CONGRESS OF THE SERBIAN GENETIC SOCIETY

BOOK OF ABSTRACTS

2024 | October 2 - 5

ZLATIBOR · SERBIA

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2024 | October 2–5

ZLATIBOR, SERBIA

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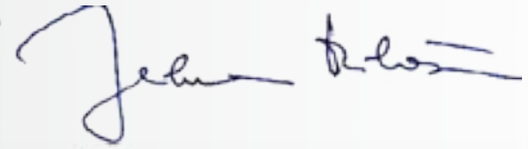
WELCOME TO VII CONGRESS OF THE SERBIAN GENETIC SOCIETY!

The Serbian Genetic Society (SGS) has been founded in 1968 and the first Congress organized by the SGS was held in 1994 in Vrnjacka Banja. Since then, the Congress of Serbian Genetic Society is held every five years.

The VII Congress of the Serbian Genetic Society has gathered over 250 scientists from different European countries and this collection of abstracts showcases cutting-edge research in the field of genetics, exploring the vast complexity of life. The abstracts contained in this book reflect the diversity and dynamism of the field. A wide range of topics have been covered including molecular genetics and genomics, medical genetics and personalized medicine, population and evolutionary genetics, microbial genetics, crop and livestock breeding, bioinformatics, genotoxicology, new technologies, and others, bringing significant contributions to our understanding of the genetic foundations that shape organisms.

We hope this collection will inspire and foster interdisciplinary collaboration, spark new ideas, and most generally, contribute to the advancement of genetics for the benefit of both science and society.



Jelena Milašin
President of the Serbian Genetic Society

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Plenary lectures

VII CONGRESS OF THE SERBIAN GENETIC SOCIETY

PL – 01 Plenary lecture

GENOMICS TECHNOLOGIES CREATE THE FOUNDATION FOR UNDERSTANDING WHO WE ARE, HOW WE FUNCTION, AND WHAT WE DRINK!

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The genome of each organism represents a complement of protein-coding genes, regulatory non-coding RNA genes, and regulatory elements (promoters and enhancers) that direct the development of an organism. This information encoded in the DNA, is critical for an organism's growth and reproduction, underlying physiological processes, behavior, and overall phenotype. In addition, DNA sequence contains information on the evolutionary history, risk factors for diseases and determines responses to the external environment. With the development of massive sequencing technologies, we can extract enormous amounts of information from the genomic data. Consequently, genomic tools are becoming essential in virtually any area of research, personalized medicine, disease prevention, agriculture, and the development of novel technologies.

In this presentation, we will show how genome sequencing projects can answer key biological questions in evolution, using our spider mite genome sequencing project, and how this genomic research leads to novel technologies for environmentally safe pest control and the development of new materials for pharmacology. Understanding the genetic relationship of the grapevine grown in one area is key for the development of the wine industry based on autochthonous varieties, combating climate change, and science-based wine marketing. Using the genomic approach, we established the full pedigree of Montenegrin grapevines and initiated similar research in Serbia that illustrates the power of genomics in this sector. Finally using human genomics, we are establishing answers to our origins showing the power of genomics in the area dominated previously by archeology and historiography, frequently producing controversial theories. Using ancient DNA (aDNA), we have shown that the genome in Serbia and other former YU republics represents the mixture of the Ancient Balkan population with Slavic migrants. Using a similar aDNA approach we are deciphering which grapevine varieties were grown before the *Filoxera* period in Fruška Gora extracting DNA from grapevine leaves discovered in Volny's Herbarium from 1812 in the Sremski Karlovci High School. These various applications of genomics illustrate how this technology is critical for the development of genomic-based science and different sectors of the economy of the 21st century.

PL – 02 Plenary lecture

EPIGENETIC MECHANISMS INVOLVED IN VASCULAR DEGENERATION INDUCED BY CHRONIC HYPERGLYCEMIA: FOCUS ON IN VITRO CELLULAR MODELS

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Type 2 diabetes (T2D) is a major contributor to cardiovascular disease, leading to macrovascular complications. A crucial mechanism underlying these issues is endothelial dysfunction, which involves complex processes associated to increased oxidative stress and inflammation.

Hyperglycemia plays a central role in endothelial dysfunction but intensive glycemic control has been proven insufficient in reducing cardiovascular complications in T2D. This suggests the need for a deeper understanding of the underlying mechanisms, including the phenomenon of 'hyperglycemic memory'. This concept is supported by observations from several in vitro studies showing that endothelial cells exposed to chronic hyperglycemia in vivo maintain a 'diabetic proinflammatory phenotype' even after exposure to normal glucose.

One potential mechanism underlying this phenomenon has been identified in epigenetic modifications, changes in gene expression that do not alter the DNA sequence. Diabetes, can disrupt epigenetic mechanisms, leading to vascular dysfunction. Recent studies have linked histone modifications to vascular complications and inflammation in diabetes. Moreover, epigenetics may play a significant role in inheritance of altered cardiometabolic traits.

