

„FIBOK 2022”

Fiatal Biotechnológusok Országos Konferenciája

Program és angol nyelvű összefoglalók



Szerkesztette

Bánfalvi Zsófia
Gócza Elen
Olasz Ferenc
Pál Magda
Posta Katalin
Várallyay Éva

Fiatal Biotechnológusok Országos Konferenciája

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**MATE GBI
Gödöllő, Szent-Györgyi A. u. 4.
2022 április 11-12.**

A KONFERENCIA SZERVEZŐI

Az MTA Agrártudományok Osztálya, **Mezőgazdasági
Biotechnológiai Tudományos Bizottságának**
társszervezésben

az MTA Biológiai Tudományok Osztálya Bioinformatikai
Osztályközi Tudományos Bizottságával,
az MTA Biológiai Tudományok Osztálya Mikrobiológiai
Osztályközi Tudományos Bizottságával,
az MTA Biológiai Tudományok Osztálya Molekuláris
Biológiai, Genetikai és Sejtbiológiai Tudományos
Bizottságával,
az MTA Kémiai Tudományok Osztálya Élelmiszer-
tudományi Tudományos Bizottságával,

INNOVATÍV MEZŐGAZDASÁGI
BIOTECHNOLÓGIÁÉRT EGYESÜLET

MAGYAR BIOINFORMATIKAI TÁRSASÁGGAL

SZERVEZŐBIZOTTSÁG

Szervező Bizottság elnöke:

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Szervező Bizottság alelnöke:

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Szervező Bizottság titkára:

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Szervező Bizottság tagjai:

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KISS Erzsébet (MATE, GBI, Gödöllő)

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Bizottság alelnöke: GÓCZA Elen (MATE, GBI Gödöllő)

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ORBÁN László (MATE, AKI, Gödöllő, Keszthely)

SOLTI László (ÁTE, Szülészeti Tanszék, Budapest)

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NAGY Tibor (MATE GBI, Gödöllő)

PONGOR Sándor (PPKE, ITK, Budapest)

SEBESTYÉN Endre (SE, ÁOK, Budapest)

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KARAFFA Levente (DE, TTK, Debrecen)

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VÁGVÖLGYI Csaba (SZTE, TTIK, Szeged)

Növény és élelmiszer biotechnológia

BÁNFALVI Zsófia (MATE, GBI, Gödöllő)

DOBRÁNSZKI Judit (DE, AKIT, Nyíregyháza)

DUDITS Dénes (ELKH, SZBK, Szeged)

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VERES Anikó (MATE, GBI, Gödöllő)

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BOROS Imre Miklós (SZTE, TTIK, Szeged)
DELI Mária (ELKH, SZBK, Szeged)
VÉRTESSY Beáta (BME, ÁBÉT, Budapest)

Fiatal Biotechnológusok Országos Konferenciája 2022

2022. április 11.

Regisztráció (8:30-9:45)

9:45 Megnyitó

Posta Katalin MATE, GBI, rektorhelyettes, intézetigazgató

Bedő Zoltán MATE Alapítvány, kuratóriumi tag

Növénybiotechnológia I. (szekcióvezető: Pál Magda, ATK)

10:00 **Dudits Dénes, Szegedi Biológiai Kutatóközpont**

A wood energy system with expanding role in mitigation of negative impacts from climate changes

10:30 **Liesel Gamarra Reinoso**, Agrártudományi Kutatóközpont: Physiological and gene expression studies for the understanding of root-based inorganic carbon uptake of *Arabidopsis*

10:45 **Oláh Dóra**, Szegedi Tudományegyetem: Nitric oxide signalling in plant responses of *Arabidopsis thaliana* to limited zinc supply

11:00 **Iman Mirmazloun**, Magyar Agrár- és Élettudományi Egyetem: Silicon treatment and downregulation of amine oxidases in *Cucumis sativus*

11:15 **Mészáros Enikő**, Szegedi Tudományegyetem: Effect of plastic contamination on early root development of rapeseed (*Brassica napus* L.)

11:30 **Hidvégi Norbert Tibor**, Debreceni Egyetem: Ultrasound effect on transcription profile of potato (*Solanum tuberosum* L.)

Ebéd szünet (11:45-12:45)

Növénybiotechnológia II. (szekcióvezető: Várallyay Éva, MATE)

12:45 **Fehér Attila, Szegedi Tudományegyetem**

In vitro regeneration of plants

13:15 **Gorcsa Teréz**, Magyar Agrár- és Élettudományi Egyetem: Promoter mutagenesis of a tomato fruit ripening related DNA demethylase by genome editing

13:30 **Tóth Liliána**, Szegedi Tudományegyetem: Production and characterization of a novel antifungal defensin from *Solanum lycopersicum* L.

13:45 **Karsai-Rektenwald Flóra**, Magyar Agrár- és Élettudományi Egyetem: Regulation and function of the GIGANTEA gene StGI.04 in potato

14:00 **Francis Wanjohi Kiemo**, Magyar Agrár- és Élettudományi Egyetem: Detection and elimination of viruses infecting sweet potatoes in Hungary

14:15 **Ali Mohammad**, Magyar Agrár- és Élettudományi Egyetem: A traceless CRISPR/Cas9 approach for precision mutagenesis in bread wheat (*Triticum aestivum*) via distant hybridization

Kávészünet (14:30-14:50)

Orvosi biokémia (szekcióvezető: Balogh Péter, PTE)

14:50 **Vértessy G. Beáta, Budapesti Műszaki és Gazdaságtudományi Egyetem:**

Genome integrity in the light on unorthodox bases in the DNA

15:20 **Antal Emőke**, Pécsi Tudományegyetem: Examination of enzymes applicable in the construction of biosynthetic pathways

15:35 **Dán Kinga**, Szegedi Tudományegyetem: Protein targets of *Neosartorya fischeri* antifungal protein 2 for new therapeutic approaches

15:50 **Kiss Benjamin**, TargetEx Kft.: Development of a new heat-stable DNA polymerase with site directed mutagenesis

16:05 **Sólyom Ildikó**, Természettudományi Kutatóközpont: Restoring the function of oncogenic proteins by improving their interaction

16:20 **Szinger Dávid**, Pécsi Tudományegyetem: CD180 stimulation affects BAFF and PI3K signalling of B cells in systemic sclerosis

16:35 **Telek András**, Budapesti Műszaki és Gazdaságtudományi Egyetem: Biocatalytic synthesis of opine-type chiral amines

Kávészünet (16:40-17:00)

Poszter előadások I. (szekcióvezető: Gócza Elen, MATE) 17:00-18:00

Alex Horánszky, BioTalentum Kft.: The effects of BPA exposure on the neural differentiation of hiPSCs

Bordás Lilla, Állatorvostudományi Egyetem: In vitro culture and vitrification of preantral follicles in different species

Dobó Diána, Magyar Agrár- és Élettudományi Egyetem: Optimalization of tetranucleotide microsatellite markers to european brown hare (*Lepus europaeus*)

Hegedűs Bettina, Magyar Agrár- és Élettudományi Egyetem: Selection of pure Pannonian (*Apis mellifera carnica pannonica*) bee colonies by molecular biology methods

Incze Dániel János, Budapesti Műszaki és Gazdaságtudományi Egyetem: Mycotoxin inactivating enzymes as feed additives

Lakatos István, Európai Ökociklus Társaság: Mycotoxin exposure during pregnancy by fallow deer

Malam Abulbashar Mujitaba, Debreceni Egyetem: Developmental Kinetics of in vitro produced sheep embryos

Mohd Adnan, Magyar Agrár- és Élettudományi Egyetem: Combined effect of cell number and toxin presence to the proliferation of Avian Primordial Germ cells

Tokodyné Szabadi Nikolett, Magyar Agrár- és Élettudományi Egyetem: Demonstration of the effect of heat treatment in the progeny of treated domestic chickens by determining the miRNA expression level of PGCs from the progeny

Urbán Martin, Magyar Agrár- és Élettudományi Egyetem: Fibroblast based genebanking in pig

Szabó-Sárvári Loretta Csilla, Széchenyi István Egyetem: Differential expression of DRD1 in experimental laying hen populations

Poszter előadások II. (szekcióvezető: Bánfalvi Zsófia, MATE) 18:00-19:00

Agyemang Evans Duah, Magyar Agrár- és Élettudományi Egyetem: Possible viral reservoir role of investigated endemic weeds on crop fields.

Fákó Vivien, Magyar Agrár- és Élettudományi Egyetem: Molecular and nutraceutical analysis of extreme purple (*Capsicum chinense* x *Capsicum annuum*) pepper hybrids

Gondor Orsolya Kinga, Agrártudományi Kutatóközpont: Comparison of different salicylic acid treatments and forms under stress conditions using large-scale analysis

Jahan Almash, Magyar Agrár- és Élettudományi Egyetem: Molecular variability of apple hammerhead viroid found apple varieties analysed and validated by hts technique

Jaksa-Czotter Nikoletta, Magyar Agrár- és Élettudományi Egyetem: Does growing fruit trees isolator house affect the detectability of their virus infection?

Nagyné Dr Galbács Zsuzsanna, Magyar Agrár- és Élettudományi Egyetem: Small RNA profiling of *Aster yellows* infected *Catharantus roseus* and Apple Proliferation Phytoplasma infected apple plants

Szabó Luca Krisztina, Magyar Agrár- és Élettudományi Egyetem: The effect of in vitro chemotherapy and heat treatment used for virus elimination from stone fruit species

Feigl Gábor, Szegedi Tudományegyetem: Counteracting effect of silicon nanoparticle priming on subsequent CuO nanoparticle-induced early stress response of monocotyledonous crops

Nehr-Majoros Andrea Kinga, Pécsi Tudományegyetem: Cyclodextrin derivatives have different effects on the proliferation and viability of CHO cells

Fogadás (19:00-22:00)

2022. április 12.

Poszter szekció (9:00-10:00)

Mikrobiológia szekció (szekcióvezető: Olasz Ferenc, MATE)

10:00 **Karaffa Levente**, *Debreceni Egyetem*

Bioreactor as a source of manganese (II) ion contamination during citric acid fermentation by *Aspergillus niger*

10:30 **Szám Dorottya**, Magyar Agrár- és Élettudományi Egyetem: Network theories of *Botrytis cinerea* extracellular secreted proteins

10:45 **Balázs Dóra Krisztina**, Magyar Agrár- és Élettudományi Egyetem: Comparison of the structure-activity relationships (SAR-s) of peptaibols from two *Trichoderma reesei* strains

11:00 **Szentpéteri Viktor**, Magyar Agrár- és Élettudományi Egyetem: Arbuscular mycorrhizal inoculation increases tomato tolerance to water deficit and heat stress

11:15 **Bartos Hunor**, Pécsi Tudományegyetem: Apple pomace as alternative substrate for succinic acid production with *Basfia succiniciproducens*

11:30 **Berek-Nagy Péter János**, Eötvös Loránd Tudományegyetem: Secondary metabolites from the dark septate endophytic fungal genus *Darksidea*

Ebéd szünet (11:45-12:45)

Bioinformatika szekció (szekcióvezető: Barta Endre, MATE)

12:45 **Pongor Sándor**, *Pázmány Péter Katolikus Egyetem*

Biotechnology and bioinformatics in the era of big data and artificial intelligence

13:15 **Fekete Zsófia**, Magyar Agrár- és Élettudományi Egyetem: Identification and characterization of circRNA-s in rabbit (*Oryctolagus cuniculus*) through bioinformatic methods

13:30 **Harmath Andrea**, Debreceni Egyetem: Competitive in-host assay to screen pathogenicity of the *Saccharomyces 'boulardii'* probiotic yeast

13:45 **Lados Botond Boldizsár**, Soproni Egyetem: Population genetic study of turkey oak (*Quercus cerris* L.) based on next generation rad-tag sequencing and SSR markers

14:00 **Maher Alnajjar**, Magyar Agrár- és Élettudományi Egyetem: Whole genome sequencing of a big family allows determining the haplotypes of the parents' chromosomes, the crossing over positions, the de novo mutations and the exact CNVs

14:15 **Takács Bertalan Vilmos**, Szegedi Biológiai Kutatóközpont: Understanding the effects of COVID-19 on the microbiome using bioinformatics and machine learning

Kávészünet (14:30-14:50)

Állatbiotechnológia szekció (szekcióvezető: Gócza Elen, MATE)

14:50 **Orbán László**, Magyar Agrár- és Élettudományi Egyetem

Zebrafish males are models of natural sex reversal in teleosts

15:20 **Bodrogi Lilla**, Magyar Agrár- és Élettudományi Egyetem: The effect of mycotoxin treatment on in vitro cultured preimplantation stage rabbit embryo

15:35 **Ecker András**, Magyar Agrár- és Élettudományi Egyetem: Characterisation and preservation of guinea fowl primordial germ cells

15:50 **Federica Lamberto**, BioTalentum Kft.: Evaluation of the effects of Bisphenol A levels on cardiomyocytes differentiation of human iPSCs

16:05 **Ninausz Nóra**, Magyar Agrár- és Élettudományi Egyetem: Genetic monitoring of grey wolves (*Canis lupus*) in Hungary

16:20 **Salamon Pál**, Pécsi Tudományegyetem: Investigation of SARS-CoV-2-induced PANoptosis

Eredmény hirdetés (16:35-17:00)

TÁMOGATÓINK

Auroscience Kft.
 Biocenter Kft.
 BioMarker Kft.
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 BIOMI Kft.
 Bio-Science Kft.
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KEY SUPPORT



SUPPORT



ASSOCIATIONS



Fiatalki Biotechnológusok Országos Konferenciája 2022

POSZTER SZEKCIÓ

2022. április 12.

