
# 6th National Conference of Young Biotechnologists

## Program and abstracts

HUN-REN ATK Martonvásár, 4-5. April 2024.

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HUN REN | ATK CENTRE FOR AGRICULTURAL RESEARCH

FIBOK2024

MAGYAR TUDOMÁNYOS AKADÉMIA · 1825 ·

# „FIBOK 2024”

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Photo: Imre Majláth

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FIBOK 2024

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# 6th National Conference of Young Biotechnologists (FIBOK 2024)

## ORGANIZED BY

**the Committee of Agricultural Biotechnology of the Section of Agricultural Sciences of the Hungarian Academy of Sciences (MTA) with**

**the Committee on Bioinformatics of the Section of Biological Sciences of MTA,**

**with the Committee on Microbiology of Section of Biological Sciences of MTA,**

**with the Committee on Molecular Biology, Genetics and Cell Biology of the Section of Biological Sciences of MTA,**

**with the Complex Committee on Food Science of the Section of Chemical Sciences of MTA,**

**with the Association for Innovative Agricultural Biotechnology and**

**with the Hungarian Bioinformatics Society and**

**the Association of Hungarian Biotechnology Students.**

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# 6th National Conference of Young Biotechnologists (FIBOK 2024)

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# 6th National Conference of Young Biotechnologists (FIBOK 2024)

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**Chair of the Scientific Committee:** Elen GÓCZA (MATE, GBI Gödöllő)

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**Plant- and food biotechnology**

Ervin BALÁZS (HUN-REN, ATK MGI, Martonvásár)
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László TAMÁS (ELTE, TTK, Budapest)
Éva VÁRALLYAY (MATE, Institute of Plant Protection, Gödöllő)
Anikó VERES (MATE, GBI, Gödöllő)

# FIBOK2024 PROGRAM

## 04.04.2024.

## Registration (8:30-9:30)

### *Opening ceremony*

9:40 **Jenő KONTSCHÁN** – HUN-REN ATK Director General
9:45 **Magda PÁL** – HUN-REN ATK MGI Chair of the Organizing Committee

### *Plant- and food biotechnology 1. section:*

Chair Person – **László SÁGI**, (HUN-REN, ATK MGI)

9:50 **Tibor JANDA** (invited speaker), HUN-REN ATK MGI: Beware of the dwarf! Or not? – EENI

10:20 **Dávid KISS-LEIZER,** MATE NTTI: PGP trait examination of six osmotolerant, soil-born bacterial strains from different genera – EN-1

10:35 **Kalpita SINGH**, HUN-REN ATK MGI: Redox modulation of hormones and metabolites in maize – EN-2

10:50 **Zita SEPSI**: Introduction of the Hungarian Biotechnology Students Association (MaBE)

*Coffee break (11:00 - 11:30)*

### *Plant- and food biotechnology 2. section:*

Chair Person – **László SÁGI** (HUN-REN, ATK MGI)

11:30 **Györgyi FERENC** (invited speaker), HUN-REN SZBK: Oligonucleotide-directed gene-specific mutagenesis: transgene-free genome editing – EENII

12:00 **Almash JAHAN,** MATE NVI: Apple luteovirus P0 protein is a suppressor of local and systemic RNA silencing – EN-3

12:15 **Jeny JOSE**, HUN-REN ATK MGI: Role of miR396 in regulating plant defense mechanisms against pathogens: insights from CRISPR/CAS9 editing in potato – EN-4

12:30 **Fanni SZABADOS,** HUN-REN ATK MGI: The role of dna demethylase demeter (DME) in regulating the methylation pattern of male and female reproductive tissues of barley – EN-5

12:45 **Rebeka PAPP,** SZTE TTIK BI: Heterologous production of a *Solanum lycopersicum* L. antifungal defensin in *Pichia pastoris* – EN-6

*LUNCH (13:00-14:30)*

***Poster presentations:***
Chair Persons – **Éva VÁRALLYAY** (MATE NVI), **Elen GÓCZA** (MATE GBI)

14:30 **Éva DOBÓVÁRI,** MATE GBI GGT: Polymorphism analysis of apricot (*Prunus armeniaca* L.) genotypes with scot markers – PEN-1

14:33 **Vivien FÁKÓ,** MATE NVI: Identification of silencing suppressor proteins encoded by *Prunus* virus F – PEN-2

14:36 **Borbála GRÓB,** MATE NVI: First detection of rubus yellow net virus on raspberries in Hungary – PEN-3

14:39 **Lilla PÉRI,** MATE NVI: Common milkweed as a virus reservoir – PEN-4

14:42 **Mirella HIRTH,** MATE HAKI: Sexing of zebrafish by genes differentially expressed in the tail fin – PEÁ-1

14:45 **Alexandra TOKÁR,** MATE ÁTI: Intraindividual variance of bull sperm morphometry parameters and their relation with field fertility – PEÁ-2

14:48 **Arnold TÓTH,** MATE GBI ÁBT: Investigating the effect of heat treatment and monitoring the molecular changes in primordial germ cells (PGC) before and after freezing – PEÁ-3

14:51 **Martin URBÁN,** MATE GBI ÁBT: The effects of X-chromosome inactivation in mouse embryonic gonads – PEÁ-4

14:54 **Szilárd GUDOR,** MOGYE GYK: Protein A affinity chromatography of biosimilar nivolumab monoclonal antibody from chinese hamster ovary (CHO) cell culture broths – PEO-1

14:57 **Maria SALINAS,** MATE GBI ÁBT: Non-invasive assessment of the quality and viability of rabbit embryos – PEO-2

15:00 **Éva MOUSSONG,** ELTE TTK BI: Effects of calcium fluoride nanoparticles on the amyloid formation and cytotoxicity of Alzheimer's amyloid-β peptide – PEO-3

15:03 **Márton Péter NYIRI,** ELTE TTK BI: Investigation of the effect of nanomaterials on the structure of amyloidogenic proteins – PEO-4

15:06 **Otília TÓTH,** HUN-REN TTK: Expression of *dUTPase* in mouse postnatal development – PEO-5

15:09 **Anna ZRINYI,** BME VBK SZKTT: Investigation and optimization of immobilization of histidine-tagged enzymes – PEO-6

15:12 **Tünde GAIZER,** PPKE ITK: Agent-based modeling of yeast communities under non-standard environmental conditions – PEB-1

15:15 **Lilla SÁNDOROVÁ,** MATE GBI GGT: T-ARMS PCR application for SNP detection in Holstein-Friesian cattle of A1 and A2 β-casein – PEB-2

15:18 **Vivien BÍRÓ,** DE TTK BTI: Manganese effect on citric acid production by *Aspergillus niger*: unlocking an efficiency boosting hidden key – PEM-1

15:21 **Susana ARAUJO NAVAS,** MATE: Preliminary results of bacterial community diversity in the rhizosphere of dragon fruit – PEM-2

15:24 **Fruzsina FÉLEGYHÁZI,** ELTE TTK BI: Investigation of soil biodiversity in alkaline grassland under regenerative agricultural practice – PEM-3

15:27 **Valentina MADÁR,** PPKE ITK: Exploring directed and multicellular growth of yeast – PEM-4

15:30 **Péter FUTÓ,** MATE GBI MABT: Ecophysiological characterisation of a *Klebsormidium* strain isolated from a cave environment – PEM-5

15:33 **Richárd MERBER,** SZTE TTIK BI: Heterologous expression and characterisation of *Neosartorya (Aspergillus) fischeri* bubble protein – PEM-6

***Coffee break (15:45 - 16:15)***

***Poster section (16:15-18:00)***

**GALA DINNER (18:00 - 21:00)**

## 05.04.2024.

***Medical and pharmacological biotechnology section:***
Chair Person – **László HIRIPI** (MATE GBI)

10:15 **Xabier OSTEIKOETXEA,** (invited speaker), SE ÁOK: Biological roles and exploitation potential of extracellular vesicles – EEO

10:45 **Dorottya Katalin HAJDÚ,** BME VBK SZKTT: Development of chemometric models for raman spectroscopy-based monitoring and control of mammalian cell cultivations – EO-1

11:00 **Nikolett NAGY,** HUN-REN TTK: Investigation of *dUTPase* expression in mouse adult neurogenesis – EO-2

11:15 **Zoé Sára TÓTH,** HUN-REN TTK MÉI: Investigation of proteinaceous inhibition of *M. tuberculosis* dUTPase – EO-3

11:30 **Gyula CSANÁDI,** BIOCENTER Kft.: Complex Biotechnology Solutions from Biocenter Ltd.

***Coffee break (11:35 - 12:00)***

***Microbial biotechnology section:***
Chair Person - **Tünde PUSZTAHELYI** (DE, MÉK)

12:00 **Miklós GYURANECZ** (invited speaker), HUN-REN ÁTKI: Development of novel methods for the control of *Mycoplasma infections* – EEM

12:30 **Dóra BALÁZS**, SZTE TTIK BI: Comprehensive investigation of bioactive peptaibols and their preparation for future agricultural application – EM-1

12:45 **Alexandra MÁRTON,** DE TTK BTI: Insights into the evolution and mutations of second alternative oxidase genes in *Aspergillaceae* – EM-2

13:00 **Zsófia BOROS,** ELTE TTK BI: *Xenorhabdus* antimicrobial products: genetic regulation of biosynthesis and perspectives of application – EM-3

13:15 **Fatma ELZHAA,** MATE ÉTTI: Incidence and antibiotic resistance of *Salmonella enterica* strains isolated from different types of egyptian cheeses – EM-4

***LUNCH (13:30-14:45)***

***Bioinformatics section:***
Chair person - **Orsolya Ivett HOFFMANN** (MATE GBI)

14:45 **Bence GÁLIK** (invited speaker), PTE SZKK: The need of reference genomes and transcriptomes - the fundation of basic research – EEB

15:15 **Esther Ijeoma IDOGWU,** MATE GBI MABT: Studying the response of *Phytophthora infestations* inoculation in different potato cultivars by a transcriptomic approach – EB-1

15:30 **Regina MARTINEK,** HUN-REN TTK MÉI: Assessment of the mutagenic effect of probably carcinogenic pesticides using a whole genome sequencing approach – EB-2

15:45 **Ákos ROZSNYÓI,** SZTE TTIK BI: Peptaibiomics: navigating biotechnological frontiers with the universal peptaibol database – EB-3

***Coffee break (16:00 - 16:30)***

***Animal biotechnology section:***
Chair person - **Elen GÓCZA** (MATE GBI)

16:30 **Nándor NAGY** (invited speaker), SE ÁOK: Bird's eye perspective of enteric nervous system development: Lessons from the avian embryo – EEÁ

17:00 **Lilla BODROGI,** MATE GBI ÁBT: Identifying the novel role of NADPH oxidase 4 in the biotransformation of T2 mycotoxin – EÁ-1

17:15 **András ECKER,** MATE GBI ÁBT: Creating FUCCI-expressing chicken primordial germ cell lines for the analysis of the cell cycle – EÁ-2

17:30 **Dániel PÉTER,** MATE HAKI: Digital PCR analysis of growth related genes in african catfish (*Clarias gariepinus*) lines – EÁ-3

17:45 **Olivér Máté SZABÓ,** MATE HAKI: A transparent zebrafish reporter line for improved visualization of gonad transformation – EÁ-4

***Announcement of results***:

18:00- (**Elen GÓCZA** and **Magda PÁL**)

# FIBOK2024

## POSTER SECTION

### Animal biotechnology

**Adrián ANTAL**, PTE TTK: Do mycotoxins influence the body conditions of free-living game species? – PÁ-1

**Réka BALÁZS**, MATE-ÁÁDI, NBGK-HGI: Molecular genetic analysis of Hungarian pannonian bee populations by microsatellite markers – PÁ-2

**Hannah Moncon FARR**, MATE HAKI: The effect of different protein supplementation during feeding of african catfish (*Clarias gariepinus*) larvae, juveniles and adults (market size) – PÁ-3

**István LAKATOS**, MATE GBI ÁBT: Mycotoxin measurements from fallow deer milk – PÁ-4

**Zsófia MOLNÁR**, MATE GBI ÁBT: Immunoassay development using functionalized chickens immunoglobulins for the detection of zearalenone and its metabolites – PÁ-5

**Katalin NAGY**, MATE ÁTI: How heat stress affects the success of embryo transfer – PÁ-6

**Jehan NAYGA**, MATE GBI ÁBT: Direct cryopreservation of avian embryonic reproductive cells for gene banking purposes – PÁ-7

### Bioinformatics

**Péter FEHÉR**, MATE GBI GGT: Kinship relationship survey of an invasive mesopredator (*Procyon lotor*) in Central Hungary – PB-1

### Medical and pharmacological biotechnology

**András BENEDEK**, HUN-REN TTK: Amide bond synthetases for a greener chemistry – PO-1

**Viktória Berta PEREY-SIMON**, BME VBK ABÉT: The crystallization of zebrafish dutpase with its interaction partners – PO-2

## Microbial biotechnology

**Barbara BRENDZSÁK**, DE MÉK: *Fusarium* mycotoxin production under aflatoxin biocontrol in corn – PM-1

**Máté FUTÓ**, MATE GBI MABT: Screening of green microalgae based on antibacterial activity and *in-vitro* testing on artificially infected fruit flowers – PM-2

**John K. KAREMERA**, SZTE TTIK BI: Antifungal activity of γ-core peptide derivatives of ascomycetous antifungal proteins – PM-3

**Lívia LÁSZLÓ**, MATE GBI MABT: Beneficial impact of arbuscular mycorrhizal fungi on plant biotic stress responses – PM-4

**Bíborka PILLÉR**, PPKE ITK: Investigating toxin production dynamics in *Saccharomyces cerevisiae* communities – PM-5

**Emelin L. RODRIGUES**, MATE ÉTTI: Isolation of moulds and evaluation of their patulin production potential from Hungarian apples – PM-6

**Dávid Busa**, DE MÉK: Effect of poultry manure-based compost teas on the growth of plant pathogens – PM-7

## Plant- and food biotechnology

**Zsombor DABOSI**, HUN-REN SZBK: Photoautotrophic and sustained $H_2$ production by the *pgr5* mutant of *Chlamydomonas reinhardtii* in simulated daily light conditions – PN-1

**Kristóf JOBBÁGY**, HUN-REN ATK MGI: Changes of stress-responsive parameters in different cereal genotypes during osmotic stress – PN-2

**Imran KHAN**, MATE NTTI: Regulation of cysteine oxidase genes in cucumber varieties during waterlogging stress – PN-3

**Roy G. KIAMBI**, MATE NVI: Characterization of virome of *Asclepias syriaca* in Hungary – PN-4

**Kitti KULMAN**, HUN-REN ATK MGI: Redox regulation of metabolism and regeneration of wheat calli – PN-5

**Diana MAKAI**, HUN-REN ATK MGI: How does whole genome duplication affect meiotic recombination and fertility in polyploid barley? – PN-6

**Azin OMID JEIVAN**, SZTE TTIK BI: Enhancing the shelf-life of fresh-cutting apple and sour cherry fruit through nano-coating based riched of apple pomace and sea buckthorn extract – PN-7

**Bánk PÁPAI**, MATE GBI GGT: Biomechanical profiling of the *Capsicum annuum frx* mutant genotype – PN-8

**Altafur RAHMAN**, HUN-REN ATK MGI: The effects of different daily light conditions on polyamine metabolism – PN-9

**Saleem SHADEN**, MATE ÉTTI: The effect of different drying methods on the banana peel pectin's extracted by ultrasound and using it as a fat replacer in reduced fat biscuits – PN-10

**Sára SIDLÓ**, MATE GBI GGT: The effect of methylation on the anthocyanin biosynthesis of pepper fruits – PN-11

**Katalin TAJTI**, HUN-REN SZBK: Exploring the plant biostimulant effects of *Kocuria arsenatis* FSP-120 and *Chlorella* sp. Macc-360 bacterial-algal combination – PN-12

**Juliana TELES CARDOSO**, MATE NTTI: Cultivar-specific responses to chilling stress in cucumber – PN-13

# PLANT- AND FOOD BIOTECHNOLOGY

## EENI#

### BEWARE OF THE DWARF! OR NOT?

***Janda, Tibor***
*HUN-REN Centre for Agricultural Research, Agricultural Institute, Department of Plant Physiology and Metabolomics, Martonvásár.*

Malnutrinution is still the main reason for death worldwide. According to the estimations, approximately 9 million people die every year of hunger or hunger-related diseases. This is more than from AIDS, malaria and tuberculosis together. Around 50-70% of crop yield reduction is the estimated direct result of abiotic stressors. Furthermore, climate change has exacerbated the frequency and severity of many abiotic stresses. In the 1950s, a so called "Green revolution" started in the global agriculture, which led to a significant increase in crop yield of the common wheat (*Triticum aestivum* L.). Dwarfing *Rht* (Reduced height) genes played an important role in this yield production. The wheat semi-dwarfing genes are widely distributed among the contemporary wheat varieties. These genes also exert pleiotropic effects on plant tolerance towards various abiotic stressors. In order to get more success in the fight against general malnutrition, a new Green revolution is needed, and the negative effects of abiotic stressors must be reduced. To achieve these goals, we must much better understand how plants may respond to the changing environment.
In a recently finished work, frost tolerance was studied in three near-isogenic lines of the facultative variety 'April Bearded', carrying the wild type allele *Rht-B1a* (tall phenotype), and the mutant alleles *Rht-B1b* (semi-dwarf) and *Rht-B1c* (dwarf), and was further compared with the tolerance of a winter type variety, 'Mv Béres'. The level of freezing tolerance was decreasing in the order 'Mv Béres' > Rht-B1a > Rht-B1b > Rht-B1c. To explain the observed differences, comprehensive approach was applied, involving targeted analyses and untargeted metabolomics screening with the help of gas chromatography/liquid chromatography—mass spectrometry setups. Several cold-related processes exhibited similar changes in the studied Rht genotypes. However, the accumulations of certain putrescine and agmatine derivatives, flavones and oligosaccharides were associated with the level of freezing tolerance in the 'April Bearded' lines, which may be the reason the different levels of frost tolerance. Further experiments based on the investigation of gibberellin-insensitive Rht mutants also provided an insight into the role of polyamines in plant growth regulation. It has been shown that polyamine metabolism and polyamine-related processes differ in Rht near-isogenic wheat lines, and especially the dwarf mutant Rht-B1c can be characterized by higher level of polyamine-catabolic activity. Furthermore, in contrast to freezing tolerance, dwarf lines could be characterised with higher level of tolerance to heavy metal cadmium, which can be due to the more efficient production of phytochelatins.

***Keywords:*** *abiotic stressors, dwarfing genes, green revolution, polyamines*

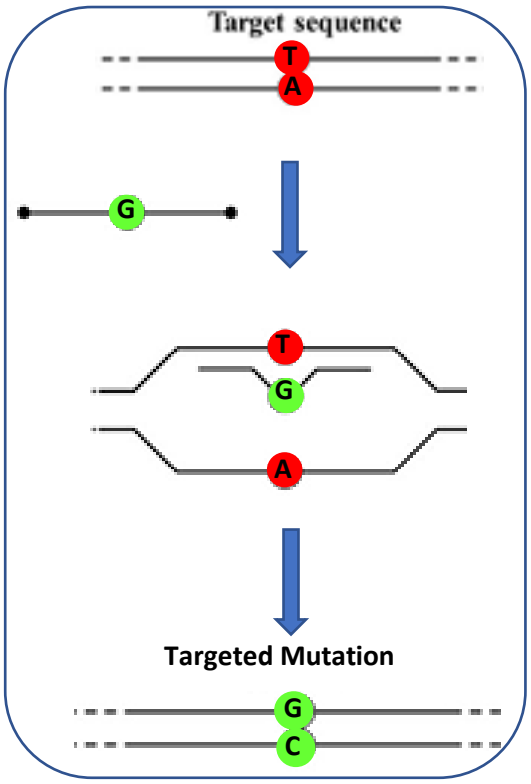
***Acknowledgement:*** *This work was supported by the National Research, Development, and Innovation Office (grant TKP2021- NKTA-06).*

# EENII#

## OLIGONUCLEOTIDE-DIRECTED GENE-SPECIFIC MUTAGENESIS: TRANSGENE-FREE GENOME EDITING

***Ferenc, Györgyi***
*HUN-REN, Biological Research Centre, Institute of Plant Biology, Szeged*

Properties of plants, such as the crop yield and its quality, abiotic and biotic stress tolerance can be increased by variety of random and targeted gene mutations. Based on the recent vote of the European Parliament, those genome edited plants will be considered equivalent to conventional plants which contain less than 20 nucleotide substitution or insertion. Oligonucleotide-directed mutagenesis (ODM) can be used for precise gene editing by using chemically synthesized DNA oligonucleotides with sequence of the target gene except of the targeted mutation. As shown in this figure, during DNA replication in the nuclei, oligonucleotides bind to the target gene and the repair enzymes incorporate the precise mutation. Later DNA oligonucleotides are digested by DNases.[1]
Our group has established an *in vitro*[2] and *in planta*[3] maize test systems for characterization of functionality of a mutagenic oligonucleotide with different chemistry and testing efficiency of various treatment combinations.