In particular, our recent unpublished data, provides proof-of-concept evidence showing the persistence of methyltransferase MLL1-dependent histone modification (H3K4me3) in offspring born to women with gestational diabetes, suggesting an epigenetic-driven transmission of maternal phenotype. This was made possible through a model of endothelial cells from the umbilical cords of women with gestational diabetes, which maintained hyperglycemia-induced alterations. In conclusion, understanding and targeting epigenetic modifications hold promise for uncovering the mechanisms underlying chronic diseases such as diabetes and developing effective treatments.

PL – 03 Plenary lecture

TRANSPOSABLE ELEMENTS DRIVE GENETIC AND EPIGENETIC NOVELTY RELEVANT FOR ADAPTIVE EVOLUTION ACROSS SPECIES

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How organisms adapt to the environment is still an open question in Biology. Whole-genome short read sequencing has allowed to explore the role of single nucleotide polymorphisms (SNPs) in environmental adaptation. However, SNPs alone can only explain a fraction of the existing ecologically relevant phenotypic variation. Among the structural variants that can now be studied, thanks to the availability of long-read sequencing, transposable elements (TEs) are likely to play a major role in adaptation. TEs can generate complex mutations, and several instances of adaptive TE insertions have been described across species. In our lab, we are systematically investigating the contribution of transposable elements to environmental adaptation in *Drosophila melanogaster*, a species that has recently colonized very distinct environments. We have generated *de novo* reference genomes and *de novo* TE annotations for 12 natural *D. melanogaster* populations. Besides new reference genomes, we have also generated transcriptomes and epigenomes that have allowed us to quantify the role of TEs in transcriptome diversification across body-parts, and to show that besides repressive histone marks TEs are also enriched for active marks that affect expression of nearby genes. For some of the adaptive TEs identified, we have linked them to their relevant fitness-related phenotype by combining several molecular approaches including CRISPR-Cas9 editing. We are currently annotating TEs in several *Drosophila* species to investigate their role in evolutionary innovations in this morphologically, ecologically and behaviorally diverse genus.

PL – 04 Plenary lecture

ALKALINE COMET AND MICRONUCLEUS CYTOME ASSAY – THE USE OF THE METHODS AND THEIR PERSPECTIVES THROUGH THE RESULTS OF THE HCOMET, HUMNAP, EDIAQI, AND BIOMOLTOX PROJECTS

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The alkaline comet assay, or single-cell gel electrophoresis, is one of the most popular methods for assessing DNA damage, and for further evaluation of genomic instability micronucleus assay (now evolved into cytome assay) has been mostly used. Although both assays are well known, with their OECD guideline protocols for some cell types, there are still open issues concerning possible new applications, the use of the assays, and even different types of samples e.g. in human biomonitoring. There are concerns about identifying factors that explain the large inter-individual and inter-laboratory variation along with standardization and harmonization among laboratories and diagnostics. Here we will explain how various projects have addressed these issues through collaborative initiatives such as the hCOMET in which many new standards and protocols have been made, with establishing a database of 19, 320 subjects with pooled data from 105 studies run by 44 laboratories in 26 countries between 1999 and 2019 that helped to establish reference values and to examine the effects of different factors. We will give an overview of using both assays in human biomonitoring studies accounting for air pollution factors from HUMNap and EDIAQI projects. Also, we will present biomonitoring results from Work Package 5 of the BioMolTox project. We will even give a brief overview of the results and conclusions of the international 52nd Congress of the European Environmental Mutagenesis & Genomics Society and Croatian Genetic Society that will be held a week before this congress in Rovinj, Croatia under the title “Fundamental genomics. New approaches. Solutions for the changing environment and human health.

PL – 05 Plenary lecture

EXPLORING PLANTS AMAZING CAPACITY TO THRIVE UNDER STRESS TO PROTECT OUR GENOMES

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Genomes are exposed permanently to stress imposed by the environment and by the cellular oxidative metabolism. Organisms have developed molecular repair mechanisms involving specific enzymes such as glycosylases, nucleases, polymerases and ligases, allowing efficient removal of damage. However, if damage is extensive, the molecular repair mechanisms are overcome, and DNA damage can be converted into mutations and possibly induction of malignancy. The highly developed secondary metabolism of plants is responsible for the high diversity of metabolites with relevant biological activities. Antioxidant activity is a common feature in secondary metabolites, allowing to potentially protect genomes from oxidative damage. However, while antioxidant activity often coincides with antigenotoxicity in many extracts, antigenotoxicity can also stem from the direct neutralization of genotoxicants or the enhancement of DNA repair mechanisms. Being fixed to the substrate, plants have developed stress responses allowing to cope with environmental stress. Therefore, it is probable that plants surviving in extreme environments have evolved secondary metabolism providing effective stress protection. Among many examples of stress-resistant plants, halophytes from the seashore are particularly resistant to salinity and intense solar radiation. The presence of the secondary metabolites (+)-catechin, (-)-epicatechin and their oligomers, and myricetin derivatives has been associated to antigenotoxicity in extracts from stress-resistant plants. Assessment of DNA single and double strand breaks using the comet assay and the yeast DEL assay, respectively, together with mutagenicity tests have disclosed remarkable antigenotoxic activities of extracts from stress-resistant plants, including halophytes, highlighting the pharmacological potential of plants adapted to extreme conditions.