Növénybiotechnológia

Bedő Janka

Magyar Agrár- és Élettudományi Egyetem

Discrimination of 'magyar kajszi' (*Prunus armeniaca* L.) clones and 'veecot' genotype cultivated in Hungary via molecular genetic methods **PN-1**

Bozóki Boglárka

Magyar Agrár- és Élettudományi Egyetem

A study of GM soybeans and coexistence in Eastern Europe **PN-2**

Carla Mota Leal

Magyar Agrár- és Élettudományi Egyetem

Insights into temporal succession of grapevine leaf pathogenic fungi under organic and conventional management **PN-3**

Desiderio Francesco

Magyar Agrár- és Élettudományi Egyetem

New putative viruses in Hungarian sour cherry genebank collection **PN-4**

Fabio Miloro

Magyar Agrár- és Élettudományi Egyetem

Identification of barley AGO4 genes and complementation assay in Arabidopsis **PN-5**

Hajnal Ádám Barnabás

Szegedi Tudományegyetem

Using mCherry marker to follow the *A. rhizogenes*-mediated transformation process in tomato root **PN-6**

Imrefi Ildikó

Eötvös Loránd Tudományegyetem

Transformation of four *Periconia macrospinos* strains to understand ecological functions of root endophytic fungi **PN-7**

Khin Nyein Chan

Magyar Agrár- és Élettudományi Egyetem

SSR based analysis on Temperate Bamboo, (*Phyllostachys* spp.) collected in Hungary **PN-8**

Kissné Bába Erzsébet

Magyar Agrár- és Élettudományi Egyetem

Nitrate stress induces ascorbate peroxidase enzyme activity and *lox* genes expression in cucumber **PN-9**

Kovács Zsófia

Magyar Agrár- és Élettudományi Egyetem

Genetic background of lilac colouration in pepper fruits (*Capsicum annuum* L.) **PN-10**

Margot Ottó

Eszterházy Károly Egyetem

Metatranscriptomic analyses reveals the role of fungal species associated with botrytis cinerea in the noble rotting process **PN-11**

Pápai Bánk

Magyar Agrár- és Élettudományi Egyetem

Gravitropic and phototropic response of procumbent (pcx) and tortuosa (tti) mutant peppers **PN-12**

Péter Dániel

Magyar Agrár- és Élettudományi Egyetem

Microsatellite marker adaptation for *Heterobranchus longifilis* **PN-13**

Turcsán Mihály

Magyar Agrár- és Élettudományi Egyetem

Virome status of different grapevine cultivars and production of healthy plants by chemotherapy **PN-14**

Állatbiotechnológia**Balázs Réka**

Magyar Agrár- és Élettudományi Egyetem

Molecular genetic investigation of Pannonian bee populations in Hungary to assess their diversity **PÁ-1**

Gál Zoltán

Magyar Agrár- és Élettudományi Egyetem

The generation of motilin knock out rabbit line as a model a gastrointestinal disease **PÁ-2**

Javkhlov Ariuntungalag

Magyar Agrár- és Élettudományi Egyetem

Oldie but goldie – the use of feulgen staining in the revival of sperm morphology **PÁ-3**

Nagy Zsófia

Magyar Agrár- és Élettudományi Egyetem

Investigation of the expression of motilin and dual oxidases in bovine tissues **PÁ-4**

Pintér Tímea

Magyar Agrár- és Élettudományi Egyetem

The effect of cholesterol-rich diet on the antioxidant system of NOX5-deficient rabbit **PÁ-5**

Tokár Alexandra

Magyar Agrár- és Élettudományi Egyetem

In vitro fertilization with ram epididymal sperm **PÁ-6**

Tóth Arnold

Magyar Agrár- és Élettudományi Egyetem

Examination of the gene expression under the embryonic gonadal development in chicken
PÁ-7

Bioinformatika**Biró Bálint**

Magyar Agrár- és Élettudományi Egyetem

Investigation of mitochondrial pseudogenes in the nuclear genomes of different mice strains
PB-1

Kalcsevszki Regina

Pázmány Péter Katolikus Egyetem

Prediction of the clinical outcome of COVID from viral mutations using machine learning techniques **PB-2**

Mikrobiológia**Adácsi Cintia**

Debreceni Egyetem

Resistance of yeasts against Fusarium mycotoxins **PM-1**

Csíkos Sándor

Eötvös Loránd Tudományegyetem

Secondary metabolites of *Ampelomyces* isolates **PM-2**

Glodia Kgobe

Magyar Agrár- és Élettudományi Egyetem

Evaluating the influence of rootstock on grapevine scion microbiome composition and diversity **PM-3**

Kondak Selahattin

Szegedi Tudományegyetem

Uptake and effect of nickel oxide nanoparticles on biomass production and reactive molecule levels in nickel-hyperaccumulator *Odontarrhena lesbiaca* ecotypes **PM-4**

ABSTRACTS

Plantbiotechnology I

A WOOD ENERGY SYSTEM WITH EXPANDING ROLE IN MITIGATION OF NEGATIVE IMPACTS FROM CLIMATE CHANGES

Dudits Dénes

ELKH Biological Research Centre, Institute of Plant Physiology, Szeged, Hungary

Key words: short rotation woody energy crops, tetraploidy, heterosis, biomass, biomethane

Selected wood species, such as willows (*Salix* spp.), can be cultivated as short-rotation coppice. These plantations are characterized by a very short, 2- to 3-year rotation, and the most productive varieties can produce up to 15 tons of oven-dried wood per hectare. The short-rotation cultivation of energy willow plants and their fermentation use for biomethane production require specific breeding and biotechnology approaches. As a first breeding step, we treated axillary buds of willow shoots with colchicine that resulted in a set of autotetraploid *S. viminalis* var. *Energó* genotypes (polyploid *Energó* [PP-E]; $2n = 4x = 76$). The duplicated genome size enlarged leaf size, and increased chlorophyll and carotenoid contents. Improved photosynthetic functions in tetraploids were also shown by greater net photosynthetic CO₂ uptake and more efficient electron transport rates of photosystems I and II. These traits can have positive impact on biomass yield or overall reduction of negative consequences of CO₂ accumulation. This autotetraploidization increased the biomass of the root system relative to diploids (details see Dudits et al. 2016). Soil salinization is one of the most damaging environmental factors limiting plant productivity. *Climate change drives soil salinization* in several ways. Therefore, we have tested the tetraploid willow genotypes for salt resistance (Cseri et al. 2020). After nine weeks of salt stress, increase in salt adaptation capability of tetraploid plants was indicated by larger biomass, leaf and root weights under salinity (1.5g NaCl kg⁻¹ soil) relative to diploids. As a further step, we integrated heterosis with polyploidy by producing triploid hybrid genotypes. We have crossed two female diploid Swedish cultivars (Inger, Tordis) with two male autotetraploid willow (*Salix viminalis*) variants and triploid hybrids (THs) developed larger leaves than their parental cultivars, and the mid-parent heterosis (MPH%) for their CO₂ assimilation rate varied between 0.84 and 25.30% (Dudits et al. 2021). Energy production by wood burning generates smoke that is made up of a complex mixture of gases and harmful particulate matter (PM). As an alternative way to convert biomass into renewable energy is the anaerobic digestion using lignocellulosic material as the substrate is a cost-effective strategy for biomethane production. In our studies on wood raw materials from the triploid willow hybrids provided positive MPH% in biomethane yield (6.38 and 27.87%) and showed negative MPH % in their acid detergent lignin contents (from – 8.01 to –14.36%). Optimization of this technology is in progress at laboratory scale in the Department of Biotechnology, University of Szeged, Szeged (Kornél Kovács, Zoltán Bagi) and, in industrial scale in the Agrár-Béta Kft. Dombóvár (István Molnár).

References:

- Cseri A., Borbély P., Poór P., Fehér A., Sass L., Jancsó M., et al. (2020). Increased adaptation of an energy willow cultivar to soil salinity by duplication of its genome size. *Biomass Bioenergy* 140:105655. 10.1016/j.biombioe.2020.105655.
- Dudits D., Török K., Cseri A., Paul K., Nagy A. V., Nagy B., et al. (2016). Response of organ structure and physiology to autotetraploidization in early development of energy willow *Salix viminalis*. *Plant Physiol.* 17 1504–1523. 10.1104/pp.15.01679.
- Dudits D, Cseri A, Török K, Sass L, Zombori Z, Ferenc G, Poór P, Borbély P, Czékus Z, Vankova R, Dobrev P, Szántó J, Bagi Z and Kovács KL (2022) Triploid Hybrid Vigor in Above-Ground Growth and Methane Fermentation Efficiency of Energy Willow. *Front. Plant Sci.* 13:770284. doi: 10.3389/fpls.2022.770284

ENI-1

PHYSIOLOGICAL AND GENE EXPRESION STUDIES FOR THE UNDERSTANDING OF ROOT-BASED INORGANIC CARBON UPTAKE OF ARABIDOPSIS

Gamarra Reinoso, L; Majláth, I; Hamow-Tajti, J; Sági, L; Éva, C

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Research on developing drought tolerance and increasing the photosynthesis performance as an alternative to improve the crop yield is emerging. The uptake of carbon dioxide through the leaf stomata is the most common way to acquire inorganic carbon in land plants, however the root-based acquisition has been also demonstrated. The aim of our project is to better understand and possibly to stimulate the root-based inorganic carbon uptake in *Arabidopsis thaliana* (ecotype: Columbia) to boost and maintain its photosynthesis during drought stress, which decreases stomatal conductance. Physiological parameters (hydroponic cultures in mutant and non-mutant plants, growth parameters, IRGA and fluorescence induction) as well as molecular biology studies (qPCR and over-expression of candidate genes) are used. In the hydroponic cultures for thale cress, two different pH were used (5.6 and 7.2) which would determine the form of inorganic carbon (CO₂ vs. bicarbonate) as well different bicarbonate concentrations (1-4 mM) were tested. Two media were evaluated, the ¼ strength Hoagland, with high pH of 7.2 supplemented with 2 mM and 3 mM NaHCO₃ promoted Arabidopsis growth and increased some of the photosynthetic parameters (fresh weight, total chlorophyll content, CO₂-fixation rate, intracellular CO₂-concentration) whereas with lower, pH 5.6 the growth decreased in all the bicarbonate concentrations. The other medium, the ¼ strength MS showed opposite results, the 2 mM bicarbonate treatment at pH 5.6 was the best combination for growth promotion. This treatment even helped the plants to alleviate the negative effects of osmotic stress caused by 6% PEG. The different composition of the media could interfere how the bicarbonate is taken up. Different KO mutants for PEP-carboxylase (PEPC3), beta carbonic anhydrase (BCA4) and carbon-permeable aquaporin channel genes (*atpip1;2*, *atpip1;3*, *atpip2;6*) were also evaluated for growth promotion. All the studied mutants showed growth promotion like the Columbia at high pH of 7.2 and 3 mM bicarbonate treatment. This suggests that the evaluated genes under the evaluated parameters are not influencing the inorganic carbon uptake. The test of the aquaporin mutants at low pH of 5.6 and 2 mM bicarbonate concentration in ¼ strength MS medium is in progress.

