as the carrier matrix to facilitate the administration of probiotics. This formulation enables the delivery of the required microorganisms in the form of a favourable cocoa-granules, ensuring high compliance among children. The cocoa powder-based granules were formulated using a continuous twin-screw wet granulation line. The final granules exhibited good to excellent flow properties (based on Carr's index and Hausner ratio), which were comparable to the commercial Nesquik® cocoa powder. Furthermore, the granules showed a narrower particle size distribution than the reference Nesquik® cocoa powder, allowing for better handling of the final formulation. The final probiotic-loaded cocoa granules contained 6.38×10^3 CFU/g of viable bacteria. These results demonstrate that the integrated, continuous twin-screw wet granulation-based manufacturing line is suitable for continuous production of probiotic cocoa-granules for children.

Keywords: *functional foods, probiotics, stabilization, twin-screw granulation*

Acknowledgement: *This work was supported by EKÖP-25-2-BME-192 University Research Fellowship Programme, TKP2021-EGA funding scheme (Project no. TKP-9-8/PALY-2021), and PD-142301 funding scheme of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund Furthermore, the János Bolyai Research Scholarship of the Hungarian Academy of Science was kindly acknowledged.*

PF#PE1

SEED AND SEEDLING-STAGE RESPONSES TO NOVEL FORMULATIONS OF (NANO)ZINC-ENRICHED PLASMA-ACTIVATED WATER UNDER OSMOTIC STRESS IN ARABIDOPSIS

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This study reports a comprehensive evaluation of seed priming using plasma-activated water (PAW) and PAW supplemented either with zinc ions (PA(W+Zn)) or zinc-oxide nanoparticles (PA(W+ZnO NP)) in *Arabidopsis thaliana*. Plasma activation in the presence of metallic Zn mitigated water acidification, extended the stability of nitrite (NO_2^-), and facilitated Zn^{2+} release, thereby generating PA(W+Zn) enriched in hydrogen peroxide (H_2O_2), nitrate (NO_3^-), NO_2^-, and Zn^{2+}. By contrast, ZnO NPs generated a less reducing environment, resulting in comparatively lower NO_2^- and higher NO_3^- concentrations, accompanied by a moderately decreased pH. All PAW formulations induced subtle alterations of the seed surface and enhanced potassium efflux, indicative of increased seed coat permeability, which was consistent with improved germination performance. Supplementation with Zn ions or ZnO nanoparticles further increased Zn bioavailability to the seeds. Seedlings exposed to osmotic stress displayed elevated nitric oxide levels, with the most pronounced increase observed following PA(W+Zn) priming. In addition, PAW and PA(W+Zn) treatments enhanced H_2O_2 accumulation in roots under osmotic stress, reflecting the stability and predominance of H_2O_2 as ROS in PAW. Although osmotic stress reduced overall seedling viability, PAW-based priming moderately stimulated cotyledon expansion and stomatal differentiation. These results confirm the germination-enhancing capacity of PAW and clarify its impact on seed coat structure, elemental makeup, redox-related signalling, and early seedling growth under abiotic stress, thereby justifying further studies in a wide range of plant species and zinc formulations.

Keywords: *plasma activated water, seed priming, zinc oxide nanoparticles*

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PF#PE2

PLASMA-ACTIVATED LIQUIDS IMPROVE OSMOTIC STRESS TOLERANCE OF PEA PLANTS

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Drought stress is an increasingly significant challenge in agriculture, making it essential to develop new strategies to improve plant resilience and yield. Plasma-activated liquids are an emerging, eco-friendly approach for seed pre-treatment with the ability to improve germination, promote plant growth, and increase tolerance to stress.
This study examines how pre-treating seeds with plasma-activated water (PAW) influences the osmotic stress tolerance of pea plants (*Pisum sativum* L. cv. Petit Provencal). The composition of reactive oxygen and nitrogen species (RONS) in PAW was altered through the supplementation of zinc ions (Zn). Seeds were treated for one day with distilled water (HP), PAW, PA(W + Zn) or Zn, then grown for 10 days before exposure to 72 hours of osmotic stress induced by 20% (w/v) polyethylene glycol (PEG8000).
Seed treatment with plasma-activated water elevates nitric oxide levels in the seed coat after a 24-hour period. All treatments improve the viability of lateral root tips exposed to osmotic stress. Under osmotic stress, plasma-activated water treatment results in larger leaf area compared to stressed control. Osmotic stress led to an accumulation of glucose and sucrose and a reduction in starch content. Application of plasma-activated water or Zn under osmotic stress lowered glucose and sucrose levels, suggesting an alleviating effect. Treatments that included Zn [PA(W+Zn) and Zn] led to higher Zn concentrations in the leaves and roots of adult plants. Osmotic stress lowered the activity of catalase (CAT) and at the same time caused a slight increase in case of peroxidase (POD) and superoxide dismutase. Our treatments increased POD and CAT activities under both control and osmotic stress conditions, suggesting the mitigating effect of plasma-activated liquids in osmotic stressed plants.

Keywords: *Plasma-activated liquids, seed pre-treatment, osmotic stress*

Acknowledgement: *The work was supported by the „Lendület" MOMENTUM project of the Hungarian Academy of Sciences (LP2023-14/2023).*

PF#PE3

MOLECULAR INSIGHTS INTO HYPOXIA TOLERANCE IN WATERLOGGED CUCUMBER

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Waterlogging creates an oxygen-deficient condition around the roots, disturbing redox balance and inhibiting cucumber growth. Understanding how hypoxia-responsive genes are regulated under waterlogging conditions can be useful to produce tolerant cultivars. In this study, we concentrated on genes related to NO signaling, ROS control, and adaptation to oxygen deficiency with a strong focus on *GSNOR*, *RBOH*, *RAP2-3*, and *Hem3* genes at the transcription level. An open-field hybrid F_1 cucumber cultivar was used in a semi-hydroponic system under control and flooding conditions with and without supplementary nitrate treatment, and a treatment with cPTIO (NO scavenger) to investigate the role of NO in the process. Physiological traits were investigated with the aim of connecting the changes to the differential expression of the studied genes. Nitrate levels were substantially decreased in flooded plants, but increased when flooded in a nitrate-containing solution. Nitrate reductase enzyme activity in leaves followed the same trend. The highest level of nitrate transporter genes (NRT) expression was detected under flooding in nitrate solution for higher NO_3^- uptake and transport, ultimately improving plant performance under waterlogging. The expression of some stress-responsive genes, such as *RAP2.3*, *RBOH*, and *Hem3,* that are partly involved in maintaining redox balance and plant tolerance to abiotic stresses, was also induced under hypoxia and, in most cases, downregulated when nitrate was available. In summary, our work demonstrated that nitrate improves waterlogging tolerance in cucumber, mainly through nitrate-derived NO production and signaling, which regulates redox homeostasis and stress response networks. This work highlights nitrate management as a promising strategy to enhance cucumber resistance to flooding.

Keywords*: Cucumis sativus L., Nitrate, Nitric oxide, Phytoglobin (Hem3), Respiratory Burst Oxidase Homolog (RBOH), S-nitroso-glutathione reductase (GSNOR), ethylene-responsive transcription factor (RAP2.3)*

PF#PE4

SMXL3 ACTIVITY IS EAR-MOTIF DEPENDENT FOR ESTABLISHING ROOT SYSTEM ARCHITECTURE IN ARABIDOPSIS

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SMAX1-like proteins (SMXLs), previously implicated in strigolactone and karrikin signaling, exhibit diverse and partially redundant roles in plant development. The divergent SMXL4 superclade, comprising SMXL3, SMXL4, and SMXL5, is not targeted by D14- or KAI2-dependent proteolysis. Although SMXL3/4/5 have been linked to key developmental processes such as phloem formation and primary root growth in Arabidopsis, their functions remain underexplored. We demonstrated that SMXL3, SMXL4 and SMXL5 could function either redundantly, or in tandem. They collectively regulate rosette growth, flowering time, and anthocyanin accumulation. The SMXL3 and SMXL4 tandem acts to suppress higher-order inflorescence branching and regulate silique and seed development via regulation of embryo development, rather than through defects in fertilization. Mutants lacking both SMXL3 and SMXL5 form spontaneous adventitious roots in the hypocotyl, a phenotype suppressed by auxin transport inhibitors and strigolactone analogs, and regulated by light and carotenoid derived signals such as anchorene. In contrast, anchor root formation and primary root tip dominance are redundantly controlled by all members of the SMXL4 superclade and require an intact EAR motif in SMXL3. This EAR motif is also essential for maintaining proper source–sink dynamics and vertical root growth trajectory. Collectively, our findings establish the SMXL4 superclade as a novel regulatory hub for developmental timing and root architecture, highlighting both EAR motif– dependent and –independent pathways in shaping plant form and function.

Keywords: *Arabidopsis, SMAX1-like proteins (SMXLs), Strigolactone, Karrikin, Hormone signaling*

Acknowledgements: *Funding support from the Hungarian National Research, Development and Innovation Office (NKFIH Grants OTKA K128644, K145907, FK146967 and FK134992; Hungarian National Laboratories Program RRF- 2.3.1-21-2022-00007). This work was completed within the framework of MTA Bolyai Fellowship (Zoltán Tolnai, BO/00618/24).*

PF#PE5

RESISTANCE AGAINST TOMATO BROWN RUGOSE FRUIT VIRUS IN WILD *SOLANUM* SPECIES

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Tomato Brown Rugose Fruit Virus (ToBRFV) is an emerging tobamovirus that poses a threat to tomato production due to its ability to overcome the widely used Tm-2² resistance, resulting in significant yield losses and market restrictions. Identifying novel durable resistance is therefore a high priority for disease-resistant breeding programs. Wild tomato relatives represent a significant source of unexplored natural resistance. Among these, *Solanum peruvianum* has been identified as a possible source of ToBRFV resistance and has shown resistance to a number of tobamoviruses. The aim of our study was to genetically map and characterize genetic resistance to ToBRFV in wild *Solanum peruvianum* species, and to identify molecular markers linked to resistance. An interspecific mapping population was generated through hybridization between *S. peruvianum* (resistant parent) and *S. lycopersicum* (susceptible parent). The first generation (F_1) displayed full resistance, while phenotyping of the F_2 population following mechanical inoculation with a Jordanian ToBRFV isolate showed a 3:1 resistant:susceptible segregation ratio, which indicates the control of the resistance trait by a single dominant gene. Molecular markers were developed for SNPs (single nucleotide polymorphisms) as well as insertions and deletions using the RNA seq data of the parental lines and the tomato reference genome (Heinze Sl3.0). Rough genetic mapping was done with 66 polymorphic markers equally distributed on the 12 chromosomes. The region responsible for the resistant phenotype was mapped on the telomeric region of chromosome 8. Fine mapping and functional validation of candidate genes will be the subject of further complementation studies. The identified gene(s) will serve as a tool in MAS and generation for the development of durable resistance breeding lines.

Keywords: *Tomato Brown Rugose Fruit Virus (ToBRFV), Solanum Peruvianum, Marker Assisted Selection (MAS)*

Acknowledgement: *This research is supported by the Stipendium Hungaricum Scholarship Program.*

PF#PE6

NITRIC OXIDE -RELEASING NANOPARTICLES ENHANCE TOMATO FRUIT DEFENSE AGAINST *BOTRYTIS CINEREA*

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Fungal pathogens are a major threat to global agriculture, causing substantial yield losses. *Botrytis cinerea*, a necrotrophic polyphagous fungus, infects over 200 plant species, including tomato, emphasizing the need for sustainable control strategies. Nanotechnology-based nitric oxide (NO) delivery systems offer a promising alternative. The natural NO donor *S*-nitrosoglutathione (GSNO) plays a key role in plant defense, and encapsulation in chitosan (CHT) nanoparticles enables controlled NO release while leveraging chitosan's antimicrobial properties. This study evaluated the efficacy of GSNO-loaded chitosan nanoparticles (GSNO-CHT NPs) in enhancing resistance of tomato (*Solanum lycopersicum* L. cv. Money Maker) fruits against *B. cinerea*. Fruits were treated as controls (without infection), infection-only (without pretreatment), and pretreated with free GSNO, GSNO-CHT nanoparticles, and empty CHT nanoparticles, followed by inoculation with B. cinerea (10^6 conidia/ml).Disease severity was assessed three days post-inoculation *via* lesion size, distribution, and infection incidence, and fungal hyphae were confirmed microscopically. Antioxidant enzyme activities (superoxide dismutase, catalase) and metabolites (lycopene, sucrose, starch, salicylic acid) were also measured. GSNO-CHT NP pretreatment significantly reduced infection and lesion size, outperforming free GSNO and empty CHT-NPs. All pretreatments decreased lesion size and shifted lesion distribution toward smaller lesions, with GSNO-CHT being most effective. Lycopene content decreased in untreated and free GSNO-treated fruits, while salicylic acid remained unchanged. Distinct antioxidant and metabolic responses were observed, including elevated catalase activity in GSNO-CHT-treated fruits. These results indicate that GSNO-CHT-NPs mitigate disease severity while inducing beneficial biochemical responses, enhancing tomato resistance to *B. cinerea* without affecting fruit quality. GSNO-loaded chitosan nanoparticles thus represent a promising sustainable plant protection strategy.