***References:***

[1.] Ferenc, Gy; Dudits, D (2017) Génspecifikus mutagenesis rövid szintetikus DNS-molekulákkal. PRECÍZIÓS NEMESÍTÉS Kulcs az agrárinnovációhoz, Agroinform,
[2.] Tiricz, H; Nagy, B; Ferenc, G; Török, K; Nagy, I; Dudits, D; Ayaydin, F (2018) Relaxed chromatin induced by histone deacetylase inhibitors improves the oligonucleotide-directed gene editing in plant cells. JOURNAL OF PLANT RESEARCH 131: 1 pp. 179-189.
[3.] Rádi, Feríz; Nagy, Bettina; Ferenc, Györgyi; Török, Katalin; Nagy, István; Zombori, Zoltán; Dudits, Dénes; Ayaydin, Ferhan (2021) *In planta* test system for targeted cellular mutagenesis by injection of oligonucleotides to apical meristem of maize seedlings. ACTA PHYSIOLOGIAE PLANTARUM 43: 5 Paper: 79

***Keywords:*** *targeted gene editing, Oligonucleotide Directed Mutagenesis, transgene-free genome editing, maize*

***Acknowledgement:*** *"Agribiotechnology and precision breeding for food security" National Laboratory, RRF-2.3.1-21-2022-00007.*

## EN-1#

### PGP TRAIT EXAMINATION OF SIX OSMOTOLERANT, SOIL-BORN BACTERIAL STRAINS FROM DIFFERENT GENERA

***Kiss-Leizer, Dávid*[1, 3]; *Molnár, Jennifer*[1]; *Máté, Rózsa*[1]; *Bereczky, Zsolt*[2]; *Papp, István*[3]**

[1] *BioFil Microbiological, Biotechnological and Biochemical Ltd., Budapest,*
[2] *Saniplant Ltd., Budapest,*
[3] *Hungarian University of Agriculture and Life Sciences, Department of Plant Physiology and Plant Ecology, Budapest,*

Abiotic stresses are economically relevant restrictive factors in agricultural production. Two of the most important environmental stresses are drought and salinity stress, which trigger closely related metabolic answers in plants. Drought stress sets back plant growth at the arable lands all over the world. The lack of water hampers nutrient uptake, root and shoot growth and development, also the efficiency of photosynthesis, all in all it has a general plant growth reducing effect that finally leads to reduced crop yields.
One effective way to reduce effects of drought and salinity stress is the application of plant growth promoting rhizobacteria (PGPR) in agricultural production. These soil-born bacteria possess several beneficial traits that can help plants to cope with environmental stresses, for example nutrient mobilization, N-fixation or osmolyte production. Many studies reported that bacterial inoculation supports plant growth in harsh environments; moreover, field experiments show that proper soil inoculation with these bacteria can increase crop production up to 110-130%.
In this study we selected six bacterial species, each from different genera (*Arthrobacter, Bacillus, Brevibacterium, Kocuria, Paenibacillus, Serratia*) based on their osmotic stress tolerance. To reveal osmotic stress tolerance, we examined the strains growth in modified media with different concentrations of NaCl (1-8%) and PEG 6000 (5-40%). We also measured the abilities of our strains for nutrient mobilization (K, P, Zn, Fe) by agar diffusion method, the EPS (exopolysaccharide) production with measuring the dry weight of slime synthetized by the bacteria, ACC-deaminase activity by spectrophotometry and production of three plant hormones (indole-3-acetic acid, gibberellic acid and trans-zeatine) with LC-MS/MS method.
The results revealed that *Serratia* spp. S130 can mobilize all the examined nutrients, *Paenibacillus* spp. S202 produces the highest amount of EPS, *Arthrobacter* spp. S153 possesses the best ACC-deaminase activity and *Brevibacterium* spp. ES85 is the best hormone producer strain.
In the future we would like to use these outstanding bacterial strains in pot experiments where we expose tomato plants to drought stress to utilize the efficiency of different PGP traits against water deprivation. Finally, the most efficient PGP strains may become used in new soil ameliorant products.

## EN-2#

### REDOX MODULATION OF HORMONES AND METABOLITES IN MAIZE

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The redox balance in plant cells is crucial for optimal growth and development. The critical balance of oxidants and reductants influences hormonal crosstalk, gene expression, and metabolism. Therefore, the study aims to understand the effect of an oxidant (5 mM $H_2O_2$) and two reductants [5 mM ascorbate (Asc) and 1 mM NaHS] on the redox-dependent growth control and modulation of hormones, antioxidants, and thiols in maize. NaHS application after 7 days significantly increased shoot length and fresh weight compared to the other compounds. Whereas, a higher level of electrolyte leakage and lipid peroxidation were observed in Asc-treated plants. Endogenous $H_2O_2$ levels differed among treatments after 7 days, with Asc-treated plants exhibiting the lowest level. The total ascorbate levels were highest in Asc-treated plants followed by $H_2O_2$ treated ones, which also had the highest cysteine concentration after 7-day treatment. Interesting results were obtained for GSH/GSSG ratio as NaHS-treated plants showed the highest ratio after 7 days, while Asc-treated plants showed the lowest ratio following 3 days. A higher antioxidant enzyme activity of dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and catalase (CAT) was recorded in Asc-treated plants along with a higher gene expression of *cytosolic DHAR*, *MDHAR*, *APX*, and *GR* after 7 days. The redox buffering capacity in cells is greatly determined by ascorbate and the glutathione pool, which significantly affect hormone signaling and crosstalk. In Asc- and $H_2O_2$-treated plants, the endogenous levels of ABA, JA, and SA were higher than in NaHS-treated plants. Moreover, the varying levels of other phytochemicals such as 3,4-dihydroxybenzoic acid, kaempferol, luteolin, ferulic acid, *p*-coumaric acid, and quercetin after the application of oxidant and reductants for 3 or 7 days further support the tight redox regulation of hormone and metabolite levels. Therefore, the study highlights the pivotal role of redox balance in regulating plant growth, stress response, and metabolic pathways, offering insights into potential strategies for enhancing plant resilience and productivity.

***Keywords:*** *Redox, phytohormones, antioxidants, maize*

***Acknowledgement:*** *This work was supported by the National Research, Development, and Innovation Office (grants K131638 and TKP2021-NKTA-06 to GK) and by the Stipendium Hungaricum program of the Tempus Public Foundation (SHE-079837-004/2022 to KS).*

## EN-3#

### APPLE LUTEOVIRUS P0 PROTEIN IS A SUPPRESSOR OF LOCAL AND SYSTEMIC RNA SILENCING

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Plants are constantly challenged in nature by various biotic and abiotic stresses including virus infections. Plant viruses are a ubiquitous and diverse group of parasites that harness the host's cellular components in the execution of viral functions like replication, translation, and movement. Numerous plant viruses cause substantial losses in agriculture and are thus a significant risk to global food and feed production.

When a virus infects a plant, viral double-stranded RNA (dsRNA) structures are recognized and processed into virus-derived small interfering RNAs (vsiRNAs) by RNase III-like enzymes, Dicer-like proteins (DCLs). These vsiRNAs are then loaded into RNA-induced silencing complexes (RISCs) containing various Argonaute (AGO) proteins to target viral RNAs for degradation.

RNA silencing is an innate antiviral immunity mechanism in plants. To counteract host RNA silencing, plant viruses commonly encode viral suppressors of RNA silencing (VSRs). Two major strategies used by VSRs are known: inhibiting the production or accumulation of locally acting siRNAs in infected tissue (local suppression) or preventing the spread of silencing signals to systemic leaves (systemic silencing suppression). Molecular plant-virus interactions provide an excellent model to understand host antiviral immunity and viral counter-defense mechanisms.

Apple luteovirus 1 (ALV-1) (family Tombusviridae, genus Luteovirus) was first detected by high-throughput sequencing in apple trees affected by rapid apple decline (RAD) disease. The disease develops gradually starting with leaf discoloration and trunk cracking, and tree vigor is severely affected. The first described isolate of ALV-1 was predicted to contain two additional ORFs (ORF0 and ORF5a) absent in other luteoviruses, with possible protein-coding activity. Polemoviruses, poleroviruses, and enamoviruses encode VSRs at the 5' end of their genomes, in a region where ORF0 of ALV-1 is predicted. The start codons of these ORF0-encoded proteins are shifted to the 5' termini, while ORF0 of ALV-1 overlaps completely with ORF1. Moreover, luteoviruses do not have any ORFs at this position and their P4 protein showed VSR activity. That this putative P0 of ALV-1 is conserved in all sequenced Hungarian variants raised the possibility of this protein acting as a VSR. In our study, we examined the potential RNA silencing suppressor activity of P0 proteins of ALV-1 isolates of Hungary. Our results revealed that the P0 protein of ALV-1 strains displayed weak local and systemic VSR activity in a transient assay.

***Keywords:*** *Tombusviridae, Luteovirus, ALV-1, viral suppressor, agroinfiltration*

***Acknowledgement:*** *A.J. is a PhD student at the Doctoral School of Plant Sciences of MATE with the Stipendium Hungaricum Scholarship of Tempus Foundation. The Hungarian Scientific Research Fund (K134895) supported our work.*

## EN-4#

### ROLE OF MIR396 IN REGULATING PLANT DEFENSE MECHANISMS AGAINST PATHOGENS: INSIGHTS FROM CRISPR/CAS9 EDITING IN POTATO

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The microRNA gene miR396 is a critical regulator in plants, controlling various developmental processes by targeting the GROWTH-REGULATING FACTOR (GRF) family of transcription factors. The role of miR396 was also shown in drought and biotic stress signalling. It is particularly significant in plant immune responses, where it regulates expression of hypersensitive induced reaction protein genes (HR) during pathogen attacks. Studies on Arabidopsis mutants (MIM396) with reduced miR396 activity demonstrate enhanced resistance to fungal pathogens, suggesting a priming effect on defense responses. Conversely, heightened expression of miR396a in tobacco increases susceptibility to *Phytophthora infestans* (Pi). In our experimental plant, potato, its miR396 gene does not target the typical targets like GRFs and HR factors but regulates the expression of the protease inhibitor multicystatins indicating that it could still have some role in pathogen resistance.
This study delves into the specific role of miR396 in potato defense mechanisms using CRISPR/Cas9 editing. Editing miR396 in 'Désirée' and 'Botond' potato cultivars resulted in reduced miR396 expression. Upon infection with *Ralstonia solanacearum* (Rs), edited 'Botond' lines 8 and 9, as well as Désirée Line 3 and 10, displayed delayed symptom development, indicating enhanced resistance. Notably, in Desiree, larger CRISPR mutations around the first target in lines 3 and 10 correlated with increased resistance. However, all edited Botond lines showed increased susceptibility to Pi, with heightened colonization in tuber assays. Among the Désirée edited lines, line 3 and 10 exhibited increased susceptibility to Pi, while the others were similar to the control. Additionally, altered expression of miR396 target genes, such as LRR receptor-like serine/threonine-protein kinase and multicystatin145, was observed. These findings underscore miR396's importance in regulating potato defense responses against pathogens and illuminate the complex interplay between miR396 and its target genes in plant immunity.

***Acknowledgement*** *National Laboratory project no. RRF-2.3.1-21-2022-00007*

## EN-5#

**THE ROLE OF DNA DEMETHYLASE DEMETER (DME) IN REGULATING THE METHYLATION PATTERN OF MALE AND FEMALE REPRODUCTIVE TISSUES OF BARLEY.**

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DNA methylation is a prominent epigenetic modification playing an important role in gene expression regulation, transposon silencing and chromatin organisation. Methylation patterns are conserved in plants and their dynamism is crucial for developmental processes as well as for adaptation to environmental stresses.

DNA methylation is regulated through mitotic and meiotic cell divisions where establishment and maintenance are ensured by methyltransferase enzymes, while removal of methyl groups is realised by passive or active demethylation, the latter catalysed by DNA glycosylases. During microsporogenesis and microgametogenesis, various epigenetic events occur, leading to the specification of tricellular pollen. Similarly, female gametes acquire specific DNA methylation patterns mediated by the DNA methyltransferase MET1 and the DNA glycosylase DEMETER (DME).

Gametes develop from mother cells which undergo meiotic cell division to halve the genetic information and distribute it into the daughter cells. Early meiotic events such as synapsis and recombination are vital for fertility and genetic diversity and are regulated by epigenetic marks, including DNA methylation and histone modifications.

Here we aimed to investigate the role of active demethylation initiated by the 5-mC DNA glycosylase DEMETER in the sexual reproduction processes of barley (*Hordeum vulgare* cv. 'Golden Promise') with an emphasis on fertility and meiosis. We have created heterozygous and homozygous *dme* mutant lines using the CRISPR-Cas9 gene editing system. Histological measurement of DNA methylation in microtome sections from ovaries and anthers showed a significantly higher DNA methylation signal intensity in the *dme* deficient meiotic samples compared to the wild type. 5-mC fluorescence intensity measured in root samples did not significantly differ from control, indicating a specific effect of the gene within the reproductive tissues. Initial screening uncovered a significant effect on plant fertility while retrospective cytological analysis revealed an increased frequency of meiotic aberrations, suggesting a significant role for DME on the sexual reproduction processes.

***Keywords:*** *Barley, CRISPR-Cas9, DNA methylation, DEMETER gene, meiosis*

***Acknowledgement:*** *The research was supported by the Hungarian Research, Development and Innovation Office (NKFIH, TKP2021-NKTA) and the Hungarian National Laboratories Program (RRF-2.3.1-21-2022-00007).*

## EN-6#

### HETEROLOGOUS PRODUCTION OF A *SOLANUM LYCOPERSICUM* L. ANTIFUNGAL DEFENSIN IN *PICHIA PASTORIS*

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The continuous spread of pesticide-resistant fungi is a serious problem in agriculture which requires introduction of new type of compounds in the plant and crop protection. Defensins of plant origin are potential candidates as these small, cationic, cysteine rich antifungal proteins with high environmental stability effectively inhibit the growth of several phytopathogenic filamentous fungi. One prerequisite of their practical application as pesticides is their industrial bulk production. Although plants secrete them in small amount, the heterologous production in a well-known expression system can overcome this problem. In the present study a novel defensin (K4CBP6) from *Solanum lycopersicum* L. was extracellularly expressed by a generally recognized safe yeast, *Pichia pastoris*. The K4CBP6 yield after purification by cation-exchange and reverse-phase high performance liquid chromatography was ~8 mg/l. Electronic circular dichroism spectroscopy indicated folded, disulphide bridge stabilized structure constituted by α-helix and β-sheet. The purified K4CBP6 was able to inhibit the growth of plant pathogenic filamentous fungi (*Fusarium* spp., *Botrytis cinerea*, *Cladosporium herbarum*) (minimum inhibitory concentrations: 25-50 µg/ml), but was inactive against the tested *Aspergillus* spp. The K4CBP6 is a non-morphogenic defensin, because it did not induce overbranching of the hyphae. K4CBP6 preserved the antifungal effect after heat treatment at 50°C and 100°C, and at slight acidic (pH=6) and basic (pH=8) conditions. The above-mentioned results indicated that K4CBP6 is a potential biopesticide to fight against phytopathogenic fungi; however, several toxicity and plant protection investigations are still required to prove it.

***Keywords:*** *plant defensin, heterologous expression, Pichia pastoris, protein structure, antifungal effect*

***Acknowledgement:*** *Present work of L.T. and L.G. was financed by the Hungarian National Research, Development and Innovation Office - NKFIH, PD 134284 and K 146131 projects.*

## PEN-1#

### POLYMORPHISM ANALYSIS OF APRICOT (*PRUNUS ARMENIACA* L.) GENOTYPES WITH SCOT MARKERS

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Apricots are widely cultivated, many varieties are available, and it is important to know their DNA fingerprint for variety preservation. Given the long juvenile phase of apricot plants, traditional breeding methods are time-consuming; however, marker-assisted selection (MAS) offers a promising approach to speed up this process.
Marker-assisted selection (MAS) is a method that allows breeders to select desirable traits in a breeding programme by analysing DNA samples early in the development of a plant. Forward genetics infers the genotype by examining the plant's morphological characteristics, while reverse genetics is about inferring the phenotype from the genotype. Molecular markers can be coding or non-coding sequences of the genome. The advantage of these markers is that they are less dependent on environmental factors or the developmental state of the plant.
In this research, our goal is to find polymorphisms among 18 apricot genotypes from the Institute of Horticultural Sciences, Cegléd using thirteen Start Codon Targeted (SCoT) markers. SCoT markers target the region flanking the start codon, a highly conserved region in plant genes. Therefore, it can distinguish genetic variation in a specific gene that is linked to a specific trait. These types of dominant molecular markers can be used in various plant species, for example for genetic diversity analysis, variety identification, and gene mapping.
The primers were selected based on the publication of Collard & Mackill (2009). Out of the 13 investigated markers, one (SCoT22) exhibited a monomorphic pattern, while two (SCoT18, SCoT31) showed limited genetic variation. Conversely, the SCoT12 primer revealed a high level (PIC 0,42) of polymorphism.
Based on our results, by increasing the number of markers and genotypes, we would like to conduct marker-trait association studies in the future that could make the breeding of new varieties resistant to different abiotic stresses, especially cold stress, more efficient.

***Reference:***
B.C.Y. Collard, B., & J.D. Mackill (2009). Start Codon Targeted (SCoT) Polymorphism: A Simple, Novel DNA Marker Technique for Generating Gene-Targeted Markers in Plants. *Plant Mol Biol Rep*, 27:86-93. doi: 10.1007/s11105-008-0060-5

***Keywords:*** *SCoT marker, polymorphism, MAS, apricot*

## PEN-2#

### IDENTIFICATION OF SILENCING SUPPRESSOR PROTEINS ENCODED BY PRUNUS VIRUS F

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Perennial woody plants can be infected by a wide range of viruses and viroids. During infection, the plant's defence system, the highly effective and specific RNA interference (RNAi) is induced. Throughout their evolution, viruses have developed strategies to block this defence process in various ways. One of these strategies involves the encoding of proteins that function as viral suppressors of RNA interference (VSR). Prunus virus F (PrVF) belongs to *Fabavirus* genus within the *Secoviridae* family, possessing a bipartite single-stranded positive RNA genome. This virus was detected in our lab in samples collected from cherry and sour cherry trees as well. Broad bean wilt virus-1 (BBWV-1), broad bean wilt virus-2 (BBWV-2), and the recently described cherry virus F (CVF), all belonging to the same *Fabavirus* genus, have been shown to encode protein with VSR activity. Our research aimed to investigate the possibility of whether PrVF encodes proteins with VSR activity.
Based on previous studies, we examined the VSR activity of different proteins encoded by the RNA2 of PrVF, using transient gene expression assay. The coding regions of movement protein (MP), large coat protein (LCP), small coat protein (SCP), as well as two combinations (MP+LCP, LCP+SCP) were cloned from the PrVF genome into a BinHA binary plasmid using the In-fusion method. The resulting recombinant BinHA constructs were transferred into *Agrobacterium tumefaciens* (strain C58C1) by triparental mating and then infiltrated into leaves of 4-week-old wild-type *Nicotiana benthamiana* together with a GFP-expressing construct. Using western blot analysis of the tagged constructs we proved that the cloned ORFs were translated into proteins at the expected size.
For the local VSR activity, GFP fluorescence signals of the infiltrated patches were examined visually under UV light at 3,5 days post inoculation (dpi). The level of GFP and test protein expression was determined by western blotting, while the level of GFP mRNA expression was determined by real-time PCR. GFP transgenic (line 16c) plants were used for the systemic VSR activity assay. The spread of the mobile silencing signal was monitored visually under UV light for a 20-day period.
In contrast to the CVF, the MP of PrVF seems not to possess VSR activity. However, similar to BBWV-1 and BBWV-2, both the LCP and SCP exhibit local VSR activity. Our ongoing research will allow us to characterize the local and systemic VSR activity of these PrVF-encoded proteins. This knowledge can be used in further experiments aimed at characterizing the role of different *Prunus*-infecting viruses in the symptom development of *Prunus* species during multiple virus infections.

***Keywords:*** *VSR, cherry, agroinfiltration*

***Acknowledgement:*** *Our work was supported by grants NKFIH K134895 and the Únkp-23-3 New National Excellence Program of The Ministry for Culture and Innovation from the source of The National Research, Development and Innovation Fund.*

## PEN-3#

### FIRST DETECTION OF RUBUS YELLOW NET VIRUS ON RASPBERRIES IN HUNGARY

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Rubus yellow net virus (RYNV) is a DNA virus of the genus *Badnavirus*. It can infect various *Rubus* species, causing net-like chlorosis of the tissue along the veins in the leaves. Its presence can be asymptomatic in many cases, but when co-infected with other viruses it can cause raspberry mosaic disease (RMD), which is usually associated with symptoms. The presence of RYNV was described in raspberry (*Rubus idaeus*), blackberry (*Rubus fruticosus*), and hybrid plants. It has been found in North America and Europe but has not been reported in Hungary.
In our study, the virome of raspberry samples collected at the Virology Station at Velence and the Variety Experimental Station at Pölöske (NÉBIH) were analysed using high-throughput sequencing. RNA was purified from the leaves of symptomatic and asymptomatic raspberries and used to prepare small RNA sequencing libraries or sent for ribodepleted RNAseq and sequenced on Illumina platform. For bioinformatic analysis, we used CLC Workbench software, which identified the possible presence of several viruses, including RYNV.
As a para-retrovirus, RYNV is known to be able to integrate into the host genome, which makes the distinction between the actively replicating and the genome-integrated form of the virus very challenging. Using published diagnostic primers, we were unable to amplify the expected product from the cDNA prepared from the isolated RNA, so we designed new primers based on the sequencing data. Using these primers, we confirmed the presence of the virus, but only when DNA purified from the plant samples was used as a template. From the circular genome (about 7,800 bp) of the virus we amplified 790-bp and 1234-bp long segments, which showed a respective 78% and 85% sequence similarity to the reference genome. Among the complete RYNV sequences in GenBank, the highest similarity (83% and 87% match) was found in the genome of a virus isolated from Canadian raspberry samples in 2013.
This is the first study to describe this virus in Hungarian raspberries. We are currently working on getting a detailed understanding of the genome of the variant to determine whether the virus is indeed present in an active replicating form in the raspberry plants studied.

***Keywords:*** *raspberry, RYNV, HTS sequencing, Badnavirus*

***Acknowledgement:*** *Our research was supported by the National Research, Development and Innovation Office (NKFIH), grant 2019-2.1.11-TÉT-2020-00124 and by Slovenian Research and Innovation Agency (ARIS), grant P4-0072.*

# PEN-4#

## COMMON MILKWEED AS A VIRUS RESERVOIR

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Common milkweed (*Asclepias syriaca*) is an invasive weed from North America. Due to its promising suitability for various purposes, it has been started to be intensively cultivated in Hungary in the late 19th century. After a while, it became evident that the plant failed to fulfill the expectations therefore its cultivation was stopped but spreading outside from its formal cultivation area it transformed to an invasive weed. However, because of its nectariferous behaviour it is still used in the agriculture. It is found mostly in disturbed, nutrient-rich soils, including cultivated areas, where it can cause great damage as a weed. Since it can act as a host plant for widespread and highly damaging plant pathogenic viruses such as cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV), its spread nearby crop fields poses the risk of a new source of infection. The development of new analytical methods, including high-throughput sequencing (HTS), allow the detection and diagnostics of any presenting viruses on common milkweed.
The aim of our study was to investigate virome of milkweeds growing in various fields using HTS to survey the real existence of its virus reservoir role. In this study, we have collected leaf samples of common milkweed growing in vineyards at Kecskemét and in a sour cherry orchard at Csopak. Some of the leaves of the examined plants showed symptoms suggesting the presence of viral disease. Total RNAs from leaves were extracted and following a DNase treatment was sequenced on Illumina platform. The resulting sequences were analysed using the CLC program. Bioinformatic analysis revealed the possible presence of several viruses, of which the presence of two of them has been confirmed by RT-PCR so far. CMV was detected at both locations, and its presence was additionally confirmed by lateral flow test. At Csopak, the presence of the recently described Wuhan aphid virus 2 (WHAV-2) was detected. RT-PCR validation of two of its four segmented genome was successful. WHAV-2 has only been detected in aphid species and green peas so far and its presence in Hungary and in common milkweed has not been described before. Validation of the other viruses detected by HTS are currently ongoing. Our results suggest that common milkweed may play a significant role as a virus reservoir, increasing its damage as an invasive weed.