In the studies of gene expression for the identification of bicarbonate pumps we found that *BOR1* and the *SULTR2;1* slightly increases in high pH bicarbonate treated roots, whereas the expression of nitrate transporter *SLAH3* greatly increased. The encoded proteins might also take up the bicarbonate ion as a secondary function, but we still must prove it. According to our hypothesis we consider that the bicarbonate pumps and the carbonic anhydrases have an important role to the root-based carbon uptake mechanism in Arabidopsis at high pH 7.2, but at lower pH 5.6 the CO₂-permeable aquaporin channels have the main role in the acquisition of inorganic carbon. In order to validate our findings, currently we are working on the vector-cloning and over-expression of full-length coding sequences of bicarbonate transporter candidate genes and aquaporin channels in transgenic Arabidopsis and bicarbonate transporter-mutant green alga *Chlamydomonas reinhardtii*.

Keywords: *Arabidopsis thaliana*, inorganic carbon, gene expression, growth promotion

Acknowledgement: The project is supported by grants NKFI FK 134874 and ATK-MATE no. 0205K0036P.

ENI-2

NITRIC OXIDE SIGNALLING IN PLANT RESPONSES OF *ARABIDOPSIS THALIANA* TO LIMITED ZINC SUPPLY

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As a microelement, zinc (Zn) is an essential regulator of optimal plant growth and development, similar to nitric oxide (NO) signal molecule. Zn deficiency has negative effect on biomass production and crops quality. In case of agricultural soils suboptimal Zn supply is a worldwide problem. Our aim was to understand the details NO signalling in *Arabidopsis thaliana* wild type (WT, Col-0) and S-nitrosogluthathione reductase (GSNOR) overproducer 35S::FLAG-GSNOR1 line grown with limited Zn supply. Plants were grown in control (15 μ M ZnSO₄), mildly (1.5 μ M ZnSO₄) and severely (0 μ M ZnSO₄) Zn-deficient medium for 7 days. During the treatment period, both *Arabidopsis* lines showed significantly reduced Zn contents in their root tips compared to control, but serious Zn deficiency was not induced by the treatments. In the WT, Zn limitation induced hydrogen peroxide (H₂O₂) and NO production, reduced GSNOR protein abundance and led to the intensification of tyrosine nitration. In contrast, in 35S::FLAG-GSNOR1 plants showing increased GSNOR protein amount and activity, reduced Zn levels did not modify NO levels, decreased H₂O₂ content, slightly decreased GSNOR protein amount and triggered a diminution in tyrosine nitration. Moreover, Zn limitation resulted in primary root shortening and decreased number of lateral roots in the WT, which was accompanied by reduced meristem cell viability and slightly reduced signals of *Cyrb1::GFP* (indicator of cell division), *DR5::GFP* (indicator of auxin), *pTCS::GFP* (indicator of cytokinin) and increased *ACS8::GUS* (indicator of ethylene) signal. The results show that Zn limitation disturbs H₂O₂ and NO homeostasis and induces nitrosative protein modification, while GSNOR overproduction prevents NO level increase and reduces H₂O₂ levels without intensifying protein nitration. As for the root growth responses to limited Zn supply, both the reduction of cell viability and the diminution of divisibility in the root meristem may contribute to primary root shortening. Additionally, the slight reduction of auxin and cytokinin levels and the increased ethylene level may be important factors in regulating root growth in case of Zn limitation. These results will be supplemented by gene expression and ICP analyses.

Keywords: *Arabidopsis thaliana*, Nitric oxide, S-nitrosogluthathione reductase, Zinc deficiency

Acknowledgement: This work was supported by National Research, Development and Innovation Office (K135303). The authors also acknowledge the provided microscopy support of the Cellular Imaging Laboratory (Biological Research Centre, Eötvös Loránd Research Network) and we thank the kind help of Éva Kapásné Török during the experimental work.

SILICON TREATMENT AND DOWNREGULATION OF AMINE OXIDASES IN *CUCUMIS SATIVUS*

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Silicon (Si) is a well-known element for its beneficial effects on several plants' growth and stress tolerance if supplied in bioavailable form. It may mitigate the effect of various stresses and may also contribute to better plant growth and development in general. Effects of Si are often linked to improvements in oxidative stress management, in particular to induction of plant's antioxidant defence mechanisms. The effects of silicon application on an open field type cucumber growth and related physiological traits were investigated. Plants were grown in a controlled environment and were treated with Hoagland solutions with and without Si supplementation.

Different physiological, genetic and phytochemical parameters (e.g. H₂O₂ and lipid peroxide content; antioxidant and redox related enzyme activities and their counterpart genes expression) associated with plant performance and fitness were investigated in the present study.

Silicon supply significantly decreased H₂O₂ content and mitigated lipid peroxidation in the leaves of treated plants. The activity of polyamine oxidase (PAO) and diamine oxidase (DAO) enzymes significantly decreased in Si-treated plants. RT-PCR analysis of selected PAO genes also revealed a decline in expression level. Several lipoxygenase (LOX) genes were similarly downregulated in Si-treated plants. The intensity of bioluminescence; an indirect indication of lipid peroxidation was significantly different between treated and non-treated samples. The silicate supplementation suppressed bio-photon emission from cucumber leaves as recorded by a Nightshade imaging system. The obtained results suggest silicon-mediated mitigation of amine oxidase activities limiting reactive oxygen species formation and revealing molecular mechanisms of silicon in maintaining redox balance in cucumber plants.

Keywords: *polyamine oxidase, diamine oxidase, reactive oxygen species, silicate, Bio-photon emission*

ENI-4

EFFECT OF PLASTIC CONTAMINATION ON EARLY ROOT DEVELOPMENT OF RAPESEED (*BRASSICA NAPUS* L.)

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Conventional abiotic stressors are considered to be key factors that negatively affect plant development and productivity. Plastic contamination represents a new emerging environmental issue, which is likely to be the longest-lasting and most limitless pollution of the Earth's biosphere. In 2019, the global use of traditional plastic cover films (e.g. polyethylene (PE) or polypropylene (PP)) reached 7.4 million tons in agriculture alone. If they are not properly removed from the soil, they break down into macro- (> 2.5cm) and meso-plastics (2.5 cm-5 mm) due to agricultural activities and natural processes. Further fragmentation results in micro- (1µm-5 mm) and nano-plastics (1-100 nm), which pose a serious threat to the natural ecosystems and become one of the major sources of agricultural plastic contamination. The use of biodegradable plastics (from various polymers with various composition) promises a potential alternative as they are able to degrade *in situ*. Their effects on the cultivated plants and soil microorganisms, however, are only superficially known.

Personal protective equipment made of plastic plays a crucial role in the human health protection during the COVID-19 pandemic. Disposable surgical masks, released into the environment due to improper waste management or careless littering, can be transformed into micro- and nano-plastics much faster than other plastic wastes owing to their fibrous material, thus challenging the life processes of both animals and plants much sooner.

Early root development of rapeseed (*Brassica napus* L.) subjected to different types of PE, PP, polylactic acid (PLA) and PP surgical mask fragments in a range of size (2.5, 2, 1, 0.5, and <0.5 cm) and concentration (0.5 and 1%) was studied in a controlled, *in vitro* semi-hydroponic system for 5 days. In the case of PP surgical mask fragments, seedlings were also cultivated in rhizotron systems, filled with controlled nutrient medium (blocking substrate) for 14 days in order to model a near-natural setting.

In the *in vitro* systems, biodegradable PLA inhibited the root growth under 2 cm fragment size, while PE and PP only had a negative effect typically at smaller fragment sizes, and in some cases even growth induction was observed. Surgical mask fragments significantly repressed the root growth under 2 cm fragment size in 1% concentration. In the microplastic range, the effects on the roots can be classified into distinct groups based on their growth responses to different amounts of plastic treatment.

In the rhizotron study, where PP surgical mask fragments were mixed into the soil, the growth response of rapeseed's root transformed over the course of the 14-day observation period. Although the root growth was not necessarily inhibited, in many cases symptoms of stress-induced morphogenic response were detected: root architecture changed significantly in order to diminish exposure to stressors.

Keywords: *plastic, biodegradable plastic, phytotoxicity, root development*

Acknowledgement: *This work was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (Grant no. BO/00181/21/4). F. G. was supported by the New National Excellence Program of the Ministry of Human Capacities (UNKP-21-5-SZTE-567).*

ENI-5

ULTRASOUND EFFECT ON TRANSCRIPTION PROFILE OF POTATO (*SOLANUM TUBEROSUM* L.)

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Ultrasound transmitted through the air (AB-US) and liquid (PE-US) can modify plant growth and development. Sound vibration, including sound and ultrasound, is one of the physical stimuli originating from the surrounding environment. To better understand, the molecular genetic mechanism underlying the physiological response of potato to the US, single-node segments of four-week-old *in vitro* plantlets were subjected to AB-US and PE-US at 35 kHz, 70 W for 20 min. The effect of these stressors (AB-US and PE-US) on the potato transcriptome was determined at 0 h, 24 h, 48 h, 1 week and 4 weeks. The RNA-seq datasets and significantly differentially expressed genes (DEGs) were analysed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) identifiers. The expression intensity of 40,430 genes was studied.

At AB-US treatment, several hundred DEGs associated with biosynthesis, carbohydrate metabolism and catabolism, cellular protein modification, and stress response were identified, and they were expressed mainly in the plasma membrane, nucleus, and extracellular region.

At PE-US treatment, we observed that 24% of up-regulated biological processes were an immediate response to the stress after the application. When we compared the five-time intervals, it was found that 138, 72, 18, 5, and 59 DEGs were significantly up-regulated, while 6, 82, 96, 172, and 107 DEGs were down-regulated, respectively. DEGs coding for universal stress protein, 21 transcriptional factors, glutathione S-transferase, and 17 heat shock proteins, mainly Hsp70, Hsp20, and Hsp90 proteins were detected.

Mechanostimulation-induced genes of plants play a role in the response to the US. Five “touch” genes (*TCH1-TCH5*) were discovered in *Arabidopsis thaliana* (L.) Heynh. ecotypes Col-0 in response to touch, wind, and rain. The *TCH4* encodes xyloglucan endotransglucosylase/hydrolase (*XTH*) 22. At our analyses of the effects of AB-US and PE-US, we assessed DEGs of *S. tuberosum* L. genes whether there might be similar or homologous sequences to *XTH* genes of *A. thaliana* (*AtXTH*) or *S. lycopersicum* (*SlXTH*), and thus putative *XTH* genes in potato, as *StXTH* genes, were identified.

AB-US and PE-US revealed several stress-related DEGs related to abiotic stress. Up- and down-regulation of DEGs related to metabolic processes and antioxidant system enzymes confirm that PE-US is a more acute abiotic stress than AB-US.

Keywords: abiotic stress, ultrasonication, mechanostimulus, xyloglucan endotransglucosylase/hydrolase

Acknowledgement: Project no. **TKP2021-EGA-20 (Biotechnology)** has been implemented with support from the National Research, Development and Innovation Fund of Hungary, financed under the **TKP2021-EGA-20** funding scheme.

Plantbiotechnology II

IN VITRO REGENERATION OF PLANTS

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Keywords: *plant cell and tissue culture, callus, organ regeneration, somatic embryogenesis, totipotency*

Plant biotechnology is based on the incredible regeneration capacity of plants: even a single somatic plant cell can regenerate the entire organism. This allows the vegetative propagation of individual genotypes in an efficient way forming the basis of many plant biotechnology applications. Rare genetic events (e.g. mutation, gene insertion) can be realised at the cell population level where millions of cells can be handled in parallel and then the cells having the desired genotype can be selected and regenerated to whole plants. Although plant cell and tissue culture has already a long history and is widely used for biotechnology purposes, our knowledge about the way of plant regeneration is still scarce. Among others, we still do not know how a differentiated somatic cell loses its differentiated functions and regains its ability to divide. Moreover, there are numerous plant species or cultivars, which are recalcitrant for in vitro regeneration. The recalcitrant species include many important crops such as cereals where only embryonic or juvenile tissues of certain genotypes are amenable for in vitro culture and plant regeneration. Understanding what causes the differential response of somatic plant cells to in vitro culture conditions would allow us to widen the available germplasm for plant biotechnology applications besides gaining insights into the flexible cell differentiation strategy of plants.