Keywords: *nitric oxide, S-nitrosoglutathione, chitosan, nanodelivery, Solanum lycopersicum, Botrytis cinerea*

Acknowledgement: *This work was supported by the National Research, Development and Innovation Office of Hungary (K 146292).*

PF#PE7

UNDER DOUBLE STRESS: INTERACTIONS BETWEEN THE EFFECTS OF HEAVY METALS AND PLASTICS ON PLANTS

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Agricultural production is of fundamental importance to society; therefore, understanding abiotic stress factors affecting plants is essential. Plant stress physiology research increasingly focuses on environmental stressors that arise from human activities. Artificial polymers pose a global environmental risk. They are capable of adsorbing various pollutants, such as toxic metal ions. In metal-contaminated agricultural areas where the use of wastewater in agriculture is widespread, several types of metals may be present in high concentrations. When this occurs simultaneously with plastic pollution, plants are exposed to complex stress, which significantly affects their growth and physiological processes.
In the present study, we investigated the effects of various types of plastics including conventional polyethylene-based materials and biodegradable polymers on the early root growth of agricultural crops in the presence of solutions containing multiple heavy metals simulating wastewater exposure
Results indicated that plastics generally promoted root growth, whereas wastewater treatment tended to reduce it, although responses were not consistent across all treatments. The responses varied substantially depending on the type of plastic applied and the plant species examined. Our findings highlight that plant reactions to pollutants are highly complex and strongly influenced by both the polymer type and the species-specific physiological traits, indicating intricate, species-dependent response patterns. Moreover, the combined presence and interaction of the two pollutants modulated root developmental responses in a complex manner. Further experiments are required to fully understand the combined effects of plastics and wastewater, as such knowledge is crucial for assessing risks and supporting sustainable agricultural practices.

Keywords: *sewage, heavy metals, plastics, root growth*

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PF#PE8

PLANT RESPONSES TO POLYETHYLENE-BASED PLASTICS: ROOT DEVELOPMENT AND NITRO-OXIDATIVE STRESS

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The widespread use of plastic materials has become a major environmental concern, with polyethylene (PE) representing one of the most persistent pollutants due to its extensive use and accumulation in landfills. Increasing plant exposure to both PE and recycled PE may disrupt root development and trigger nitro-oxidative stress, ultimately affecting growth and productivity. Understanding these physiological responses is essential for assessing the ecological risks posed by polyethylene-based plastics and for informing strategies to mitigate their impact on terrestrial ecosystems.
In this experiment, we investigated the effects of polyethylene plastic and its recycled form on early root development and germination of rapeseed (*Brassica napus* L.) and garden cress (*Lepidium sativum* L.) under *in vitro* conditions, as well as their nitro-oxidative responses underlying these growth patterns. The plastic fragments were cut to a size of 0.5 cm and applied at concentrations of 0.5% and 1%.
Based on the morphological data, the two species exhibited distinct responses. In rapeseed, root growth was influenced primarily by the type of plastic, whereas in garden cress a concentration-dependent stress response developed. This may be due to the increased production of reactive molecules in the stress response underlying negative growth responses.
Our findings show that PE plastic materials induce stress during the early developmental stages of the two examined crops, suggesting that residual PE in soils may pose agricultural risks. To fully assess their broader implications, additional studies are needed to determine the long-term consequences for crop performance and ecosystem stability.

Keywords: *PE, recycled plastics, plant stress responses, root development*

Acknowledgement: *The project was supported by the National Research, Development and Innovation Office, Hungary (NKFIH FK 142475) and by the Lendület "Momentum" Program of the Hungarian Academy of Sciences (LP2025-8/2025 to G.F).*

PF#PE9

WEEDS AS VIRUS RESERVOIRS: VIROME OF A MAIZE FIELD IN THE NAGYCSEPELY REGION

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Plant-infecting viruses are capable of infecting not only crops directly, but also a wide range of weed species, turning them into virus reservoirs. This research examined the relationship between weeds and viruses in a maize field located in the Nagycsepely region (Somogy county). After surveying the weed species present in the field, samples were collected at three locations within the selected maize field from both weed and maize individuals showing virus-like symptoms (such as chlorosis or striping). Following total nucleic acid extraction, the virome of these plants was determined using high-throughput sequencing (HTS). Seven weed species (*Echinochloa crus-galli, Setaria viridis, Chenopodium hybridum, Chenopodium album, Datura stramonium, Convolvulus arvensis, Sorghum halepense*) were examined beside the collected maize plants. Altogether, 63 samples were processed.

Based on the virus hit list generated by comparing the HTS-derived sequences with NCBI plant virus reference genomes, a total of nine virus species were detected. The virus hit list included wheat streak mosaic virus (WSMV), a pathogen causing severe damage in cereals. Its presence was confirmed by an independent RT-PCR in several *Echinochloa crus-galli* and maize samples. After purification and cloning of the PCR products, Sanger sequencing enabled the determination of the exact WSMV sequences found in the samples, and phylogenetic analysis revealed the variability and relatedness of the detected WSMV variants. Barley virus G (BVG) was also confirmed by RT-PCR and was detected not only in *Echinochloa crus-galli* but also in *Setaria viridis* and *Sorghum halepense*. As a continuation of the research, PCR-based confirmation of additional viruses identified through bioinformatic analysis is currently ongoing.

This study identified the dominant weed species present in maize fields and clarified which of them can function as significant virus reservoirs. It also revealed the diversity of viruses infecting both maize and the associated weeds. Overall, these findings provide a clearer understanding of the epidemiological role of weeds in virus persistence and transmission.

Keywords: *plant virus, WSMV, Echinochloa crus-galli, Zea mays, HTS, BVG*

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PF#P1

DETECTION AND MOLECULAR CHARACTERIZATION OF *ORYZA SATIVA* ENDORNAVIRUS IN RICE CULTIVARS COLLECTED FROM SZARVAS, HUNGARY

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Rice is a staple food for over half of the world's population, underscoring the critical need for its sustainable production and emphasizing the importance of understanding the diverse viral communities associated with this crop. Although some viruses cause severe disease, others, including persistent and asymptomatic agents, form important components of the rice virome. To investigate the virome of cultivated rice, a field survey was conducted in Szarvas, Hungary, to identify viral pathogens potentially infecting local rice (*Oryza sativa*) cultivars. Nine plant samples were collected, including eight rice plants representing three different cultivars (Tunde, ARS, and FEK) and one *Cyperus* sp. weed sample. Total nucleic acid was extracted from all samples, followed by DNase treatment. High-throughput RNA sequencing was performed, and subsequent bioinformatic analysis of the data (FastQ files) using CLC Genomics Workbench revealed the presence of sequence reads corresponding to *Oryza sativa* endornavirus (OsEV). Following cDNA synthesis and quality verification through actin gene amplification, specific primers targeting OsEV were designed. Gradient PCR optimization was then carried out to establish suitable amplification conditions. Subsequently, conventional PCR screening demonstrated that seven out of the nine samples tested positive for OsEV, with all positive signals originating from the rice cultivars (Tunde, ARS, FEK), while the *Cyperus* sp. weed sample tested negative. The amplified PCR products were purified from agarose gels and submitted to Sanger sequencing. The obtained sequences confirmed the presence of OsEV in the positive samples.
These findings provide the first molecular evidence for the presence of OsEV in local rice cultivars collected from Szarvas, Hungary. Ongoing research aims to expand sampling and assess the genetic diversity of OsEV isolates, thereby contributing to a broader understanding of the virus's distribution, host range, and potential implications for rice production in Hungary.

Keywords: *Plant virus, rice cultivars, Oryza sativa endornavirus, High throughput sequencing.*

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PF#P2

CHARACTERISATION THE PROMOTER REGION OF *SP6A* RESPONSIBLE FOR REGULATING TUBERIZATION IN THE POTATO CULTIVAR 'DÉSIRÉE'

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Tuber formation in potato (*Solanum tuberosum* L.) is a complex developmental process regulated by the *SP6A* gene, a phloem-mobile tuberization signal and a homolog of the *Arabidopsis* florigen FT. The expression of *SP6A* is influenced by environmental factors such as temperature and abiotic stresses, affecting tuberization in both short-day tuberizing and day-length independent tuberizing cultivars like 'Désirée'. Overexpression of *SP6A* can accelerate tuberization but may reduce shoot growth and increase tuber number. We assume that fine-tuning *SP6A* expression could enhance earliness of tuberization and stress tolerance of cultivated potatoes without compromising canopy development or tuber yield. To investigate this, we designed PCR primers based on the *S. phureja* genome sequence (https://spuddb.uga.edu/) and isolated, sequenced and compared the *SP6A* gene of 'Désirée' to that of *S. phureja*. We found minor nucleotide differences in exons 1–3 and complete identity in exon 4. Analysis of the *SP6A* promoter region showed conservation between the two species but also identified genetic variations that could impact promoter activity under different environmental conditions. In silico analysis of a 2-kb region of *SP6A* promoter using the Plant Transcriptional Regulatory Map database (https://plantregmap.gao-lab.org/) identified binding sites for Dof, ARF, E2F/DP, and MYB-related transcription factors. 'Désirée' lacked SBP-binding sites but had additional M-type MADS-box and Nin-like binding motifs, suggesting cultivar-specific regulation of *SP6A* expression under certain environmental conditions. To test the activity of the *SP6A* promoter *in planta* the amplified promoter fragments were cloned into the pCAMBIA1391Z GUS fusion vector for transformation into 'Désirée'. Plant genes are generally under positive/negative regulation. Targeted mutagenesis may eliminate negative regulator binding sites to enhance tuber initiation. Genetic modifications targeting negative regulators of *SP6A* could potentially enhance tuber initiation, offering a promising approach for developing resilient potato cultivars.

Keywords*: potato tuberization, SP6A gene, promoter motifs, regulation*

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PF#P3

IMPROVING THE EFFICIENCY OF OLIGONUCLEOTIDE DELIVERY IN MAIZE VIA PEG-MEDIATED TRANSFORMATION AND LAYERED DOUBLE HYDROXYDE (LDH) NANOSHEET

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Climate change can cause several problems, including changes in the precipitation patterns that contribute to the rising temperature and prolonged drought periods. Maize, as an important commodity crop, has become highly susceptible to drought stress, resulting in a significant reduction of maize productivity. The acceleration of climate change underscores the urgent need for an advanced strategy to improve drought tolerance in maize. Mutations in the gibberellic acid-related gene *GA20ox* have shown considerable potential to improve drought tolerance in this crop.
In this study, we aim to enhance drought tolerance by targeting the *GA20ox* gene in the SZ17 maize line using the CRISPR/Cas9 system, as a reference method and Oligonucleotide-directed mutagenesis (ODM) as a more precise method. The CRISPR/Cas9 construct is introduced to calli via *Agrobacterium*-mediated transformation and to maize protoplast via Polyethylene glycol (PEG)-mediated transformation. Specific mutation frequency is assessed using Next Generation Sequencing (NGS). In parallel, we are working on the optimization of the ODM method in our experiment.
ODM is a simple gene editing method that uses short, single-stranded oligonucleotides to introduce precise mutations into the target genes; however, its efficiency is low. Therefore, further methodological improvements are needed, such as increasing the efficiency of DNA delivery. As a test system, we are correcting the STOP codon in the *mutant GFP* (*mGFP*) gene by oligonucleotides, which leads to GFP-positive cells that could be detected by fluorescence microscopy in a few days. We have also designed an oligonucleotide to introduce a codon stop into the *GA20ox* gene, thereby reducing the Gibberellic acid content in the plants, which can lead to drought tolerance improvement. To help the selection of the transformed cells, we deliver the designed oligonucleotides into maize protoplasts via PEG-mediated transformation together with a fluorescent protein coding plasmid (mCherry).
We further refine our strategy by employing the use of layered double hydroxide (LDH) nanosheet coated with lysozyme to deliver the oligonucleotide. The lysozyme coating temporarily disrupts the cell wall structure, facilitating the transport of DNA. LDH- loaded with FAM labelled, *GA20ox* gene targeting oligonucleotide are introduced to maize cell suspensions, and the delivery is followed by fluorescence microscopy.

Keywords: maize, *GA20ox*, *Oligonucleotide Directed Mutagenesis (ODM)*

Acknowledgement: *This work was supported by the National Research, Development and Innovation Office of the Hungarian Government through the RRF-2.3.1-21-2022-00007 grant for the "National Laboratory Program of Agro-Biotechnology and Precision Plant Breeding to Support Food Safety".*

PF#P4

EVALUATING REGENERATIVE PERFORMANCE AND BIOACTIVE COMPOUND VARIATION IN SWEET BASIL THROUGH MICROPROPAGATION"

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Ocimum basilicum L, belongs to the family of *Lamiaceae* and is commonly known as sweet basil and an annual herb that is used as fresh and dried for food flavorings, pharmaceuticals, and essential oil extraction. *In vitro* micropropagation of *O. basilicum* is crucial to keep the phytochemical and biological activity uniform due to the genetic diversity within the same species of the *Lamiaceae* family. Our aim is to establish an optimized protocol for basil *in vitro* micropropagation and measuring the composition of biochemical compounds such as total phenolics, flavonoids content, and antioxidants capacity. We also aim to measure the leaf area among the experimental plants.

For the experiment of shoot induction nodal explants were placed on 7 treatments of MS medium supplemented with different amounts of cytokinin (BAP, 0-1.5mg/L) solo and in combination with auxin (IBA, 0 or 0.25 mg/L). Root formation of the newly generated shoots was done by using three different treatments of auxin (IBA, 0 - 0.5 mg/L).