***Keywords:*** *common milkweed, CMV, HTS, invasive weed*

***Acknowledgement:*** *Our work was funded by the NKFIH K146087 grant.*

## PN-1#

### PHOTOAUTOTROPHIC AND SUSTAINED $H_2$ PRODUCTION BY THE *PGR5* MUTANT OF *CHLAMYDOMONAS REINHARDTII* IN SIMULATED DAILY LIGHT CONDITIONS

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We studied the photoautotrophic hydrogen ($H_2$) production capacity of the *pgr5* mutant of *Chlamydomonas reinhardtii* under simulated daily light conditions (stepwise increase between 0 and 1000 µmol photons $m^{-2}$ $s^{-1}$). We have developed an automated thin cell layer system to monitor $H_2$ production, enabling regular nitrogen flushing, pressure adjustment, and automated gas sampling. In our automated system, the *pgr5* mutant outperformed the wild-type strain by 100% in terms of $H_2$ production under varying light intensities, and its photosynthetic apparatus and hydrogenase activity were maintained. Immunoblot analysis revealed that the mutant preserved its photosynthetic subunits, including PsbA, PSBO, CP47, PetB, and PsaA, and maintained approximately 29% of its original hydrogenase activity after 85 hours of $H_2$ production. The study suggests that the *pgr5* mutant is a promising candidate for sustained and efficient $H_2$ production under adverse light conditions encountered in bioindustry settings. The findings highlight the potential of the *pgr5* mutant for bioindustry applications and the need for further investigation and optimization of culture conditions for economic viability and industrial-scale application.

***Keywords:*** *Chlamydomonas, biohydrogen, photoinhibition, PGR5, photosynthesis, thin cell layer cultures*

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## PN-2#

### CHANGES OF STRESS-RESPONSIVE PARAMETERS IN DIFFERENT CEREAL GENOTYPES DURING OSMOTIC STRESS

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Imbalances in the water regime result in osmotic stress leading to alterations in cellular processes and physiological functions. The aim of our experiments was to compare the osmotic stress-induced changes of various protective mechanisms in five cereal genotypes with different drought tolerance. These include the possible activation of the ROS-scavenging ascorbate-glutathione cycle, the accumulation of polyamines and the induction of stress response-related genes. Following a 7-day 15-% polyethylene-glycol-induced (PEG) osmotic stress, discernible changes in the investigated parameters were detected in the studied cereal genotypes (*Aegilops biuncialis* Vis 642 – *Ae. b*. 642, drought-sensitive; *Ae. b*. 382, drought-tolerant; *Triticum aestivum* L. Cappelle-Desprez – C.D., drought-sensitive; Plainsman V – Pl., drought-tolerant and Martonvásári 9kr1 - Mv9kr1, drought-tolerant).
The treatment decreased the fresh weight of all genotypes and the shoot length in most of them. The antioxidants and subsequently the amount of $H_2O_2$ were greatly affected by osmotic stress. The glutathione reductase activity decreased in shoots of all genotypes, while the ascorbate peroxidase activity was lower only in shoots of wheat. The reduced γ-glutamyl-cysteine (glutathione precursor) content increased in shoots of *Ae. b.* 382 and 642, and in the roots of Mv9kr1. The amount of reduced glutathione (GSH) increased in the shoots of C.D. and Mv9kr1, while the level of its oxidised form (GSSG) decreased in the two *Aegilops* genotypes. Investigating the roots, the GSH content increased in all genotypes, except for *Ae. b.* 382, while the GSSG level became greater in all genotypes except for *Ae. b.* 642. The $H_2O_2$ content decreased in the shoots of C.D. and increased in that of Mv9kr1. Interestingly, it decreased in the roots of both *Aegilops* genotypes but increased in that of Mv9kr1.
Among the polyamines, the putrescine levels increased in the shoot of *Ae. b.* 642 and Pl., and also in the root of Mv9kr1. The spermidine levels only became greater in the roots of Mv9kr1. The spermine content increased in shoots of all genotypes except for Mv9kr1, while it increased only in the root of *Ae. b.* 382 and Mv9kr1. The amount of 1,3-diaminopropane increased in shoots of all genotypes, while in the roots it increased in Mv9kr1 and decreased in Pl.
The expression of antioxidant genes such as *ascorbate peroxidase* and catalase was larger in shoots compared to roots. The *gibberellin 20 oxidase 1-D* transcription decreased in the roots of all genotypes. The expression of genes encoding different chaperones did show a general increase in shoots, but a general decrease in roots except for the *dehydrin 3* gene.
The results show that the investigated two species display distinct trends and changes in the investigated parameters, mostly irrespective of the drought tolerance of the individual genotypes. Some of the studied protective components were only induced in the *Aegilops* genotypes, thus, their genes can be introduced into wheat to increase its stress tolerance.

***Keywords:*** *osmotic stress, cereals, antioxidants*

***Acknowledgement:*** *The work was funded by NKFIH (K131638 and TKP2021-NKTA-06).*

## PN-3#

### REGULATION OF CYSTEINE OXIDASE GENES IN CUCUMBER VARIETIES DURING WATERLOGGING STRESS

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Commercially grown cucumber F1 hybrids (*Cucumis sativus* L.), whether bred for cultivation in open field or for greenhouses, are subjected to distinct environmental conditions. In greenhouse setups, often in soilless media, the confined rhizosphere and intensive fertigation practices can cause waterlogging and subsequent hypoxia stress, but surprisingly achieving high productivity. This study aims to elucidate the underlying molecular traits contributing to this phenomenon. To this end two representative cucumber hybrids (‘Joker’ and ‘Oitol’), typically grown under open-field and greenhouse conditions respectively, were subjected to waterlogging stress in a semi-hydroponic system with perlite/rockwool substrates. The plants were flooded with nutrient solution at four-leaf stage. Oxygen levels declined in the media and both hybrids exhibited elevated alcohol dehydrogenase activity in response to waterlogging. This was indicative of anaerobic metabolism activation, albeit less pronounced in the greenhouse-type hybrid, 'Oitol.' Through qRT-PCR analysis the induction patterns of *cysteine oxidase* (*CysOx*) genes, crucial components of the hypoxia sensing pathway were investigated. The results revealed hybrid-specific responses. 'Joker' exhibited significant upregulation of all four annotated *CysOx* genes, particularly *CysOx1* and *CysOx4*, displaying approximately 17- and 5-fold increases, respectively. In contrast, 'Oitol' showed negligible changes in *CysOx1* and *CysOx4* expression and modest upregulation in *CysOx2* and *CysOx3*. These findings reveal a key step of hypoxia sensing pathway in cucumber and establish hybrid specific induction of cysteine oxidase genes in flooded roots.

## PN-4#

### CHARACTERIZATION OF VIROME OF *ASCLEPIAS SYRIACA* IN HUNGARY

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Common milkweed or butterfly weed (*Asclepias syriaca* L.) member of the genus *Asclepias*, is an invasive weed that originates in North America. It is a perennial rhizomatous herbaceous plant reaching heights of approximately six feet and has clusters of pinkish-purple flowers. This species has become a major concern in Hungary due to its ability to spread rapidly and outcompete native plants. It spreads aggressively in natural and semi-natural habitats, orchards and field crops. Therefore, it is essential to establish its host potential for economically destructive viruses.
This research aims to shed light on the role of viruses in the biology and spread of *A. syriaca* and provide a deeper understanding of the viral ecology within this invasive weed species. We aimed to determine viromes of *A. syriaca* plants growing at an orchard and at natural habitats using HTS-based metagenomics sequencing, alongside bioinformatics analysis tools. A survey was conducted in a plum orchard (Tapiobicske), wet natural habitat (Tapiobicske) and sandy, dry natural habitat (Nagykata) in Hungary. Four leaves of ten plants from each location, showing different virus like symptoms, were collected and used for total isolation of nucleic acid. Three pools, mixing up nucleic acid originating from the same location were prepared, DNase treated and sent for sequencing as a unified pool. Bioinformatic analysis of sequenced reads was commenced using CLC Genomics workbench (QIAGEN Aarhus, Denmark) with data importation as fastq file, followed by trimming and generated FastQC reports. Contigs were prepared from paired reads, and followed by BLAST of contigs to the reference genomes of the currently known viruses infecting plants, fungi and insects. The reads were directly mapped to reference genomes of viruses for which contigs with E value of zero has been found.
Currently validation of the presence of these viruses are ongoing. Using cDNAs prepared from the original nucleic acid extracts we will be able to check their real existence in *A. syriaca*, and determine the number of the infected plant at all locations.

***Keywords:*** *Asclepias syriaca, plant virus, high throughput sequencing (HTS), invasive weeds*

***Acknowledgement:*** Our work was funded by the *by the NKFIH K146087 grant. Kiambi Roy is a PhD student of the MATE Doctoral School of Plant Sciences and supported by the Stipendium Hungaricum scholarship of TEMPUS.*

## PN-5#

### REDOX REGULATION OF METABOLISM AND REGENERATION OF WHEAT CALLI

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Redox changes in plants affect growth and development by reprogramming metabolism. Reactive oxygen species and antioxidants are involved in these processes as components of the signalling networks. We hypothesized that ascorbate (Asc) and hydrogen peroxide ($H_2O_2$) may influence shoot and root formation and redox-dependent metabolic processes in the calli. In this study, we used *Triticum aestivum* L, cv. Chinese Spring calli, which were induced from immature embryos. Calli were treated with 0, 10, 20 and 40 mM Asc and $H_2O_2$ for one week. We investigated the effect of these treatments on shoot regeneration, the activity of antioxidant enzymes, the levels of hormones and thiols.

Based on our results, Asc and $H_2O_2$ treatments decreased glutathione levels in a concentration-dependent manner. In addition, $H_2O_2$ also changed the redox state of glutathione. Addition of 10 and 40 mM Asc increased the levels of the studied hormones. The activity of the measured antioxidant enzymes was significantly lower after using the higher concentrations of Asc and $H_2O_2$. Treatment with 10 mM Asc increased the activity of ascorbate peroxidase and glutathione reductase, and treatment with 10 mM $H_2O_2$ increased the activity of dehydroascorbate reductase and monodehydroascorbate reductase. Shoot regeneration from calli was stimulated by 10 mM Asc and 20 mM $H_2O_2$ treatments.

Asc and $H_2O_2$ altered the redox state of the calli, leading to changes in metabolism and hormone levels as well as enhanced shoot regeneration if applied in lower concentrations.

***Keywords****: ascorbate, hydrogen peroxide, callus, antioxidant*

***Acknowledgement****: The NKFIH-K131638 and TKP2021-NKTA-06 grants supported this research.*

## PN-6#

### HOW DOES WHOLE GENOME DUPLICATION AFFECT MEIOTIC RECOMBINATION AND FERTILITY IN POLYPLOID BARLEY?

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Whole genome duplication (WGD) is a key driver in biodiversity evolution, fostering the emergence of new gene functions and facilitating adaptation to harsh environments. Polyploidy, a condition characterized by multiple sets of chromosomes, is a common phenomenon in plants, with all angiosperms experiencing at least one ancient WGD event.
However, WGD is not without its challenges. The process of polyploidization induces substantial alterations in cellular biology, including an increase in cell size, a modified correlation between cell volume and nuclear DNA content, and changes in chromosome behavior during meiotic cell division. In the context of diploid meiosis, pairs of homologous chromosomes identify each other, synapse, and undergo recombination. This process not only generates new allele combinations but also ensures precise chromosome segregation. The introduction of an additional pair of homologous chromosomes in newly formed autotetraploids disrupts this pairing equilibrium, leading to chromosome instability and infertility.
In this study, we explored the impact of WGD on the fertility and meiotic chromosome behavior of barley (*Hordeum vulgare*), with an emphasis on synapsis and recombination. We observed a notable decline in fertility in comparison to diploids. Fertility negatively correlated with the frequency of chromosome segregation errors during the first and second stages of meiosis. Interestingly, fertility variations in the autotetraploids spanned a broad range, with some plants exhibiting complete sterility while others maintained relatively high spikelet fertility. Changes were also noted in chromosome pairing and chiasma formation, with a significant per-chromosome decrease in class I mature crossovers, as indicated by the frequency of HEI10 immunofoci.

***Keywords:*** *polyploidy, recombination, barley*

***Acknowledgement:*** *The research was supported by the Hungarian Research, Development and Innovation Office (NKFIH, 2021-1.2.4-TÉT-2021-00033, TKP2021-NKTA) and the Hungarian National Laboratories Program (grant number RRF-2.3.1-21-2022-00007).*

## PN-7#

# ENHANCING THE SHELF-LIFE OF FRESH-CUTTING APPLE AND SOUR CHERRY FRUIT THROUGH NANO-COATING BASED RICHED OF APPLE POMACE AND SEA BUCKTHORN EXTRACT

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This research emphasizes the critical role of fruits in human nutrition, offering essential nutrients and health benefits. However, preserving their freshness and nutritional value, especially for fresh-cut and berry fruits, is challenging due to issues like browning and microbial contamination. Fresh-cut fruits like apple are clean, convenient, and often have lower pesticide residues. The following study explores how edible coatings can extend the shelf life of fruits, reduce waste, and enhance health benefits. It specifically highlights the suitability of apples and sour cherry fruits, which contain abundant phenolic compounds such as phenolic acids, flavonoids, anthocyanins, and an excellent source of antioxidants such as vitamins, for this approach. Apple is a popular and diverse fruit that may offer various health benefits due to their nutrient content. However, the mechanical processing can damage their natural structure, making them more susceptible to environmental factors. In response to the numerous calls to decrease the deterioration rate of cherries, the concept of food preservation has begun to develop new technologies to extend their shelf life in order to meet consumer demands. Applying the edible coatings to delay the development of physiological disorders during storage of cherries is one efficient approach that greatly helps to slow down the respiration rates of cherries. The use of natural polymers, plant-based materials, and agricultural waste as eco-friendly packaging materials is discussed as an alternative to non-biodegradable plastics. Millions of tons of apple pomace are generated annually worldwide as a byproduct of juice, cider, or puree production. This waste material has high moisture and biodegradable organic content, making it a suitable resource for bioplastic production. Other beneficial agriculture by-product is sea buckthorn extract, which is a rich source of bioactive compounds with potential health benefits. When processing sea buckthorn into products like juice and jam, a substantial amount of waste (pomace) is generated that often used for animal feed due to the lack of processing facilities. New extraction techniques have been developed to utilize its pomace from various fruits to capture valuable nutrients. Additionally, the use of nanotechnology for protection and the incorporation of natural plant extracts to enhance biopolymer films are presented as promising solutions. Ultimately, the study underscores the importance of preserving fruit quality and nutritional benefits while addressing environmental concerns in food packaging. It highlights the potential for a biodegradable and sustainable approach to fruit conservation and packaging through the development of edible coatings and alternative packaging materials.

***Keywords:*** *Edible coating, Fresh-cut and Berry fruit, Agricultural waste*

***Acknowledgement:*** *This work was supported by Food Science Doctoral School of Hungarian University of Agriculture and Life Sciences*

## PN-8#

### BIOMECHANICAL PROFILING OF THE *CAPSICUM ANNUUM FRX* MUTANT GENOTYPE

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Upon conducting resistance experiments of *Capsicum annuum* L. plants against *Meloidogyne sp.* parasites, some individuals were found to possess extremely fragile, glass-like vegetative organs, including roots and stems. According to breeding experiences, this fragile phenotype, designated as 'fragile plant' (*frx*), has been determined to be monogenic and recessive. In this study, we aim to unveil the biomechanical properties of the *frx* trait by examining the maximal breaking force (N) of three different regions of the stems.

In our measurements, we selected stems that showed no apparent signs of disease or damage caused by pests. Three-point bending experiments were conducted using a Universal Testing Machine to evaluate the mechanical characteristics of the samples. Both of the genotypes (fragile, not fragile) were grown under greenhouse conditions until they reached fully maturation. Then the plant stems were collected and each sample was precisely cut to a uniform length and positioned on a pedestal, and then a consistent force was exerted on the samples until they either fractured or reached their maximum flexure point. This provides valuable information about their fruit bearing capacity and potential applicability in different cultivation styles.

Notably, the maximum force required for breakage was lower in the case of the *frx* mutants in every region of the stem, with fractures occurring earlier and abruptly. The breaking point in *frx* stems consistently displayed a distinct and clear-cut separation as it was previously recorded during the breeding processes as well. Conversely, in the control genotype, 'Garai Fehér' a heightened force requirement for breakage was observed. Additionally, in certain instances, plant stems exhibited flexure without complete fracture, which never happened in case of the *frx* plants.

Comparison of the average value and deviation of each group revealed significant differences between the two genotypes, with substantial deviations in such cases. Consequently, we conclude that *frx* mutants break easier and faster, not just in case of the stems, but also in case of the pedicel, suggesting their potential use in mechanical harvesting technologies. Our experimental findings provide insights into their fruit-bearing capabilities and potential applications in alternative and sustainable methods for vegetable cultivation.

***Keywords:*** *Capsicum annuum, mutant, mechanical,*

***Acknowledgement:*** *Supported by the ÚNKP-23-3-II-MATE-33 new national excellence program of the ministry for culture and innovation from the source of the national research, development and innovation fund.*

## PN-9#

### THE EFFECTS OF DIFFERENT DAILY LIGHT CONDITIONS ON POLYAMINE METABOLISM

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Light plays a pivotal role in plant growth and stress resilience, while polyamines (PAs) are crucial compounds in these processes. Numerous studies demonstrate the interplay between PAs and photosynthesis, with PAs impacting photosynthesis at various levels and vice versa, as PA biosynthesis responds to light regulation. The primary objective of this study was to deepen our understanding of how light quality and quantity impact PA metabolism at both metabolite and gene expression levels. Additionally, we sought to explore the role of daily rhythms on PA metabolism in wheat plants. A special question was how PA treatments influence PA metabolism under these various light conditions.
Our results revealed positive correlation between light intensity and the levels of putrescine (PUT) or spermidine (SPD) levels under different light intensity conditions (50, 250, and 500 μmol $m^{-2}s^{-1}$). However, light intensity did not significantly affect PA metabolism at the gene expression level. Light spectra (three spectral compositions were applied, which were referred to by their most typical characteristic: blue, red, and the combination of blue and red lights) had minimal impact on the actual PA pool, although blue light decreased, while red light increased the expression of PA metabolism-related genes. PA treatments showed varying effects on PA metabolism under different light conditions, aiding plant adaptation. Further experiments were performed with three genotypes under white light at 250 μmol $m^{-2}s^{-1}$ light intensity, where the most characteristic PA treatment-induced changes were observed.
Daily monitoring of PA contents (sampling were performed after 1, 3, 5, 7, 9 and 13 hours after the beginning of illumination) revealed an increase in PUT and SPD levels, while the level of the catabolite product, 1,3 diaminopropane (DAP) decreased. These daily changes in PA levels are also found under different light periods (16 h light/ 8 h dark or 8 h light/ 16 h dark). In addition, extended daily exposure to light (16 h light/ 8 h dark) itself promoted PA synthesis, while concurrently inhibited degradation compared to the 8 h light/ 16 h dark conditions.
In conclusion, results suggested that light quantity and quality play important role in the adjustment of PA metabolism. Changes in PA levels of different genotypes can be partly generalised, and it can be stated that: more light – more PA. The fine-tuning of the synthesis, back-conversion and terminal catabolism could be responsible for changes in the actual PA pool, leading to successful adaptation to different light conditions.

***Keywords:*** *light, polyamine, wheat*

***Acknowledgment:*** *This work was funded by the National Research Development and Innovation Office, Hungary (NKFIH K134395).*

## PN-10#

### THE EFFECT OF DIFFERENT DRYING METHODS ON THE BANANA PEEL PECTIN'S EXTRACTED BY ULTRASOUND AND USING IT AS A FAT REPLACER IN REDUCED FAT BISCUITS

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Nowadays the agro-industrial by-products reuse represent a renewable source for some food additives or even derive new added-value ingredients with functional properties, which will aid the entire food system. Banana is among the most produced, traded and consumed fruits globally with massive amount of peel as by-product, which is rich in essential nutrients constituents and it is also a source of valuable natural compounds such as antioxidant, cellulose, and pectin.
Recently ultrasound assisted extraction (UAE) gets more attention as an alternative to pectin's conventional extraction method by mineral acids with high temperature in order to improve its quality, and to save the environment. There are also various drying techniques could be used for pectin powder production, more studies are needed to choose the best one as it is a vital step in pectin production affecting its properties. Several carbohydrate-based fat replacers including pectin have been used to mimic the textural and sensory attributes provided by fat, with a fewer amount of calories. So, this study aims to evaluate and compare functional properties of banana peel pectin which will be extracted by ultrasound technique and dried by vacuum oven, freeze dryer, spray dryer and hot air oven, and then assess the impact of using it as partial fat replacers in reduced fat biscuits.
Extraction pectin from banana peel powder will be done in ultrasound bath using citric acid under various conditions of temperature and time. The pectin precipitates will be dried using different techniques**,** and then the dried pectin samples properties (solubility, holding water capacity) will be evaluated. Finally, pectin powder with the best properties will be used as a fat replacer in reduced fat biscuits with different percentages to study its nutritional value, physical and sensory properties. These results offer detailed information about pectin extracted from banane peel, using ultrasound techique under different conditions and with four different drying methods in order to reveal how the applied methods influenced the yield and properties, and to increase the ability to use this by-product as a fat replacer in biscuitsThis can be useful to introduce an effective alternative pectin source in the food industry.