Plant regeneration may follow various pathways of organogenesis or embryogenesis with or without intermediate callus formation. Furthermore, the initial conditions that can trigger regeneration in various species/explants are strikingly different. Recent discoveries using up-to-date research methodologies have given unprecedented insight into the cellular and molecular processes underlying in vitro plant regeneration especially in the case of the model plant *Arabidopsis thaliana*. Nevertheless, it is becoming more and more obvious that generalizations should be avoided. Moreover, the meaning of plant cell and tissue culture processes has to be revisited in light of the current research achievements. Some of the basic terms such as “callus”, “dedifferentiation”, “totipotency”, “and somatic embryogenesis” might mean different things today than several decades ago when they were first used and defined.

In this lecture, I attempt to review the meaning and use of the above traditional terms in light of current results of modern plant biology to disconfirm some plant cell and tissue culture dogmas and to overcome potential “terminology-raised” barriers in plant regeneration-based biotechnologies.

ENII-1

PROMOTER MUTAGENESIS OF A TOMATO FRUIT RIPENING RELATED DNA DEMETHYLASE BY GENOME EDITING

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Tomato is one of the most popular vegetable worldwide and consumed in large quantities, which makes this plant very important both economically and agronomically. Genomic DNA methylation is an important epigenetic mechanism that influences gene expression without modifying the DNA sequence. In plants, DNA methylation happens on a cytosine nucleobase and the process is directed by DNA methyltransferases. This modification on gene promoters inhibits gene expression. The active removal of methylation marks has major importance during plant development and plant cell reprogramming. Genomic DNA methylation can be actively removed by Demeter-like DNA demethylases (DMLs), of which four can be found in tomato (SIDML1, SIDML2, SIDML3, SIDML4). Tomato fruits undergo a dramatic loss in DNA methylation during ripening and SIDML2 was found to be a core regulator in the start of this process. SIDML2 has several ripening related transcription factor targets like the Ripening Inhibitor (RIN) MADS-box transcription factor and the Squamosa Promoter Binding-Like Protein (SPL). The aim of our project was to create a series of mutations in the *SIDML2* promoter to identify and understand *cis*-regulatory elements that regulate the expression of this gene. By modifying the promoter region, the protein structure itself is not affected, and this way we can expect subtle phenotypic changes, like variations in the timing, pattern, and level of gene expression. To achieve this, we used a multiplex CRISPR/Cas9 genome editing system targeting the proximal and distal part of the *SIDML2* promoter and obtained a high number of independent mutant lines. To our surprise, we found various phenotypes which are not limited to fruit ripening. We have several interesting lines carrying large deletions either in the proximal or distal part of the *SIDML2* promoter. The fruit ripening time was similar to the wild-type in most of the lines, however, some lines showed significantly shorter ripening time while others ripened longer. Developmental defects were more obvious, however. For example, many lines produced seedless fruits more frequently than the wild-type tomato plants. Many lines have fruits with more than two locules. Three lines showed serious developmental defects: they looked like plastic plants with no trichomes (only glandular type 6), very stiff leaves, and cauliflower-like apical and axillary meristems. Most of them produced fruits, mostly seedless, but one line was so sick that it never produced any flowers. Its phenotype suggested that it had a meristem developmental defect.

Keywords: DNA demethylation, fruit ripening, CRISPR

Acknowledgment: NKFI OTKA K-129171

ENII-2

ANTIFUNGAL EFFECT OF A NOVEL DEFENSIN FROM *SOLANUM LYCOPERSICUM* L., AND ITS γ -CORE PEPTIDE DERIVATIVES

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Management of crop losses to pesticide resistant filamentous fungal strains is a recent challenge for agriculture. There is an essential need to develop new prevention and treatment strategies applying novel antifungal compounds which differ in mechanism of action from the conventional chemical pesticides. Previous studies already demonstrated that the highly stable plant defensins effectively inhibit the growth of plant pathogenic fungi; and their evolutionarily conserved γ -core regions (GXC-X_[3-9]-C) play a key role in it. It was also demonstrated that synthetic peptides spanning their γ -core regions possess the same antifungal effect as that of the full-length defensin. These features render them to be promising candidates as biopesticides. In this work we heterologously expressed a novel *Solanum lycopersicum* L. defensin in *Penicillium chrysogenum*, and chemically synthesized its peptide motifs spanning the two γ -core regions. An *in vitro* microdilution susceptibility testing method was applied to investigate their antifungal efficacy against *Botrytis cinerea* SZMC 21472, *Cladosporium herbarum* FSU 1148, *Fusarium oxysporum* f.sp. *lycopersici* CBS 123668, and *Fusarium oxysporum* SZMC 6237J. The full-length recombinant defensin reduced the growth of *Cladosporium herbarum* FSU 1148 and *Fusarium oxysporum* SZMC 6237J. A γ -core peptide derivative showed high antifungal efficacy when it was hydrophilic and highly positively charged. The fungal cell killing efficacy of the most effective peptide derivative was investigated on the conidia of the most susceptible fungal strain, *C. herbarum* FSU 1148 applying fluorescence-activated cell sorting (FACS) coupled with propidium iodide staining. FACS analysis indicated that the antifungal active peptide is able to destruct the plasma membrane integrity of fungi within 16 hours.

Taken together, our results demonstrated that plant defensins and their peptide derivatives represents a promising compounds to fight against phytopathogenic fungi.

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

Acknowledgement: Present work of L.T. is financed by the PD 134284 project of the Hungarian National Research, Development and Innovation Office - NKFIH.

ENII-3

REGULATION AND FUNCTION OF THE *GIGANTEA* GENE *StGI.04* in potato

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GIGANTEA (*GI*) is a plant-specific gene encoding a nuclear protein with diverse functions (e.g., flowering time regulation, control of circadian rhythm, stress responses, etc.) detected in many plant species. In potato (*Solanum tuberosum* L.), however, only its role in tuberisation has been demonstrated thus far. Based on the role of *GI* in the model plant *Arabidopsis* and other plant species, we hypothesised that the function of *GI* in potatoes is not restricted only to tuberisation. To test this hypothesis a search for the *A. thaliana* *GI* homologue in the *S. tuberosum* Group Phureja genome sequence has been carried out. Two *GI* genes were identified in potato, one on chromosome 4 and the other on chromosome 12 (*StGI.04* and *StGI.12*). We started our analysis with *StGI.04*. To get information on the regulation of *StGI.04* the promoter region of the commercial potato cultivar ‘*Désirée*’ was cloned and sequenced. The PlantRegMap data analysis platform predicted more than ten transcription factor families binding to the *StGI.04* promoter including those involved in flowering, circadian clock regulation and abscisic acid response and a binding site for the POTATO HOMEBOX 20, which is presumably involved in tuber initiation.

Organ-specific expression of *StGI.04* was tested using RT-qPCR. *StGI.04* mRNA was detected in each tested organ, however, in different amounts. The stress responses of *StGI.04* were tested on detached leaves of greenhouse-grown ‘*Désirée*’ plants and found that the osmotic stress, heat and cold up-regulated *StGI.04*. To get information on the function of *StGI.04* the technique of antisense repression of gene expression was applied in ‘*Désirée*’. The highest level of repression reached around 50%. Nevertheless, this level did not influence tuber formation and yield but did cause a reduction in tuber colour. ‘*Désirée*’ is a red-skinned potato whose skin colour is determined by anthocyanins. Thus, we concluded that *StGI.04* influences the anthocyanin content of tuber skins.

To explore the effects of *StGI.04* repression on the leaf transcription profile RNA-seq analysis was performed with a selected antisense line. We found that, as *AtGI* in *Arabidopsis*, *StGI.04* influences the expression of the key genes of the circadian clock, flowering, starch synthesis, and stress responses. No alterations in the mRNA levels of the key genes in anthocyanin synthesis or catabolism were detected. Thus, our result supports the earlier hypothesis that the anthocyanins are synthesized during the transport process to tubers and not in the leaves.

Keywords: *Solanum tuberosum*, *GIGANTEA*, transcription factor, transcriptome analysis

Acknowledgement: This work was supported by the National Research Development and Innovation Office, Hungary, Grant No. NN_124441. KO and JJ are sponsored by the Stipendium Hungaricum Scholarship.

Detection and elimination of viruses infecting sweet potatoes in Hungary

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Abstract

Sweet potato, the third most important root and tuber crop globally has been grown in Hungary for over three decades. Its increasing popularity could be attributed to nutritional benefits such as prevention of diabetes, cancer, obesity and blindness, considering its low glycemic index, high anthocyanins, fibre and beta-carotene contents, respectively. In the years 2019 – 2021, over 100 sweet potatoes were collected from farmers' fields, retail stores, researchers and gene bank in different parts of Hungary. They were grown in a greenhouse at the MATE Institute of Genetics and Biotechnology and symptoms were evaluated for 12 weeks. RNA and DNA were extracted from the samples to check for 15 important viruses (eleven RNA and four DNA viruses) using universal and specific primers. Five RNA viruses were detected by qPCR: *Sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato virus G* (SPVG), *Sweet potato virus C* (SPVC), *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato virus 2* (SPV2). Two DNA viruses were detected by PCR: *Sweet potato leaf curl virus* (SPLCV) and *Sweet potato pakakuy virus* (SPPV). The lack of virus-free sweet potato propagation materials contributed to the spread and persistence of these viruses in the fields. Currently, planting of pathogen tested or virus-free sweet potatoes is the only efficient control method; but there is no program for the production of virus-free sweet potatoes in Hungary. Therefore, six sweet potato genotypes were selected to undergo heat treatment for removal of viruses. Virus elimination was successfully conducted in five out of six sweet potato genotypes important for Hungarian farmers. Four genotypes labelled *T96*, *92R*, *105R* and *12R* were obtained from the National Centre for Biodiversity and Gene Conservation of Hungary while two genotypes labelled *Blk*, and *Ylw* were provided by a producer farmer. Genotype *105R* could not be cleaned of SPVG and SPVC. This study reported for the first time the occurrence of SPCSV and SPLCV in Hungary. SPCSV is a quarantine virus and has been previously reported in Spain and Portugal in the European Union. SPFMV and SPPV are the most common viruses in sweet potatoes. They are mostly symptomless. Sweet potato virus disease (SPVD), the most economically important disease of sweet potatoes caused by co-infection of SPFMV and SPCSV was also reported for the first time in Hungary. Other SPCSV co-infections such as SPCSV-SPPV and SPCSV-SPV2-SPPV complexes were also found. It would be interesting to investigate the interaction between these pathogens based on their economic importance. SPCSV forms synergies with other RNA and DNA viruses causing severe symptoms in sweet potatoes. Understanding its mechanism of action and interaction would pave way for designing methods to control it. SPPV was unique because it could neither be removed from sweet potatoes by heat treatment and meristem tip culture nor could it be transmitted by grafting to *Ipomoea setosa*. This raised serious concerns about the presence of SPPV in 'virus-free' plants supplied by sweet potato pre-basic seed producers, especially in cases where they do not check for the virus. We postulated that SPPV is a persistent virus in the meristem of sweet potatoes. It possibly has a symbiotic relationship with sweet potato due to its persistence and presence in the meristem and seeds. Although different varieties of sweet potatoes respond differently to different viral infections and co-infections, we would like to focus our research on the mechanism of co-infection of the most important virus economically (SPCSV) and either one or both of the most widespread viruses (SPPV and SPFMV). In the meantime, setting up a national or international scheme to produce virus-free sweet potatoes for farmers in Hungary and Europe would be a good start to alleviate farmers' losses and the risk to food and nutrition security.

Keywords: heat treatment, *Ipomoea batatas*, sweet potato virus

Acknowledgement: We are grateful to all the farmers, Dr Melinda Tar and the National Centre for Biodiversity and Gene Conservation of Hungary for giving us some of the plant samples used in this study. Hungary helps agency funded this study through the Scholarship for Christian Young People.

A TRACELESS CRISPR/Cas9 APPROACH FOR PRECISION MUTAGENESIS IN BREAD WHEAT (*Triticum aestivum* L.) VIA DISTANT HYBRIDIZATION.

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Abstract: CRISPR/Cas9 system is widely used to generate mutations and study their impact on crop improvement. The basic CRISPR/Cas system requires two components: a Cas nuclease, such as Cas9, Cpf1, and a programmable guide RNA (gRNA). The efficiency of genome editing is greatly affected by the mode of execution. It is most effective to incorporate the genes encoding the two major elements into the plant genome and then remove them after mutations are created. However, short foreign DNA fragments may remain in the plant genome. No matter how transgene is integrated into the plant genomes raises important legislative concerns regarding genetically modified organisms. Another concern is the delivery of genome editing machinery because established methods show strong genotype-dependency.