In the analytical determination of three phytochemical compounds including TPC, TFC, and TAC was done among three different groups of plants (*in vitro*, acclimatized *ex vitro* and *in vivo)* was done by spectrophotometer. Leaf area was also checked among these groups.

In case of shoot induction, the best treatment was not supplemented with hormones for shoot length, leaf number, number of nodes, and regeneration percentage. We observed that all treatments combined with auxin performed significantly poorly compared to all the others. Root induction treatment had (0.5 IBA, 0.25 IBA mg/L) no difference for promoting root length. We observed that for rooting we do not need any hormone supplementation, but it can enhance the process.

Among the three groups of plants, we found that the in *vitro* group had the highest amount of Total phenolic and flavonoid content. This group had the highest antioxidant capacity as well. In the case of TFC we documented significant differences among the three plant groups.

Leaf areas of the acclimatized *ex vitro* plants were higher and more expanded compared to *in vitro* and *in vivo* leaves.

In our experiment, we found that micropropagation increases the number of leaves and nodes, producing high-quality basil plants with consistent beneficial traits and considerable economic value.

Keywords: *Basil, Micropropagation, Bioactive compounds, Ocimum basilicum, Leaf area*

PF#P5

INVESTIGATING THE ROLE OF *WAT-1* AND *OSML-15* IN THE DISEASE RESPONSE OF POTATO TO *RALSTONIA SOLANACEARUM* INFECTION

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Potato (*Solanum tuberosum*) is a widely grown food crop globally, facing a serious threat from *Ralstonia solanacearum* (*Rs*), the bacterium causing bacterial wilt. *Rs* uses a type III secretion system to deliver effectors that suppress plant immunity, colonize the xylem, and disrupt water and nutrient transport, resulting in significant yield losses ranging from 20% to 100% worldwide. Potato's defense against *Rs* involves two main layers: pattern recognition receptors triggering pattern-triggered immunity (PTI) and resistance proteins inducing effector-triggered immunity (ETI) upon detecting bacterial effectors. To control bacterial wilt in susceptible cultivars prompts the exploration of molecular strategies targeting susceptibility and resistance genes. In this study, transgenic lines of the potato cultivar Désirée were generated with antisense silencing of the susceptibility gene *WAT-1*, associated to auxin transport and tryptophan synthesis during infection and overexpression of the pathogenesis-related gene *OSML*-15, related with drought response during infection. The transgenic constructs, driven by the constitutive 35S promoter, were introduced into potato through *Agrobacterium tumefaciens*-mediated leaf transformation. Based on RT-qPCR measurements, three lines with reduced *WAT-1* expression and three lines with *OSML-15* overexpression were selected for testing against *Rs* in pot-grown plants. Disease index and mortality ratio were used to assess tolerance to *Rs*. While two *WAT-1* silenced lines showed a slight decrease in disease and mortality index compared to the control, the differences were not statistically significant in the 26-day test. In contrast, one *OSML-15* overexpressing line remained symptom-free even 45 days after infection, indicating potential tolerance to *Rs*. Further studies will focus on confirming the tolerance of this line, evaluating tuberization, and assessing *OSML-15* expression in roots under different stress conditions.

Keywords: *Antisense Gene silencing, Bacterial wilt, Désirée, Overexpression, and Tolerance*

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PF#P6

EXAMINATION OF HEAT-INDUCED FERROPTOTIC CELL DEATH

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A recent study described a heat-induced form of programmed cell death (PCD) called ferroptosis, which was first discovered in tumor cells. This type of PCD is primarily caused by iron-dependent lipid peroxidation. The accumulation of iron ions via the Fenton-reaction increases the levels of reactive oxygen species (ROS) and lipid peroxides, thereby causing oxidative stress. The characteristics of ferroptosis include the depletion of the antioxidant glutathione (GSH) pool and mitochondrial shrinkage.
The aim of our work was to investigate the involvement of membrane-attached glutathione peroxidase-like (GPXL) antioxidant isoenzymes in this process using *Arabidopsis thaliana* (L.) plants overexpressing the *AtGPXL5* gene. To achieve this, we optimized a method for specifically inducing this phenomenon. Six-day-old *Arabidopsis thaliana* Col-0 ecotype and AtGPXL5-overexpressing seedlings were pretreated with the ferroptosis inhibitors ciclopirox (CPX) or Ferrostatin-1 (Fer-1). This pretreatment was followed by a 10-minute exposure to a 55 °C heat shock. After three hours of further incubation at room temperature, H_2O_2 levels, cell viability, and GSH levels were examined by fluorescence microscopy. Lipid peroxidation was detected using Schiff's reagent. The expression of selected PCD-related genes was measured by quantitative real-time PCR.
Our results showed a drastic decrease in viability in both pretreated and control plants after the heat shock. H_2O_2 and GSH levels increased significantly during stress in both control and CPX-pretreated plants, while Fer-1 pretreatment prevented the elevation of lipid peroxidation. Overexpression of AtGPXL5 attenuated the increases in H_2O_2 and GSH levels after the 55 °C heat shock. The expression of the ferroptosis-marker genes *asparagine synthetase 2* and *3 (ASN2, ASN3)*, as well as the cation transport regulators *CCL2* and *CCL3*, and the cell death-inducing gene *kiss of death* (*KOD*) did not change in either the wild-type or the AtGPXL5-overexpressing plants. However, different endoplasmic reticulum (ER) stress-responsive genes, such as *bZIP28*, *BIP1*, *BIP3*, *IRE1a*, and the hypersensitive response factor *WRKY33*, showed significant changes in response to heat shock.
In conclusion, our results do not support the occurrence of ferroptosis in *Arabidopsis* seedlings after heat shock. Instead, they suggest the formation of a ferroptosis-like cell death. Moreover, AtGPXL5 may be an important factor in the stress responses of plants under high-temperature conditions.

Keywords: *ferroptosis, cell death, ROS, heat stress*

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PF#P7

INVASIVE PLANT, INVISIBLE COMPANIONS: THE SECRET VIROME OF ASCLEPIAS SYRIACA

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Asclepias syriaca L. (common milkweed) is an invasive perennial plant native to North America and widely distributed across Hungary, where it poses ecological and agricultural concerns. Despite its increasing prevalence, the viral diversity associated with this species remains poorly characterized.
To explore its potential role as a viral reservoir, *A. syriaca* asymptomatic and symptomatic leaves were collected from orchards and vineyards at different regions in Hungary. In total, 29 leaf samples were subjected to RNA extraction, DNase treated and three pools were prepared for sequencing based on location. A comprehensive characterization of the virome was performed using Ribodepleted RNA High-Throughput Sequencing (HTS). Sequenced reads were assembled and analyzed for viral signatures using all plant-infecting viral sequences available in the NCBI GenBank database as reference.
Bioinformatic analyses revealed a diverse virome comprising 13 plant viruses, including representatives of the families *Potyviridae*, *Tombusviridae*, *Rhabdoviridae*, and *Geminiviridae*. The presence of these viruses were validated using RT-PCR assays. Presence of most of the HTS-diagnosed viruses were validated, including economically important plant pathogens such as *Alfalfa mosaic virus* (AMV) and *Cucumber mosaic virus* (CMV). In addition, putative novel virus sequences showing low similarity to known taxa were identified, suggesting the presence of previously undescribed viral species. Some of the detected viruses were closely related to pathogens infecting economically important crops, indicating that *A. syriaca* may function as a potential reservoir host.
These findings provide a comprehensive insight into the *A. syriaca* virome diversity in Hungary and suggest that this invasive plant species may serve as a host or reservoir for a wide range of plant viruses with possible ecological and phytosanitary implications for surrounding agricultural systems.

Keywords: *plant virus, Asclepias syriaca, High Throughput Sequencing (HTS)*

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K.R. and L.D.P. are PhD students of the MATE DSAFS and DSNS, respectively.

PF#P8

DISSECTING THE ROLE OF WRKY AND MAPK KINASE FAMILY MEMBERS IN RESISTANCE BREEDING TO *RALSTONIA SOLANACEARUM* INFECTION IN POTATO

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Bacterial wilt or brown rot disease is caused by *Ralstonia solanacearum* (*Rs*), a significant soilborne phytopathogen. This bacterium enters the plant through the roots and spreads through the xylem vessels. Transcription factors such as WRKYs and MAP kinases (MAPKs) are crucial in responding to pathogens. However, limited research exists on MAPK signalling and WRKY transcription factors in potato defense against *Rs*. Previous studies showed downregulation of *WRKY22* and *WRKY24* in *Rs*-resistant potato cultivars 'Calalo Gaspar' (CG) and 'Cruza 148' (CR) upon *Rs* infection. Additionally, the expression of MAP kinase *MAPK9* and MAP kinase kinases *MEKK1* in CG, *MEKK7* in CR, and *MEKK EDR1* in both cultivars decreased after *Rs* infection, suggesting a negative role in *Rs* defense. Our study aims to investigate the role of MAPK9, MEKKs, WRKY22, and WRKY24 signalling during *Rs* infection in potato through gene silencing. Fragments of the selected genes were cloned in antisense orientation into the pCP60 vector for constitutive expression from the 35S promoter. Transgenic potato cultivar 'Désirée' plants were generated using *Agrobacterium*-mediated transformation with constructs 10WRKY22, 01WRKY22, 06WRKY24, 05MAPK9, 11MEKK7, and 12MEKK ERD1. Currently, we are assessing gene silencing levels in transgenic plants through RT-qPCR to identify plants with high repression levels. Selected plants will be propagated, potted, and evaluated for *Rs* resistance.

Keywords: *transcription factors, xylem vessels, negative regulators*

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PF#P9

UNRAVELING ASCORBATE TRANSPORT IN CHLOROPLASTS: INVESTIGATING THE ROLE OF PHT4;2 TRANSPORTER

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Ascorbate is an essential metabolite for both plants and animals. In plants, ascorbate plays a multifunctional role; however, to date, only two ascorbate transporters have been characterized in plants: AtDTX25 and AtPHT4;4. Since ascorbate has multiple functions in photosynthesis and the protection of the photosynthetic apparatus, it is surprising that *Arabidopsis thaliana* mutants lacking the chloroplast-localized PHT4;4 transporter do not show a clear phenotype, suggesting that PHT4;4 is not the only ascorbate transporter in the chloroplast envelope membrane. Therefore, the goal of this research is to identify additional ascorbate transporters in the chloroplast. Previous experiments on *pht4;4* mutant plants showed a significant increase in *PHT4;2* transcript levels under high light stress. A similar upregulation of *PHT4;2* expression was also observed in a mutant with low ascorbate content (*vtc2-4*). These results suggest that PHT4;2 may be involved in ascorbate transport into the chloroplasts, even though it has been reported to be more highly expressed in root plastids. To investigate the ascorbate transporter activity of PHT4;2, we used wild-type (Col-0), *vtc2-4, pht4;4-3,* and *pht4;2-1* single mutants and created homozygous double *pht4;4-3 x pht4;2-1* mutant lines, as confirmed via PCR. Then, we determined the rate of electron donation from ascorbate to PSII via chlorophyll *a* fluorescence to estimate the relative chloroplastic ascorbate content. A detailed characterization of photosynthetic activity under normal and high light conditions is being carried out in single and double *pht4;2-1 pht4;4-3* mutants. To this end, we use chlorophyll a fluorescence measurement (direct and modulated) to measure electron transport rates and determine the proton motive force in the chloroplast. Leaf samples are also collected for measuring total cellular ascorbate and anthocyanin contents by HPLC, phosphate and ATP contents, and for immunoblotting of various photosynthetic subunits. We also measure NPQ, which is highly sensitive to changes in chloroplastic ascorbate content. We would also like to express the PHT4;2 gene in yeast, check its ascorbate transport activity, and use confocal microscopy techniques to identify the exact location of the PHT4;2 transporter in chloroplasts. Based on these experiments, we expect to be able to conclude whether PHT4;2 acts as a chloroplastic ascorbate transporter. If confirmed, this would enhance our understanding of ascorbate transport mechanisms in chloroplasts and their role in photosynthesis and responses to oxidative stress.

Keywords: *Ascorbate, Chloroplastic ascorbate transporters, Chlorophyll a fluorescence, Non-photochemical quenching, Photosynthesis,* PHT4;2 *transporter*

Acknowledgement: *This work was supported by grants from the National Research, Development, and Innovation Office (K146791), the Momentum (Lendület) Program of the Hungarian Academy of Sciences (LP2024/21), and the Stipendium Hungaricum Fellowship Program. The authors thank Soujanya Kuntam and Gábor Rigó (Institute of Plant Biology, HUN-REN Biological Research Centre, Szeged) for scientific support.*

PF#P10

GENOME EDITING OF THE CML30-TYPE AND THE EFFECT OF ITS KNOCKOUT ON THE ANTHOCYANINS SPECTRUM IN POTATOES

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Signalling pathways involving calcium are essential for various cellular functions. Calcium (Ca2+) is a vital and dynamic component that acts as one of the key messengers in the biochemical processes of plant cells. A large group of proteins that act as Ca^{2+} chelators includes EF-hand domain proteins, among which are calmodulin-like proteins (CML).
Potatoes are one of the main components of many people's daily diet due to their valuable nutritional properties. In addition to the carbohydrate composition of potato tubers, potatoes are also valued for their content of high-quality proteins, vitamins, dietary fibre, minerals, anthocyanins and xanthophylls.
The development of certain qualitative characteristics in potential commercial varieties can be stimulated using traditional or molecular breeding methods. CRISPR-Cas9 genome editing tools can be used to enhance or suppress gene expression depending on their role in plant metabolism.
In this work, a CML30-type gene was used for genome editing of *Solanum tuberosum* Desiré potatoes. For this purpose, a plasmid vector based on pKSE401 was cloned. Eight lines of mutant plants were obtained, in which the knockout of the target gene was confirmed by T7 endonuclease assay and sequencing of the selected region. The tubers obtained from the harvest of mutant lines were characterised by a reduced content of anthocyanins in the skin, and a reduced content of anthocyanins was also noted in the leaves of these plants. Two of the eight lines studied had completely yellow potato skins, unlike the original pink ones. As it turned out, according to the results of analysis by electrospray ionization mass spectrometry (VION-TOF), the yellow colour was due to the absence of pelargonidin formation. The effect of Ca^{2+} concentration on changes in the composition of some types of anthocyanins has been described previously, but changes in the metabolic pathway of pelargonidin in potatoes were detected for the first time.