***Keywords:*** *banana peel waste, fat replacer, biscuit*

***Acknowledgement:*** *This work was supported by Food Science Doctoral School of Hungarian University of Agriculture and Life Sciences*

## PN-11#

### THE EFFECT OF METHYLATION ON THE ANTHOCYANIN BIOSYNTHESIS OF PEPPER FRUITS

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Pepper (*Capsicum annum*) is one of the most important cultivated vegetables in the world. Its economic value is largely determined by the colour of the fruit. In the course of our work, we examined the anthocyanins responsible for the formation of the purple colour. Anthocyanins can protect the plant against many biotic and abiotic stresses, and are also beneficial for the human body. Retrotransposon insertion or even some post-translational regulatory mechanisms, such as methylation can influence their biosynthesis. Several research have described a retrotransposon (LINE-1) insertion in the promoter of the pepper *MYBa* gene, which resulted in extreme anthocyanin accumulation in pepper tissues. The plants we examined here, came from a controlled cross where the paternal parent was homozygous for the retrotransposon insertion, few plants from the F2 generation showed sectoral purple discoloration in their leaves and fruits.
The main goal of our research was to examine the different coloured sectors of pepper fruit. We examined the expression of genes involved in the anthocyanin biosynthetic pathway in both pigmented and non-pigmented regions of the fruits. We also measured the methylation pattern of the different sectors. Analytical measurements were also carried out to determine the antioxidant capacity, total polyphenol content, and total monomeric anthocyanin content of the same sectors.
Overall, expression pattern of 15 genes was studied, which were generally more strongly expressed in the purple sectors, but we also experienced outliers. We observed more methylation differences between the purple and green sectors as well. We have mostly found cases where the DNA isolated from the purple sector is not methylated, while it is methylated in the green sector. The methylation of the 3' UTR region of the retrotransposon was also higher in the green/non-pigmented regions. As of the analytical measurements, significantly higher values were scored in the purple sectors compared to the green sector of the same fruit.
In conclusion we can state that the amount of biologically active compounds was significantly higher in the purple-coloured sectors. These compounds are also useful for plants themself and organisms that eat plants, providing protection against stress and maintaining health. Methylation and gene expression studies can help to better understand the genetic background of anthocyanin biosynthesis.

## PN-12#

### EXPLORING THE PLANT BIOSTIMULANT EFFECTS OF *KOCURIA ARSENATIS* FSP-120 AND *CHLORELLA* SP. MACC-360 BACTERIAL-ALGAL COMBINATION

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Past empirical research has demonstrated that bacteria and algae in the soil environment closely interact with plants through mutually beneficial relationships. We investigated the effects of *Kocuria arsenatis* FSP-120 bacteria and *Chlorella* sp. MACC-360 microalgae treatment on tomato plants and their rhizosphere. Bacterial species were isolated from the rhizosphere surface and bulk soil of drought-tolerant plants and tested their PGP (Plant Growth Promoting) properties. In addition to the algae, we also detected a high level of exopolysaccharides (EPS) synthesis in the *Kocuria arsenatis* FSP-120 strain. We conducted experiments where *Kocuria arsenatis* bacterial species were co-cultivated with *Chlorella* sp. MACC-360 green algae. Pairwise algal-bacterial combinations were cultivated for five days in MM medium. We have investigated the effects of bacterial phylogenetic relationship and growth rate on specific algal growth and biomass yield. Also, we have tested selected stable algal-bacterial combinations in plant biostimulation studies. We have observed that appropriately selected combinations of bacteria and green algae, specifically *Kocuria arsenatis* FSP120 and *Chlorella* sp. MACC-360 microalgae had significant positive effects on the mass of the tomato roots, the number of flower buds, the flowering kinetics and tomato stress resistance compared to plants treated solely with algae or bacteria, respectively. These results show that this microalgae-bacteria combination might be used as an efficient biostimulant treatment to not only promote growth but also enhance plant's tolerance to various stresses. The metagenomic results showed that *Chlorella* sp. MACC-360 treatment resulted in the enrichment of a specific *Pseudomonas putida* bacterium in the tomato rhizosphere.

***Keywords:*** *biostimulant, Kocuria, Chlorella, exopolysaccharide, tomato, plant*

## PN-13#

### CULTIVAR-SPECIFIC RESPONSES TO CHILLING STRESS IN CUCUMBER

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Current cultivation techniques consider biotic and abiotic stress tolerance while selecting cultivars to suit specific growing systems. This study focused on two cucumber cultivars: open field variety 'Joker' and greenhouse variety 'Grafito'. These cultivars represent divergent genotypes with supposedly different chilling stress tolerance due to their respective cultivation practice. In order to characterize the two F1 hybrids for this trait, malondialdehyde (MDA) and proline levels were investigated as relevant plant responses to low temperature. To induce chilling stress plants were subjected to 8 °C temperature in a phytotron chamber during their early growth phase. Proline levels varied significantly among cultivars and treatments, approving the presumed role of this metabolite in cold stress response. In contrast, MDA levels did not differ significantly between cultivars or treatments. These findings improve our understanding on how different cucumber genotypes respond to low temperature stress at the molecular level. The study reveals that proline levels are impacted by both cultivar type and chilling stress exposure, whereas MDA levels are reasonably stable under the investigated conditions and time frame. The results shed light on cucumber plants' biochemical responses to chilling stress, emphasizing the role of cultivar-specific features for tolerance. Our findings provide useful insights for future cucumber chilling stress studies, demonstrating that MDA levels alone should not be considered as a exclusive stress marker, but cultivar specific properties and other metabolic responses should also be addressed.

***Keywords:*** *chilling, cucumber, stress markers*

# MEDICAL AND PHARMACOLOGICAL BIOTECHNOLOGY

HUN REN ATK CENTRE FOR AGRICULTURAL RESEARCH
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# EEO#

## BIOLOGICAL ROLES AND EXPLOITATION POTENTIAL OF EXTRACELLULAR VESICLES

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Extracellular vesicles are phospholipid bilayer-enclosed structures released by all cells in nature. They are either released from the endosomal compartment or from the plasma membrane of cells. They play roles in various biological processes, from the maintenance of homeostasis to intercellular signaling. Accumulating evidence support the role of extracellular vesicles in plants and in the communication between plant cells and pathogens.
Extracellular vesicles carry important internal cargo (proteins, nucleic acids, metabolites) as well as an externally adsorbed biomolecular corona. Over the past two decades, research on extracellular vesicles has illuminated their potential for exploitation. They can serve as prognostic/diagnostic biomarkers or therapeutic agents/vehicles. Internal/external cargo loading, chemical surface modifications and engineering of extracellular vesicles place them in the research spotlight as future theranostic agents.

***Keywords:*** *extracellular vesicles, engineering, therapy*

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# EO-1#

## DEVELOPMENT OF CHEMOMETRIC MODELS FOR RAMAN SPECTROSCOPY-BASED MONITORING AND CONTROL OF MAMMALIAN CELL CULTIVATIONS

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Monoclonal antibodies have become increasingly prevalent therapeutics in the pharmaceutical industry, with many ranking among the top-selling products. Their production, however, necessitates the application of complex technology. The expression of antibodies employs bioreactor cultivation of mammalian cells, most commonly Chinese hamster ovary cells. Chinese hamster ovary cell culture processes involve the use of costly multicomponent nutrient media and exhibit high sensitivity to environmental changes, both of which significantly impact protein quantity and quality.
In this study, innovative process analytical technology tools were employed to address these challenges. By monitoring and controlling critical process parameters, we ensured robust and efficient production of monoclonal antibodies. Our research focused on bioreactor cultivation of Chinese hamster ovary cells producing adalimumab, with inline Raman spectroscopy and dielectric spectroscopy playing pivotal roles in monitoring and controlling the cultivation process. For the inline monitoring of critical nutrients and byproducts chemometric models were developed by evaluating spectral data obtained from the bioreactor using different multivariate regression methods, such as Partial Least Squares, Support Vector Machine or Artificial Neural Networks. Calibration models for various nutrient component concentrations demonstrated strong predictive capabilities, enabling real-time monitoring of a bioreactor cell cultivation.
Moreover, our study led to the development of dynamic feeding strategies for the automatic control of multi-component feed supplementation based on nutrient concentrations determined by Raman spectroscopy. Implementation of these strategies resulted in prolonged cultivation times, increased viable cell density, and subsequently elevated antibody titers. These outcomes underscore the effectiveness of inline Raman spectroscopy as a suitable method for real-time monitoring and control of biotechnological processes. The access to real-time information not only facilitates a deeper process understanding but also supports ongoing process optimization efforts.

***Keywords:*** *pharmaceutical biotechnology, monoclonal antibodies, process analytical technology, process optimization, dynamic feeding strategies, Raman spectroscopy, data analysis*

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## EO-2#

### INVESTIGATION OF DUTPASE EXPRESSION IN MOUSE ADULT NEUROGENESIS

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The enzyme dUTPase has an important role in maintaining genomic integrity by hydrolysing dUTP into dUMP, which is the substrate of thymidylate synthase in the *de novo* dTTP synthesis (Vértessy BG, 2009). The penultimate step of dTTP and dUTP biosynthesis is catalyzed by dTMP kinase, which loss-of-function has been shown to cause severe postnatal neurodegenerative disease (Vanoevelen JM, 2022). Through its catalytic activity dUTPase keep the cellular dUTP/dTTP ratio low. If dUTP is available in the nucleotide pool it can be misincorporated into the DNA instead of dTTP. Cytosine deamination is also resulted in uracil base in the genome; however, it is a mutagenic process. Irrespectively of the source enzymes of the base excision repair mechanism excises uracil bases. If the genomic uracil level increases, the overactivation of the repair process leads to single- and double-strand breaks and can lead to cell death. The *Dut* gene encodes a nuclear and a mitochondrial isoform, which are generated via alternative splicing and alternative promoter usage. Previously, we developed an optimized RT-qPCR method for the isoform-specific determination of dUTPase mRNA expression in different mouse tissues (Rácz GA, 2019). We found relatively high mRNA expression of the nuclear isoform of dUTPase in the brain, and several studies suggested that dUTPase activity is high in the brain (Spector R 1983, Kan L 1999, Focher F 1990). The elevated expression was unexpected, as dUTPase is known to play an important role in proliferating cells and mass production of neural cells is completed by adulthood. Therefore, we aimed to determine the localisation of dUTPase in different brain regions, cell types and subcellular levels with immunostaining in 3-month-old adult mouse brain. Our results showed that dUTPase expression is observed in the progenitor cells in the germinative zones of the brain, i.e., in the subventricular and the subgranular zone, suggesting a potential role of dUTPase in cell differentiation. We showed that dUTPase is expressed in the intensely proliferating intermediate progenitor cells, presumably from the start of the mitotic activity, and is present during mitosis. These novel findings suggest that dUTPase may have a role in cell differentiation and indicate that accurate dTTP biosynthesis can be vital, especially in neurogenesis.

***Keywords:*** *expression, dUTPase, development*

***Acknowledgement:*** *K135231, FK137867, NKP-2018-1.2.1-NKP-2018-00005, 2022-1.2.2-TÉT-IPARI-UZ-2022-00003 to BGV*

## EO-3#

### INVESTIGATION OF PROTEINACEOUS INHIBITION OF M. TUBERCULOSIS DUTPASE

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The dUTPase enzyme plays a key role in the maintenance of genomic integrity in various species by preventing the uracil incorporation into DNA. The decreased activity of the enzyme can indirectly lead to DNA double-strand brakes and to cell death (1). The design of species-specific dUTPase inhibitor could help in defence against human pathogens, like Mycobacterium tuberculosis, as the emergence of multidrug-resistant strains causes an increasing problem in the treatment of tuberculosis (2). A known dUTPase inhibitor, the staphylococcal protein Stl strongly inhibits the Mycobacterium tuberculosis dUTPase (MtDUT) (3,4). The understanding of their interaction may help in the design of efficient MtDUT inhibitors.

For this reason, we have crystallized the complex of MtDUT and a truncated Stl protein variant, Stl1-159 and obtained an X-ray dataset at Elettra Trieste with 3.4 Å resolution. Bio-layer interferometry measurements were performed in favour of the analysis of the protein-protein binding kinetics. In order to examine whether shorter, truncated versions of Stl possess a more effective inhibitory effect on MtDUT activity than the full-length protein, we tested the effect of three truncated versions of Stl by steady-state activity measurements.

According to the result of the Bio-layer interferometry and steady-state activity measurements, the full-length Stl protein possesses the highest affinity to MtDUT and has the most effective inhibitory effect on dUTPase activity. The structural model of the Stl1-159-MtDUT complex reveals the main amino acid residues involved in the interaction. However, there may still be unknown interacting residues on the C-terminal region of Stl, which contribute to the formation of the strong complex explaining the most effective inhibition of MtDUT.

***References***:

[1] Vértessy, B.G., and Tóth, J. (2009). Acc. Chem. Res. 42, 97–106.
[2] Heyckendorf, J., Lange, C., and Martensen, J. (2014). Elsevier, 239–253.
[3] Maiques, E., Quiles-Puchalt, N., Donderis, J., Ciges-Tomas, J.R., Alite, C., Bowring, J.Z., Humphrey, S., Penadés, J.R., and Marina, A. (2016). Nucleic Acids Res. 44, 5457–5469.
[4] Hirmondó, R., Szabó, J.E., Nyíri, K., Tarjányi, S., Dobrotka, P., Tóth, J., and Vértessy, B.G. (2015). DNA Repair (Amst) 30, 21–27.

## PEO-1#

### PROTEIN A AFFINITY CHROMATOGRAPHY OF BIOSIMILAR NIVOLUMAB MONOCLONAL ANTIBODY FROM CHINESE HAMSTER OVARY (CHO) CELL CULTURE BROTHS

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Due to advancements in recombinant DNA technology, the significance of targeted therapeutic monoclonal antibodies (mAbs) has dramatically increased in recent times, occupying a pivotal position in the global biotechnological pharmaceutical industry. The synthesis of these biosimilar products occurs in mammalian expression systems, primarily in Chinese hamster ovary (CHO) cell cultures. With the evolution of upstream processes, the production yield of monoclonal antibodies has escalated from a few milligrams to grams per liter. This advancement exerts significant pressure on downstream processes, necessitating modifications to achieve greater efficiency. One prevalent and primary chromatographic purification step in the downstream process of monoclonal antibodies is Protein A affinity chromatography.
The aim of our research is to characterize the structure of biosimilar Nivolumab, an IgG4 monoclonal antibody, expressed in CHO mammalian cell lines. This involves purification using Protein A affinity chromatography, determining correlations between different passages, confirming size using MALDI-TOF-MS and SDS-PAGE separation, as well as employing native SDS-PAGE.
During purification, we worked with samples from four increasing passages to investigate temporal correlations. Our results confirm that the concentration of produced antibodies increases proportionally with the increasing number of passages. The characterization of the structure of biosimilar Nivolumab monoclonal antibodies has been achieved.

***Keywords:*** *Monoclonal antibody purification, Protein A affinity chromatography, biosimilar Nivolumab*

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Új Nemzeti Kiválóság Program

# PEO-2#

## NON-INVASIVE ASSESSMENT OF THE QUALITY AND VIABILITY OF RABBIT EMBRYOS

***Salinas, María[1,2]; Tokodyné, Szabadi, Nikolett[1,2]; Dévai, Gréta[1,2]; Urbán, Martin[1,2], Nemes, Annamaria[3], Fancsovits, Péter[3]; Bodrogi, Lilla[1,2], Gócza, Elen[1,2]***

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miRNAs are short, non-coding RNA molecules capable of regulating numerous key biological processes through post-transcriptional gene expression regulation and gene silencing. In our experiments on rabbit embryos, we developed a novel miRNA detection protocol suitable for detecting miRNAs present in minimal quantities in both embryo samples and their corresponding culture medium samples.
Initially, we compared the efficiency of two RNA isolation kits, and based on the test results, we found that the RNAqueous™-Micro Total RNA Isolation Kit is more suitable for producing clean RNA isolates from lysed embryo samples. We examined the expression of five development-specific miRNAs (miR-24-3p, miR-92a-3p, miR-103a-3p, miR-191-5p, and miR-378a-3p) in 4-day-old and 6-day-old rabbit embryos and their culture media. We found significant differences in the expression levels of miRNAs extracted from 4-day-old compared to 6-day-old embryos, while the miRNA expression pattern in medium samples indicated less distinction between 4-day-old and 6-day-old embryos. Nevertheless, we were able to identify weak-quality embryos based on the miRNA expression profile in the culture medium collected from the individually cultured embryos.
Therefore, the combination of the five miRNAs examined could likely be used to assess embryo quality based on the miRNA profile of the culture medium. However, for a more accurate classification of embryos, need further investigation into additional miRNAs beyond the five studied is required.
We anticipate that our innovative miRNA detection method and the biomarker combination of these miRNAs will facilitate the non-invasive assessment of human IVF embryo quality and viability. This will be achieved through the identification and evaluation of development-specific miRNAs in the culture medium.

***Keywords:*** *miRNA, rabbit, blastocyst, culture medium*

***Acknowledgment:*** *This study was supported by grants RRF-2.3.1-21-2022-00007*

## PEO-3#

### EFFECTS OF CALCIUM FLUORIDE NANOPARTICLES ON THE AMYLOID FORMATION AND CYTOTOXICITY OF ALZHEIMER'S AMYLOID-B PEPTIDE

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Application of nanoparticles is becoming common in biotechnological and medical fields. For instance, calcium-fluoride nanoparticles ($CaF_2$ NPs) are used in dental care applications as a remineralization agent. Proteins might go through conformational changes upon interacting with nanoparticles. Nanoparticles can also influence aggregation processes of amyloidogenic proteins and peptides. The amyloid-β (Aβ) peptide is able to form amyloid fibrils in the brain in Alzheimer's disease. We synthesized and characterized $CaF_2$ NPs, and studied their effects on the structure, aggregation, and cytotoxicity of in-house expressed Aβ(1-42). We investigated the kinetics of Aβ amyloid formation by assessing Thioflavin T fluorescence in the presence of nanoparticles of different concentrations. $CaF_2$ NPs reduce lag time of the aggregation in a concentration dependent manner. We used circular dichroism spectroscopy and the BeStSel method to determine the secondary structure composition of the monomeric and the amyloid form. Aβ monomers form amyloid with a high β-structure content in 30 hours at 37°C, both with and without $CaF_2$ NPs. However, the presence of $CaF_2$ NPs results in higher β-sheet content and a change in the proportion of antiparallel and parallel β-sheets. We performed cytotoxicity assays on the mouse hippocampal mHippoE-14 cell line to evaluate toxicity of Aβ, nanoparticles, and their combination. While Aβ was highly cytotoxic, nanoparticles were usually less toxic or even neutral. Additionally, we observed that $CaF_2$ NPs might reduce cytotoxicity of Aβ if applied in appropriate concentrations.

***Keywords:*** *amyloid-β, nanoparticles, CD spectroscopy*

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## PEO-4#

### INVESTIGATION OF THE EFFECT OF NANOMATERIALS ON THE STRUCTURE OF AMYLOIDOGENIC PROTEINS

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Nanoparticles are fragments of materials in the nanometer size range. In this range, interactions between molecules and different materials can change the physicochemical parameters and properties of molecules. Interest in the study of nanoparticles has increased in recent times because of their enormous potential and threat. Micro- and nanoplastics are present in our everyday environment and have been detected in several living organisms and in products for human consumption, such as honey. They have also been detected in several human organs and tissues, including the human placenta. Immune responses and inhibition of cell development have been described as effects of particles. The complex of symptoms caused by micro- and nanoplastics is named as *plasticosis*. They have the potential to affect proteins in the body and in the environment (Hildebrand et al., 2018). Interactions with nanoparticles can lead to conformational changes and the denaturation of proteins (Roach et al., 2005). Therefore, understanding the consequences of proteins and nanoparticle interactions is essential to discribe their environmental effects. We investigated the effects of nanoplatics on protein structure using ThT fluorescence, CD and SRCD spectroscopy, electron microscopy, and cytotoxicity measurements. Our results indicate that polystyrene nanoparticles, even in small amounts, affect the aggregation process of the amyloid-β protein involved in Alzheimer's disease. We also revealed that the particles can rapidly and in relatively large amounts bind the monomeric form of the protein on their surface affecting the aggregation capabilities of the protein. Our results also suggest that the presence of nanoparticles may reduce the toxicity of amyloid-β in cellular medium. In our recent measurements, we observed that pre-grown amyloid fibrils can be dissociated by polystyrene particles.

***Keywords:*** *amyloid, nanoparticles, nanoplastics, CD spectroscopy, Alzheimer's disease*

***Acknowledgement:*** *This work is supported by the National Research, Development and Innovation Office of Hungary (grants PD135510, K138937, 2019-2.1.11-TÉT-2020-00101, and 2019-2.1.11-TÉT-2019-00079), the New National Excellence Program (ÚNKP-23-3) and Doctoral Excellence Program (DKOP-23) of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund.*

## PEO-5#

### EXPRESSION OF DUTPASE IN MOUSE POSTNATAL DEVELOPMENT

***Tóth, Otília*[1,2.], Nagy, Nikolett[2,3.], Rácz, Gergely Attila[1,2.], Hádinger, Nóra[4.], Acsády, László[4.], Pintér, Tímea[5.], Gál, Zoltán[5.], Gócza, Elen[5.], Hiripi, László[5,6.], Vértessy G, Beáta[1,2.]**

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The dUTPase enzyme has an important role in nucleotide metabolism by hydrolyzing dUTP into dUMP and pyrophosphate (Vértessy BG, 2009). The produced dUMP is a precursor of *de novo* dTTP synthesis. Through the catalytic activity of dUTPase enzyme it decreases the dUTP/dTTP ratio in cells. If the dUTP/dTTP level is high, dUTP can be incorporated into the DNA. Cytosine deamination is also resulted in uracil which leads to mutations. Therefore, uracil bases are excised via the base excision repair (BER) mechanism. As the genomic uracil level increases in the DNA, the overactivation of BER leads to single- and double-strand breaks and causes cell death. It was shown previously that knockout of the *Dut* gene in mouse leads to early embryonic lethality (Pálinkás HL, 2019). Two isoforms of human dUTPase were described in the literature, the nuclear and the mitochondrial isoforms, which generated via alternative splicing and alternative promoter usage (Ladner RD, 1996). Previously, a highly reliable RT-qPCR method was optimised to detect the isoform-specific mRNA expression of dUTPase in different mouse tissues (Rácz GA, 2019). Our aim was to investigate the tissue-specific mRNA expression of the different dUTPase isoforms during development in mouse. We determined the expression in brain, gonad and liver derived from 14,5-day embryos and in heart, kidney, ovary, testis, thymus, lung, liver and brain derived from postnatal 2-, 4-, 10-week- and 13-month-old mice. We found that the mRNA expression of the nuclear isoform in the lymphoid organs, i.e., the thymus and the spleen, shows uniformly high level throughout development. These results are in good accordance with the well-established role of dUTPase in dividing cells, hence in these organs extensive lymphocyte production happens. In the reproductive organs, we found also an elevated mRNA expression level, as folliculogenesis and spermatogenesis occur in the gonads. Moreover, we found relatively high mRNA expression of nuclear dUTPase in the mouse brain. Regarding the mitochondrial isoform, we observed lower and more constant expression levels during development than in case of the nuclear isoform. We found the highest expression of mitochondrial dUTPase in the heart, which is known to be rich in mitochondria. Our observations underline that dUTPase has an important function in cell proliferation and in cell differentiation.