We devised hybridization-based editing technology (Hi-Breeder) to overcome these problems, which enables direct editing of wheat lines via a single cross with transgenic barley (*Hordeum vulgare* L.) line. In the first step, wheat is crossed with a ‘Golden Promise’ barley carrying a reporter gene (*DsRed*) and a wheat-specific (*TaMlo* gene) guide RNA-Cas9 CRISPR construct. In the next step, F1 hybrid plants carrying the CRISPR construct are selected from the hybrid population based on the expression of the reporter gene and using barley chromosome-specific PCR markers. These hybrid plants are backcrossed with the original wheat parent, resulting in wheat with diploid genome and monosomic chromosome additions of barley. In the F1BC1 generation plants that still contain the chromosome(s) carrying the CRISPR construct are simply selected based on the phenotype of the reporter gene to allow another round of genome editing in the recurrent (paternal) wheat genome. Following self-fertilization, the genome-edited diploid wheat plants without barley chromosomes can be identified in the progeny.

To justify our system all three *TaMlo* homoeologues, *TaMlo-A1*, *TaMlo-B1*, and *TaMlo-D1* of wheat were targeted. *Mildew resistance locus o (Mlo)* gene is a member of an ancient eukaryotic gene family that is conserved throughout the plant kingdom. In barley, natural and induced loss-of-function mutations of (*Mlo*) gene confers broad-spectrum resistance against all *Blumeria graminis* f.sp. *hordei* (*Bgh*) isolates. We identified a large number of *TaMlo* mutant hybrids based on PCR/restriction enzyme assay in our preliminary experiment. In a further experiment, only 4 mutated hybrid plants were generated in F1 generation out of 11 plants carrying the Cas9 though the efficiency is very less as compared to our preliminary experiment. These plants were backcrossed with wild-type wheat. Among the F1BC1 generation, the mutation was confirmed by PCR/restriction enzyme assay in 1 plant out of 3 plants carrying the Cas9 gene. In the next-generation analysis, we want to select plants lacking all the barley chromosomes with the desired mutation(s) in wheat.

Keywords: CRISPR/Cas9, Traceless, Distant hybridization, Precision mutagenesis

Acknowledgment: This work is supported by [grant number- NKFI-FK 134264 and ELKH (ATK-MATE) 0205K0036P].

Medical biochemistry

GENOME INTEGRITY IN THE LIGHT ON UNORTHODOX BASES IN THE DNA

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BME VBK ABÉT and ELKH TTK Institute of Enzymology

Faithful preservation and replication of genomic information is essential to maintain cells and organisms. Multiple pathways cooperate to maintain genomic integrity, and the fine-tuned regulation of DNA repair pathways and nucleotide metabolism are interlinked at several points to allow smooth and efficient maintenance. In the recent years, however, independent pieces of experimental evidence became accumulating that show a signaling capacity of erroneous bases in the DNA not just in damage response, but in other physiological processes as well.

The presentation will address the role of uracil in DNA in several such processes and will also review the current capacities for a true and chemically correct understanding of genomic DNA.

EO-1

EXAMINATION OF A MALONYL-COA-REDUCTASE ENZYME FOR METABOLIC ENGINEERING PURPOSES

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Determination of the three-dimensional structure of proteins is a very important area of molecular biology and structural biology [1]. This can ease such important proteomics tasks like protein engineering or structure-based drug design. The Protein Data Bank (PDB)[2], which contains 3D structures determined by experimental methods, is the primary basis for structural-based proteomic studies. The main steps of homology protein modeling are the selection of the template sequences, local alignment, main chain-, loop- and side chain prediction, structure optimization and evaluation [3]. When an atomic 3D structure of a protein is available, it is possible to examine features such as shape, surface properties, and presence of cavities. In addition to examining the properties of the protein itself, information on the interaction of the protein with other molecules (such as various small ligands or other biological macromolecules, proteins or nucleic acids) is also important [4]. Molecular docking is one of the molecular modeling tools a method that predicts a preferred arrangement of a molecule (usually a ligand) in a stable complex bound to another (usually a biological macromolecule) [5]. The docking program used in our work is based on the spatial matching technique, and it treats the target protein and the docking molecule as complementary interfaces.

The homology-based models were made by using the Modeller program from the online platform Andrej Sali Laboratory, the models contain the two active sites of the malonyl-CoA-reductase (Mcr) enzyme namely the McrN, McrC domains. Molecular docking was performed with AutoDock[6] using a rigid model for the macromolecule. AutoDock4 uses a semiempirical free energy force field to predict binding free energies of small molecules to macromolecular targets. Since the active site was not previously known, the grid was set with a spacing of 0.375 Å and 58 points of evaluation in each direction. Nine possible binding sites were proposed by AutoDock for both models and those that showed lower binding energy were selected for further analysis. We also examined the presence of hydrogen bonds between the substrate and the nearby amino acids in hope to determine the catalytic amino acids.

Keywords: homology modelling, docking, malonyl-CoA-reductase, Modeller, Autodock

EO-2

PROTEIN TARGETS OF *NEOSARTORYA FISCHERI* ANTIFUNGAL PROTEIN 2 FOR NEW THERAPEUTIC APPROACHES

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As a consequence of the increasing number of infections caused by drug-resistant *Candida* strains, there is an urgent need to develop new, effective and safely applicable anti-*Candida* treatment strategies for the medicine. The *Neosartorya fischeri* antifungal protein 2 (NFAP2) of an ascomycetous origin is a promising candidate from this respect. NFAP2 is a low molecular weight, cationic, highly stable, extracellular protein, which effectively inhibits the growth of different *Candida* species both *in vitro* and *in vivo*. One of the key steps in its reliable and safe medical application as antifungal drug is an understanding the molecular mechanisms behind the antifungal activity. Considering this, the present work aimed to identify the intracellular protein targets of NFAP2 in *Candida albicans* SC5314, which can be involved in the antifungal mechanisms. We applied different *in vitro* and *in vivo* mass spectrometry-coupled target-fishing methods to realize this aim, such as Far-Western and label transfer protein interaction analyses, and application of an *in vivo* chemical crosslinker, NHS-SS-Diazirine. Our result from these methods indicated that NFAP2 is able to interact with triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase and enolase 1 from *C. albicans* SC5314. These enzymes are important parts of the metabolic pathway of glycolysis, supposing that inhibition of the glycolysis is an antifungal mode of action of NFAP2. These NFAP2-specific protein targets provide templates for further antifungal drug development targeting the fungal glycolysis.

Keywords: antifungal protein, *Candida albicans*, target-fishing, mode of action, glycolysis

Acknowledgement: Present work of L.G. is financed by the FK 134343 project of the Hungarian National Research, Development and Innovation Office - NKFIH.

EO-3

DEVELOPMENT OF A NEW HEAT-STABLE DNA POLYMERASE WITH SITE DIRECTED MUTAGENESIS

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Nowadays, PCR based molecular diagnostics became part of our everyday life. The PCR based method can be used to quickly and reliably identify pathogens from different types of samples. Salt and inhibitor tolerance have to be important properties for a heat stable DNA polymerase, which applied in PCR reactions.

Based on previously described and published *Taq* DNA polymerase mutations in this work we designed and produced four different mutants in the *Thermus sediminis* DNA polymerase I enzymes. The designed mutations were made by *QuickChange* mutagenesis. Recombinant protein expression and subsequent purification led to enzymes that did not contain PCR inhibitors and DNA/RNA degrading enzymes.

The mutant DNA polymerases were compared in short and long gene amplification reactions. The F749I and I707M+F749I mutants were further characterized using an *EvaEZ* activity assay. The salt tolerance of these enzymes was measured in long PCR reactions. In all assays we used a *Taq* DNA polymerase and a *TseM1* (previously described mutant version of *T. sediminis* DNA polymerase I) as a reference. Ellagic acid, a small molecular weight polyphenol, was applied to prove the inhibition of the PCR reaction.

All the produced mutant enzymes had DNA polymerase activities. In the long gene amplification PCR reaction, E708K and I707M underperformed compared to the other mutants. The polymerase activity was improved in the case of F749I and I707M+F749I double mutants at 72 °C. The salt tolerance of the I707M+F749I mutant was improved as well. Surprisingly, the heat stability of I707M+F749I was better than F749I and *TseM1* and showed significant difference from the native *Taq* DNA polymerase.

Keywords: rational enzyme design, PCR, DNA polymerase

EO-4

RESTORING THE FUNCTION OF ONCOGENIC PROTEINS BY IMPROVING THEIR INTERACTION

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The KRAS protein is one of the most important oncogenes, with a particularly high prevalence in high-risk tumour types such as pancreatic cancer, colorectal cancer and lung cancer. KRAS belongs to the small GTPase family of proteins and is involved in signalling pathways related to cell proliferation and apoptosis. It acts as a molecular switch in these intracellular signalling pathways: GTP is capable of signal transduction when bound and GDP is inactive when bound. The protein therefore has its own GTPase activity, but this activity is rather low, so it is regulated by helper proteins: GAP (GTPase activating protein) proteins accelerate GTP hydrolysis, thereby promoting KRAS inactivation, and GEF (guanine nucleotide exchange factors) proteins catalyse the exchange of GDP for GTP.

The most common and most significant KRAS mutations include G12C and G12D mutations. Glycine at position 12 is located on the surface that interacts with the GAP helper protein, and its point mutation therefore stoically interferes with the proper interaction of the two proteins. This also impairs the GTPase activity of the mutated KRAS, leading to a continuously active state and thus uncontrolled cell division.

The idea of our research group was that the defective behaviour of oncogenic KRAS mutants could be eliminated by creating point mutations in the GAP protein that could potentially improve the interaction of the two proteins, and thus even restore the GTPase activity that ensures normal signal transduction. We aimed to design, produce and test GAP mutants that might be able to accelerate the activity of mutant KRASs more than wild-type GAP.

Investigating the interaction of the two proteins is therefore crucial to better understand the behaviour of mutant KRAS and to consider potential therapeutic options. My research involves the investigation of the interactions of KRAS protein and its major mutants with GAP using biolayer interferometry (BLI). This technique is based on binding one of the partners, in this case GAP, to the end of a sensor, and then immersing it in a solution containing KRAS, the formation of binding is detected as a wavelength shift. Association and dissociation curves are recorded to determine the interaction between k_{on} and k_{off} , and from these the K_D .

Keywords: *protein-protein interaction, cancer, biolayer interferometry, mutagenesis, oncogene*

Acknowledgement: *project1; project2; text*

EO-5

CD180 STIMULATION AFFECTS BAFF AND PI3K SIGNALLING OF B CELLS IN SYSTEMIC SCLEROSIS

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Systemic sclerosis (SSc) is a fibrosing rheumatic autoimmune disease characterized by immune dysregulation and early B cell activation. CD180, a TLR-like surface molecule, is shown as a potent activator of B cells, utilizing the PI3K/AKT pathway. TLR9 stimulation driven mTOR phosphorylation is impaired in B cells from SSc patients; thus, exploring mTOR's relation to CD180 is pertinent. Also, BAFF-R and TACI are BAFF receptors participating in B cell survival, activation and class-switching, and their expression increases by TLR9 or TLR4 stimulation, encouraging the investigation of CD180's effect on them. BAFF has elevated concentration in SSc patients, adding further relevance to this inquiry.

Peripheral blood samples from the SSc and healthy control (HC) groups were evaluated with and without anti-CD180 stimulation. We investigated BAFF-R and TACI expression change of B cells with flow cytometry. We also performed phospho-specific flow cytometry to measure phosphorylation change of AKT and the mTOR substrate, S6, in B cells isolated with MACS. Preliminary data shows BAFF-R+ B cell ratio reduced in SSc compared to HC, and stimulation does not reduce it, unlike in HC. The ratio of TACI+ cells is elevated in SSc, but stimulation increased it only in HC. CD180 stimulation increases the phosphorylation level of AKT and S6 in both groups, but AKT's increase is higher in HC.

Differences between SSc and HC group's response may support the substantial effect of CD180 in B cell regulation, making it a valuable target for further research to understand B cell behaviour and consider novel therapeutic approaches.

Keywords: Systemic Sclerosis, Scleroderma, TLR, CD180, B cell, BAFF, BAFF-R, TACI, AKT, mTOR, S6, activation

Acknowledgement: The research was supported by the Hungarian National Research, Development and Innovation Fund (OTKA FK-139028, TKP2021-EGA-10), the Medical School, University of Pécs (PTE ÁOK-KA 2021/19), the New National Excellence Program of the Hungarian Ministry for Innovation and Technology (ÚNKP-21-5-PTE-1340).