Keywords: *calmodulin-like proteins, Solanum tuberosum, pelargonidin*

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PF#P11

PGPR ISOLATED FROM THE ROOT ZONE OF HALOPHYTIC PLANT *PETROSIMONIA TRIANDRA* ENHANCE SALT TOLERANCE IN OTHER PLANT SPECIES

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Cultivated soils are negatively affected by rising heat and declining rainfall caused by global warming. These can result in harmful effects like salinization and salt accumulation, which reduces the yield by inhibiting the absorption of water and nutrients of the plants. Plant breeding and biotechnology programs focus on creating crop plants more tolerant to stress resulting from extreme weather conditions, alternative strategies can be developed to reduce these damaging effects. Plants can naturally adapt to adverse environments like salty soils, by developing different survival strategies. One possible mechanism is the formation of a community between their roots and salt-tolerant bacteria found in saline soils. Bacteria inhabiting the root zone of halophytic plants can alleviate damaging effects of salinity are collectively referred to as plant growth-promoting rhizobacteria (PGPR). Many PGPR strains enhance plant growth by increasing salt or drought tolerance, promoting root development, or stimulating the production of osmoprotectants and protective proteins. We have isolated 24 bacterial strains from the rhizosphere of the halophytic *Petrosimonia triandra*, which grows on saline soils in Cluj County, Romania. These bacterial strains could be cultivated *in vitro* on medium supplemented with 2 M NaCl. We identified the bacterial strains based on 16S rRNA gene sequencing, and tested their plant growth-promoting effects using *Arabidopsis thaliana* and *Brassica napus* (rapeseed). Six bacterial strains were found to promote *Arabidopsis* root and shoot growth in *in vitro* conditions on medium containing 125 mM NaCl. Additionally, we tested the root and shoot development of rapeseed plants in greenhouse experiments and found that some bacterial strains enhanced plant growth in saline conditions. Our preliminary data obtained with *Arabidopsis* and rapeseed suggest that several of the isolated PGPR strains improve salt tolerance of higher plants.

Keywords: *global warming, salinization, PGPR, Arabidopsis, rapeseed*

Acknowledgement: *NKFI ADVANCED-151222.*

PF#P12

ARBUSCULAR MYCORRHIZAL INOCULATION AND SUSCEPTIBILITY OF TOMATO TO A. ALTERNATA, S. SCLEROTIORUM OR E. NEOLYCOPERSICI

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Arbuscular mycorrhizal fungi (AMF) establish a pervasive mutualistic symbiosis with the roots of most terrestrial plants, widely recognized for enhancing crop sustainability. The primary advantage of this association lies in improved water and nutrient acquisition – particularly the uptake of immobile soil phosphates and nitrogen – and heightened resilience against abiotic stresses like drought and salinity. However, the benefits of this interaction extend beyond growth promotion to include Mycorrhiza-Induced Resistance (MIR). Despite this potential, the protective efficacy of MIR is not universal. Since necrotrophic pathogens and biotrophic pathogens exploit different host vulnerabilities, it remains unclear how AMF-mediated modulation of defence pathways influences these contrasting infection strategies. This study evaluates whether AMF inoculation provides broad-spectrum protection or acts differentially against these divergent fungal lifestyles.
To investigate this topic, tomato (*Solanum lycopersicum* L.) seeds were germinated and grown in sterilized substrate (1:2 sand and peat) under controlled greenhouse conditions. Half of the plants were inoculated with a mixture of *Rhizophagus intraradices* and *Rhizophagus clarus* (AMF+) using standard inoculation procedures, while the other half were left uninoculated as controls (AMF-). After 5 weeks, the tomato plants (AMF+ and AMF-) were exposed to either *Alternaria alternata*, *Sclerotinia sclerotiorum* or *Erysiphe neolycopersici* infection to simulate biotic stress conditions. Plants were harvested after 1 and 2 weeks of growth to evaluate vegetative growth parameters. Disease severity was determined by quantifying conidial production using a Bürker counting chamber. Additionally, the extent of AMF root colonization was assessed to verify symbiotic establishment.

Keywords: *arbuscular mycorrhizal fungi, fungal pathogen, necrotrophic fungi, biotrophic fungi*

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PF#P13

IMPROVING CATIONIC POLYMER–BASED DNA DELIVERY IN MAIZE PROTOPLASTS

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Maize (*Zea mays*) is one of the most important crops in agriculture, both domestically and globally. It plays an important role as a food, feed, and industrial raw material, but due to climate change, increasingly frequent and intense dry and hot periods are causing significant crop yield losses and food security problems. Therefore, the development of more stress-tolerant maize varieties is becoming an even more important task today. Precision gene editing techniques, such as CRISPR/Cas9 system or oligonucleotide directed mutagenesis (ODM), offer new opportunities for the targeted improvement of drought-tolerance traits.
The aim of our work is to develop and optimize an efficient maize protoplast-based DNA delivery method using cationic polymer for maize cell lines capable for regeneration. During our research, we successfully optimized protoplast isolation protocol that resulted in viable and proliferating *calli* from which we were able to regenerate plants. To monitor DNA uptake and help the selection of the successfully transformed cells, GFP-expressing plasmid was co-transformed with oligonucleotides. The transformation resulted in GFP-positive, dividing *calli*. GFP fluorescence proved to be a more reliable indicator of DNA uptake than FAM-labeled oligonucleotides, which produced intense fluorescent signals mainly in damaged or dead cells. The optimized system provides an opportunity to increase the efficiency of the delivery of plasmid-based CRISPR constructs and oligonucleotides encoding target mutations into maize protoplasts. The development of a protoplast-based transformation platform may open up new opportunities for targeted gene editing applications in maize. In the future, this method may contribute to the production of varieties that are more resistant to environmental stresses, especially drought.

Keywords: *maize, ssDNA, CRISPR, protoplast, cationic polymer*

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PF#P14

ASSESSMENT OF ESSENTIAL COMPOUNDS IN *IN VITRO* MICROPROPAGATED ORIGANUM PLANTS

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Origanum vulgare L. (*Lamiaceae*) is an important medicinal and aromatic plant used widely in culinary, pharmaceutical and agricultural applications. This study aimed to establish an efficient *in vitro* micropropagation protocol for *O. vulgare* and to compare bioactive compound profiles among *in vitro*-derived, adapted, and seed-germinated plants. The experiment was conducted in two stages: shoot induction and rooting. During shoot induction stage, internode explants were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of 6-Benzylaminopurine (0, 0.5, 1, and 1.5 mg/L BAP) with or without 0.25 mg/L Indole-3-butyric acid (IBA). The results reported no significant effects on the regeneration percentage (%), number of nodes and leaves. However, the highest regeneration percentage (85%) was achieved at 0.5 mg/L BAP + 0.25 mg/L IBA. The maximum shoot elongation (8.57 ± 2.9 cm) was observed at 1.5 mg/L BAP. Unwanted calluses were formed and induced the highest at 0.25 mg/L IBA (7.20 ± 1.7). Root development during the shoot stage was most effective at 0.25 mg/L IBA (10.00 ± 1.6 roots). For the rooting stage, the effect of IBA at three concentrations (0, 0.25, and 0.5 mg/L) were studied. IBA did not show a significant effect on the number of roots and length of roots. Phytochemical analysis showed no significant differences in the total phenolic or flavonoid content among *in vitro*, adapted and seed-germinated plants. However, seed-germinated plants showed the highest antioxidant capacity (95.99 ± 20.06 µmol As/g).

Keywords: *Origanum vulgare, in vitro micropropagation, bioactive compounds, shoot induction stage, rooting stage, 6-Benzylaminopurine (BAP), Indole-3-butyric acid (IBA), Total Phenolic Content, Total Flavonoid Content, Antioxidant Capacity, in vitro plants, adapted plants, seed-germinated plants.*

PF#P15

GIBBERELLIN-OXIDASE GENE EXPRESSION AND ITS ASSOCIATION WITH STEM ELONGATION IN TTI (TORTUOUS INTERNODII) MUTANT PEPPER

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A decumbent stem is one that lies or trails along the ground for most of its length before turning upward at the tip. Irregular stem elongation can also produce such a growth habit, which may appear disadvantageous in cultivation, yet it can be exploited in alternative horticultural systems. The *tti* plants exhibit elongated hypocotyls and develop long, spiraling, slender stems; although abnormal, this stem morphology may offer potential for ornamental use or vertical cultivation.
Gibberellins are known to play a major role in internode elongation. Mutations affecting genes involved in gibberellin biosynthesis often result in substantially longer stems compared to wild types, which may also compromise stem rigidity. Notably, the GA3ox and GA20ox enzymes act in the cytoplasm to convert biologically inactive gibberellins into their active forms. Overexpression of the genes encoding GA20ox and GA3ox has been shown in several plant species to promote stem elongation.
In our study, we compared the expression levels of these genes across three stem regions of the *tti* mutants and evaluated their relationship with stem length and thickness. We found that *GA3ox*, *GA20ox*, and *GA20ox1* were all overexpressed in the *tti* mutants; however, this overexpression was consistently restricted to the basal stem region. This pattern is positively associated with stem length, as our measurements confirmed that *tti* plants develop significantly longer internodes than the controls. In contrast, we found no difference in stem thickness between *tti* and control plants, indicating no clear relationship between gene expression and stem diameter.
These findings suggest that the spatially restricted overexpression of gibberellin-biosynthetic genes underlies the characteristic elongated, spiraling stem morphology of the *tti* mutants. Such a growth habit, although atypical, could be exploited in specialized cultivation systems where flexible or elongated stems are advantageous. The unique stem architecture may also hold ornamental value or serve as a model for studying hormone-regulated internode development in crop improvement programs.

Keywords: *gibberellin, stem-mutation, pepper*

PF#P16

INVESTIGATION OF TOXICITY AND APLICABILITY OF TWO *SOLANUM LYCOPERSICUM* L.-DERIVED DEFENSINS

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The spread of fungicide-resistant phytopathogenic fungi poses serious problem in agriculture. Due to the decreasing effectiveness of chemical-based pesticides, and their strict regulation by the EU, new environmentally friendly antifungal strategies need to be developed. Plant-derived defensins represent promising biofungicide candidates because of their board-spectrum antifungal activity against several filamentous fungi at relatively low inhibitory concentrations, as well as their lack of cytotoxicity to plants, and mammalian cells.
Our research group identified and heterologously produced two *Solanum lycopersicum* L.-derived defensins (B1N680 and K4CBP6). These recombinant defensins inhibited the growth of phytopathogenic fungi, such as *Botrytis cinerea*, *Cladosporium herbarum*, *Trichoderma harzianum*, and several *Fusarium* species at low minimum inhibitory concentrations (MIC: 6.25 – 25 μg ml^{-1}). These findings indicate that B1N680 and K4CBP6 could be promising biofungicides; however, to support their broader agricultural application, further investigations into their toxicity and applicability are required.
In this study we monitored the toxicity of these defensins on tomato (*Solanum lycopersicum*) and *Medicago truncatula* seeds, as well as on tomato fruits and leaves. Furthermore, we examined the applicability of B1N680 and K4CBP6 on tomato fruits against *C. herbarum* and on detached tomato leaves against *B. cinerea*. According to our results the defensins at a concentration of 400 μg ml^{-1} did not impair primary and lateral root development of *Medicago truncatula* seedlings, although they slightly reduced the primary root length of *S. lycopersicum* seedlings. B1N680 and K4CBP6 showed no toxicity on tomato leaves and fruits even at 400 μg ml^{-1}. Moreover, the defensins inhibited the spread of *B. cinerea* on tomato leaves and *C. herbarum* on tomato fruits. Based on these results, B1N680 and K4CBP6 appear to be promising biofungicides, due to their lack of toxicity and *in planta* antifungal activity.