***Keywords:*** *expression, dUTPase, development*

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# PEO-6#

## INVESTIGATION AND OPTIMIZATION OF IMMOBILIZATION OF HISTIDINE-TAGGED ENZYMES.

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During my research work, my goal was to investigate the selective enzyme immobilization methods previously used in our research group. During selective enzyme immobilization, histidine-tagged enzymes were immobilized on surface-modified polymer supports. Previously, optimization experiments were carried out, during which a solid polymer containing epoxy groups was modified with a mixture of amines and bisamines. Metal ion complexing groups were formed via the modification of bisamines with ethylene diamine dianhydride. Continuing this work, I expanded the range of applied surface modification agents, and I investigated the effect of surface modification on the immobilized biocatalysts. In the first experiments, phenylalanine ammonia-lyase from parsley was used as a model enzyme.
This enzyme immobilization method is based on the recombinant proteins labeled with histidine and the side chains of this amino acid selectively form a complex with the metal ion immobilized on the surface. After the quick complexation, the covalent bond formation takes place through the epoxy groups of the support.
During the immobilization, cobalt ions were used in all cases to form the metal-protein complex. After the immobilization, the biocatalysts were tested in the ammonia elimination reaction of phenylalanine. Based on the results, while the quality of the bisamines barely affected the activity, the monoamines had a significant effect on the final biocatalyst activity.

***Keywords:*** *metal ion affinity chromatography, enzyme immobilization, biocatalysis*

***Acknowledgement:*** *The research was performed in the frame of project no. RRF-2.3.1-21-2022-00015 has been implemented with the support provided by the European Union.*

## PO-1#

### AMIDE BOND SYNTHETASES FOR A GREENER CHEMISTRY

***Benedek, András*[1], *Tasnádi, Gábor*[2], *Vértessy G., Beáta*[1]**

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Amide bond formation is the most heavily utilized transformation in pharmaceutical industry. Alarmingly, although a wide range of coupling reagents exist, their toxicity, high cost and low atomic efficiency raises serious environmental and economical concerns. A promising solution for greener amide bond synthesis would be to utilize enzymes. Amide-bond synthetases (ABSs) are functional in water-based solutions under mild reaction conditions (e.g. 30 °C at pH 7.5). Our aim is to explore new ABSs from sequence databases and test them with several pharmaceutically important substrates, establishing an enzyme library.
I used 8 published ABS enzyme genes as search templates in the NCBI Genome database to identify so far uncharacterized homologues. Out of the several hundred identified hits, I only choose those ones to be tested in the lab possessing divergent active site characteristics. I map the ABS active site with the software PyMOL via representing existing X-ray diffraction structural models of the reference enzymes.
I have proposed 11 new members of the ABS library to be gene synthetized and I have produced them using *Escherichia coli* bacteria in our lab. I purified and we partially tested 7 of these new candidates and 4 reference enzymes for their substrate preferences. Search for new candidates, production and testing of the existing ones are still in progress.
We see that the studied enzyme homologues have different preference for the applied test substrates, raising the promise that our growing enzyme library will be able to cover a wide range of pharmaceutically important substrate pairs.

***Keywords:*** *enzyme library, amide bond, green chemistry*

***Acknowledgement:*** *AB wishes to thank Les Laboratoires Servier and Servier Research Institute of Medicinal Chemistry for their financial and professional support.*

## PO-2#

### THE CRYSTALLIZATION OF ZEBRAFISH DUTPASE WITH ITS INTERACTION PARTNERS

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In addition to the four canonical bases within DNA, uracil is one of the most frequently occurring non-orthodox constituent in the genome. Its appearance can be traced back to several reasons, on the one hand, it can happen due to the oxidative deamination of cytosines or due to an inappropriate dTTP:dUTP ratio. The reason for the latter case is that DNA polymerases cannot distinguish between the two building blocks, so it depends on their cellular concentration which one is incorporated into the DNA during polymerization [1]. Among other things, the dUTPase enzyme helps to maintain the proper ratio. It catalyses the cleavage of dUTP into dUMP and inorganic pyrophosphate, and by its effect effectively prevents the misincorporation of uracil into DNA, at the same time providing a dUMP substrate for de novo thymidylate biosynthesis [2]. We found dUTPase interacting partners from two yeast hybrid experiments. In the future we want to investigate these interactions and determine these structures. As a first step for this we crystallized the zebrafish dUTPase with a substrate analogue and then define the structure with x-ray crystallography.
The zebrafish as a model organism is very popular among researchers today, as it is easy to maintain and requires little cost, their development is quite fast, and they show good survival against different post-fertilization procedures. In addition to these, it has the additional advantage of being a vertebrate species that shows 70% homology with the human genome [3]. As a result of these benefits, we use the zebrafish recombinant dUTPase.

***References*:**

[1] Róna G, Scheer I, Nagy K, Pálinkás HL, Tihanyi G, Borsos M, Békési A & Vértessy BG (2016) Detection of uracil within DNA using a sensitive labeling method for in vitro and cellular applications. Nucleic Acids Res 44, e28.
[2] Vértessy BG and Tóth J (2009) Keeping uracil out of DNA: physiological role, structure and catalytic mechanism of dUTPases. Acc Chem Res 42, 97–106.
[3] Gutiérrez-Lovera C, Vázquez-Ríos AJ, Guerra-Varela J, Sánchez L & de la Fuente M (2017) The potential of zebrafish as a model organism for improving the translation of genetic anticancer nanomedicines. Genes (Basel) 8.

# MICROBIAL BIOTECHNOLOGY

## EEM#

### DEVELOPMENT OF NOVEL METHODS FOR THE CONTROL OF *MYCOPLASMA* INFECTIONS

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Mycoplasmas are successful minimal pathogens, which represent the simplest life forms capable of self-replicating outside of a host. The *Mycoplasmataceae* family encompasses more than 100 species, many of which are pathogenic for humans and a wide range of animals, causing major concern to the medical and veterinary professions. Mycoplasmal diseases lead to enormous economic losses in the livestock industry all around the world.
Control of mycoplasma infection consists of three general aspects: eradication followed by prevention of the infection, vaccination, or medication. Our research group was established in 2012 with the support of the Momentum program of the Hungarian Academy of Sciences. In the past decade, the focus of our research projects was the development of novel methods for the control of various *Mycoplasma* infections.
Firstly, we started to work with ruminant mycoplasmas, particularly *M. bovis*. We developed genotyping methods, tested the antibiotic susceptibility profiles of various strains, and were the first who performed a complex study to discover the mutations responsible for antibiotic resistance and to develop cost-effective molecular diagnostic assays for their rapid detection.
Probably our largest achievement in avian mycoplasmology was the development of molecular DIVA (differentiating infected from vaccinated animals) tests for the live *M. gallisepticum* and *M. synoviae* vaccine strains. After publishing papers in highly-ranked scientific journals, we further improved the methods and finally sold the tests under a royalty agreement to BioChek B.V., the global market leader company in the field of poultry diagnostics.
Antimicrobial resistance is an emerging problem. I realized that as a veterinary researcher, one of the most effective ways to fight against antimicrobial resistance is if we focus on vaccine development to replace the application of antibiotics with active immunization in livestock production. We were the first who successfully developed a live-attenuated vaccine candidate against *M. anserisalpingitidis*, which causes reproductive disorders in geese. Prior to publication, the candidate was patent deposited at Leibniz-Institute DSMZ, Germany under ID number DSM 33810 and a patent was submitted under number P 21 00357.
Currently, we are working on the development of a live-attenuated *M. gallisepticum* vaccine candidate as well as we seek solutions against the porcine pathogen *M. hyorhinis* in the forms of inactivated, live-attenuated, and mRNA vaccine candidates.

***Keywords:*** *avian, bovine, Mycoplasma, porcine, veterinary*

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## EM-1#

### COMPREHENSIVE INVESTIGATION OF BIOACTIVE PEPTAIBOLS AND THEIR PREPARATION FOR FUTURE AGRICULTURAL APPLICATION

***Balázs, Dóra; Marik, Tamás; Szekeres, András; Vágvölgyi, Csaba; Tyagi, Chetna; Kredics, László***

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Extreme weather conditions and newly appearing pathogens are causing an increasing problem in agriculture, which calls for the search for new approaches and solutions. One possible way can be provided by bioactive, secondary metabolic products that can support plant cultivation with their wide-ranging effects. Such secondary metabolites are the peptaibols produced by filamentous fungal *Trichoderma* species. Peptaibols can be characterized by a high degree of amino acid variability in their sequences and their ion channel-forming ability in the cell membrane. Due to their characteristic properties, peptaibols can exert inhibitory effects against several plant pathogenic bacterial and fungal species, furthermore, they can also provide growth-supporting effects on plants through different mechanisms. However, for their future practical application, a deeper understanding of their background modes of action is essential, by performing modern computer modeling techniques.
During our research, a comprehensive investigation of the peptaibol production of 6 *Trichoderma* strains belonging to the Longibrachiatum clade was performed. The total peptaibol production of the strains was determined using the HPLC-ESI-MS method. The effects of peptaibol extracts were studied against eleven commonly known Gram-positive and Gram-negative bacterial strains and the minimal inhibitory concentrations (MIC, mg $ml^{-1}$) were determined. The growth-slowing effects of the extracts were studied on 4 plant pathogenic fungal species with effectivity tests (EC, mg $ml^{-1}$). In parallel, computer modeling investigations were performed by accelerated molecular dynamics (aMD) simulations with the most produced sequences. The results of the laboratory tests and computer modeling simulations were compared by examining structure-activity relationships (SARs). Based on our results, the most characteristic differences between the peptaibols are the ‘Gly-Leu-Aib-Pro’ and ‘Gly-Aib-Aib-Pro’ amino acid motifs, which have a significant effect on the structure dynamics and stability and appear to affect the expressed bioactivity. With a more detailed knowledge of the action of peptaibols, we can facilitate a targeted and rapid selection of *Trichoderma* strains producing bioactive sequences and lay the foundation for their future application and biotechnological large-scale production.

***Keywords:*** *peptaibol, Trichoderma, bioactivity*

***Acknowledgement:*** *The research is supported by the ÚNKP-23-4-SZTE-544 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund and this study was supported by the Hungary-Serbia IPA Cross-border Co-operation Programme project FERTILEAVES (HUSRB/23S/11/027).*

## EM-2#

### INSIGHTS INTO THE EVOLUTION AND MUTATIONS OF SECOND ALTERNATIVE OXIDASE GENES IN *ASPERGILLACEAE*

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Alternative oxidase (Aox) is a branched mitochondrial terminal oxidase, that bypasses Complex III and IV. Aox accepts electrons directly from ubiquinol and reduces oxygen to water without contributing to the proton gradient used for ATP synthesis. Aox is known to have various functions, including helping cells manage stress conditions, regulating cellular metabolism, and maintaining redox balance. In certain organisms, such as plants, it can play a role in preventing excessive reactive oxygen species production under stress conditions.
Aox is nearly ubiquitous in fungi, yet the presence of multiple *aox* genes is uncommon. However, a second *aox* gene (*aoxB*) is present in some taxa of *Aspergillaceae*. Paralogous genes typically originate from duplication events and are passed down vertically. We offer evidence of four separate duplication events along the lineage that resulted in *aox* paralogues (*aoxB*) in contemporary *Aspergillus* and *Penicillium* taxa. In certain species, three *aox* genes are co-expressed, yet there are entire sections and series within *Aspergillus* that lose transient *aoxB* content. Within the subgenus *Nidulantes*, we have identified seven instances of independent *aoxB* gene loss and two instances of gain. The paralogous clades originate from widespread *aoxA* parent genes but never replace them, *aoxA* remains permanent across filamentous fungi.
Within the database, *Aspergillus niger* strains possess six different alleles of the *aoxB* gene. Besides the wild type, we found five different mutations that caused errors in the gene product. A full-length AoxB is encoded in the acid producer ATCC 1015 strain.
The investigation of alternative oxidase genes is crucial for a deeper understanding of citric acid production or clinical aspects. Fermentation occurs based on highly complex biochemical relationships, wherein the *aox* gene plays a crucial role. Understanding a gene well significantly contributes to strain development or possible clinical treatment.

***Keywords:*** *alternative oxidase, phylogenetic analyses, mutations, gene duplication, gene loss, Aspergillaceae*

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## EM-3#

### XENORHABDUS ANTIMICROBIAL PRODUCTS: GENETIC REGULATION OF BIOSYNTHESIS AND PERSPECTIVES OF APPLICATION

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Antimicrobial production of bacterial symbionts (EPB) of entomopathogenic nematodes (EPN) is positively controlled by the *hfq* gene. Deletion of *hfq* turns off the expression of numerous biosynthetic gene clusters (BGC) encoding biosynthetic cooperating enzymes responsible for the production of antimicrobial end products. If any of the *hfq*-controlled BGCs are reactivated in a Δ*hfq* mutant, then the strain will produce only one type of antimicrobial. The so-called "easy Promoter Activated Compound Identification" (easyPACId) approach by Bode et al. revolutionized the search for EPB-produced drug candidate molecules. We also use this reproducible technique for drug-hunting in *Xenorhabdus szentirmaii* (EMC), and *X. budapestensis* (EMA) discovered and first characterized in our labs. We recently published that the cell-free conditioned media (CFCM) of the wild-type strains of both species severely inhibit the growth of the chytrid fungus *Batrachochytrium dendrobatidis* (Bd), the causative agent of the epidemic amphibian disease, chytridiomycosis. Furthermore, EMC-CFCM efficiently reduced the Bd-infection load on juvenile common toads (*Bufo bufo*) without harmful side effects. The CFCM of each of the *hfq*-deleted (EMA, and EMC) strains performed significantly ($P<0.005$) lower antifungal activity than the respective wild-type strain *in vitro*. We reactivated several BGCs one by one in our Δ*hfq* mutants and plan to test their antagonistic potentials on Bd as well as on different Gram-positive and Gram-negative bacteria, and eukaryotic pathogens of clinical, veterinary, and plant pathogenic significance to identify drug-candidate bioactive compounds. We already investigated the antitumor activity of the CFCM of wild type, Δ*hfq*, and phenazine BGC reactivated strain of EMC against a human leukemia cell line and a human ovarium carcinoma cell line, and our results suggest that phenazines produced by EMC effectively inhibit proliferation of tumor cells. Based on our results, *Xenorhabdus* antimicrobial products, and the genetic regulation of their biosynthesis will provide a wide range of applicability of these new therapeutic agents.

***Keywords:*** *Xenorhabdus, chytridiomycosis, antitumor activity*

***Acknowledgement:*** *OTKA K 128203, NKFIH RRF-2.3.1-21-2022-00007*

## EM-4#

### INCIDENCE AND ANTIBIOTIC RESISTANCE OF SALMONELLA ENTERICA STRAINS ISOLATED FROM DIFFERENT TYPES OF EGYPTIAN CHEESES

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Cheeses are nutrient-dense dairy products, which possess a high consumption level around the world, including Egypt. Hence, there is a growing concern regarding the regular evaluation of the microbial quality and safety of cheeses. The current study aimed to investigate the incidence of different serovars and virulence genes and antimicrobial resistance profile of *Salmonella enterica* strains in the regularly consumed cheese types in Egypt.
At intervals between October 2021 and May 2022, two hundred ten random samples of Ras (Rumi), pasteurized Kareish, and soft cheeses (70 of each) were collected from the Mansoura hypermarkets (in Egypt) and directed for microbiological, serological, and molecular analyses.
Strains of *Salmonella enterica* were detected in 18 (8.5%) cheese samples, of which 16 (22.8%) were derived from Rumi cheeses and two (2.8%) from pasteurized Kareish cheeses. *S.* Typhimurium, *S.* Enteritidis, *S.* Infantis, *S.* Tsevie and *S.* Virchow were the most prevalent serovars. Virulence-encoding genes typical to *Salmonella*, such as *invA, hilA, stn, and spvC*, were detected in 100%, 94.4%, 88.8%, and 27.7% of retrieved isolates, respectively. Among the investigated *Salmonella* isolates, 5.5%, 16.7%, and 61.1% were classified as pan-drug resistant (PDR), extensively drug-resistant (XDR), and multidrug-resistant (MDR).
This study reported the widespread distribution of virulent, XDR and MDR *Salmonella enterica* strains isolated from Egyptian Rumi and pasteurized Kareish cheeses, which may be of public health concern. Hence, strict hygienic practices must be applied during the manufacturing, distribution, and handling of these types of cheeses.

***Keywords:*** *Egyptian cheeses, Salmonella serovars, antibiotic resistance.*

***Acknowledgement:*** *This work was supported by the Food Science Doctoral School of the Hungarian University of Agriculture and Life Sciences.*

## PEM-1#

### MANGANESE EFFECT ON CITRIC ACID PRODUCTION BY *ASPERGILLUS NIGER*: UNLOCKING AN EFFICIENCY BOOSTING HIDDEN KEY

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Achieving high yields of citric acid requires a unique combination of culture conditions, with the deficiency of manganese(II) ions in the growth medium being especially crucial. Concentrations exceeding 5 µg/L (= 5 ppb) result in a reduction of around 25% in the final citric acid yield. Because of its characteristics, this organic acid finds utility across a spectrum of industrial sectors, spanning from food and beverage to detergent and pharmaceutical industries. The predominant method for citric acid production involves large-scale industrial fermentations utilizing the filamentous fungus *Aspergillus niger*. When compared to alternative hosts, *A. niger* stands out for its ability to achieve remarkably high yields, with potential outputs reaching up to 95 kg of citric acid per 100 kg of sugar. Technical-scale production of citric acid predominantly uses stainless steel tank fermenters. However, glass bioreactors, commonly used for process development, also incorporate stainless steel components, where manganese serves as a crucial alloying element. Our study reveals that manganese(II) ions leach from these bioreactors into the growth medium during citric acid fermentation. This leaching phenomenon leads to alterations in fungal physiology and morphology, resulting in a significant decrease in citric acid yields. The extent of manganese(II) ion leaching depends on factors such as fermentation duration, the acidity of the culture broth, and the sterilization method employed.
Moreover, CexA is the main citrate exporter of *A. niger*. The citric acid production was examined in overexpression mutant strains of *cexA* under manganese deficiency and sufficient conditions. This leads to citric acid accumulation even in the presence of high manganese(II) ion concentrations. Additionally, the impact of CexA on fungal morphology was elucidated through microscopic analysis.

***Keywords:*** *Aspergillus niger, citric acid, fermentation, cexA*

***Acknowledgement:*** *VB was supported by the Ph.D. Excellence Scholarship from the Count István Tisza Foundation for the University of Debrecen. AM was also supported by the ÚNKP-23-4-I-DE-362 New National Excellence Programs of the Hungarian Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund.*

# PEM-2#

## PRELIMINARY RESULTS OF BACTERIAL COMMUNITY DIVERSITY IN THE RHIZOSPHERE OF DRAGON FRUIT

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In Ecuador, pitahaya (dragon fruit) is an exotic and economically significant fruit. This crop is a new alternative to agricultural production with a significant economic potential for producers. However, the fruit is prone to many diseases incited by fungi, bacteria, viruses, and nematodes leading to heavy losses in plant production. The main role in that could be intensive agriculture, leaving a negative print on soil microbiome, but missing information about microbial diversity of dragon fruit rhizosphere.
We aim to better understand the composition of bacteria in the rhizosphere of yellow dragon fruit crops and how microbial diversity responds to different agricultural management.
We investigated the bacterial community's diversity in *Selenecereus megalanthus* crops using high-throughput sequencing of the 16rRNA. The tested experimental fields are located in Palora, in Morona Santiago province Amazon region. The rhizosphere soils of dragon fruit were sampled from eight farms, four focused on conventional (monoculture) and four on organic management. In total 2764 amplicon sequence variants (ASV) were identified in rhizosphere soils of all types of farm management. The bacterial abundance showed a significant difference between conventional, organic farms and one of the organic farms with a special system management showing *Xanthomonadales* as the predominant taxon order. The dominant phyla were Proteobacteria, Acidobacteria, Choroflexi, and Actinobacteria in all types of management. In addition, alfa bacterial diversity showed lower diversity in the special organic farm with a different production system compared with the others.
Our preliminary result is the first step to see in more detail the microbiome in dragon fruit's rhizosphere.

***Keywords:*** *Dragon fruit (Selenecereus megalanthus), rhizosphere, bacterial community*

***Acknowledgement:*** *This research was funded by the industrial research and development projects in Hungarian–Vietnamese cooperation, grant number 2019-2.1.12-TÉT_VN-2020-00001. We also thank the Stipendium Hungaricum program and Prof. Lisbeth Espinoza who provided farmers contacts.*

## PEM-3#

### INVESTIGATION OF SOIL BIODIVERSITY IN ALKALINE GRASSLAND UNDER REGENERATIVE AGRICULTURAL PRACTICE

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Properly characterizing soil health is crucial to understanding the processes and decisions necessary for regenerating degraded soils. The European Soil Monitoring System reports that at least 60% of European soils are currently affected by some form of degradation. Intensive farming not only causes soil degradation but also reduces biodiversity.
Soils can contain over half of the earth's microbial biodiversity. However, the relationship between the soil microbiome and soil type diversity, or pedodiversity, is not well understood. Digital soil mapping methodologies and advanced molecular biology studies can help uncover new, previously unknown relationships.
The sample site is an alkaline grassland in the Great Hungarian Plain, currently under regenerative agricultural use. During the summer of 2023 a field survey was carried out. 13 soil profiles were prepared. The international standard Word Reference Base soil classification system (WRB) was used to describe and classify the sections. Additionally, a complete physical and chemical laboratory analysis of the genetic soil horizons was performed to create a precision soil map. This map enables accurate delineation of the various soil types present.
Soil samples were collected during the summer from meadow soil and solonyec soil. The sampling was repeated in November 2023 from fresh soil sections, ranging from the genetic levels of the soil section to a depth of 110 and 140 cm. Trap plants were then planted in the soil samples, and the percentage of root colonization was determined. Through an examination of the colonization potential of various genetic soil types, we were able to identify the soil properties that significantly affect the presence of arbuscular mycorrhizal fungi (AMF). Our analysis also revealed how the capacity for AMF colonization varies within the soil profile, providing a better understanding of how depth affects AMF communities.
Additionally, we conducted molecular biology analyses of the entire profile of soil sections. The obtained results can demonstrate the variation of soil biodiversity throughout the entire soil section, aiding in the comprehension of soil biodiversity.