EO-6

BIOCATALYTIC SYNTHESIS OF OPINE-TYPE CHIRAL AMINES

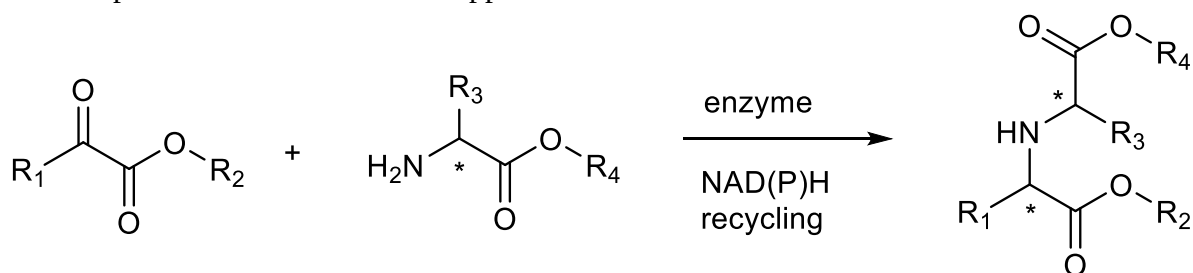
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Opines and opine-type secondary amine dicarboxylic acid derivatives are not only valuable natural products, but are building blocks of various fine chemical products, agrochemicals and pharmaceuticals including peptidomimetics.(1) Their synthesis involves the reductive coupling of an amino acid (or ester) to an α -keto acid (or ester). Traditional chemical synthesis lacks stereoselectivity and often requires expensive reducing agents. Biocatalysis offers a stereoselective and more sustainable alternative. Our research focuses on the development of a biocatalytic platform for the synthesis of a range of opine-type chemicals. To this end we screen enzymes from various families that are reported to reduce imines or perform reductive amination. These include imine reductases (IREDs), reductive aminases (RedAms), opine dehydrogenases (ODHs), amine dehydrogenases (AmDHs) and amino acid dehydrogenases (AADHs)(2). The tested enzymes are either literature-known biocatalysts or derived from metagenomes. In a collaborative effort we want to further extend the available enzyme space by performing metagenome mining. The substrate scope of the screened enzymes is explored with a so far unreported combination of substrates where an amino ester is used as an amine donor in an enzymatic reductive amination of an α -keto acid. Initial hits can be used to drive more targeted metagenomic mining and be subjected to enzyme engineering. Selected substrates and enzymes are used to optimize reaction conditions with the aim to develop a scalable process towards industrial applications.



Keywords: biocatalysis, reductive amination, opine

Acknowledgement: Prepared with the professional support of the Doctoral Student Scholarship Program of the Co-operative Doctoral Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund.

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Microbiology

BIOREACTOR AS A SOURCE OF MANGANESE(II) ION CONTAMINATION DURING CITRIC ACID FERMENTATION BY *ASPERGILLUS NIGER*

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The filamentous Ascomycete fungus *Aspergillus niger* is a superior producer of citric acid (2-hydroxy-propane-1,2,3-tricarboxylic acid), a compound widely used in food, cosmetics and pharma industries. This had led to the development of a fermentation technology that today accounts for over 95% of the global production. High-yield citric acid production requires a combination of unusual nutritional conditions, of which maintaining a low manganese (II) ion concentration (<5 ppb) in the culture broth is a key feature. In this lecture we provide evidence that in addition to contaminations in the liquid, sugar-containing medium, the bioreactor itself can also be the source of manganese (II) ions to such an extent that the corrosion-driven leaching could surpass the critical level, reducing molar citric acid yield by ~30%. We show that leaching of manganese from the stainless steel components is predominantly associated with standard autoclaving and the strongly acidic (pH <2) culture broth characteristic for citric acid fermentation. We also demonstrate that the application of anti-corrosion treatments and modified sterilization protocol can fully restore citric acid yield.

Keywords: citric acid, manganese (II) ion, *Aspergillus niger*

Acknowledgement: This research was supported by the Hungarian National Research, Development and Innovation Fund (grants NN128867 to LK and K138489 to EF). VB was supported by the New National Excellence Program of the Hungarian Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund (grant ÚNKP-21-3-I-DE-322).

EM-1

NETWORK THEORIES OF BOTRYTIS CINEREA EXTRACELLULAR SECRETED PROTEINS

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The discipline of network medicine has been founded for less than a decade and a half and now it is at the frontiers of life science research. Two Hungarian researchers of Transylvanian origin, Albert-László Barabási and Réka Barabási, are credited with the creation of the new discipline: the term was used by them in an article written in 2007. They have discovered the presence of scale-free networks in a number of very distant biological systems since then and also the therapeutic potential of knowledge of the structure in them. A scale-free network model like this describes the development of cancer, the spread of the COVID-19 epidemic, the structure of some neural networks, and numerous protein-protein interaction networks that have been mapped.

In our research extracellular protein patterns of Botrytis cinerea grown on Furmint grapes in the laboratory at four successive botrytis stages was investigated to gain a deeper understanding of the metabolic processes responsible for nematode rot. Our results suggested that the Barabási-Albert scale-free network model provides a good approximation of the extracellular protein network of B. cinerea. We determined the main topological parameters of the extracellular protein network and identified the main components of the network and the key proteins involved in the network and thus in nematode rotation.

Keywords: Botrytis cinerea, extracellular networks, scale-free networks

Acknowledgement: GINOP-2.3.2-15-2016-00061

EM-2

COMPARISON OF THE STRUCTURE-ACTIVITY RELATIONSHIPS (SAR-S) OF PEPTAIBOLS FROM TWO *TRICHODERMA ROSSICUM* STRAINS

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Members of the genus *Trichoderma* play important roles in biotechnology and agriculture due to their several beneficial properties including their secondary metabolites produced. Through their antagonistic abilities, certain species of the genus are able to displace various plant pathogenic microorganisms from their habitats, thus providing protection, as well as to promote the growth of plants. One of the most important secondary metabolites produced by *Trichoderma* species are peptaibols, which belong to the group of peptaibiotics. Peptaibols have characteristic properties and the sequences are characterized by a high degree of variability due to the fact that non-ribosomal peptide synthetases (NRPSs) are responsible for their production. In our research, two strains (TUCIM 889 and TUCIM 3235) belonging to the species *Trichoderma rossicum* were examined. The peptaibol production of the strains were identified previously. Based on their *tefla* sequences, the two *T. rossicum* strains are closely related with each other, however, although their peptaibol composition similar, the length of the sequences produced by the two strains differs by one amino acid. After large-scale extraction of peptaibols, their minimal inhibitory concentration (MIC, mg ml⁻¹) values were determined against 11 commonly known Gram-positive and Gram-negative bacteria. Comparing the two *T. rossicum* strains based on the MIC-values of the peptaibol extracts, *T. rossicum* TUCIM 889 producing 19 residue peptaibols showed a stronger inhibitory effect to Gram-positive bacteria, moreover it was also able to inhibit the Gram-negative *Agrobacterium vitis* at the concentration of 5 mg ml⁻¹. The effect of the two peptaibol extracts was similar only in the case of *Rhodococcus fascians* (MIC: 0.312 mg ml⁻¹). The peptaibol sequences were modelled using accelerated molecular dynamics (aMD) simulations. Modern modeling techniques such as aMD improve the knowledge of the folding process and channel-forming properties of peptaibols. Based on the aMD results, the different amino acid motifs of the peptaibol sequences determine the folding behaviour and the energetically stable states of the peptaibols, thereby have an effect on bioactivity and inhibitory effect against microorganisms.

The structure-activity relationships (SAR-s) of peptaibols can be investigated by the comparison of results obtained during bioactivity tests and computational modeling, which can lead to the better understanding of the background of their bioactivity. Results obtained during SAR-s can lead to an efficient selection of peptaibiotic compounds for the application in plant treatment.

The authors wish to Thank Prof. Irina Druzhinina (Nanjing Agricultural University) for the access to the examined *T. rossicum* strains.

Keywords: *Trichoderma*, peptaibol, bioactivity, modeling

EM-3

ARBUSCULAR MYCORRHIZAL INOCULATION INCREASES TOMATO TOLERANCE TO WATER DEFICIT AND HEAT STRESS

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Arbuscular mycorrhizal (AM) symbiosis can alleviate abiotic stresses in the host plant. With global climate change on the rise this characteristic is increasing in importance. Our goal was to monitor the effects of *Funneliformis mosseae* on water deficit and heat stresses exposed tomato plants. In order to achieve this we set up an experiment where tomato plants were grown in a climatic chamber for seven weeks. This was followed with a 2 week stress period where plants were subjected to single water deficit, single heat and combined water deficit and heat stresses. For the application of water stress the water level of the growing system was reduced for the whole two week period, heat stress was applied only on the second week of the stress period, while combined stress contained both the two week long water deprivation and the one week long heat stress.

Colonization seemed to be not influenced by the different conditions as there were only slight differences in the AM abundance among the stress treatments. Shoot and root biomass of mycorrhizal plants was increased under all conditions. Relative water content of water stress imposed (single and combined) plants was improved by AM compared to their non-AM counterparts. Mycorrhizal plants in general had lower levels of the observed stress markers than non-AM ones, however the difference was not always significant. Defence enzymes also appeared to follow this trend. The gene expression of a transcription factor HSFA2 and a chaperon HSP70, and two aquaporin genes PIP2.5 and PIP2.7 were also studied with qRT-PCR, as these four genes are main participants in heat and water stress response. The levels of HSFA2 and HSP70 were significantly reduced during heat and combined stress imposition when comparing non-AM and AM plants within a treatment. Under water deficit, PIP2.5 showed significantly higher expression in mycorrhizal plants, while combined stress reduced the expression in non-mycorrhizal and mycorrhizal plants as well. PIP2.7 expression had the same trend in all treatments showing a lower level in mycorrhizal plants.

At first glance the previously mentioned alleviating effects were the most prominent during heat stress, however heat stress did not induce that severe changes than the other two treatments in the first place. All together, mycorrhizal inoculation was effective in mitigating the imposed stress treatments.

Keywords: *Arbuscular mycorrhiza, Tomato, Abiotic stress*

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.*

EM-4

APPLE POMACE AS ALTERNATIVE SUBSTRATE FOR SUCCINIC ACID PRODUCTION WITH *BASFIA SUCCINICIPRODUCENS*

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Four-carbon dicarboxylic acid, succinic acid traditionally is synthesised from petroleum based materials by the hydrogenation of maleic acid, carbonylation of ethylene glycol or oxydation of 1-4, butanediol. Succinic acid also is a widespread biosynthesised precursor which was listed in US Department of Energy's Top 12 bio-based molecules. It is a platform molecule is widely used as detergents, surfactants, biodegradable plastics and also in the production of different foods and pharmaceuticals. Numerous microbial species were selected and applied in the production from different substrates like glucose, glycerol. Various experiments were performed by research groups all over the world which resulted extensive knowledge on behavior and metabolism of these microorganisms. *Basfia succiniciproducens* member of *Pasteurellaceae* family was recognised from its high natural succinic acid production ability. Ordinary substrates, as listed earlier were applied in biosynthesis.

Apple pomace generated in food industry contains consumable carbohydrates which can be used as substrate, whey as by-product of the dairy industry is also a possible starting material. Wastes considered as alternative substrates are useful substances in the production of high-added value components.

In our work we investigated different pretreatment methods for choosing high carbohydrate content substrates which were followed by bioreactor set-up fermentations using *Basfia succiniciproducens* strain.

Keywords: succinic acid, alternative substrates, *Basfia succiniciproducens*.

SECONDARY METABOLITES FROM THE DARK SEPTATE ENDOPHYTIC FUNGAL GENUS *DARKSIDEA*

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Endophytic fungi, colonizing plants internally and asymptotically, are considered valuable but unexplored sources of diverse natural products, because of their interactions with plants and other microorganisms via secondary metabolites (SMs). During investigations on root endophytic fungal communities of semiarid sandy grasslands in Hungary, we frequently isolated representatives of the dark septate endophytic fungal genus *Darksidea*, characterized by melanized and septate hyphae. We also obtained numerous congeneric isolates from similar ecosystems in different regions of the world, resulting in a remarkable collection of *Darksidea* isolates. These isolates represent different known and presumably novel species. As the metabolite composition of this common and widespread fungal genus has not been determined yet, we aimed to identify SMs of *Darksidea* by metabolite profiling all available isolates.