Keywords: *plant defensin, antifungal activity, toxicity, phytopathogenic fungi, Solanum lycopersicum L.*

Acknowledgement: *Present work of L.G. was financed by the Hungarian National Research, Development and Innovation Office - NKFIH, K 146131 project.*

PF#P17

SMALL PARAQUAT RESISTANCE (SPQ) PROTEIN REGULATES ABIOTIC STRESS RESPONSES IN ARABIDOPSIS

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Plants face significant challenges imposed by abiotic stresses, such as drought, salinity, and oxidative stress, often resulting in the unavailability of essential nutrients due to reduced movement and uptake, as well as competition with other nutrients in the soil, which severely affect plants' health and productivity. Iron is an essential micronutrient for plant development and is required in many enzymatic processes involved in respiration and photosynthesis, and heme biosynthesis, and thus tightly regulated. Small Paraquat Resistance (SPQ) protein is a novel protein identified in the salt- and drought-tolerant plant *Lepidium crassifolium*. To study the gene function, SPQ overexpression lines tagged with either GFP or HA were generated, and a T-DNA insertion mutant was obtained from the SALK mutants' collection. Another mutant allele was generated via CRISPR/Cas9 mutagenesis.
Subcellular localization of GFP-tagged SPQ showed that the fusion protein is associated with the endomembrane system, including the vacuolar membrane as well as chloroplasts, suggesting its involvement in diverse cellular processes. In Arabidopsis, SPQ overexpression confers drought, salt, and paraquat tolerance. RNA sequencing suggested that the protein can be involved in iron homeostasis via the regulation of group Ib bHLH transcription factors and the shoot-specific lncRNA CAN OF SPINACH, both implicated in iron metabolism.
Our results suggest that SPQ is involved in the regulation of various stress-response pathways, partly through iron regulation. These insights display SPQ as a promising candidate for engineering abiotic stress resilience in crops.

Keywords: *Arabidopsis, Lepidium, drought, salinity, iron metabolism, mutant,*

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PF#P18

PURPLE VS. GREEN *CAPSICUM ANNUUM* LEAVES RESPONSE TO *ALTERNARIA ALTERNATA* IN DETACHED LEAF ASSAY

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Anthocyanins are phenolic compounds with strong antioxidant activity, helping to mitigate damage caused by reactive oxygen species (ROS) and contributing to antifungal defense mechanisms. Pepper (*Capsicum annuum*) is susceptible to fungal pathogens such as *Alternaria alternata*, the causal agent of black mold. Previous studies have indicated that anthocyanin pigments may inhibit this pathogen. The 'Azteco' genotype of *C. annuum* exhibits a natural variation in pigmentation, producing anthocyanin-rich purple leaves and green leaves within the same plant. This unique characteristic provides an excellent model to evaluate the potential protective role of anthocyanins against *A. alternata*. The aim of this study was to determine whether anthocyanin-rich tissues exhibit enhanced resistance to *A. alternata.*
Purple and green leaves from the same *C. annuum* 'Azteco' plants were selected, disinfected, and inoculated with *A. alternata*. Infected leaves were incubated for 1, 3, and 6 days. Biochemical analyses included total monomeric anthocyanin content, total polyphenolic content (TPC), and antioxidant capacity (FRAP). Enzymatic antioxidant responses were assessed by measuring superoxide dismutase activity and catalase and peroxidase (POD) activity. Fungal and plant DNA quantification was performed to evaluate pathogen proliferation. Gene expression analysis was focusing on genes involved in the polyphenolic biosynthetic pathway. ROS production and localization were visualized using Nitroblue Tetrazolium and 3,3'-Diaminobenzidine staining.
Purple leaves exhibited consistently higher polyphenolic content and antioxidant capacities than green leaves across all sampling days. Peroxidase activity (POD) increased sharply in purple leaves from day 0 to day 3 and remained high at day 6. Green leaves responded less intensively, exhibiting lower POD values at both day 3 and day 6, with all between-colour differences significant ($p < 0.05$), indicating stronger ROS-associated signalling in purple tissues. Quantification of fungal colonisation based on the ratio of fungal to pepper genomic DNA revealed a colour-dependent difference. Green leaves harboured 30–80-fold higher fungal genomic DNA compared with purple leaves. The difference between the two colours was statistically significant ($p < 0.05$), demonstrating lower *Alternaria alternata* proliferation in purple leaves under detached-leaf assay conditions. Taken together, purple leaves demonstrated higher antioxidant capacity, stronger oxidative enzyme activity, and drastically reduced fungal colonisation relative to green leaves, suggesting that anthocyanin pigmentation contributes strongly to enhanced resistance against *A. alternata*.

Keywords: *anthocyanin, artificial infection, pepper, alternaria*

PF#P19

OPEN-FIELD TRIALS OF MYCORRHIZAL AND BACTERIAL INOCULATION ON YIELD AND SOIL PARAMETERS IN SUNFLOWER PRODUCTION IN DIFFERENT SOIL TYPES

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Sunflower (*Helianthus annuus* L.) is a globally significant and commercially valuable arable crop of major economic importance in agriculture worldwide. However, modern agricultural challenges, including climate change, soil degradation, water management, and the use of synthetic chemicals, such as pesticides and fertilisers, make alternative solutions necessary to improve productivity besides cost effectiveness. This study aims to investigate an environmentally friendly and sustainable variation through the application of a commercial, seed-coating product containing mycorrhizal fungus (*Rhizoglomus irregularis*) and beneficial soil bacteria (*Azospirillum* sp.) in field conditions.
Trials were conducted to evaluate the consortium's effects on sunflowers in three distinct soil types through integrated chemical and microbiological assessments, like the evaluation of mycorrhizal root colonisation and the measurement of plant growth performance. Soil functional diversity was characterised using Biolog EcoPlate™, while leaf enzymatic activity provided insight into plant physiological responses.
Microbial inoculation visibly enhanced sunflower growth parameters (plant height, stem diameter, leaf number, head diameter) and increased yields compared to untreated controls. Responses differed among the three soil types at different Hungarian sites, indicating location-specific effects. Final analyses of seed quality parameters, leaf enzymatic activities and nutrient dynamics are in progress.
These findings indicate that applied microbial product can enhance crop productivity and most likely the quality through enhanced nutrient uptake efficiency, but the effectiveness of the treatment varies according to soil type. Our results may help to expand the possibilities for applying mycorrhizal and beneficial soil bacteria inoculants and increase our knowledge under open-field conditions.

Keywords: *Helianthus annuus* L.*, mycorrhiza, beneficial soil bacteria, yield enhancement, crop quality, soil-microbe interaction*

Acknowledgement: *This research was funded by the Agri-biotechnology and Precision Breeding for Food Security National Laboratory, grant number RRF-2.3.1-21-2022-00007; and supported by the Flagship Research Groups Programme of the Hungarian University of Agriculture and Life Sciences.*

PF#P20

INTRODUCTION OF TRANSFORMING VECTOR AND MUTAGENIC SYNTHETIC OLIGONUCLEOTIDES INTO MAIZE APICAL MERISTEMS

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The shoot apical meristem (SAM) of maize is an explant that may be particularly useful for gene editing. Since it can be isolated from young shoots sprouted from readily available mature seeds, maize genotypes resistant to *in vitro* culture can be also targeted. SAM contains in the L2 layer the developmental precursors to the plant's germ cells (pollen and ovules) therefore gene editing events may be heritable. The aim of the research is to develop a gene editing method that can be effectively applied to the shoot apical meristem of maize.
Gene editing in maize is performed using two methods. One method is the Agrobacterium-mediated delivery of the CRISPR/Cas9 system, which can allow gene knockout with acceptable efficiency. Transformation is followed by fluorescence microscopy as the vector expresses *mCherry* gene, too. Red fluorescent cells can be detected in the meristem of the explants; however, the results show that the dividing central cells are negative.
The other method is Oligonucleotide-Directed Mutagenesis (ODM), which is suitable for introducing precise mutations. The oligonucleotides encoding the mutation were delivered into SAM using sugar-mediated uptake. With this method, oligonucleotide molecules accumulated predominantly in the cell walls. Oligonucleotides were observed in the nucleus only in a few cases, and only in non-viable cells.
These observations indicate that the accessibility of the inner cells of the apical meristem is significantly limited, and that further experiments will require the application of alternative methods or treatments.

Keywords: *maize apical meristem, CRISPR, ODM*

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PF#P21

GRAPEVINE-ASSOCIATED BACTERIA AND YEASTS IN CONTRASTING VINEYARD SYSTEMS

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The study aimed to characterise the microbial diversity of wine grapes (*Vitis vinifera* L.) in two distinctly managed vineyards (conventional and organic) in the Badacsony wine region, Hungary. Sampling included two white grape varieties: the locally characteristic Kéknyelű (blue steam) and the globally cultivated Pinot Gris, both grown under both management systems. Sampling in late May targeted inflorescences and shoots/leaves, while in September, additional samples were collected from berries, along with shoots and leaves. The working hypothesis was that microbial abundance and community composition differ among plant tissues, grape varieties, and cultivation systems, driven by the effects of vineyard management practices.
In 2025, a total of 361 microorganisms were isolated from the two sampling periods and subsequently incubated under aerobic and anaerobic conditions on various culture media. Based on colony-forming unit (CFU) counts, higher microbial biomass was observed in the case of shoots and leaves compared to berries in the Kéknyelű variety, whereas the opposite trend was detected in Pinot Gris, where berries exhibited higher CFU values than shoots and leaves. In case of the isolates, 191 (52.9%) were identified using Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight mass spectrometry (MALDI-TOF MS). Preliminary bacterial data indicated that the dominant genera were *Bacillus*, *Pseudomonas*, *Lactococcus*, *Pantoea*, and *Weissella*. *Lactococcus* and *Pseudomonas* were primarily associated with inflorescences, whereas *Bacillus*, *Weissella* and *Pantoea agglomerans* were more frequently isolated from shoots and leaves. Yeast communities were dominated by *Aureobasidium*, followed by *Hanseniaspora*, *Metschnikowia*, and *Filobasidium*.

Keywords: *grapevine, conventional, organic, microbiome, bacteria, yeast, colony-forming unit, MALDI-TOF MS*

Acknowledgement: *This work was supported by the Flagship Groups Research Programme of the Hungarian University of Agriculture and Life Science.*

Bioinformatics

BI#PLEN

DATA-TO-DIAGNOSIS: INTEGRATING GENOMICS, BIOINFORMATICS, AND CLINICAL WORKFLOWS

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Modern clinical laboratories increasingly rely on high-throughput molecular testing, yet the diagnostic value ultimately depends on how efficiently wet-lab assays are linked to robust bioinformatics and clinically interpretable reporting. Here we present a modular, end-to-end 'data-to-diagnosis' framework established at the University of Pecs that integrates sample-to-result molecular genetics workflows (qPCR/ddPCR and NGS) with standardized dry-lab pipelines across infectious disease surveillance, oncology, and reproductive genetics.
The workflow covers harmonized preanalytics (decontamination; DNA/RNA extraction from liquid and solid matrices; manual or automated handling), assay execution (multiplex RT-qPCR, fluorescent probe-based detection, droplet digital PCR quantification), and sequencing-based applications. For low-input cfDNA applications, we implemented whole-genome amplification strategies followed by Illumina NovaSeq sequencing (2x150 bp). Bioinformatics processing includes read-level QC (FastQC), adapter and quality trimming (Cutadapt/Trim Galore), alignment to GRCh37 (BWA-MEM), BAM processing (SAMtools), copy-number variation calling against reference controls, and functional interpretation using curated resources (UNIQUE, Genetic Alliance, CDO). Outputs are delivered as karyograms, QC dashboards, and structured reports; for surveillance and multimodal use cases, results are linked to phylogenetic platforms (Nextstrain) and to radiology-derived 3D models (3D Slicer) combined with molecular biomarkers (e.g., miRNA field alterations).
In regional SARS-CoV-2 diagnostics and monitoring, the integrated pipeline enabled standardized RT-qPCR testing and spatial-temporal tracking via an interactive dashboard. In reproductive genomics, the non-invasive preimplantation workflow was applied to spent embryonic culture media from IVF-PGD cycles, with ddPCR-based discrimination of embryonic cfDNA from contaminating DNA and downstream NGS-based CNV profiling. Analytical sensitivity versus trophectoderm PGD was 88.2-94.7% depending on WGA strategy, with diagnostic performance of 83.3-91.7%, and 95.8% concordance between amplification pipelines. Overall, this integrated framework improves reproducibility and turnaround while enabling scalable, clinically governed deployment of genomics and bioinformatics from routine diagnostics to translational research.

Keywords: *clinical genomics, bioinformatics pipeline, cell-free DNA*

Acknowledgement: *RRF-2.3.1-21-2022-00012 (National Laboratory of Human Reproduction, Hungary); Thematic Excellence Program 2021 Health Sub-programme of the Ministry for Innovation and Technology in Hungary, within the framework of the EGA-13 project of the University of Pécs; EU-RESPONSE Consortium.*

BI#E1

COMPUTATIONAL MODELING OF AUXIN-BINDING ABILITY IN PHI CLASS GLUTATHIONE TRANSFERASES

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Glutathione transferases (GSTs, EC 2.5.1.18) are a heterogeneous superfamily of proteins involved in various intracellular processes. Their most characteristic feature is their glutathione-dependent activity, as these enzymes facilitate the nucleophilic attack of reduced glutathione (GSH) on the electrophilic sites of numerous molecules. GSTs are regulated by diverse stimuli, including environmental factors (such as biotic and abiotic stresses), and their activity is activated through stress signaling - often mediated by phytohormones. Notably, GSTs may also modulate phytohormone levels, as certain members, particularly within the Phi class (GSTFs), have been shown to directly bind small phytohormones like auxin. However, the structural details of these interactions remained unclear. It has been shown through X-ray crystallography that an *Arabidopsis thaliana* GSTF, AtGSTF2, contains unique non-catalytic ligand-binding sites (L1 and L2 sites). In our work, we focused on the the potential link between this non-catalytic ligand-binding function and phytohormones. During this analysis, we investigated the potential non-catalytic auxin-binding ability of the previously mentioned AtGSTF2 along with the orthologous tomato (*Solanum lycopersicum*) GSTFs (SlGSTFs). Using *in silico* methods, including protein modeling with AlphaFold, molecular docking (GNINA), and molecular dynamics (MD) simulations (OpenMM engine and AMBER force fields), we provide the first structural evidence for the possible non-catalytic auxin-binding ability of GSTFs in their L sites. Specifically, our findings demonstrate the specific binding of indole-3-acetic acid to the L1 site of AtGSTF2 and indole-3-butyric acid to the L2 site of SlGSTF5. These results suggest a novel phytohormone-binding function, thereby expanding the known roles of plant GSTs.