# PEM-4#

## EXPLORING DIRECTED AND MULTICELLULAR GROWTH OF YEAST

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During evolution, multicellularity and directed growth have evolved along numerous pathways. This altered lifestyle strategy provided ecological advantages over unicellular forms of life. In our research, we model this evolutionary strategy shift using baker's yeast (*Saccharomyces cerevisiae*). This unicellular eukaryote serves as an excellent model organism and is easily modifiable.
Previous studies have demonstrated that knockout of the ACE2 (Activator of CUP1 Expression 2) gene leads to significant phenotypic changes. In its absence, the daughter cell fails to fully separate from the mother cell, resulting in their remaining together in a clonal multicellular structure.
The BUD8 and BUD9 (BUD site selection) genes determine the bipolar budding pattern of yeast cells. In the absence of one of them, this pattern is disrupted, and the mother cell only produces a daughter cell at one pole. Combining this with the knockout of ACE2, we obtain a directed growth of a multicellular structure.
We perform these modifications in several yeast strains and examine the physiological changes compared to the unicellular ancestor. Through these experiments, we get closer to understanding this phenomenon of evolution and comprehend the ecological advantages or disadvantages associated with such structural changes.

***Keywords:*** *multicellularity, yeast, colony formation, growth pattern*

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## PEM-5#

### ECOPHYSIOLOGICAL CHARACTERISATION OF A *KLEBSORMIDIUM* STRAIN ISOLATED FROM A CAVE ENVIRONMENT

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Members of the *Klebsormidium* genus play a major role in the formation of biological soil crusts (BSC). BSCs contribute to the stabilization of the soil surface, improve soil structure, and influence the water retention of the soil. Due to their high brassinosteroid content and key involvement in forming BSCs, *Klebsormidium* species have potential biotechnological applications. In our work, we explored the photosynthetic characteristics and evaluated the brassinosteroid content of a *Klebsormidium* strain isolated from a Hungarian cave. The temperature and light optimum of the algal strain were tested by oxygen yield measurements and pulse-amplitude-modulated fluorescence. Additionally, we evaluated the specific growth rate of the strain from 10 to 40°C. We have shown that the studied microalgae performed net photosynthesis over the 5 to 45°C temperature range. Regarding photosynthetic activity, the maximum oxygen yield was observed at 30-40°C, while chlorophyll fluorescence measurements peaked at 35-40°C. Our findings also revealed a broad temperature range for microalgal growth within 10 to 40°C, with a 20-25° temperature optimum, aligning with the measured brassinosteroid levels. The strain exhibited high light utilization factors (α) and low light adaptation parameters ($I_k$). Our investigation demonstrated, despite its cave origin, that the examined *Klebsormidium* strain can grow and perform photosynthesis over a broad temperature range, highlighting its potential for biotechnological applications.

***Keywords:*** *algal growth, chlorophyll fluorescence, oxygen evolution, temperature optimum, brassinosteroid*

***Acknowledgement:*** *this research was supported by the National Research, Development and Innovation Office of Hungary (K 140351, RRF-2.3.1-21-2022-00014, MKI-2018-00034, KKP 144068, ÚNKP-22-3). We express our special thanks to Máté Burányi for the technical support.*

## PEM-6#

### HETEROLOGOUS EXPRESSION AND CHARACTERISATION OF *NEOSARTORYA (ASPERGILLUS) FISCHERI* BUBBLE PROTEIN

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As a consequence of the limited number of effective antifungal drugs, and the global emergence and spread of (multi)drug-resistant fungal strains, there is an urgent need to identify new antifungal compounds with different modes of action than that of the conventional ones. In our previous studies, we already demonstrated that *Neosartorya* (*Aspergillus*) *fischeri* NRRL181 can produce two small, molecular weight, cysteine-rich, highly stable proteins with antifungal activity; namely *N.* (*A*) *fischeri* antifungal proteins 1 and 2 (NFAP and NFAP2). The genome analysis of *N.* (*A.*) *fischeri* NRRL 181 indicated the presence of a third antifungal protein [*N.* (*A.*) *fischeri* bubble protein, NFBP] belonging to the "bubble protein" class of the ascomycetous antifungal proteins. Considering that the already isolated and characterized members of this antifungal protein class are potential antifungal compounds, in the present study we aimed the heterologous production of NFBP in a *Pichia pastoris*-based expression system, and characterization of the recombinant protein. Western blotting indicated that the applied *P. pastoris* KM71H strain secreted the His-tagged NFBP into the culture broth. After ultrafiltration and purification by His-tag affinity and reversed-phase high-performance liquid chromatography, the NFBP yield was ~ 84 mg/l. Mass spectrometry indicated the presence of additional EAEA amino acids from the Kex2 cleavage site at the N-terminus. Electronic circular dichroism spectroscopy confirmed the folded, disulphide bond-stabilized structure of NFBP constituted by β-strand and loop regions. Antifungal susceptibility tests showed slight antifungal efficacy of NFBP in comparison with NFAP and NFAP2. NFBP was able to inhibit the growth of different fungi at relatively high concentrations, such as *Cryptococcus neoformans* IFM 5844 (minimum inhibitory concentration, MIC: 100 µg/ml), *Candida albicans* SC5314 (MIC: 200 µg/ml), and *Botrytis cinerea* SZMC 21472 (MIC: 200 µg/ml). *Cladosporium herbarum* FSU1148, *Aspergillus fumigatus* CBS 101355, *Fusarium oxysporum* CBS123668 and the native producer, *N. fischeri* NRRL 181 proved to be not susceptible to NFBP even at the highest concentration (400 µg/ml) applied in the susceptibility test. Based on these results, we can conclude that NFBP is not a such potent antifungal protein as NFAP and NFAP2.

***Keywords:*** *Neosartorya (Aspergillus) fischeri, bubble protein, heterologous expression, protein structure, antifungal activity*

***Acknowledgement:*** *The present work of L.G. was financed by the Hungarian National Research, Development and Innovation Office - NKFIH FK 134343 and K 146131 project.*

## PM-1#

### *FUSARIUM* MYCOTOXIN PRODUCTION UNDER AFLATOXIN BIOCONTROL IN CORN

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Mycotoxins are secondary metabolites produced by fungi that often occur as contaminants in food and feed. Due to their varying degrees of toxicity, their investigation is of paramount importance. *Fusarium* toxins are most detected in food including fumonisins, zearalenone, and trichothecenes (*e.g.*, deoxynivalenol, T2/HT2).
In our micro-plot field experiment, plants of two maize hybrids (Orpheus, GEIX1770) were inoculated with *Aspergillus flavus* aflatoxin-producing toxinogenic or atoxinogenic strains or their mixture. Our experiment aimed to see how the application of biocontrol *A. flavus* affects Fusaria and their toxin formation in the two different maize hybrids. Is there an inhibition on *Fusarium* mycotoxin production in *A. flavus* treatments or not?
T2/HT2, deoxynivalenol, zearalenon, and its derivatives, and fumonisins were detected through immunoassay with fluorescence detection. Differences between irrigated and non-irrigated micro-plot fields were also tested in the Tukey test.
In fumonisin production, there were no significant differences between all treatments but irrigated atoxinogenic and toxigenic *A. flavus*. Moreover, a strong effect of the maize hybrid was found. For deoxynivalenol and its derivatives, there were no significant differences found under non-irrigated conditions, however, irrigation caused significant alterations in toxin production. Zearalenone production was not affected by irrigation or by *A. flavus* treatments.
Biological control of aflatoxin production with atoxigenic *A. flavus* has a partly inhibitory effect on Fusaria mycotoxins, especially deoxynivalenol and its derivatives.

***Keywords:*** *Fusarium, Aspergillus, mycotoxin*

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## PM-2#

### SCREENING OF GREEN MICROALGAE BASED ON ANTIBACTERIAL ACTIVITY AND IN-VITRO TESTING ON ARTIFICIALLY INFECTED FRUIT FLOWERS

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In recent years, alternative and biological defense methods have gained prominence in combating plant diseases. One potential source of biological pesticides is microalgae, whose excellent adaptability is supported by a series of metabolites produced to survive extreme conditions. There are many microalgae, *e.g., Chlorella* sp., and *Scenedesmus* sp. with proven antibacterial effects presumably associated with polyphenols, alkaloids, terpenes, polysaccharides, fatty acids, sterols, lactones, and proteins.
The purpose of our study was to estimate the putative inhibition effect of metabolites produced by selected algae on widely occurring facultative pathogens (*Escherichia coli* NCAIM B.01992, *Staphylococcus aureus* NCAIM B.01055, *Pseudomonas aeruginosa* NCAIM B.01057 and *Erwinia amylovora* NCAIM B.01975).
First, we performed the biological screening of organic solvent extracts of single-cell freshwater green microalgal cultures originating from the collection of Albitech Biotechnology Ltd. The cultures were identified at the Department of Microbiology of Eötvös Lóránd University using molecular biological methods based on the 18S rRNA gene and then maintained in active and cryopreserved forms.
Two different organic solvents – ethanol and diethyl-ether – were used to create the extracts from the lyophilized biomass. The antibacterial effect of the extracts was determined using the agar gel diffusion method. The minimum inhibitory concentration was measured by broth microdilution assay in a cell culture plate, as positive control antibiotics were used.
We have confirmed the antibacterial effects of four single-cell freshwater microalgae strains against facultative pathogenic bacteria in vitro. The *E. coli* strain was the least susceptible to treatments and *S. aureus* was the most sensitive. Using artificially infected various pear blossoms we were able to detect a significantly reduced extent of *E. amylovora* infection due to antibacterial algal extracts.

***Keywords:*** *microalgae, antibacterial, agar diffusion, microdilution*

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## PM-3#

### ANTIFUNGAL ACTIVITY OF Γ-CORE PEPTIDE DERIVATIVES OF ASCOMYCETOUS ANTIFUNGAL PROTEINS

***Karemera, K. John*** [1,2]***; Váradi, Györgyi*** [3]***; Tóth, K. Gábor***[3,4]***; Rákhely, Gábor***[1,5]***, Galgóczy, László*** [1,6]

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The increasing prevalence of drug-resistant fungal strains to conventional antifungal drugs has led to the escalating rise in fungal infection cases worldwide. Therefore, there is an urgent demand for new compounds with different antifungal modes of action from that of conventional antifungals. Antifungal peptides are potential candidates. To date, several antifungal peptides have been investigated from both natural and synthetic sources and have shown the ability to kill fungal pathogens. A detailed phylogenetic analysis indicated the presence of different antifungal proteins (AFPs) in the fungal class, Eurotiomyces. Despite the high differences in their primary structure, all of them contain the evolutionary conserved so-called γ-core motif with GXC-X [3-9]-C amino acid order, where X can be any amino acid. The synthetic peptide derivatives spanning this motif can effectively inhibit fungal growth. In the present study, we investigated how the physicochemical properties of the γ-core peptide derivatives (Pγ) influence their antifungal efficacy. To this aim, we investigated the antifungal efficacy of 21Pγs of AFPs with different physicochemical properties against four filamentous fungi (*Aspergillus fumigatus* CBS 101355, *Botrytis cinerea* SZMC 21472, *Cladosporium herbarum* FSU 1148, *Fusarium oxysporum* f.sp. *lycopersici* CBS 123668), and two yeasts (*Candida albicans* SC5314, *Saccharomyces cerevisiae* SZMC 0644). A broth microdilution susceptibility test indicated that the antifungal activity of a Pγ highly depends on the fungal species investigated on. We observed that, not just positively charged Pγs are antifungal active as we expected before, but the neutral ones can also have antifungal effects. Our findings suggest that the antifungal activity of a Pγ highly depends on the balance between the charge and grand average hydropathy value.

***Keywords:*** *antimicrobial peptides, γcore, antifungal activity*

***Acknowledgment:*** *This work was financed by Stipendium the Hungaricum Scholarship (JKK). The present work of L. G. was financed by the Hungarian National Research, Development and Innovation Office - NKFIH, FK 134343 project.*

## PM-4#

### BENEFICIAL IMPACT OF ARBUSCULAR MYCORRHIZAL FUNGI ON PLANT BIOTIC STRESS RESPONSES

***László, Lívia*[1]; *Sáray, Réka*[2]; *Hegedűsné Pintye, Alexandra* [2]; *Salánki, Katalin*[2]; *Posta, Katalin*[1]**

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Arbuscular mycorrhizal fungi (AMF) play pivotal roles in shaping plant-microbe interactions, influencing plant growth, and enhancing abiotic stress tolerance. However, a detailed understanding of their impact on plant responses to biotic stress remains a critical research frontier. This research aims to gain broader knowledge about the AMF-mediated biotic stress response of plants in the scope of fungal or viral infections.
Our research employs an integrative approach, combining classical methodologies and advanced techniques such as quantitative real-time PCR to unravel the effects of the AMF-mediated biotic stress responses to powdery mildew or cucumber mosaic virus (CMV) infection. We investigate the impact of biotic stress through altered physiological parameters in tomato (*Lycopersicon esculentum* Mill.) plants inoculated with AMF (*Rhizophagus irregularis*).
Our preliminary data in the AMF-mediated biotic stress response implies that AMF-inoculated, infected plants exhibit enhanced biotic stress tolerance, which manifests in elevated fresh and dry weights and height, as well as less severe disease symptoms compared to the non-inoculated, infected tomato plants.
The outcomes of this research hold significant implications for sustainable agriculture practices, with the potential to harness AMF as bio-enhancers for plant protection against fungal or viral infections.

***Keywords:*** *arbuscular mycorrhizal fungi, biotic stress, powdery mildew, cucumber mosaic virus*

***Acknowledgement:*** *This research was funded by National Research, Development and Innovation Office, grant number OTKA142974, and an industrial research and development projects in Hungarian–Vietnamese cooperation, grant number 2019-2.1.12-TÉT_VN-2020-00001.*

## PM-5#

### INVESTIGATING TOXIN PRODUCTION DYNAMICS IN *SACCHAROMYCES CEREVISIAE* COMMUNITIES

***Pillér, Bíborka; Gaizer, Tünde; Görög, Nóra; Szintai-Major, Eszter; Csikász-Nagy, Attila***

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Microbial populations found in natural environments form complex communities comprising multiple strains, which possess the capacity to mutually influence each other. One particular form of interaction stems from the secretion of toxins by killer active yeast strains. This toxin production holds significant importance in various fields, such as food and beverage fermentation. With the help of these killer active strains, the microbial community can be controlled during the fermentation process, guaranteeing the desired outcome.
Our study aims to explore the distinct growth characteristics of various toxin-producer strains of *Saccharomyces cerevisiae* by investigating yeast culture growth, both individually and within mixed populations. We seek to investigate the impact of different toxin-producing strains on the growth of other laboratory strains. We carefully selected several strains of *Saccharomyces cerevisiae* and labeled them with fluorescent proteins. To identify the specific types of toxins produced by each strain, we utilized a PCR-based approach. Subsequently, we evaluated the toxin-producing ability of each selected strain on various types of solid media and in liquid culture. Our preliminary findings indicate that varying conditions significantly affect the efficiency of toxin production and the susceptibility of other strains to the toxin produced.

***Keywords:*** *Saccharomyces cerevisiae, interaction, toxin production*

***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 the Thematic Excellence Programme (TKP2020-NKA-11).*

## PM-6#

### ISOLATION OF MOULDS AND EVALUATION OF THEIR PATULIN PRODUCTION POTENTIAL FROM HUNGARIAN APPLES

***Leandro Rodrigues, Emelin*[1]; Kosztik, Judit[2,3]; Tóth, Ákos[4]; Csernus, Olivia[1]; Bata-Vidács, Ildikó[2,3]**

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Apples, a globally popular fruit, are a key crop in Hungary, where they are grown in the largest quantity. Although being robust, many biological illnesses can cause damage to the fruit, fungal infection being one of the concerns. Another concern that has been gaining awareness of apple producers in recent years is the contamination by mycotoxins, especially patulin, regularly found in apples and their products.
In this study, fungal colonies were isolated from 40 apple samples, which consisted of three pieces of apple fruits in a paper bag, harvested the day before. Samples arrived from 7 Hungarian locations. For each apple, mould was cultured on Malt Extract Agar plates. Fungal colonies were selected, and the morphological features of the colonies, and spore characteristics of the isolates made it possible to group the isolates into four clusters: *Aspergillus*, *Alternaria*, *Penicillium*, and others. From representatives of the clusters, DNA was extracted and identified by ITS sequencing. A total of 183 moulds were identified: 61 isolates belonging to the genus *Alternaria*, 47 to the genus *Aspergillus*,13 to the genus *Penicillium* and 62 belonging to other species.
As the genera *Aspergillus* and *Penicillium* are recognized as patulin producers, the following experiments were realized for the strains belonging to these clusters. The strains were grown in Malt Extract Broth (MEB), DNA extraction was performed, and a PCR reaction was carried out to amplify the *IDH* gene that encodes a key enzyme of patulin production. The strains were grown again in MEB under the same conditions as before for 7 days. PAT extraction was carried out from the culture media with ethyl acetate as solvent. The solvent was evaporated, and the samples were re-suspended in acetonitrile. After the extraction, the samples were developed on TLC to check for patulin.
The location had a higher influence than the cultivation method on the distribution of fungus. The only *Aspergillus* strain that presented a positive result was strain B9/6, which originated from the cultivar Golden Reinders grown in Debrecen-Pallag by integrated cultivation. Of the *Penicillium* isolates only one strain, B10/6, presented a band for the *IDH* gene of the right size (500-600 bp). This result was confirmed with the TLC tests. These results allow us to conclude that patulin contamination of the apples is low since from 183 mould isolates, 60 among them belonging to possible patulin producers, only two had the potential to produce patulin.

***Keywords:*** *apple, mycotoxin, patulin, IDH gene*

***Acknowledgement:*** *The research was supported by project 2020-1.2.4-TÉT-IPARI-2021-00001 – Tempus Public Foundation –Stipendium Hungaricum Scholarship. The authors acknowledge the Doctoral School of Food Science of the Hungarian University of Agriculture and Life Sciences for the support of this study.*

## PM-7#

### EFFECT OF POULTRY MANURE-BASED COMPOST TEAS ON THE GROWTH OF PLANT PATHOGENS

**Boczonádi, Imre [1,2]; Busa, Dávid [3]; Gorliczay, Edit [1,2]; Csótó, András [1]; Csüllög, Kitti [1]; Tamás, János [1,2]**

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Compost teas, often abbreviated as CTs, are liquid solutions derived from composted materials, well known for their effectiveness in combating plant diseases (Souleymane et al., 2010). This study investigates various aspects of poultry manure-based compost teas and their ability to inhibit the growth of plant-pathogenic filamentous fungi such as *Aspergillus niger* (MW314791) and *Macrophomina phaseolina* (OQ319832) strains. We studied three poultry manure-based CTs labelled A, B, and C. Stress sensitivity tests involved creating 1:10 solutions, agitating them at 220 rpm for 24 hours at 25°C. Subsequently, we supplemented Potato Dextrose Agar (PDA) medium with varying concentrations of the CTs (0,1% up to 20%) and assessed colony diameters after a 5-day incubation at 25°C. Stress tests showed that CTs inhibited *A. niger* growth. At 5%, Teas A and B inhibited *A. niger* by about 50%, while Tea C only by 20%. Similar results were seen with 15% concentration on PDA medium, achieving 90% inhibition for Teas A and B, but only 20% for Tea C. Even at 1%, all CTs significantly inhibited *M. phaseolina* growth. Tea B was notably more effective, inhibiting around 90% even at 0.3% concentration. Overall, all three CTs (A, B, and C) show promise, with their microbial composition capable of phosphate solubilization, crucial for plant growth. They effectively combat *A. niger* and *M. phaseolina* under in vitro conditions.

***References***

[1] Souleymane B. Koné; Antoine Dionne; Russell J. Tweddell; Hani Antoun; Tyler J. Avis (2010). Suppressive effect of non-aerated compost teas on foliar fungal pathogens of tomato. 52(2), 167–173.

***Keywords:*** *Plant pathogens, Compost teas (CTs), Inhibitory effect*

***Acknowledgement:*** *The research presented was carried out within the framework of the Széchenyi Plan Plus program with the support of the RRF 2.3.1 21 2022 00008 project.*

# BIOINFORMATICS

## EEB#

### THE NEED OF REFERENCE GENOMES AND TRANSCRIPTOMES - THE FUNDATION OF BASIC RESEARCH

***Gálik, Bence*[1]; *Urbán, Péter*[1]; *Herczeg, Róbert*[1]; *Schmalz, Dániel*[1]; *Kun József*[1]; *Gyenesei, Attila*[1]**

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Nowadays numerous research projects rely on a *de novo* reference genome and/or transcriptome. As the result of the advanced stage of second and third generation sequencing technologies, applications and the rapid development of the corresponding bioinformatic solutions, the assemble a genome is not that demanding. However, we have to take into account other factors like the genome size, complexity, repeat content and the financial manners and possibilities of the project as well.
In the current lecture we aimed to present a “reference guide” that helps the researchers to design their own genome project. The workflow will guide through the main steps and challenges of building a draft Eukaryotic genome, including some essential wet-lab and sequencing aspects, but it focuses mainly on the bioinformatic parts (e.g. assembly, gene prediction, annotation and quality control). The workflow emphasizes the bioinformatic tools that could be applied for genome assembly, scaffolding and gap-filling steps and the dedicated sequencing method. We should not stop at the genome sequence itself, the transcriptome as crucial as a good draft genome. The workflow will point out to the RNA-related procedures as well.
Our recommendations may help those young researchers and research groups who does not have that much experience in this field. As a Core Facility our goal is to support these future projects in a collaborative way.