Metabolite composition of the fungal isolates was analysed by high-performance liquid chromatography hyphenated with diode array and high-resolution tandem mass spectrometry detection (HPLC-DAD-HRMS/MS). In addition to the HRMS/MS identifications, chemical structures of isolated compounds were confirmed by nuclear magnetic resonance (NMR) spectroscopy.

As a result, we identified numerous SMs of the genus *Darksidea* for the first time. These compounds represent diverse structural groups, such as polyketides, terpenes, quinones and amino acid derivatives. The genus *Darksidea* proved to be a valuable source of several rarely occurring and also of some novel compounds. Furthermore, correlations were found between the phylogeny and metabolite diversity of this genus, confirming the practical utility of closely related isolates for the high-yield production of certain compounds.

SMs and correlations between the phylogeny and metabolite diversity of *Darksidea* will be presented on the conference.

Keywords: *Darksidea*, endophytic fungi, secondary metabolites

Acknowledgement: This work was supported by the National Research, Development and Innovation Office, Hungary (grants: OTKA NKFIH KH-130401, NKFIH K-135712, EFOP-1.8.0-VEKOP-17-2017-00001, ELTE Thematic Excellence Programme 2020, TKP2020-IKA-05), the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (G. Tóth, D.G. Knapp) and the Bolyai + New National Excellence Program of the Ministry for Innovation and Technology (G. Tóth, D.G. Knapp).

Bioinformatics

BIOTECHNOLOGY AND BIOINFORMATICS IN THE ERA OF BIG DATA AND ARTIFICIAL INTELLIGENCE

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Bioinformatics, the science of biological information has become a crucial scientific infrastructure of the life sciences and perhaps the most important interface between data and biological knowledge. In a classical scenario, biological data are processed, annotated and stored in public databases in a one-by-one fashion and they can be accessed with standard, widely available software tools. With the advent of high throughput data collection methodologies, as well as the parallel growth of data storage capacities, this is no longer possible. Today, an ever growing amount of data is stored in raw, unprocessed or partially processed form in various databases that are not directly accessible with standard tools. This is a typical “big data” situation brought about by such automated methodologies as high throughput DNA sequencing, genomics and metagenomics, mass-spectrometry based proteomics, genetic profiling etc. In a typical project, data from various sources need to be collected first in order to prove that the goal is novel, and this check needs to be repeated a few times during the project. Biotechnology – famously defined by Lee Hood as “the industrial use of biological information” – is deeply affected by these complexities that are further aggravated by privacy and IP issues.

On the computer science side, the emergence of artificial intelligence i.e. machine learning technologies contributed a great deal to the solution of these problems. Classical machine learning methods such as neural networks, support vector machines, Hidden Markov Models, have been in use for many years in difficult classification and prediction tasks of bioinformatics. The recent advent of deep learning methods brought about revolutionary changes in various fields of computer sciences ranging from natural language processing to chess playing. Bioinformatics applications are currently under development in various fields. AlfaFold2 is a notable success story in predicting protein 3D structure from amino acid sequence. Novel machine learning strategies such as automated machine learning (AML) transfer and few-shot learning help us to tackle problems where only limited number of training material are available.

This presentation is meant to give an overview on trends, educational programs and institutional frameworks of bioinformatics in Hungary, with special regard to the needs of researchers interested in entering this field.

Keywords: *bioinformatics, big data, machine learning, deep learning*

Acknowledgement: This work was supported by grants of the Hungarian National Development, Research and Innovation (NKFIH) Fund, OTKA PD (138055) and Thematic Excellence Programme (TKP2020-NKA-11).

EB-1

IDENTIFICATION AND CHARACTERIZATION OF CIRCRNA-S IN RABBIT (*ORYCTOLAGUS CUNICULUS*) THROUGH BIOINFORMATIC METHODS

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Circular RNA-s (circRNAs) are RNA molecules that form covalently closed circles. They are mostly produced from protein coding genes during splicing and can contain exonic and intronic sequences. CircRNA-s, originally thought to be a product of splicing errors, have received growing attention in recent years, and as of today, a wide range of circRNA sequences and their functions have been confirmed in various species. However, our knowledge of circRNAs is far from complete.

Our goal was to identify and characterize as wide as possible a range of circRNAs data in rabbit (*Oryctolagus cuniculus*), using bioinformatics methods. The rabbit, despite its important role as a human disease model, is currently not featured in available circRNA databases.

From publically available data, we selected samples of 9 tissue types of 7 domestic rabbit breeds, where rRNA depletion was used for library preparation, followed by paired end short read sequencing. We identified over 8000 reliable circRNAs on the rabbit reference (OryCun2.0) genome, which we then characterized considering various aspects.

We identified a number of exon-intron (EIciRNA) and intronic circRNAs as well as ones that originate from regions where there is no known gene annotated. We predicted possible functions based on primary structure and alignment coverage, such as the regulatory function of EIciRNAs, or miRNA sponging capability. We also investigated the putative secondary structure of the identified circRNAs and compared it to secondary structures of known mouse and human circRNAs as it could point to functional similarities despite possibly highly different sequences.

Furthermore, we investigated the flanking regions of circRNAs and found that the flanking introns are enriched in Lagomorph specific transposable elements which coincides with published data in other species.

Keywords: *circRNA, rabbit, gene regulation, genome analysis, novel gene identification*

Acknowledgement: *This work was supported by the “Új Nemzeti Kiválósági Program” (ÚNKP-21-3-I-MATE-16) scholarship for PhD students*

EB-2

COMPETITIVE IN-HOST ASSAY TO SCREEN PATHOGENICITY OF THE *SACCHAROMYCES 'BOULARDII'* PROBIOTIC YEAST

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The yeast *Saccharomyces 'boulardii'* is applied as a probiotic microorganism worldwide in more than 80 countries (McFarland, 2017). Today, these yeast-containing probiotics are used to protect the intestinal flora, which plays a role in supporting the immune system. They are also able to cure or prevent antibiotic associated and traveler's diarrhea, and *Clostridioides difficile* infections, and reduce the symptoms of IBS and Crohn's disease (Szajewska *et al.*, 2020; Florez *et al.*, 2018; Choi *et al.*, 2011). However in the recent years, more and more studies have reported yeast probiotic-derived fungemia. Infants, elderly and immunocompromised patients, and patients who have undergone prolonged hospitalization are at increased risk, and long-term antibiotic and probiotic treatment is also a predisposing factor (Ventoulis *et al.*, 2020, Imre *et al.*, 2019). Thus research on probiotic yeast has to focus on reducing the incidence of such cases.

The occurrence of yeast probiotic-derived infections raises the question of the genomic and phenotypic properties that allow *S. 'boulardii'* to enter or survive in the mammalian bloodstream. During our work we utilized the probiotic isolate collection of the Department of Molecular Biotechnology and Microbiology at the University of Debrecen (containing both commercial and clinical *S. 'boulardii'* isolates), and the sequenced genomes of these yeasts to reveal the characteristics that enable the probiotic yeast to cause bloodstream infections. We identified copy number variations and various single nucleotide polymorphisms in the genomes of the probiotic yeast isolates, and compared these among commercial, mycosis-causing and non-mycosis human isolates. The yeasts differed in a few thousand polymorphic sites, and all isolates showed various mutational patterns, fungemia isolates did not have unique common ones. As the bloodstream survival could not be linked to well-defined genomic adaptations, we designed a pathogenicity screen that involves a competitive in-host assay. A control probiotic isolate is planned for these assays with a constitutive expression of a fluorescent/visible color marker that can be injected to laboratory test animals simultaneously with tested isolates. The competitive assay relies on identifying colonies on an isolate-level visually. This enables a rapid identification of relative growth/survival rates in the bloodstream, and this data may subsequently be correlated with observable phenotypic differences, to unequivocally link phenotypic characteristics (as in Imre *et al.*, 2021) with pathogenic potential.

Keywords: *Saccharomyces 'boulardii'*, fungemia, pathogenicity, fluorescence, bloodstream

Acknowledgement: NKFIH FK138910

POPULATION GENETIC STUDY OF TURKEY OAK (*QUERCUS CERRIS* L.) BASED ON NEXT GENERATION RAD-TAG SEQUENCING AND SSR MARKERS

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Promoting the adaptation of our forests to the increasing climatic changes is one of the most urgent issues of present-day forestry management. Although forest tree species dynamically shift their ranges in response to local site factors, this process takes a long time in most cases. Due to the present rate of climate change, forestry management must focus on the range shift potential of native forestry tree species, which can contribute to increase resilience of the forest ecosystems through carefully selected provenances. Within the large distribution zones of our native forest trees, we can identify sites that are sufficiently similar to our environment projected for the future. Presumably, the populations living in such sites are adapted to the local conditions. Molecular genetic approaches can separate populations of different sites, reveal patterns along ecological factors, these can help us to get more information about the spatial distribution of adaptive characters. Based on population genetic patterns we can delimit new seed zones from where we can obtain more resilient reproductive materials.

Turkey oak (*Quercus cerris* L.) is a keystone forest tree species in Europe, in Hungary its share in forest cover is the largest among native oak species. It has a major ecological role in the forest ecosystems of the Carpathian Basin. The species' distribution range extends throughout the Apennine and Balkan Peninsula, eastwards to Asia Minor, and northwards to Carpathian Basin. In this study, we examine 88 individuals from 8 autochthonous Central- and Southeast-European populations, three from Bulgaria, Kosovo and five from Hungary. In the following period, we aim to expand this set with samples deriving from other Central and Southeast-European populations. During the sampling, we plan to pay particular attention to the populations living close to the drought tolerance limit of turkey oak in the forest-steppe zone and the outstanding ones in terms of productivity.

To the genetic investigation, we apply two distinct approaches. We examine the nuclear genome with double digest restriction site-associated DNA sequencing (ddRAD-seq) method and conventional simple sequence repeat (nSSR) markers. In addition, we also perform SSR marker analysis on the chloroplast DNA. ddRAD-seq can provide high-resolution genome-wide single nucleotide markers (SNPs) for studying adaptive potential of the species, while neutral SSR markers can yield phylogeographic inference on populations. Especially, by the maternally inherited chloroplast haplotypes new information can be obtained about the glacial refugia and postglacial migration routes of turkey oak. The latter approach is capable of delimiting populations of larger geographic regions therefore we examine whether it is suitable for the tracking of reproductive material transfer in forestry practice.

Keywords: *Quercus cerris* L., population genetics, ddRAD-seq, nSSR, cpSSR

Acknowledgement: This study was partly supported by the Ministry of Agriculture of Hungary (Kaán Károly project, no.: EVgF/549/2018, EGF/178/2019)

EB-4

WHOLE GENOME SEQUENCING OF A BIG FAMILY ALLOWS DETERMINING THE HAPLOTYPES OF THE PARENT'S CHROMOSOMES, THE CROSSING OVER POSITIONS, THE DE NOVO MUTATIONS, AND THE EXACT CNVS.

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We whole genome sequenced a rabbit family with eight children. We processed the raw reads with standard pipelines using OryCun3 as the reference genome sequence. After determining the SNPs, indels and CNVs, we have started to analyze the inheritance of individual variations. According to the Mendelian laws, considering that the rabbit is a diploid organism, if one parent is homozygous reference while the other one is homozygous alternate, every child will be heterozygous. More interesting examining all sites in a chromosome, where for example the mother heterozygous while the father is homozygous reference. In this case, theoretically half of the children will inherit the mother's A allele, while the other half the B allele. If A is the reference allele, only the children inherited the B allele will be heterozygous. If we go to the next variation, three cases can be observed. If the same children will be heterozygous then the mothers two B alleles were on the same chromosome. If there is a swap between the heterozygous and homozygous children, the two B alleles were in different chromosomes. Finally, if only one child jump from the heterozygous group into the homozygous group or vice versa, it indicates a crossing over event. Using this approach, we have developed a new algorithm which can determine the near exact haplotypes of the parents' chromosomes and positions of the crossing over events.

Having known the variations for the whole family, we could also determine the *de novo* variations. Surprisingly, we found a large number of such mutations. We have also found an unexpectedly large number of variations, which were genetically inconsistent.

Copy Number Variations (CNVs) are larger deletions or duplications in the genome relative to the reference genome sequence. Detecting CNVs is difficult, even having a nowadays usual 30x coverage whole genome sequencing. The programs used for this usually give only a statistical probability for a CNV and different programs often have different results. Having sequenced a family, makes it possible to verify the results of these CNV predictions by analyzing their Mendelian inheritance.