Keywords: *protein-ligand interactions, molecular docking, molecular docking simulations*

Acknowledgement: *This study was funded by the Hungarian National Research, Development and Innovation Office (Grant Number: NKFIH K 138589).*

BI#E2

ROLE OF IN SILICO METHODS THROUGH REVEALING THE EFFECTS OF MOLECULAR INTERACTIONS BETWEEN BIOMOLECULES AND LIGANDS IN PREDICTION OF PROTEIN FUNCTIONS

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Recent advancements in informatics have made widespread application of *in silico* methods for biological research possible. Introduction of AI-based algorithms and improvement of computer performance specifically let us make more accurate predictions of how supra- and infra-individual biological systems work.
The functionality and structural stability of proteins heavily depends on the possible intra- or intermolecular interactions between the amino acid sidechains and other ligands or heteroatoms. Changing one or more amino acid with mutation can change the whole protein's functionality, blocking certain interactions or making them possible.
Computational methods let us not just model the 3 dimensional structure of proteins, but also understand the interactions that stabilize these structures. Therefore, the functional difference between protein variants can be predicted with them.
Several of these methods are usually combined to build system that can be used to simulate molecular interactions. Molecule modelling lets us predict the secondary and tertiary structure of proteins, and with docking, we can assemble several peptide chains into a protein complex. Molecular dynamics can be used to simulate the movement and gyration of the molecule's atoms and bonds in an explicit solvent.
Below are three examples of *in silico* modelling of the molecular interactions of proteins:
1) Dimerization and DNA-binding of *Brachypodium distachyon* LBD transcription factors. In a previous work, we started to model the LBD protein family of *Brachypodium distachyon* (purple false brome), a model organism of monocot plants. Molecular dynamics was used to determine whether the examined proteins function as homo- or heterodimers (and in the latter case, which other LBD protein they dimerize with) and how they bind to the DNA.
2) The simulation of certain mutations in the Rubisco activase enzyme of *Zea mays*. Ribulose-1,5-bisphosphate (RuBP) carboxylase-oxygenase (Rubisco) enzyme is the limiting step of photosynthetic carbon fixation, and its activation is regulated by its co-evolved chaperone, Rubisco activase (Rca). We were investigating the distal effects of mutations in the conserved Walker A and Walker B regions of the ATPase on the hexameric structure of the protein, that were also important for the increased Rubisco activase function of the mutant proteins at elevated temperature.
3) The investigation of certain phosporylation sites of the E2F-DP-RBR protein complex of *Arabidopsis thaliana*. It was previously demonstrated that hyper-phosphorylation of RB controls its interaction with E2F and inhibits its tumor suppressor properties. In silico methods can be used to reveal how RB activation signals are integrated in a phosphorylation code that determines the diversity of RB activity.

Microbial biotechnology

MI#E1

PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF MAIZE (*ZEA MAYS* L.) TO TOXIGENIC AND ATOXIGENIC STRAINS OF ASPERGILLUS FLAVUS UNDER FIELD CONDITIONS

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Aflatoxin contamination by toxigenic *Aspergillus flavus* remains a significant constraint to maize (*Zea mays* L.) production due to its impacts on food safety, grain quality, and human and livestock health. The deployment of atoxigenic strains as biological control agents has emerged as a promising field-based strategy for reducing aflatoxin accumulation. This study evaluated the physiological and biochemical responses of maize inoculated with toxigenic and atoxigenic strains of *A. flavus* under field conditions. A complete randomised design was used with three treatments: control (no inoculation), inoculation with a toxigenic strain, and inoculation with an atoxigenic strain. Measurements included kernel number per ear length, fungal counts (log CFU/g), aflatoxin B1 (AFB1) concentration, starch, protein, and total polyphenol content of the kernels. Results demonstrated that inoculation with the toxigenic strain significantly increased fungal proliferation and AFB1 accumulation, while reducing kernel number per ear length and starch content. In contrast, the atoxigenic strain resulted in significantly low AFB1 levels, comparable to the background contamination of the control group, and showed improved kernel production and substantially higher starch content ($p < 0.05$). Irrigation enhanced protein and polyphenol accumulation ($p < 0.05$), while nitrogen rate showed minimal influence on biochemical parameters ($p > 0.05$). These results provide in-field evidence that, in addition to its anti-aflatoxigenic activity, the atoxigenic strain interacts with the maize plant differently from the toxigenic strain and affects the kernel development.

Keywords: *aflatoxin, Aspergillus flavus, atoxigenic, kernel development*

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MI#E2

SENSING PROTEIN DEVELOPMENT USING CLICK DISPLAY TECHNOLOGY

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The development of artificial binding proteins using directed evolution is a significantly evolving field due to their broad range of applications in biosensors, disease diagnosis and control and research. The stable D3 domain of Salmonella flagellin serves as an excellent scaffold protein for developing specific binding variants through directed evolution, which, when reassembled, form nanorods with high binding site density and can be employed in various applications such as sensing elements in biosensors, virus neutralizers, targeted drug development, affinity purification methods and diagnostic procedures for the specific detection and selection of particular components from complex biological samples. Click display, a novel protein display method, overcomes limitations of traditional display techniques, allowing in vitro selection from large protein libraries. In this project, click display is used to select and further develop specific binding proteins entirely in vitro from a protein library derived from the D3 domain of Salmonella flagellin. A model system using anti-GFP-sfGFP is established to optimize click display experimental conditions.

Keywords: *Binding proteins, sensing, D3 domain, flagellin, click display, sfGFP, aGFP*

MI#E3

INTEGRATED ASSESSMENT OF EXTRACELLULAR ORGANIC MATTER-ENHANCED BIOREMEDIATION IN USED LUBRICANT OIL-CONTAMINATED SOILS: MICROBIAL RECOVERY, HYDROCARBON REMOVAL AND SPECIES-SPECIFIC TOXICITY RESPONSES

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Used lubricant oil (ULO) contamination introduces a wide range of hazardous compounds into soils, resulting in persistent environmental deterioration and impaired soil functionality. Under such stress conditions, soil microorganisms, including hydrocarbon degraders, may transition into a low- or zero-activity viable but non-culturable (VBNC) state. Therefore, sustaining microbial activity or reactivating VBNC cells is essential for efficient bioremediation. Resuscitation-promoting factor (Rpf)-containing extracellular organic matter (EOM) derived from *Micrococcus luteus* has previously shown early-stage stimulatory effects in ULO-biodegradation. In the present study, we aimed to enhance the biostimulation (BS) treatment of ULO-contaminated *ex situ* soil microcosms by supplementing EOM at the beginning of the experiment (Day 0) and again following the completion of the first biodegradation phase (Day 20), when the initial EOM effect had diminished. After 60 days, the initial extractable petroleum hydrocarbon concentration (EPH=30,300 mg/kg) was reduced by 46% in soils treated with a single EOM application (BS+EOM), whereas sequential EOM treatment (BS+2×EOM) resulted in a more pronounced 56% reduction. In comparison with the control treatments, sequential EOM supplementation also led to elevated soil respiration and higher colony-forming units. To evaluate soil toxicity, an integrated assessment was applied, including plant germination tests, collembolan survival and soil enzyme activites. Despite improved ULO bioconversion and increased microbial and enzymatic activities, the germination index of oilseed rape (*Brassica napus* L.) and the vitality of root apical meristems declined in all biostimulated treatments. In contrast, EOM supplementation significantly improved the survival rate of the collembolan *Folsomia candida*. Overall, the results indicate that sequential EOM application can effectively boost microbial activity, microbial biomass, thereby accelerating hydrocarbon bioconversion, while yielding species-specific ecotoxicological responses: soil fauna benefited from remediation progress, whereas plant-based phytotoxicity remained elevated. It is essential to emphasize that ecotoxicological assessments should not rely on a single test organism. Evaluating multiple species and applying an integrated toxicity assessment provide a more comprehensive and reliable understanding of remediation success in complex hydrocarbon-polluted soils.

Keywords: *used lubricant-polluted soils, environmental rehabilitation, extracellular organic matter, ecotoxicology*

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MI#E4

RHIZOSPHERE MICROBIAL DYNAMICS ACROSS THREE MENTHA SPECIES AND THEIR ASSOCIATION WITH SOIL TRAITS AND ESSENTIAL OIL COMPOSITION

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Mentha species are rich in essential oils (EOs) and valued for their antioxidant properties, low toxicity, and strong antimicrobial activities. Although *Mentha* produces diverse EOs and secondary metabolites that can influence soil microbial composition, their effects on rhizosphere microbiota and their relationships with soil physicochemical properties remain poorly understood. To address this gap, we investigated the rhizosphere microbiota of three closely related taxa: *Mentha* × *villosa* (B10), *M. spicata* (B17), and *M. suaveolens* (J17), which were grown under uniform field conditions. Rhizosphere and bulk soils were analyzed for physicochemical parameters, microbial community composition (16S rRNA and ITS sequencing), essential oil profiles (GC–MS), and arbuscular mycorrhizal (AM) colonization.
The three *Mentha* species differed significantly in soil chemical properties and EO composition, which strongly influenced their rhizosphere microbial communities. Despite their close genetic relatedness, each species hosted a distinct microbial assemblage: *M. suaveolens* J17 exhibited the highest bacterial diversity, associated with its γ-terpinene–rich EO profile, whereas *M. spicata* B17 showed the most divergent community, with the lowest bacterial diversity but the highest AM colonization. Microbial community structure was strongly associated with humus content, phosphorus, potassium, and sodium levels. Correlation analysis revealed that humus and exchangeable sodium were positively associated with bacterial richness, whereas fungal diversity showed weaker and sometimes negative associations with pH and sodium. Redundancy analysis further demonstrated that humus, available phosphorus, and potassium were the strongest predictors of both bacterial and fungal community structure. Integration of EO profiles revealed clear genotype-dependent chemical signatures: B10 and B17 were rich in L-carvone and limonene, whereas J17 was dominated by cis-piperitone epoxide and piperitenone oxide. These chemical patterns were significantly linked to microbial variation.
Together, these findings demonstrate that subtle differences in plant chemical traits and soil properties can drive pronounced shifts in rhizosphere microbiota, even among closely related *Mentha* cultivars. This work highlights how EO chemistry, soil nutrients, and AM fungi interact to structure microbial communities in aromatic plant systems.

Keywords: *Rhizosphere Microbiome, Mentha Species, Essential Oil Composition*

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MI#E5

MICROALGAE POPULATIONS ARE GROWING DIFFERENTLY THAN EXPECTED

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The growth of bacterial populations has been described as a dynamic process of continuous reproduction and cell death. Unfortunately, still nowadays the population growth is characterized according to Malthusian principles from back in 1798. However, this is far from the reality. In a well fed, growing bacterial population, the stationary phase inevitably occurs, and it is not due to accumulated toxins or persistent cell death. In the nature a fast reproducing population spends the most time in the stationary phase, the period of the exponential phase is usually negligible comparing to the stationary phase. In the natural environment, continuous bacterial growth is seldom found, in contrast to laboratory conditions, when bacteria are cultured in rich media at an optimal temperature and parameters.
Based on earlier literatures, when nutrients become limited or other environmental conditions restricted the population growth the bacterial cultures enter the stationary phase. Here we provide novel principles of population development. We reported that during a population growth the cells went through different differentiated phases where the phenotype of the cells was changing from the proliferating to the terminally differentiated one losing the colony forming ability (CFU), meanwhile the total cell concentration remained constant. Therefore, we suggest that a bacterial population can be considered as a virtual tissue as a result of a specific differentiation processes, in which the exponential-phase cells develop to stationary-phase cells and eventually reach the unculturable (VBNC), terminally differentiated form. The richness of the nutrient had no effect on growth rate or on stationary cell density. The generation time seems not to be a constant value, rather it depended on the concentration of the starter cultures. Inoculations with serial dilutions of stationary populations reveal a so-called minimal stationary cell concentration (MSCC) point, up to which the cell concentrations remain constant upon dilutions; that seems to be universal among unicellular organisms. Briefly, Ughy, Nagyapati et al. (2023) suggest to reconsider the 225 year old dogma of bacterial population growth.