***Keywords:*** *short reads, long reads, genome, transcriptome, gene prediction, functional annotation*

## EB-1#

### STUDYING THE RESPONSE OF *PHYTOPHTHORA INFESTANS* INOCULATION IN DIFFERENT POTATO CULTIVARS BY A TRANSCRIPTOMIC APPROACH

***Idogwu, Esther Ijeoma*[1]; Taller, János[1]; Nagy, Erzsébet[1]; Wolf, István[2]; Polgár, Zsolt[2]; Frank, Krisztián[2]***

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Late blight caused by the oomycete fungus *Phytophthora infestans* (Mont.) De Bary, is one of the most devastating diseases of potatoes. In the wild potato species *Solanum demissum* 11 race-specific *P. infestans* resistance genes are known, which have been utilized in different breeding programs. Some of these R genes have already been cloned (R1, R2, R3a, R3b, R8), but most of them are not yet isolated. Previous analyses using the Black differential set indicated that in the cultivar White Lady (WL) most of these R genes are present, conveying a high-level *P. infestans* resistance to the cultivar. Besides WL, the cultivar Kastia with reported (Gergely, 2004) good horizontal resistance, and a susceptible cultivar, Sárvári borostyán were used, to compare their reaction to *P. infestans* inoculation via transcriptome analysis. Experimental plants were grown and inoculated under controlled conditions in a phytotron. One drop of 15,000 sporangium/mL containing solution of the *P. infestans* isolate MP-1548 (obtained from Poland) was added to the abaxial leaf surface of vigorously growing plants. Leaf samples for transcriptome analysis were taken before inoculation and 18, 24, 48 and 72 hrs after inoculation. Transcriptome sequencing on the 3x(1+4) samples was done on a NextSeq 500 (Illumina, USA) platform.
SOAPdenovo-Trans was used for assembling the transcriptome and quantification was done with the "index" and "quant" commands of the Salmon program. Differentially expressed genes (DEGs) were identified with the DESeq2 Bioconductor package in the R environment. DEGs were annotated based on the KEGG database using the KOBAS-i tool.
The inoculation test proved the expected resistance of WL and the susceptibility of Sárvári borostyán, but Kastia produced typical symptoms of late blight infection, which can be due to the possibly different avirulence gene content of the MP-1548 isolate compared to the *P. infestans* isolate that was used 20 years ago at Keszthely, and to the controlled environmental conditions compared with the reported foil-house experiment. By crossing experiments using the same paternal line the sensibility of Kastia and Sárvári borostyán to the MP-1548 *P. infestans* isolate was proven, too.
The more than 100,000 transcripts of each transcriptome were filtered using different approaches and these gene collections were compared among the three cultivars. In these analyses transcripts were collected according to: 1) expression triggered in different timepoints after the inoculation; 2) up- and downregulated with a threshold of two-fold change; 3) NB-LRR motif containing transcripts; and 4) transcripts with high sequence similarity to transcription initiation factors. Results will be introduced during the presentation.

***Keywords:*** *Phytophthora infestans, potato, transcriptome analysis, resistance*

***Acknowledgement:*** *This research was supported by the INN_139994 and INN_139993 OTKA projects of the National Office of Research, Development and Innovation, Hungary.*

## EB-2#

### ASSESSMENT OF THE MUTAGENIC EFFECT OF PROBABLY CARCINOGENIC PESTICIDES USING A WHOLE GENOME SEQUENCING APPROACH

***Martinek, Regina*[1,2]*; Lózsa, Rita*[1]*; Póti, Ádám*[1]*; Németh, Eszter*[1]*; Várady, György*[1]*; Szüts, Dávid*[1]

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Pesticides are significant environmental pollutants, and many of them possess mutagenic potential, which is closely linked to carcinogenesis. Here we tested the mutagenicity of all six pesticides classified probably carcinogenic (Group 2A) by the International Agency of Research on Cancer: 4,4'-DDT, captafol, dieldrin, diazinon, glyphosate and malathion. Whole genome sequencing of TK6 human lymphoblastoid cell clones following 30-day exposure at subtoxic concentrations revealed a clear mutagenic effect of treatment with 200 nM captafol or 100 μM malathion. Each pesticide induced a specific base substitution mutational signature: captafol increased C to A mutations primarily, while malathion induced mostly C to T mutations. 4,4'-DDT, dieldrin, diazinon and glyphosate were not mutagenic. Whereas captafol induced chromosomal instability, H2A.X phosphorylation and cell cycle arrest in G2/M phase, all indicating DNA damage, malathion did not induce DNA damage markers or cell cycle alterations despite its mutagenic effect. Hypersensitivity of REV1 and XPA mutant DT40 chicken cell lines suggests that captafol induces DNA adducts that are bypassed by translesion synthesis and are targets for nucleotide excision repair. The experimentally identified mutational signatures of captafol and malathion could shed light on the mechanism of action of these compounds. The signatures are potentially suitable for detecting past exposure in tumour samples, but the reanalysis of large cancer genome databases did not reveal any evidence of captafol or malathion exposure.

***Keywords:*** *pesticide, DNA damage, mutagenesis, mutational signature*

## EB-3#

### PEPTAIBIOMICS: NAVIGATING BIOTECHNOLOGICAL FRONTIERS WITH THE UNIVERSAL PEPTAIBOL DATABASE

***Rozsnyói, Ákos*[1], Balázs, Dóra[2], Tyagi, Chetna[2], Terna, Gergő[1,2], Szekeres, András[2], Vágvölgyi, Csaba[2], Marik, Tamás[2], and Kredics, László[2]**

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Peptaibols are secondary metabolites synthesized mainly by *Trichoderma* species, a group of bioactive molecules that show outstanding potential in biotechnology, environmental biology, health research and bioinformatics. These non-ribosomal peptides are capable of self-assembling into transmembrane ion channels that become incorporated into both prokaryotic and eukaryotic cell membranes, ultimately culminating in cell death. Their history dates back to the mid-20$^{th}$ century since when about 1500 different peptaibol sequences have been catalogued from *Trichoderma* strains.
The growing number of newly identified peptaibol sequences and publications in the past decades has made it necessary to create a centralized database. In response to this growing need, the "Peptaibol Database" was created in 1997 and the "Comprehensive Peptaibiotics Database" in 2013. The exponential increase in peptaibol knowledge (thanks to biotechnological and bioinformatics innovations in recent years) has made these databases outdated, which has led to the need for the creation of the "Universal Peptaibol Library". This dynamic database, built on the MySQL open-source platform, aims to bring together all existing peptaibol data while making it easier for researchers to add newly discovered sequences. For easy access to the library, our team develops a website using HTML, PHP, CSS and JavaScript. The key features include various search functions for known peptaibol sequences, similarities, access to publication data and information on source fungi, as well as comprehensive data on their molecular and mass spectrometric properties. In addition, we will introduce an immersive peptaibol structure visualization feature based on previously published 3D structures using NGLview, an interactive molecular structure visualization software. Our further aim is to add bioactivity data of peptaibol sequences to the database in the future.
The aim of the Universal Peptaibol Library is to bring together all known peptaibol data in a centralized, easily accessible location, thereby, enabling further research in microbiology, biotechnology and molecular biology, and assisting the future practical application of peptaibols in both agriculture and human health.

***Keywords:*** *peptaibol, database, library*
***Acknowledgement:*** *This study was supported by ÚNKP-23-3- SZTE-501 New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund, and by the Hungarian National Research, Development and Innovation Office (project No. 2022-1.2.6-TÉT-IPARI-TR-2022-00009).*

## PEB-1#

### AGENT-BASED MODELING OF YEAST COMMUNITIES UNDER NON-STANDARD ENVIRONMENTAL CONDITIONS

***Gaizer, Tünde*** [1]***; Pillér, Bíborka*** [1]***; Juhász, János*** [1,2]***; Metzing, Máté*** [1]***; Bélteki, Zsófia*** [1]***; Madár, Valentina*** [1]***; Szakadáti, Helga*** [1]***; Pongor, Csaba*** [1]***; Csikász-Nagy, Attila*** [1]

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Microbial colonies are routinely grown on agar plates in everyday experimental settings. Standardized conditions ensure these colonies grow in a reproducible fashion. We follow such standards, while in nature microbes are under a constantly changing environment, yet we are not sure how condition changes affect colony growth characteristics. To identify the factors of colony growth dynamics, we have developed and parameterized a quantitative agent-based model of yeast colony growth by synergizing mathematical modeling with laboratory experiments with *S. cerevisiae*. Our model successfully reproduces the selection of non-standard environmental conditions. Specifically, we introduced experimental methods that mimic the effects of humidity and nutrient gradients. Through colony growth experiments and model fitting, we demonstrate the influence of various environmental factors, including moisture, nutrient availability, and initial colony inoculation conditions.
Our results revealed that colony growth is controlled by the initial spread of microbes, the wetness of media and diffusion of nutrients. The presented model can be used to simulate the growth of colonies at various initial settings and can reveal which cells proliferate in a growing colony. The model is extended to multiple strains and interactions to predict yeast community growth. These preliminary results will be presented. Our results can help researchers to design and control colony growth experiments as well as yeast communities more precisely than before.

## PEB-2#

### T-ARMS PCR APPLICATION FOR SNP DETECTION IN HOLSTEIN-FRIESIAN CATTLE OF A1 AND A2 B-CASEIN

***Sándorová, Lilla*[1]; *Ninausz, Nóra*[1]; *Fehér, Péter Árpád*[1]; *Nagy, Barbara Katinka*[2]; *Somoskövi, András*[3]; *Fodor, Dániel*[3]; *Szabari, Miklós*[4]; *Holló, Gabriella*[4]; *Bodnár, Ákos*[5]; *Póti, Péter*[5]; *Mezőszentgyörgyi, Dávi*[4]; *Bodó, Szilárd*[4]; *Stéger, Viktor*[1]**

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The research is anchored in the growing market interest for milk and dairy products with specific nutritional value, notably featuring the A2 beta-casein protein, characterized by its rich composition, appropriate fatty acid profile, and distinct technological attributes. The A1 form of the bovine milk *β-casein 2* (CSN2) gene is different from the original A2 form, which influences several negative physiological effects, such as inflammation, unwanted immune responses, eczema, respiratory dysfunction, digestive problems. The A1 and A2 beta-casein variants exhibit dissimilarities at the 67$^{th}$ amino acid position, where proline is substituted by histidine in the A1 type. Furthermore, the distribution of A1 and A2 alleles in Holstein-Friesian cows varies regionally.
This study is dedicated to the development and application of Tetra-ARMS Polymerase Chain Reaction (T-ARMS PCR) for the precise detection of Single Nucleotide Polymorphisms (SNPs) associated with A1 and A2 β-casein types in Holstein-Friesian cattle. Widely recognized for its specificity and sensitivity, the T-ARMS PCR technique employs four primers in a single PCR reaction. Total genomic DNA was extracted from ear cartilage samples using MagCore Genomic DNA Tissue Kit according to the manufacturers' protocols. Genomic DNA samples from Holstein-Friesian cattle were subjected to T-ARMS PCR, producing distinct band patterns corresponding to A1 and A2 *β-casein* genotypes. The amplified products underwent gel electrophoresis, enabling the identification of specific SNP patterns linked to each β-casein type. The ARMS-PCR generated an internal control fragment of 586 bp, a 384 bp fragment indicative of the A1 allele, and a 256 bp fragment corresponding to the A2 allele. Heterozygous individuals for A1A2 exhibited both control fragments and amplicons of 256 bp and 384 bp.
In conclusion, this study offers profound insights into the genetic variations of A1 and A2 β-casein types in Holstein-Friesian cattle, laying the groundwork for enhancing the nutritional quality of milk and dairy products. The application of T-ARMS PCR emerges as a precise and effective method for SNP detection, enabling the identification and selection of cattle with desired *β-casein* profiles. This research contributes significantly to ongoing efforts to elevate both the health-related attributes and market value of dairy products.

***Keywords:*** *A2 β-casein, T-ARMS PCR, SNPs, genetic variations, Holstein- Friesian cattle*

***Acknowledgement:*** *this research is funded by mate 2020-1.1.2-piaci-kfi-2021-00305. The development of technologies to support the production of widely accepted industrial milk, using molecular biology tools for nutritional physiology, is related to the consortium proposal. We would like to express our gratitude to Bóly Agricultural Producer and Trading Ltd. for the samples.*

## PB-1#

### KINSHIP RELATIONSHIP SURVEY OF AN INVASIVE MESOPREDATOR (*PROCYON LOTOR*) IN CENTRAL HUNGARY

***Fehér, Péter*[1,3], Scsepkó, Nikolett[1], Sándor, Lilla[1], Katona, Krisztián[2,3], Biró, Zsolt[2,3], Galambos, László[4], Horváth, Zsolt[2], Szabó, László[2,3], Bócsi, Balázs[2], Heltai, Miklós[2], Stéger, Viktor[1,3]***

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Invasion in a biological sense is a principal component of global change, because scientists recognised it as a threat to biodiversity and natural resources on a global scale. The raccoon (*Procyon lotor*), native to North America, was introduced to Europe in 1934. Researchers consider Germany to be the centre of European distribution. Raccoon populations have increased and spread substantially over the past century. Raccoon population size in Hungary is on an increasing trend, according to hunting estimates, which may indicate their permanent establishment. The aim of this study was to investigate the genetic relationships within a raccoon population around Ócsa. Muscle tissues were obtained from free-ranging animals (n = 13) legally shot between 2015 and 2023. Ten microsatellites were adapted from other publications, optimized for multiplex PCR, and used for genotyping. To identify parental and kinship relations among individuals the parentage assignment package Colony2 was used. The software detected several siblings and offspring-parent relationships among the samples from Ócsa. The software identified a male individual as the probable father of two individuals (a male and a female) being full siblings. In addition, the software found several half-sibling relationships between the samples. The relatively high number of assumed kinship relationships suggests that there are no, or very few immigrant individuals in this population, which may cause inbreeding. The study was funded by the National Research, Development and Innovation Office in Hungary (RRF-2.3.1-21-2022-00006).

***Keywords:*** *raccoon, Hungary, kinship relationship*

***Acknowledgement:*** *National Research, Development and Innovation Office (National Laboratory for Health Security, RRF-2.3.1-21-2022-00006).*

# ANIMAL BIOTECHNOLOGY

# EEÁ#

## BIRD'S EYE PERSPECTIVE OF ENTERIC NERVOUS SYSTEM DEVELOPMENT: LESSONS FROM THE AVIAN EMBRYO

***Nagy, Nándor***

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Among birds, the domesticated poultry species, like chicken, quail, turkey and duck, have been specifically used in enteric nervous system (ENS) research since the early nineteenth century. Later, the chicken and the quail embryo became important model organisms in developmental biology of the gastrointestinal tract and its associated nervous system. The avian ENS, similar to mammals, is a complex network of neurons and glial cells that controls gut motility, secretion, absorption, and local blood flow. Precursors of enteric neurons and glia arise from vagal neural crest cells (NCC), a highly invasive population of multipotent stem cells that delaminate from the neural tube, enter the gut, and colonize its entire length.
In selecting the "optimal" biological model, scientists have always focused on easy access, low-cost sustainability, rapid embryonic development, easy microsurgical manipulation, and resistance to experimental interventions. Although the histogenesis and development of the ENS has been studied in a number of animal model systems, the avian embryo has proven to be one of the most suitable for ENS related embryological studies. For example, the developmental mechanism of the ENS in chicken and quail embryos is highly similar to the human fetus: both enteric plexuses are formed in the early stages of development, while the submucosal plexus in the colorectum of mouse and rat embryos only appears after birth. The avian embryo is well suited for studying the molecular regulation of ENS formation. Poultry eggs are plentiful, inexpensive, and easy to maintain under laboratory conditions. In addition, many features of ENS development are conserved in avians and mammals. Avian embryos are accessible during all stages of embryogenesis and a large repertoire of methodologies, including tissue grafting, retroviral-mediated gene transfer, electroporation, and embryo culture, can be used to perturb and analyze gene function during development.
While classical embryo manipulation techniques made the avian embryo an important tool in ENS developmental biology, it had been at a considerable disadvantage for developmental genetic studies. One reason for this is that targeted mutations, conditional mutants, which can be carried out in mammalian and amphibian embryos, were not possible in birds. However, using novel gene manipulation methods described in recent years (retroviral transfection techniques, electroporation, CRISPR/Cas9 gene editing), targeted loss-of-function and gain-of-function mutations can now be induced in avian embryos to study the ontogeny of ENS. These innovations have brought the avian embryo back to the forefront, as transgenic techniques in mice are costly and time-consuming.

***Keywords:*** *avian, embryo, developmental biology*

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# EÁ-1#

## IDENTIFYING THE NOVEL ROLE OF NADPH OXIDASE 4 IN THE BIOTRANSFORMATION OF T2 MYCOTOXIN

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Due to the impacts of global climate change, the presence, potency, and distribution of mycotoxins in food and feed have emerged as persistent health concerns. Central to the toxicity of many mycotoxins is the generation of reactive oxygen species (ROS), which contribute to the onset of pathologies and modulate various physiological processes. NADPH oxidases (NOX), key generators of ROS within the body, exert significant control over cellular functions and developmental pathways. Among the NOX isoforms, NOX4 is the most widely distributed and plays a pivotal role in maintaining physiological redox balance by catalyzing ROS production in vivo. Utilizing a genome-edited rabbit model deficient in functional NOX4 (NOX4-KO), previously developed by our research team, we investigated the redox regulation of rabbit embryonic fibroblast models exposed to T2 mycotoxin *in vitro*. Employing the RNA sequencing technique, we identified several enzymes involved in the detoxification process, encompassing both phase 1 and phase 2 enzymes. Comparative analysis between NOX4-KO and wild-type cells unveiled distinct patterns in early-phase gene expression dynamics of biotransformation enzymes upon mycotoxin exposure. Notably, DUOX1, another significant isoform of the NOX family, exhibited alterations in expression following T2 mycotoxin exposure. While a dose-dependent increase was observed in wild-type cell cultures, the absence of NOX4 resulted in upregulated DUOX1 expression but seemingly showed a pattern characteristic in the case of hormesis. These findings illuminate the possible interplay between NADPH oxidases, particularly NOX4 and DUOX1, in the biotransformation process of T2 mycotoxin. Our findings contribute to a deeper comprehension of the involvement of NOX enzymes in T2 mycotoxin exposure, offering insights that may inform strategies for mitigating its adverse effects.

***Keywords*****:** *NADPH oxidase 4, DUOX1, T2 mycotoxin, biotransformation, NOX4 knock-out genome edited rabbit fibroblast*

***Acknowledgement:*** *Project no. RRF-2.3.1-21-2022-00007Agribiotechnology and Precision Breeding for Food Security National Laboratory; Project no. TKP2021-NKTA-34 has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-NKTA funding scheme*

# EÁ-2#

## CREATING FUCCI-EXPRESSING CHICKEN PRIMORDIAL GERM CELL LINES FOR THE ANALYSIS OF THE CELL CYCLE

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Chicken primordial germ cells (PGCs) can be practical tools for gene preservation and transgenesis. Strong, well-proliferating cultures are essential for these purposes. Our goal was to establish a reliable transgenic cell line using the Fluorescence Ubiquitination-based Cell Cycle Indicator (FUCCI) as an alternative and improved method for monitoring cell health *in vitro.*
We utilized Hungarian White breed embryos to establish PGC lines. The PGCs were then electroporated with FUCCI transgene containing plasmid. Transgenic cells were collected individually, and one-cell-derived cultures were established. Once the appropriate cell number was reached, the cell lines were frozen. Ten tubes per line were frozen to ensure their availability for future projects.
The success of the transgenesis was confirmed through DNA sequencing, confocal analysis, chromosomal examination, and timelapse imaging.
We analysed the integration sites of the FUCCI transgene. In the FCF5 (ZW, female genotype) and FCM5 (ZZ, male genotype) one-cell-derived PGC lines, we found that they contain the complete FUCCI transgene complex.
The transgenic cells were also injected back into 56-hour-old embryos, and their presence in the 10-day-old gonads was confirmed.
The chicken PGC lines expressing FUCCI can be utilized for further experiments, such as exploring the effects of heat stress or toxin treatments on the cells.

***Keywords:*** *chicken, PGC, FUCCI*

***Acknowledgement:*** *This study was supported by grants RRF-2.3.1-21-2022-00007*

# EÁ-3#

## DIGITAL PCR ANALYSIS OF GROWTH RELATED GENES IN AFRICAN CATFISH (*CLARIAS GARIEPINUS)* LINES

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Hungary is leading of the intensive breeding of African catfish (*Clarias gariepinus)* in Europe. The expenditures of intensive fish breeding are increasing year by year. The most expensive part of the production is the feed, because of the high content of fish meal and oil ingredients. The replacement of animal-based meals can cause decreased growth and development of catfish. However, scientific experiments suggest that selection through generations for lowered content of fish meal and oil feed can be a solution.
*Clarias gariepinus* lines were selected (selected groups) for higher body mass through 4 generations using low fish meal containing feed (partially fish meal replacement with soy meal). In comparison, the control group was maintained on conventional feed (high fish meal content fish feed). Three hundred fish from the fourth generation were used in a mixed comparative /demonstration trial. After 6 weeks rearing the smallest and the largest fish's brain, liver, and muscle were sampled for digital PCR based gene expression analysis of growth-related genes.
Our early results suggest that the expression of growth hormone, growth hormone receptor and somatostatin genes differ by fish groups and feed groups. The control fish group had similar expression in the case of *gh* and the *sst* on control feed in small and large fish. However, in brains and livers, the *ghr* had 2,5x higher expression in the small fish group. The growth hormone receptor gene had similar expression patterns in both brains and livers in all fish groups.
During the continuation the muscle tissue and other growth-related genes (insulin- like growth factor – I, insulin-like growth factor – II, activin receptor 1, activin receptor 2b, follistatin, myostatin) will be included in the dPCR investigations. Using the same samples, whole transcriptome analysis will be done among the groups.

**Keywords**: *growth genes, Clarias gariepinus, fish feed, digital PCR, gene expression*

***Acknowledgement:*** *The work was supported by the iFishIENCi project (European Union's Horizon 2020 research and innovation program under grant agreement No 818036), and by the National Research Development and Innovation Office (NKFIH) Hungary, grant number 2017-2.3.3- TÉT-VN-2017-00004.*

# EÁ-4#

## A TRANSPARENT ZEBRAFISH REPORTER LINE FOR IMPROVED VISUALIZATION OF GONAD TRANSFORMATION

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The primary goal of our research is to understand sexual development of teleosts using the zebrafish model. During the development of zebrafish gonad, a 'juvenile ovary' forms in most future males and later transforms into a testis [1]. This process is not fully understood yet. We have been using the *tg{ddx4::egpf}* (earlier *tg{vas::egfp}*; [2-3]) transgenic line to visualize gonad transformation, since the maternally encoded *ddx4* transcripts and the fluorescent protein segregate with the primordial germ cells (PGCs). However, the presence of pigments interferes with *in vivo* observation of Egfp signals derived from ovaries. For this reason, we introgressed the transgene into the double pigment mutant zebrafish line, *casper* [4] with a more efficient selection procedure, than those published earlier [5-6]. The new homozygous transgenic line on *casper* background was named *gáspár* (pronounce: ghaashpar). Currently we are working on the characterization of this line and its potential application as an alternative model for studying gonad development. Our preliminary data clearly show that lack of black and reflective pigments in the trunk area enables visualization of Egfp-tagged germ cells with higher resolution. Although PGCs play an essential role in early gonad formation [7], we have observed that occasionally they do not reach the gonadal ridge during their migration. To reveal potential effects of this mis-migration process on sexual development of zebrafish, we studied the migration of PGCs *in vivo* with time-lapse imaging. Despite their obvious advantages and benefits, transgenic reporter lines with ovary-enhanced Egfp production are not ideal for studying testicular processes. Therefore, we started to work on the second transgenic construct that can be introduced into *gáspár* to visualize the testis development with a different reporter gene. So far, we have found six marker gene candidates, whose regulatory region can be potentially suitable for marking the testis *in vivo*. We propose that the *gáspár* line should be considered in those experiments where signals from the gonad are detected - especially in larvae/juveniles - as its use will help researchers to gain a better understanding on the sexual development of zebrafish.