The mother rabbit used for this analysis is from a commercial breed. We found that her genome contains a large number of heterozygous variations. After we carried out the haplotype analysis, we found an even more unexpected phenomenon. The mother's Alt alleles, which are different from both the reference and the father's (dwarf hobby rabbit) sequence, are in long stretches in one chromosome usually and these runs of ALT alleles are jumping between the mother's parental chromosomes. This indicates strong selection steps during the breeding and makes our approach suitable for identifying regions, which are important for selecting useful traits.

Keywords: Haplotype, recombination, Copy Number Variants, De Novo Mutations

Acknowledgement: K132814; TKP2021-NKTA-34; 2017-1.3.1-vke-2017-00026, DOCTORAL SCHOOL OF BIOLOGICAL SCIENCES AT MATE.

UNDERSTANDING THE EFFECTS OF COVID-19 ON THE MICROBIOME USING BIOINFORMATICS AND MACHINE LEARNING

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The respiratory tract is a major portal for microbial and viral exposure and its microbiome plays an important role in respiratory health. Respiratory viral infections affect not only the microbiome of the respiratory tract but also of the gut, which has a role in influencing how the body reacts to respiratory diseases. Although SARS-Cov-2 attacks mostly the respiratory tract it can also affect the gut and so the effect of the virus on the gut microbiome has become a hot topic in microbial research during the current epidemic. To gain insight on how the virus affects the microbiota during and after infection, we analysed the microbial composition of patients during and after COVID-19 and compared these results with the microbiota of healthy patients. For each patient, three microbial samples were taken from different areas of the body: nose, pharynx and gut, aiming for the major infection portals of the virus. We used statistical, bioinformatics and machine learning methods to analyse the changes in microbial composition caused by the infection on the level of species abundance and also on the level of genes, using functional metagenomics. The following results are preliminary and a broader study with more participants is on the way.

With these methods we could show that COVID-19 causes an observable change in the microbial composition of all three niches and that this change doesn't disappear with the disappearance of the viral particles, but lingers on, during recovery. We observed the most significant differences in the *Campylobacter*, *Schaalia* and *Escherichia* genera and these differences were observable not only for the infected but for recovered patients as well.

With functional metagenomics we could demonstrate that these changes do not only affect the relative abundance of the microbial species but can also be observed on the level of genes as well. We found that the viral infection resulted in under-representation of several genetic pathways connected to the healthy microflora, while other, dysbiosis and bacterial infections connected, pathways were over-represented.

Our results indicate that the COVID-19 infection changes the composition of the nasal, pharyngeal and gut microbiome, and this difference lingers even after recovery from COVID-19. Our results help to gain better understanding to the effect of a viral infection on the body and the recovery of the microbiota after such an event. These results can be beneficial for designing therapeutic methods for the recovery from a viral respiratory infection with probiotics and bacterial microbiota transplantation.

Keywords: metagenome, coronavirus, infection, bioinformatics, machine learning

Animal biotechnology

ZEBRAFISH MALES ARE MODELS OF NATURAL SEX REVERSAL IN TELEOSTS

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Keywords: sex determination, gonad differentiation, fish reproduction

Acknowledgement: The work described in the Hungarian lab is supported by the Frontline Research Excellence Grant of the National Research, Development and Innovation Office of Hungary (KKP 140 353). Earlier data are from studies funded by the National Research Foundation, Prime Minister's Office, Singapore under its Competitive Research Programme (Award No: NRF-CRP7-2010-001; 2012-2017), and by internal research grants from Temasek Life Sciences Laboratory, Singapore.

Abstract:

In domesticated zebrafish lines sex is determined through Polygenic Sex Determination (PSD), resulting in sex ratios that are stable for a given breeding pair, but can be dramatically different across a range of families. Zebrafish males start to develop a 'juvenile ovary' during the early phase of their sexual differentiation. Then, the developing gonad undergoes a rapid transformation involving an apoptotic wave, eventually producing a differentiating testis.

We have been studying this 'gonadal transformation' process in order to gain a deeper understanding about the involvement/interplay of genetic and environmental processes. We have been utilizing a transgenic reported line called *Tg(ddx4:ddx4-EGFP)^{zf45Tg}* that shows sexually dimorphic expression of the transgene between the female and the male gonad as early as 3-5 weeks post-fertilization. The primary platform of our investigations was comparative microarray-based transcriptomic analysis, but other high throughput 'omic technologies' have also been utilized.

In this talk, I will present the current status of this research area, including results obtained by our former Singapore-based research team. In addition, I will also reveal hitherto unpublished data generated by our lab based at the Georgikon Campus of MATE.

EÁ-1

THE EFFECT OF MYCOTOXIN TREATMENT ON IN VITRO CULTURED PREIMPLANTATION STAGE RABBIT EMBRYO

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Fusarium toxins are widely distributed in the European food chain. With the change of local environments and shifts in climatic conditions, mycotoxin contamination of food and animal feed is a rising problem all over the world including temperate climate. Animal models show that mycotoxins pass through the placenta affecting the development of the offspring as well. There are very little data presented with the concern of mycotoxin exposure of the mammalian offspring in the timeframe from ovulation of the oocyte through fertilization until the implantation of the embryo. To our knowledge evaluation of long-term effects of low level and short duration mycotoxin exposure (relevant to real-life exposure) at this very early developmental stage is not available at all.

Aim: Study the effects of mycotoxins on early embryonic development to set up a sublethal concentration of the toxin, namely T2 toxin, zearalenone (Zea), and the combination of the two for future studies to monitor mycotoxin effect on the development of non-communicable diseases later in life.

Methods: Rabbit (New Zealand white rabbit) embryos were derived from superovulated does 10 hours after insemination (time0). An increasing amount of mycotoxin was administered to the culturing media (T2 toxin 2,5;5;10 ng/ml. Zearalenone 5;10;20 ng/ml), combined (T2)2,5/(Zea)10 and 20 ng/ml). Embryos were cultured in RDH media at optimal environmental conditions (37C, 5% CO₂, 85%). Development was constantly monitored under a microscope and photo-documented for a period of 96 hours. Control groups (solvent and no treatment controls) were also monitored. The developmental stage of the embryos was recorded every 24 hours. The ratio of certain developmental stages was compared to the non-toxin control group.

Results: An optimal concentration of 2,5 ng/ml was determined for T2 toxin for future studies. In the case of zearalenone there was no significant effect of the toxin on rabbit embryo development in the studied range (5-20 ng/ml). The combination of T2 toxin and zearalenone had a synergistic effect on rabbit embryo development based on blastocyst development rate.

Keywords: preimplantation rabbit embryo, mycotoxin, T2 toxin, zearalenone

Acknowledgement: TUDFO/51757-1/2019-ITM, TKP2020-NKA-24

CHARACTERISATION AND PRESERVATION OF GUINEA FOWL PRIMORDIAL GERM CELLS

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Guinea fowl is a traditional farm animal of the Hungarian culture and carries important attributes like heat resistance. Unfortunately, their part in the modern poultry industry is small, so the tool of gene preservation is needed to conserve this species' genetic information. For avian species one of the most promising preservation methods is the freezing of primordial germ cells (PGCs). We tried to create a new culturing medium based on our knowledge regarding the chicken and goose PGC cultures.

We opened 60-hour-incubated eggs and isolated PGCs from the circulation via blood sampling. For the culturing we tried two media: one was the chicken PGC culture medium without serum, and one was the same, conditioned over the tissue culture of 10-day-old guinea fowl embryos' gonads for 1/2/3 days. We cultured the cells for 4-6 weeks depending on their proliferation. Gradient centrifugation and immunostaining were also done on these PGC lines.

Despite the huge amount of time and energy devoted to this project, we could not create a medium that is able to maintain a stable guinea fowl PGC culture yet. Our work is also held back by the very seasonal egg laying nature of the guinea fowl. However, in spite of all difficulties, we successfully froze 6 samples from the gonadal PGC cultures for the gene bank, and we were also able to immunostaining them with 30B6, CVH and ToPro3 markers and take pictures of a stained guinea fowl PGC.

Keywords: Guinea fowl, gene preservation, PGC, primordial germ cell, cell culture

Acknowledgement: This research was funded by VEKOP-2.3.2-16-2016-00012, TUDFO/51757-1/2019-ITM, TKP2020-NKA-24 and 2019-2.1.11-TÉT-2019-00036.

EVALUATION OF THE EFFECTS OF BISPHENOL A LEVELS ON CARDIOMYOCYTES DIFFERENTIATION OF HUMAN IPSCs

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Over a lifetime, individuals are exposed to a variety of environmental chemicals which have been linked to the onset of non-communicable diseases including cardiovascular diseases (Developmental Origins of Health and Disease (DOHaD) concept). Bisphenol A (BPA), widely used in the manufacturing of plastics for food storage and other purposes, has been linked to heart developmental alterations. The present study aims to investigate the effects of BPA exposure during the earliest stages of heart development, using a human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes model. hiPSC-derived cardiomyocytes have been established and validated with the positive expression of cardiac development genes (e.g., NKX2-5, GATA4) and cardiac structural markers (e.g., Sarcomeric α -Actinin, Troponin T), both in 2D and 3D *in vitro* systems. Additionally, spontaneously beating activity has been observed and analyzed through video-based system. The effects of BPA exposure are evaluated starting from the undifferentiated stage (iPSC) through the cardiomyocyte differentiation process, with the aim to investigate maladaptive changes on gene expression, functionality and phenotype of cardiomyocytes during their early development.

In conclusion, our *in vitro* model will help to identify early biomarkers that may be linked to the onset of cardiovascular diseases, in correlation with human clinical observations and animal studies in the DOHaD field.

Keywords: Heart development, DOHaD, cardiomyocytes differentiation, hiPSCs, Bisphenol A.

Acknowledgement: European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No. 812660 (DohART-NET).

GENETIC MONITORING OF GREY WOLVES (*CANIS LUPUS*) IN HUNGARY

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Grey wolves (*Canis lupus*) are historically among the most controversial large carnivores. Their numbers declined in the past, but due to conservative programs, they are making a comeback in the mountainous regions of Europe, and they have established a population in Hungary too. For the monitoring of the Hungarian grey wolf population we established non-invasive sampling methods, which are suitable for species and individual identification and also for population survey and characterisation with genetic methods.

For genetic analyses, buccal swab samples from prey, hair, urine, and stool samples proved to be viable. Individual identification and population structure analysis was performed using 14 tetranucleotide microsatellite loci.

Genetic clustering was performed by STRUCTURE software, identification and relationship analysis was done with COLONY software.

Our results show the population structure of grey wolf packs in Hungary, the differences between the animals in different regions of the Northern Hungarian Uplands and the areas they inhabit. Our methods also proved to be useful in forensic investigations, as in identifying the species of the predator when preying on farm animals.

Keywords: genetics, wolf, predator, microsatellites, species conservation

Acknowledgement: We are thankful for the colleagues of both the Bükk National Park Directorate and the Aggtelek National Park Directorate for non-invasive sample collecting and providing information about them.

This work has been supported by the HUSKROUS/1702/6.1./0010 project, provided by Aggtelek National Park Directorate. We are also thankful for the support of the Ministry of Agriculture.

STUDY OF SARS-COV-2-INDUCED PANOPTOSIS, A NEWLY DESCRIBED INFLAMMATORY PROGRAMMED CELL DEATH

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The innate immune sensor, ZBP1, is a positive regulator of PANoptosome formation and activation. ZBP1-dependent PANoptosome includes ZBP1 (sensor), RIPK3 and RIPK1 (necroptotic proteins), NLRP3, ASC and CASP1 (inflammatory/pyroptotic proteins), CASP8 (apoptotic protein) and CASP6. The aim of our research was to determine the expression pattern of PANoptosome components (RIPK3, RIPK1, NLRP3, ZBP1, CASP1, CASP6, CASP8 proteins) after infection with SARS-CoV-2 virus. In SARS-CoV-2-infected asymptomatic individuals, the immune response and molecular background are not yet known. A further aim of the research is to explore the molecular pathway that activates/inhibits SARS-CoV-2-infected individuals (which are the key molecules) in order to control the disease asymptotically or occurs with severe symptoms (even fatalities).

Keywords: SARS-CoV-2, PANOPTOSIS, immune response, molecular pathway

Acknowledgement: This work was supported by the ÚNKP-20-3 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development, and Innovation Fund.

Abstracts of posters

(in preparation)