Keywords: *bacterial growth phases, bacterial differentiation, genetic regulation*

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MI#E6

BOOSTING ANAEROBIC LIGNOCELLULOSE UTILIZATION VIA SYNTROPHIC INTERACTIONS

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Lignocellulose-based biomass represents one of the most abundant renewable resources, holding great promise for sustainable energy generation and as a raw material for the chemical industry.
Our research aims to develop a stable, efficient microbial consortium capable of degrading lignocellulose under artificial conditions, relying on tight syntrophic interactions. To this end, we investigated the degradation efficiency of rumen-derived anaerobic fungi (AGF), methanogenic archaea (MA), and bacteria (BAC), cultivated on straw. Using SEM, HPLC, and metagenomic approaches, we observed intensive microbial proliferation and close cell-to-cell associations among AGF, MA, and BAC. Metagenomic sequencing provided insights into dynamic community shifts, while HPLC analyses revealed the composition of degradation products.
In line with previous findings on biomass pretreatment, we also applied biological pretreatment in our system. Both the anaerobic fungus–methanogen (AGF-MA) and the fungus–methanogen–bacterium (AGF-MA-BAC) consortia generated substantial methane yields when supplied with hydrogen. Notably, the AGF-MA-BAC community produced 27% more methane than AGF-MA alone, and under optimal microbial configurations, methane production increased by 50–60%. Subsequent biogas fermentation trials confirmed that the AGF-MA-BAC consortium consistently achieved the highest methane output. These results support our hypothesis that this minimal microbial community enhances structural breakdown of plant biomass and maximizes methane generation.
Since methane is the primary energy carrier in biogas and directly usable as a renewable fuel, the synergistic action of this microbial system offers a promising route for energy-efficient processing and utilization of lignocellulose-rich biomass and agricultural residues.

MI#E7

EVOLUTION IN MULTICELLULAR YEAST SYSTEMS

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The emergence of multicellularity has independently occurred across multiple evolutionary lineages. This lifestyle provided several advantages over unicellular forms, such as division of labor among cells and protection against predators. Nevertheless, many environments exist in which multicellularity is not beneficial, and thus unicellularity has been retained throughout evolution, indeed in extreme habitats (high pressure, extreme temperatures, etc.) it is often the only viable strategy.
In this work, we investigate the process by which a subset of cells within a species and population begins to grow in an aggregated morphology, forming cell clusters within an otherwise unicellular environment. We use Saccharomyces cerevisiae as a model organism, a species capable of facultative multicellularity through the formation of pseudohyphae. Our aim is to make this trait permanent and to establish obligately multicellular lineages.
In our experiments, we aim to determine how these structural changes alter the fitness and growth patterns of the strains, and how they influence interactions. Furthermore, through experimental evolution, we investigate the limits of invasive and multicellular growth.

MI#E8

CHALLENGES IN THE PRACTICAL APPLICATIONS OF BIOACTIVE PEPTAIBOLS

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Peptaibols are short, linear, helical antimicrobial peptides synthesized by non-ribosomal peptide synthetases, mostly by members of the filamentous fungal genus *Trichoderma*. Non-proteinogenic amino acids, such as α-aminoisobutyric acid (Aib) or D-isovaline are present in their backbone, increasing the stability of peptaibols. The N-terminus is acetylated, and the C-terminus contains an amino-alcohol group. Due to these properties, peptaibols are able to form voltage-dependent ion channels in the phospholipid bilayers of the cells, through which they exert their biological activity. They can inhibit several pathogenic microorganisms, especially Gram-positive bacteria and filamentous fungi. Peptaibols can also induce systematic resistance in plants. Different *Trichoderma* strains produce different kinds of peptaibols, which can be categorized based on their length and amino acid composition. Based on the different structures of different kinds of peptaibols, their bioactivities are also different among the peptaibols produced by various *Trichoderma* strains. The aim of our group is to identify the peptaibiome of the widest range and types of selected strains by HPLC-HRMS methods, to purify the peptaibols by semi-preparative HPLC and to test their biological activities on various bacteria and filamentous fungi. A deeper understanding of their mode of action is facilitated by accelerated molecular dynamic simulations. However, the practical use of peptaibols faces several challenges. They dissolve in water poorly or not at all, and a large-scale, cost-effective fermentation system has yet to be developed. Therefore, an affordable method for large-scale peptaibol fermentation was established using different grains as substrates and incubation methods. To increase the water-solubility of peptaibols, amino acid substitutions using accelerated molecular dynamic simulations were carried out showing promising results.

Keywords: *peptaibol, Trichoderma, bioactivity, structure-activity relationships*

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MI#PE1

RAPID MONITORING OF THE PRESENCE AND BIOACTIVITY OF PEPTAIBOLS PRODUCED BY *TRICHODERMA* STRAINS FROM A HISTORICAL COLLECTION

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Increasing environmental problems causing significant damage in agriculture are prompting the search for new, innovative biological solutions. Several species of the filamentous fungal genus *Trichoderma* are already used in practice as biocontrol agents, and their secondary metabolites may also open new opportunities. Peptaibols are small, bioactive peptides produced mainly by *Trichoderma* species, which have a versatile effect on both microorganisms and plants.
A set of 18 strains selected from a historical *Trichoderma* collection established during the early 1990s was involved in our study. The isolates originated primarily from soil samples collected in different areas of Hungary. The dried material of the isolates was transferred into glycerol solution in glass tubes, from which they were revived by the inoculation to malt extract medium supplemented with yeast extract. More than 70% of the strains were able to grow after inoculation. The species level identification of the *Trichoderma* strains was originally performed based on micro- and macromorphological characteristics. As the taxonomy of the genus *Trichoderma* has substantially improved during the past 3 decades, we carried out molecular reidentification based on the sequence analysis of 3 diagnostic markers (ITS, *tef1-α* and *rpb*), which revealed that the strains belong to the species *T. harzianum*, *T. atroviride*, *T. longibrachiatum*, *T. simmonsii*, *T. koningiopsis*, *T. virens* and *T. gamsii*.
Peptaibol extractions were performed from both the mycelium of the revived strains and the media using methanol-chloroform solvents. The bioactivity of the crude extracts was tested against bacterial strains. Extracts were found that showed activity, and in several cases, extracts obtained from the culture medium also exerted a significant inhibitory effect. The peptaibol content of the extracts was also investigated by mass spectrometry measurements, and the total peptaibol production was determined. Several *Trichoderma* strains produced bioactive peptaibols, which will be subjected to further examinations to comprehensively determine their bioactivity profiles. Furthermore, results deriving from the examined historical *Trichoderma* collection can be compared with data obtained from recent sets of isolates, which may shed light on eventual population structural, ecophysiological and microevolutionary processes.

Keywords: *Trichoderma, peptaibol, biocontrol*

Acknowledgement: *This study was connected to the Interreg-IPA Hungary-Serbia Cross-border Cooperation Programme (PLANTSVITA project, HUSRB/1602/41/0031) and the Scholarship Program of the Ministry of Culture and Innovation, Financed from the National Research, Development and Innovation Fund (EKÖP-570-SZTE and EKÖP-578-SZTE).*

MI#PE2

OPTIMIZATION OF LARGE-SCALE AND COST-EFFICIENT EXTRACTION METHODS FOR PEPTAIBOL COMPOUNDS

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Fungi produce numerous secondary bioactive metabolites, most of which are peptide antibiotics. Peptaibols form a group of peptaibiotics with significant economic and commercial potential. They are secondary metabolites produced by fungal strains of the genus *Trichoderma*, typically consisting of peptides 5–20 amino acids in length, with molecular masses ranging from 500 to 2200 Da. Peptaibol sequences usually contain non-proteinogenic amino acids and are assembled by the non-ribosomal peptide synthetases (NRPS). Their characteristic components include α-aminoisobutyric acid (Aib) and isovaline, as well as an acetylated N-terminus and 1,2-aminoalcohols. Peptaibols possess enormous potential in the defense against phytopathogenic and human pathogenic microbes. They exhibit antibacterial, antifungal, and antiviral activities, and they are also able to induce plant resistance against a wide range of plant pathogens. They represent a new generation of alternatives to agrochemical agents and human therapeutics. For the large-scale production of peptaibol-based products, it is also necessary to develop an efficient and simple extraction method. Although peptaibols can be extracted using various solvents, for large-scale production the cost and toxicity of each solvent, as well as the purity of the resulting extract, must be taken into account.
In our work, we investigated the peptaibol production of *T. longibrachiatum f. bissettii* SZMC 12546 with the aim of developing a cost-effective and simple extraction method that would allow the production of large quantities of peptaibol products. After the cultivation of the fungus, we carried out the extraction of peptaibols using several different solvents and various solvent mixtures. The amount of extracted peptaibols was determined by a highly sensitive bioactivity assay against *Micrococcus luteus*. During the evaluation of the results, we found that, in addition to the chloroform:methanol 2:1 ratio previously used by our research group, other solvents and solvent mixtures also proved effective for peptaibol extraction. Chloroform, although an efficient solvent, is extremely expensive and highly toxic; therefore, it is worth considering that other solvents (e.g. dichloromethane, acetonitrile) are significantly cheaper and less toxic, making them suitable alternatives for extraction. Based on the bioactivity assays, peptaibol extracts obtained with the most promising solvents will also be quantified using HPLC–HRMS analyses.

Keywords: *Trichoderma,* peptaibol, extraction, solvent

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MI#PE3

ENVIRONMENTAL REGULATION OF KILLER TOXIN DYNAMICS IN YEAST POPULATIONS

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Killer toxins produced by certain yeast species play a key role in shaping microbial community structure, especially during fermentation. Using fluorescently labelled *Saccharomyces cerevisiae* strains, we conducted a detailed investigation into how environmental factors influence both toxin synthesis and strain vulnerability. Our experimental approach examined multiple environmental variables—including temperature, pH levels, and nutrient composition—to determine their effects on toxin production and efficacy.
We employed time-lapse microscopy to observe competitive interactions between mixed strain populations under diverse conditions. Comparative experiments in solid versus liquid media revealed distinct patterns in toxin potency and strain behaviour, including variations in growth rates and competitive advantage. Killer phenotype expression proved highly sensitive to temperature, with maximum toxin production occurring at cooler temperatures. Additionally, elevated glucose concentrations in growth media intensified the susceptibility of vulnerable strains to killer toxins.
Our results offer practical strategies for engineering microbial communities to achieve desired fermentation characteristics while advancing our understanding of how killer toxins function ecologically in wild yeast populations.

Keywords: *Saccharomyces cerevisiae, toxin production, interactions*

Acknowledgement*: The research has been supported by the Hungarian National Research, Development and Innovation Office (NKFI/NRDI) through the Hungarian Scientific Research Fund (OTKA-K20-134489) and by the EKÖP-25-4 University Research Scholarship Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund.*

MI#PE4

ENVIRONMENTAL AND EVOLUTIONARY DRIVES OF AFLATOXIN DETOXIFICATION IN LACTIC ACID BACTERIA

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Lactic acid bacteria represent promising biological agents for mycotoxin detoxification in food systems, and their performance is known to vary substantially among different strains under varying environmental conditions. However, the specific mechanisms by which pH, temperature, and evolutionary adaptation interact to influence both bacterial growth and aflatoxin B1 (AFB1) detoxification remain poorly understood, limiting optimization of these biocontrol strategies. Therefore, we systematically examined how pH (4, 6, 7), temperature (25, 30, 35°C), and evolutionary state—adaptive-derived (AD) versus parental (P0)—influence growth and AFB1 detoxification in three lactic acid bacteria: *Levilactobacillus brevis* AMKT5/3, *Loigolactobacillus coryniformis* AMKB6/3, and *Lactiplantibacillus pentosus* AMK6/4. Growth assays were paired with detoxification analyses partitioning AFB1 into supernatant, cell-associated, and degraded fractions. Strains exhibited distinct pH-dependent growth optima: *L. brevis* grew fastest at pH 7, *L. coryniformis* at pH 6–7, and *L. pentosus* across pH 5–7. Under optimal pH conditions, temperature responses diverged markedly among strains. Growth increased with temperature in all strains, but *L. coryniformis* improved steadily up to 35°C, whereas *L. brevis* and *L. pentosus* plateaued between 30–35°C, indicating narrower thermal performance windows. A composite Z-score integrating detoxification percentage, degraded mass, and residual toxin ranked *L. coryniformis* as the strongest detoxifier, followed by *L. brevis* and *L. pentosus*. Although measurement uncertainty prevented statistical significance testing, effect sizes and standardized performance indices revealed clear biological patterns: AD variants of *L. brevis* and *L. coryniformis* tended to detoxify more AFB1 through degradation, while the AD variant of *L. pentosus* tended to detoxify less compared with its P0 strain. AFB1 detoxification was driven primarily by degradation rather than binding, and evolutionary improvements were strongly strain specific. These findings demonstrate that environmental conditions and evolutionary history shape bacterial mycotoxin elimination performance in highly strain-specific ways, providing crucial insights for optimizing biocontrol applications.