***References***:

[1] Orbán et al., Mol. Cell. Endocrinol. 312: 35-41 (2009); [2] Krovel and Olsen, Mech. Dev. 116: 141-151 (2002); [3] Krovel and Olsen, Dev. Biol. 271: 190-197 (2004); [4] White et al., Cell Stem Cell 2: 183–189 (2008); [5] Presslauer et al., Reproduction 152: 507-517 (2016); [6] Akhter et al., Zebrafish 13: 155–160. (2016); [7] Tzung et al., Stem Cell Reports 4: 61-73 (2015)

***Keywords:*** *natural sex reversal, apoptosis, PGC, transgenic reporter line, casper*

***Acknowledgement:*** *The work was supported by the Frontline Research Excellence Grant (KKP 140 353 to LO) and the New National Excellence Program (ÚNKP 23-2-I to OMSz) of the National Research Development and Innovation Office (NKFIH) of Hungary.*

# PEÁ-1#

## SEXING OF ZEBRAFISH BY GENES DIFFERENTIALLY EXPRESSED IN THE TAIL FIN

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In wild-type zebrafish sex is determined by a ZW-ZZ sex chromosomal system [1], whereas in domesticated zebrafish lines a polygenic sex determination was proposed [1-2]. As such, no genomic marker is available for sexing domesticated zebrafish individuals. Although phenotypic markers of sexual dimoprhism can be utilized in most cases, their use does not yield reproducible results in some of the domesticated lines. Currently, the only remaining option for sexing in these cases are transgenic reporter lines, e.g. *tg{ddx4::egfp}* [3] or *tg{piwil1::egfp}* [4], where the transgene is differentially expressed in the gonads.
Here, we tested the potential use of differentially expressed genes in the tailfin of the two sexes of adult zebrafish as minimally invasive sex markers for non-transgenic individuals. Although others described such genes earlier [5], they have not attempted to develop markers from them. We isolated RNA from the tailfin of male and female adult zebrafish and analyzed their transcriptome by RNAseq. We have identified 387 differentially expressed genes (DEGs), out of which 87% were from the male fins. Based on bioinformatic analyses and practical considerations, we have identified 12 potential markers; their characterization and PCR-based testing is in progress.
We are hoping to identify markers showing differential expression prior to the appearance of first phenotypic dimorphic signs. As zebrafish belongs to the cyprinid clade of teleosts, potential extension of the markers to related fish species might have commercial importance.

***References:***
[1] Wilson et al., Genetics 198(3): 1291-1308 (2014);
[2] Liew et al., PLoS ONE 7(4): e34397 (2012);
[3] Krøvel and Olsen, Mech. Dev. 116: 141-151 (2002);
[4] Leu and Draper, Dev. Dyn. 239(10): 2714–21 (2010);
[5] Hosseini et al., BMC Genomics 20: 341 (2019)

***Keywords:*** *zebrafish, sex determination, expressed sex markers*

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# PEÁ-2#

## INTRAINDIVIDUAL VARIANCE OF BULL SPERM MORPHOMETRY PARAMETERS AND THEIR RELATION WITH FIELD FERTILITY

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The routine semen analysis procedure in the production of commercial frozen bovine semen is based mainly on automated instrumental measurements, thanks to the development of modern technology. However, the use of older methods, such as morphological evaluation and morphometric measurement of spermatozoa, still have their importance.
20 samples of frozen semen of commercially available Hungarian Simmental bulls were analyzed. The only background information we had about the bulls was that 12 animals had poor fertility ("bad" bulls), while 8 had acceptable fertility ("good" bulls). We measured the samples by flow cytometric assays for viability, mitochondrial activity and plasma membrane asymmetry, and we also stained smears with Feulgen staining for microscopic examination. We chose Feulgen stain for the staining procedure because this staining method does not cause any size change of the damaged cells during the staining procedure. Feulgen kit stains the chromatin of the sperm, which in the case of bull spermatozoa has a very compacted structure. Other dyes, on the other hand, stain the membrane and the acrosome, which in damaged cells can cause the swell of the cell and may distort the measurement results. We performed morphometric measurements Feulgen-stained slides using a free software tool ImageJ (https://imagej.net/ij/). The software allows us to measure several parameters of the sperm heads, so we could evaluate the perimetric, area, length and width of the sperm heads. 60-200 cells were measured in each sample.
Descriptive statistics and non- parametric Mann–Whitney U-test were done with R Commander (v. 2.6-2.). Statistical significance was set at $p<0.05$.
No significant difference was found between the results of the "good" and "bad" bulls based on the automated, flow cytometric measurements. Moreover, there was no significant difference between the "bad" and "good" bull sperm samples in the mean values of sperm head area, head perimetric, head length and head width, and there were no significant differences in the standard deviation values of head area and head width. The "good" bull group, however, showed significantly smaller standard deviation values for head length and head perimetric indicating smaller sperm head intraindividual variance, meaning that this bull group had more uniform sperm heads.
This means that the observations from a hundred years ago are still valid; Williams and Savage found in 1925 that bulls with higher fertility have more uniform sperm heads, – our findings indicate that such differences in intramale sperm head variance still exist in domestic bulls, affecting field fertility.

***Keywords:*** *morphometry, bull sperm, sperm head, fertility*

***Acknowledgment:*** *This study was supported by the National Scientific Research Fund, Hungary (NKFI OTKA K139145).*

# PEÁ-3#

## INVESTIGATING THE EFFECT OF HEAT TREATMENT AND MONITORING THE MOLECULAR CHANGES IN PRIMORDIAL GERM CELLS (PGC) BEFORE AND AFTER FREEZING

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Nowadays, a modern, *in vitro* method for gene preservation is cryopreserving and storing primordial germ cells in gene banks. In birds, this is possible because the PGC migration takes place in the extra-embryonic blood vessels, so its isolation from embryonic blood is possible. The actuality of the topic is given by the preliminary heat treatment of PG cells at a higher temperature, which can enhance the effectiveness of deep cooling the cells through the activation of heat shock proteins.
The goal of our project was to deepen our understanding on the molecular level during the cryopreservation of the germ cells, and to try a new heat treatment method to prepare the cells for the freezing.
In the experiment 2–2 male and female Partridge-coloured Transylvanian Naked neck chicken primordial germ cell lines were used. These cell lines were newly developed and had not been frozen before. We used three experimental groups: absolute control, where cells did not receive any treatment; control group where the cells were subjected to the same treatment as the heat-treated group but at 38°C; and the heat-treated group.
The heat treatment was carried out for 3 hours at 42°C in 1.5ml Eppendorf tubes, 6x100.000 cells/group, in 500 µl medium each tube. An Eppendorf Thermomixer device was used to heat treatment at 350 rpm to prevent cell settling. The PGCs were collected immediately after treatment, after 48 hours, and after two weeks 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*

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# PEÁ-4#

## THE EFFECTS OF X-CHROMOSOME INACTIVATION IN MOUSE EMBRYONIC GONADS

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To investigate X chromosome inactivation, male mice from the XGFP#4 strain were crossed with CD1 females to monitor X chromosome inactivation in female embryos during embryonic development. Inactivation was initially observed in the trophectoderm of blastocysts; subsequently, it occurred randomly in the somatic cells of embryos. Crosses were also performed with males from the EGFP#5 strain, wherein EGFP was linked to an autosome.
Eight-cell-stage embryos were retrieved from the oviducts of pregnant females and further cultured *in vitro* until the blastocyst stage. Upon placing XGFP#4 blastocysts on gelatine-coated plates, low EGFP expression was noted in the attached inner cell mass (ICM) cells. By the third day post-attachment, intense EGFP expression was detectable in giant trophoblast cells and primordial germ cells (PGCs).
Additionally, 14.5-day-old embryos underwent dissection, and their gonads were harvested and immersed in a 4% paraformaldehyde (PFA) solution for fixation. The fixed gonads were then sequentially transferred to sucrose solutions with concentrations of 10%, 20%, and 30%. Following this, the organs were embedded in a 7.5% gelatine matrix and sectioned using a *Microm 500 OM* Cryostat. Subsequently, the sections underwent staining utilizing the haematoxylin-eosin staining method. Immunostaining was then performed to assess GFP expression within the genital ridge of XGFP#4 and EGFP#5 mouse embryos, facilitating comparative analysis.

***Keywords:*** *X-chromosome inactivation, mouse, embryology*

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# PÁ-1#

## DO MYCOTOXINS INFLUENCE THE BODY CONDITIONS OF FREE-LIVING GAME SPECIES?

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Mycotoxins are secondary metabolites of Molds, which can cause critical problems in human and animal health. Several mycotoxins can be harmful for the body conditions of domestic animals, directly or indirectly. The tested mycotoxins were Aflatoxin, Zearalenone, Fumonisin B1, Deoxynivalenol and T-2. We analysed the correlation between the concentration of these mycotoxins in kidney, body weight and kidney fat index of roe deer does, and female brown hare individuals harvested legally on agriculture areas. We could not prove significant influence of the mycotoxins to the body conditions. We concluded that the mycotoxin concentration exceeded the threshold of the influence acceptably only. We also concluded game managers and hunters are not able to select the individuals suffer of mycotoxicosis observing the body condition.

***Keywords:*** *Aflatoxin; Zearalenone; Fumonisin B1; Deoxynivalenol; T-2; Lepus europaeus; Capreolus capreolus*

# PÁ-2#

## MOLECULAR GENETIC ANALYSIS OF HUNGARIAN PANNONIAN BEE POPULATIONS BY MICROSATELLITE MARKERS

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Several subspecies of the honey bee are known in Europe, such as *Apis mellifera ligustica, Apis mellifera buckfast, Apis mellifera carnica, Apis mellifera mellifera*. They play an important role in the ecosystem and economy, but their populations are declining all over the world, for example in America and in Europe. In Hungary, only the Pannonian bee (*Apis mellifera carnica pannonica*) can be bred as the one officially recognized breed since 2012. In our country, their numbers haven not decreased significantly in the last 10 years, but there is a risk of losing genetic variability and hybridization with native/endemic species. For this reason, many researches investigate the unique reproductive biological and genetic traits of the bees; that support gene conservation.
The aim of our study is to determine the genetic diversity of the Pannonian bee populations based on microsatellite markers, furthermore to detect the presence of possible foreign genetic material and to set up a molecular marker set for breed/population identification.
Bee colonies that had passed the morphological variety certification successfully were compared to the groups of the failed, reference and Carnica bee samples from other European countries. More than 300 individuals were genotyped using multiplex microsatellite marker sets, and the fragment analysis was performed. The data of the four groups were analyzed by population genetics statistical programs. The basic diversity measures (heterozigosity, inbreeding) were determined within populations and the genetic differentiation between populations, moreover the genetic structure of the bee colonies was assessed. Based on our results so far, the bee populations examined are close to the Hardy-Weinberg equilibrium state, which means that their breeding seems to be appropriate. The level of inbreeding as well as the genetic differentiation is quite low; there is no clear structuring between the populations.
Several unique and distinctive alleles were detected. The groups of the failed, reference and Carnica samples had a higher proportion of heterozygous individuals, and higher genetic differentiation than the successfully certified stocks. The reference group was largely distinct from the others while those overlap quite a lot. Based on the population statistics of the results obtained from the fragment analysis, a very relevant sample collection could be chosen among the groups investigated for further high-resolution marker research (mtDNA, SNP), in order to set up a correct marker panel for identification. Our results may provide help in beekeeping and gene conservation maintaining the genetic variance.

***Keywords:*** *honey bee, microsatellite, genetic diversity*

***Acknowledgement:*** *VEKOP-2.3.2.-15-2016-00012; Hungarian Beekeepers National Association*

# PÁ-3#

## THE EFFECT OF DIFFERENT PROTEIN SUPPLEMENTATION DURING FEEDING OF AFRICAN CATFISH (CLARIAS GARIEPINUS) LARVAE, JUVENILES AND ADULTS (MARKET SIZE).

***Farr, Hannah Moncon*[1]*; Nyulas-Zeke, Ildikó*[2]*; Urbányi, Béla*[1]**

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In recent times, the fastest-growing food production sector has been aquaculture. With the rapid growth of the human population, the demand for fish is also constantly increasing. Fish is highly nutritive and rich source of animal protein. Natural water fishing is no longer able to meet the needs. Today, 50% of the fish consumed by humans is produced on farms, but this figure could reach 60-70% by 2030 (Subsinghe et al., 2009). As the aquaculture sector grows, so does the demand for full-fledged feed. For the improvement of fisheries and to achieve maximum yields from resources of fresh water, it is necessary to provide artificial feed, by which fish grows rapidly and attains maximum weight in shortest possible time. Among commonly used feed ingredients, fishmeal is considered to be the best ingredients due to its compatibility with the protein requirements of fish (Alexis et al, 1996). The high content of protein and amino acids in the fishmeal makes it the preferred choice of manufacturers for fish feed. To produce one tonne of fishmeal, about four and a half tonnes of live fish are needed, which are still derived from natural water, including sea fishing. It is clear that the production of fishmeal from natural water fisheries is unsustainable. Research shows that marine and ocean life has declined dramatically in recent decades, partly due to overfishing. As a result, more research activities are being conducted to replace fishmeal as a feed component. Although fishmeal is one of the most commonly used sources of protein in fish farming, its high price is a major constraint in feed formulation when used in the formulation of feed, it leads to an increase in the cost of feed and a corresponding rise in fish production (Ekelemu et al, 2000)
Early research on fish farming occasioned by the demand for fish protein which is caused by the high population growth and the Increasing demand for fish protein has placed pressure on the demand for fish from capture and marine productions. This study therefore seeks to address the issue of efficient aquaculture production so as to resolve and address the gap of inadequate fish for the fish protein needs of developing countries. This study will critically analyze and explore the possibilities of using aquaculture as a means to address the issue of fish protein gap through sustainable aquaculture in developing countries.
The objective will be to analyze the effect of protein feed supplementation in the intensive rearing of African catfish larvae, juveniles and adults (market size) groups through the examination of the following parameters: survival of fish; fish growth (body weight/body length); examination of the feeding coefficient; analysis of feed coefficient; meat quality analysis; determination of feeding costs.

***Keywords***: *Aquaculture, Protein, African Catfish, Lavae, Juveniles, Adults, Supplementation*.

# PÁ-4#

## MYCOTOXIN MEASUREMENTS FROM FALLOW DEER MILK

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The adverse effects of climate change also have an impact on agriculture. Increase in temperature and water activity promotes harmful mould species colonization plants of and result in an increasing occurrence of mycotoxins. Long-term exposure to mycotoxins can lead to the development of chronic diseases. These include liver and kidney damage, immune system disfunctions, hormonal disorders, reproductive biological abnormalities, cancer, and neurological diseases. It is important to note that mycotoxins have no taste or smell and so animals cannot detect those in contaminated food. Strict rules for Aflatoxin M1 (AM1) are already in place in many EU countries and measures are planned to regulate the maximum allowable concentration of Ochratoxin-A (OTA) and Zearalenone ZEA. Before 2010, aflatoxins were only present in tropical and subtropical areas, but because of climate change, mycotoxin-contaminated cereals have become increasingly common in Hungary. The transmission rate of aflatoxins from feed to milk in cattle has been found to vary between 0.35% and 3.5% in several studies. Zearalenone and its metabolites have been detected in milk and dairy products, as demonstrated in several studies. We studied the phenomenon in one of the most important Hungarian game species, the fallow deer (*Dama dama*) to estimate the transmission of Zearalenone, deoxynivalenol (DON), Aflatoxin M1 and Fumonisin B1 (FB1) to the calves.
In the analysis of the fallow deer hinds breast milk samples, the maximum zearalenone toxin concentrations measured in the samples are low. The results of this study show that the toxin Fumonisin B1 can be detected to a greater extent in samples of Hind's milk. The transmission probability was extremely high, presumably passing rapidly into breast milk but slowly excreted from the body. Overall, the results of this study show that the concentration (?) of Fumonisin-type toxins in milk is very low, but they may be present at higher levels in some fallow deer milk, and further studies are planned. Although detectable in milk, deoxynivalenol is rapidly metabolized in ruminants. In Prelusky's 1987 experiment, where ewes were fed high doses, high concentrations were also measured in their milk. The results from the milk samples of fallow deer indicated that the DON retained in the milk was 5.68%, so the concentrations in the milk of fallow deer were similar to those measured in this study. This research shows that feeding is a key issue not only in conventional livestock production but also in outdoor and wild game feeding. Feeding should aim to contain the lowest possible concentration of contaminants in order to maximise the development of foetal and neonatal fallow deer calves.

# PÁ-5#

## IMMUNOASSAY DEVELOPMENT USING FUNCTIONALIZED CHICKENS IMMUNOGLOBULINS FOR THE DETECTION OF ZEARALENONE AND ITS METABOLITES

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It is well known that mycotoxin manifestation, which is also increasing due to climate change, and related human and animal populations, such as farm animals or wildlife, represent an increasing economic (agricultural) and health risk and damage.
The focus of our research is the study of persistent mycotoxins of natural origin, produced by certain molds, which are environmentally damaging.
In addition to the identification of exposure sources (e.g., contaminated feed, grain, etc.), the detection of toxin (or derivatives) accumulation in living systems, organs, and tissues, the (quantitative) detection of the relevant agents, and the investigation of the associated short- and long-term physiological effects are of fundamental economic and societal interest.
Among the common mycotoxins that are also regulated by law, zearalenone (ZEN) is particularly dangerous because of its immunotoxic, carcinogenic, or hepatotoxic effects, estrogen-like nature, and induced reproductive biological abnormalities.
Once deposited in the body, ZEN can be converted into, among others, α- or β-zearalenol metabolites, which can have a variety of adaptive physiological and/or even behavioural effects.
To the best of our knowledge, few market procedures or services can simultaneously measure mycotoxins (for example, ZEN) and their metabolites from animal samples, body fluids, organs, tissues, and (also) measure accumulation.
For this purpose, and also for relevance, dedicated immunoassay-based ELISA-like analytical assays are being developed to achieve both early (even at low concentrations) detection of exposure (exposure) and accumulation (ideally of ZEN and its metabolites together) within the body.
In our procedures, we often use 'home-produced' polyclonal poultry immunoglobulins, which can be isolated cheaply from egg yolk, as specific anti-ZEN binding agents on different high-throughput measurement platforms.

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# PÁ-6#

## HOW HEAT STRESS AFFECTS THE SUCCESS OF EMBRYO TRANSFER

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Embryo transfer is a widely used reproductive technology worldwide to accelerate genetic progress and is also an effective means of increasing fertility in cases of heat stress, as it can help avoid damaging oocytes and early embryos from hyperthermia. After exposure to high temperatures, the oocyte can suffer the effects of heat stress for at least 105 days (Torres-Júnior et al., 2008) and remains sensitive to high temperatures even after maturation. Exposure to heat stress during the two following days after fertilisation may result in a reduction in embryo development, but this effect is less profound for the following three to seven days. Until the seventh day, embryos at the morula or blastocyst stage are highly resistant to damage by maternal hyperthermia. Surprisingly, despite the reduction in the number of embryos produced during heat stress, the season in which the embryos were produced had no significant effect on the survival of the embryos after the transfer. Thus, it appears that embryos that were able to survive the heat stress-induced damage and the initial critical stage of development were able to maintain gestation at a similar rate as embryos produced during cooler months of the year (Vieira et al., 2014).
In our preliminary study, we observed the results of embryo transfers performed during heat stressed and non-heat stressed period. *In vivo* embryo production was performed using Holstein-Friesian donor heifers from the MATE Kaposvár Campus Teaching Facility. The embryos were graded according to the IETS manual. Afterwards, first and second grade embryos were cryopreserved or immediately transferred. During the heat stressed period six of the thirteen freshly transferred embryos retrieved from five embryo transfer programmes maintained pregnancy (46%). By transplanting five cryopreserved embryos from the same programmes, two cows remained pregnant at the 60-day rectal palpation examination (40%). In the non-heat stressed period, embryos were produced from four programs. From eight embryos five were freshly transferred; one cow remained pregnant (20%), and from three embryos transplanted after cryopreservation, two animals remained pregnant (67%).
Finally, from the two periods pregnancy outcomes were better in the heat-stressed period, although there was no significant difference between the rates.

# PÁ-7#

## DIRECT CRYOPRESERVATION OF AVIAN EMBRYONIC REPRODUCTIVE CELLS FOR GENE BANKING PURPOSES

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In the recent scientific era, studies on gene preservation on avian species have continuously emerged to maintain their biodiversity. Research has been mainly focused on chicken, therefore there is a need to develop and adapt methods and knowledge for other avian species as well. The quail (*Coturnix coturnix*), notably, has emerged as a prevalent experimental model across diverse scientific domains, serving as a platform for transgenic technology and a source of high-quality eggs and meat. In the present study, single-cell suspension of reproductive cells derived from cryopreserved gonads of both sexes was injected into recipient quail embryos and gonadal chimerism was analyzed. Male donor cells were detected in both male and female recipient embryos resulting in germline chimeric gonads at a rate of 81.0%. The concentration of PGCs in the female gonad is lower than in males and that might be the reason female cells could not be found in the recipient gonads, thereby emphasizing the need for further methodological refinement. Even with this limitation the *in vitro* cryopreservation method based on the direct freezing of gonads has proven to be applicable to quail. This is a low-tech, cost-effective, and efficient method of cryopreservation which - after further research - might be an important addition to the efforts of quail genetic conservation.

***Keywords:*** *Coturnix coturnix*, quail, cryopreservation, primordial germ cells (PGCs), germline chimerism, gene banking

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