Keywords: *Aflatoxin B1; Detoxification; pH; Temperature; Adaptive-derived strains.*

MI#P1

INVESTIGATION OF THE SURFACTIN PRODUCTION OF A *BACILLUS LICHENIFORMIS* STRAIN

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Surfactins are cyclic lipopeptides produced by *Bacillus* species that have a significant scientific attention due to their versatility and potential for sustainable industrial applications. Unlike synthetic surfactants, these biosurfactants offer superior biodegradability and effectiveness at lower concentrations. Due to the advantageous properties of surfactins, these molecules are outstanding candidates in the fields of environmental sustainability, where they facilitate the bioremediation of contaminated soil and water; in agriculture, they serve as eco-friendly biopesticides; and in medicine, where they function as potent antibacterial, antifungal, antiviral, and antitumor agents. Therefore, monitoring surfactin production across multiple *Bacillus* species can support the identification of suitable candidates for future biocontrol applications. In this study, we successfully performed the taxonomic identification of the *Bacillus* strain 27614 isolated from fungal compost. The taxonomic determination was based on the *rpoB*, *eub*, and *gyrA* gene sequences' PCR products from the purified template DNA of the bacteria. Subsequently, High-Resolution Mass Spectrometry (HRMS) analysis was performed to examine surfactin production and identify each individual surfactin variant produced by the SZMC 27614 *B. licheniformis* strain. The analysis of the surfactin variants was based on the characteristic fragmentation patterns of surfactin molecules. In the HRMS method, surfactin molecules were observed according to the mass of the sodium adduct form of the parental ion (*m/z* 1016, *m/z* 1030, *m/z* 1044, *m/z* 1058, *m/z* 1072, *m/z* 1086, *m/z* 1100, *m/z* 1114). A total of 22 individual surfactin variants were identified. Following the spectrometric analysis, inhibition assays were performed to determine the antimicrobial activity of the strain against phytopathogenic bacteria and fungi. In the cases of the phytopathogenic fungi, the examined strain showed moderate effectiveness only against *Fusarium culmorum* SZMC 11041 strain. For the phytopathogenic bacteria, moderate effectiveness against *Xanthomonas campestris* SZMC 6183, *Rhizobium radiobacter* SZMC 14555, and *Erwinia amylovora* SZMC 21402 was observed. The presented *Bacillus licheniformis* strain holds promise as a candidate for future biocontrol applications against phytopathogenic bacteria aimed at reducing chemical pesticide use and supporting more sustainable plant protection strategies.

Keywords: *surfactin, bioremediation, antibacterial, mass spectrometry, inhibition assay*

MI#P2

SOLVENT-BASED SEPARATION OF *TRICHODERMA* PEPTAIBIOTICS

***Henriett L. Hunkár*[1,2], Dóra Balázs[1], Fanni Kovács[1,2], Gergő Terna[1,2], Ákos Rozsnyói[1,2], András Szekeres[1], Mónika Varga[1], Tamás Papp[1], Csaba Vágvölgyi[1], László Kredics[1], Tamás Marik[1], Chetna Tyagi[1]**

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Members of the genus *Trichoderma* are widespread filamentous fungal species that are highly competitive within soil microbial communities due to their efficient enzyme production and diverse bioactive secondary metabolites. Due to these advantageous traits, *Trichoderma* species have gained significant prominence in biotechnology over the past few decades. They produce numerous antimicrobial secondary metabolites, including peptaibiotics, which play a key role in their biological activity against other microorganisms. The largest and most characteristic subgroup of peptaibiotics is the peptaibols, alongside lipopeptaibols, lipoaminopeptides, and cyclic peptaibiotics. Separating these different peptaibiotic groups is essential for their targeted investigation and the evaluation of their bioactivity.
This study investigates the fractionation of peptaibiotics produced by *Trichoderma byssinum* SZMC 28389 and *T. helicum* SZMC 27992 using different solvents. After large scale growth cultivation, the mycelium was harvested, and peptaibiotics were extracted using solvents of different systems: chloroform : methanol (2:1), hexane : methanol (1:1) and hexane. The resulting extracts were separated into phases and the bioactivity of crude extracts from the fractions were analyzed using agar diffusion assay for their bioactivities against *Micrococcus luteus*. The samples were further analyzed by mass spectrometry to obtain a comprehensive profile of both peptaibols and lipopeptaibols present. Based on these results, the separation of lipopeptaibols was successful only in the case of *T. byssinum*, which indicates, that the method may be specific to a certain group of lipopeptaibols. In the future, we plan to further optimize the separation procedures and to identify an appropriate solvent system for all peptaibiotics produced by *Trichoderma* species.

Keywords: *Trichoderma, peptaibol-lipopeptaibol separation, HPLC-MS, solvent fractionation*

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MI#P3

COMPARATIVE RESISTANCE DEVELOPMENT OF *CANDIDOZYMA AURIS* TO AN ANTIFUNGAL PROTEIN (NFAP2), ANIDULAFUNGIN, AND AMPHOTERICIN B

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Since its first identification in 2009, the opportunistic pathogen *Candidozyma auris* has rapidly spread worldwide, and the number of multidrug-resistant isolates has risen rapidly, posing a serious global public health threat. This situation underscores the urgent need for novel, safe antifungal agents with a low propensity for resistance development. The *Neosartorya (Aspergillus) fischeri* antifungal protein 2 (NFAP2) has emerged as a particularly promising candidate.
In this study, we examined the potential for resistance development against NFAP2. The *C. auris* NCPF 8971 strain was subjected to a microevolution experiment in which gradually increasing concentrations of NFAP2 was applied to evaluate its adaptive capacity. Two clinically approved antifungal drugs were included as comparators: anidulafungin (ANI), an echinocandin, one of the most commonly prescribed agents against *C. auris*, and amphotericin B (AMB), which likely shares the closest mode of action with NFAP2 (cell membrane integration disruption). Six independent evolutionary lineages were maintained for each treatment. We observed that *C. auris* was capable of developing resistance to the 8× minimum inhibitory concentration (MIC) of NFAP2 and ANI, while it developed resistance even at 32× MIC of AMB. To characterize cross-resistance profiles, antifungal susceptibility of all evolved strains to NFAP2, AMB and ANI was determined using a modified CLSI M27-A3 microdilution assay. NFAP2- and ANI-resistant strains exhibited reduced susceptibility to AMB. ANI-resistant strains showed increased susceptibility to NFAP2, whereas AMB-resistant strains displayed elevated tolerance to ANI.

Keywords: *Candidozyma auris, antifungal resistance, NFAP2, microevolution, cross-resistance*

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MI#P4

IDENTIFICATION OF NEW PEPTAIBIOTIC SEQUENCES FROM *TRICHODERMA VELUTINUM* ISOLATES

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Trichoderma is a soil-dwelling fungal genus that can be found all over the world. They enhance plant development through several ways, such as root colonization, improved nutrient solubilization, increased tolerance to abiotic stresses, and are widely recognized for producing diverse secondary metabolites, including peptaibiotics. Peptaibiotics are linear amphipathic polypeptides synthesized through the nonribosomal peptide synthetase pathway. They are rich in non-standard amino acids like α- aminoisobutyric acid (Aib) and isovaline, and are typically composed of 4-21 amino acids. These metabolites exhibit antimicrobial, antiviral, and antifungal properties, which make them a chemically and pharmacologically significant group. Our study focused on the peptaibiotic production of 2 strains of *Trichoderma velutinum*, a soil-borne filamentous anamorphic fungus described taxon within the Pachybasium section of the genus *Trichoderma*. In order to identify the entire peptaibiome, an optimized HPLC separation coupled with high-resolution mass spectrometric (HRMS) detection was used. The method highlights all the peptaibiotics produced by a *Trichoderma* strain on the chromatograms with high accuracy. Our findings demonstrate that, three groups of peptaibiotics were identified from the *T. velutinum* strains, including six 14-residue peptaibols, ten 18-residue peptaibols, and forty-six 7-residue lipopeptaibols. Moreover, all the 18-residue peptaibols and 7-residue lipopeptaibols appeared to be potentially novel compounds, while the 14-residue peptaibols corresponded to previously reported Hyporodocin sequences. Overall, this study shows diverse range of peptaibiotics production and provides a foundation for future work aimed to characterizing their biological activities.

Keywords: *Trichoderma velutinum, peptaibiome, peptaibiotics, peptaibols, lipopeptaibols*

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MI#P5

ISOLATION OF BENEFICIAL MICROBIAL STRAINS FROM DROUGHT-AFFECTED REGIONS OF HUNGARY

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Due to global climate change, drought stress is occurring with greater frequency and intensity, leading to substantial declines in agricultural productivity. It is becoming increasingly evident that traditional agricultural systems alone are insufficient to effectively cope with these emerging stressors.
One of the most promising of these solutions is the exploitation of the symbiotic benefits conferred by plant growth-promoting rhizobacteria (PGPR). These microbes through the solubilization of organic phosphates, make otherwise inaccessible forms of phosphorus available to the host. Additionally, by fixing atmospheric nitrogen, they provide an essential nitrogen source even in nitrogen-deficient soils. Indole-3-acetic acid (IAA), a phytohormone produced by many PGPR strains, promotes root system development, thereby enhancing water and nutrient uptake efficiency. Moreover, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, an enzyme produced by certain strains, degrades the ethylene precursor ACC, reducing stress induced ethylene levels an especially vital function under drought or salt stress.
The isolation and identification of such microorganisms are essential steps toward the development of effective microbial inoculants. Strains isolated from soils naturally exposed to drought or from the rhizosphere of drought-tolerant plant species are particularly promising, as they are already adapted to extreme environmental conditions.
Research and targeted isolation of drought-tolerance-enhancing soil microbes therefore represent not only a scientific challenge but also a practical opportunity to advance environmentally sustainable and resource-efficient agricultural practices.

Keywords: *Plant growth-promoting rhizobacteria (PGPR), drought tolerance, Phosphate solubilization, Nitrogen fixation, ACC deaminase, Indole-3-acetic acid (IAA)*

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MI#P6

ANTAGONISTIC EFFECT OF *CANDIDA ZEYLANOIDES* SZMC 26644 AGAINST PLANT PATHOGENIC MICROORGANISMS

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Plant pathogenic bacteria and filamentous fungi are responsible for considerable economic losses not only in agricultural fields but also during the post-harvest storage phase. In order to decrease the detrimental effects of these microorganisms, there has been a paradigm shift towards environmentally sustainable methods, such as the utilization of biocontrol organisms that are safe, rather than the application of synthetic chemical compounds. Several papers evidence the efficacy of yeasts as biocontrol agents, demonstrating their capacity to suppress the growth of various bacteria and filamentous fungi. The mechanism underlying this biocontrol efficacy is frequently facilitated by the synthesis of volatile organic compounds.
The inhibitory activity of *Candida zeylanoides* SZMC 26644 against fungi responsible for food spoilage by producing volatile substances was previously documented by our research group. In this study we examined the effect of this strain on the growth of plant pathogenic bacteria (*Agrobacterium tumefaciens*, *Burkholderia phytofirmans*, *Erwinia amylovora*, *Pseudomonas syringae pv. panicii*, *Pseudomonas tolaasii*, *Xanthomonas campestris pv. vesicatoria*) and filamentous fungi (*Alternaria alternata*, *Aspergillus flavus*, *Botrytis cinerea*, *Rhizoctonia solani*) on the surface of solid media. For filamentous fungi, the diameter of colonies developed in the presence of *C. zeylanoides* was compared to the diameter of colonies developed on control plates. Regarding bacteria, the decrease in cell number was examined in the presence of *C. zeylanoides* in contrast to control conditions. The results indicated that *C. zeylanoides* SZMC 26644 is able to inhibit the growth of both bacterial and fungal species. Our microscopic observations revealed that both bacterial and filamentous fungal cells undergo morphological changes in the presence of *C. zeylanoides*.
We employed gas chromatography-mass spectrometry (GC_MS) to identify volatile metabolites, resulting in the identification of 92 distinct compounds. Among these, four compounds were exclusively detected in the *C. zeylanoides* culture, while two additional compounds exhibited more than tenfold increase in their concentration compared to the control samples containing only the culture medium. The two compounds, phenylethyl alcohol and 3-methylbutanoic acid, have been documented to have antimicrobial properties in the literature data, and may thus be responsible for the observed growth inhibition.
The effect of phenylethyl alcohol and 3-methylbutanoic acid was studied on the abovementioned bacterial strains. Inhibitory activity was observed in the presence of 3-methylbutanoic acid; however, phenylethyl alcohol exhibited growth inhibition only against *X. campestris,* where not only growth but also the production of pigment was affected as well. Study is currently in progress to elucidate the impact of these compounds on filamentous fungi.

Keywords: *Candida zeylanoides, volatile compound, biocontrol*

MI#P7

ALTERATION OF THE FUNGAL ENDOPHYTE SECRETOME INDUCED BY SODIUM BUTYRATE

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Bioactive secondary metabolites produced by endophytic fungi hold significant potentials for the pharmaceutical, food industries and the agriculture. The genes responsible for synthesizing these compounds often remain inactive when the fungi are cultured under standard laboratory conditions, mostly due to the absence of the specific environmental cues or nutrients provided by their natural host organisms. However, the epigenetic modifiers can be used to alter chromatin structure and consequently activate silent biosynthetic pathways.
In our study, we investigated the influence of sodium butyrate as epigenetic modifiers to identify the potential changes in the secondary metabolite profiles of an endophytic fungus. The fungal strain was passaged on sodium butyrate-supplemented solid medium and secondary metabolites were extracted from liquid cultures after each passage. Secondary metabolites were first extracted with ethyl acetate and subsequently with the mixture of chloroform and methanol. Then mass spectrometric analysis was employed to characterize the secondary metabolites of the treated fungal strains. The changes in the metabolite profile were separately analysed in the mycelium and in the culture medium and were examined which metabolites increased or decreased due to the epigenetic modifier. Additionally, the occurrence and abundance of metabolites detected in both environments were also investigated and determined.
Our findings suggest that epigenetic modification can serve as a potent strategy to unlock the biosynthetic potential of endophytic fungi, offering new avenues for the discovery of bioactive compounds.

Keywords: *endophytic fungi, secondary metabolites; epigenetic modification; metabolomics*

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