PL – 06 Plenary lecture

FUTURE OF GENETICS IN MEDICINE

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The rapid evolution of genetic technologies over the past decade has dramatically enhanced our understanding of the role of pathological genetic variability in the etiology of human diseases. These advancements have significantly impacted early diagnosis, individualized treatment, and prevention across nearly all medical fields, ushering in revolutionary changes in medical practice. However, health systems often resist swift, radical changes and face challenges such as underfunding. Integrating genomics into medical practice is a complex undertaking that requires coordinated efforts from genetic professionals, national professional societies, and other key stakeholders, including non-genetic healthcare professionals, patients, and policymakers. Developing a comprehensive national strategy tailored to the specific needs of individual health systems is essential. The responsibility and initiative for this integration primarily rest with geneticists, who must lead these efforts to ensure the successful implementation of genomic medicine.

PL – 07 Plenary lecture

USE OF PHENOMIC AND GENETIC DATA IN BUCKWHEAT BREEDING – ECOBREED EXAMPLE

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The marine environment is one of the most diverse habitats on the Earth, it shows singular environmental conditions, these features stimulate the inhabitants to activate unusual metabolic pathways.
Natural products are the most prolific source of bioactive compounds, more than 100 natural products or molecules synthetized on natural products scaffolds are in clinical trials, particularly as anti-cancer agents and anti-infectives.
Last decades have been characterized by an increasing development of the Multi-Drug Resistant (MDR) bacteria to many antibiotics present on the global market, leding us to the need of new antibiotics.
Our team has managed to isolate several bacteria of diverse taxa from sediments of Ria Formosa's lagoon, Portugal. One of the most interesting isolated bacteria was identified to be a *Vibrio* sp., which showed a high 16S rRNA gene sequencing similarity with *Vibrio spartinae*. Our analysis revealed the presence of prodigiosin and cyclo prodigiosin as major metabolites, followed by many other peaks belonging to prodigiosin-like molecules. The presence of these secondary metabolites, in the past has been often mistaken for blood droplets on bread, in fact observations of prodigiosin formed the bases for the Miracle of Bolsena in 1263.
The family of natural red pigments, called prodigiosins, is characterised by a common pyrrolyl pyrromethene skeleton and a deep-red colour. Prodigiosin is the most known component of this family, its wide range of biological activities includes antimalarial, antifungal, immunosuppressant and antibiotic activities, but it has recently received renewed attention for its anticancer effect against many cancerous cell lines, showing a very lower cytoxicity on the normal cell lines.
The objective of this talk will be to tell more about this family of natural products, searching for the best condition for their production and going through the discovery of new prodigiosin-derivatives.

Acknowledgements: This research was supported by the European Union's Horizon 2020 Project ECOBREED - Increasing the efficiency and competitiveness of organic crop breeding under grant agreement number 771367.

BUCKWHEAT, GENETIC RESOURCES, BREEDING, MAS

PL – 08 Plenary lecture

APPROACHES AND ACHIEVEMENTS IN GRAPEVINE BREEDING

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The grapevine (*Vitis vinifera* L.) is one of the most important economic plants cultivated in almost all continent. Today there are around 8 million hectares of vineyards worldwide. About 71% of grape production is used for wine, 27% as fresh grapes and 2% as dried grapes or processed into juice. Consuming an adequate amount of grapes, as well as wines rich in nutrients and bioactive compounds, is considered necessary for a healthy human life. Grapevine germplasm is quite diverse and consists mainly of *Vitis vinifera* varieties (about 13,600), followed by interspecific hybrids (about 8,300) and varieties of species other than *Vitis vinifera* (about 1,500). Today, about 6,000 grapevine varieties are really grown. The most widely cultivated variety in the world is Kyoho, followed by Cabernet Sauvignon, Sultanina and Merlot. Climate change and the rapid adaptation of invasive pathogens are putting constant pressure on breeders to develop improved varieties. The objectives of grapevine breeding vary according to its usage and are often region-specific. Most breeding programs aim to combine high yields and high grape quality with improved resistance to diseases and pests and/or increased adaptation to adverse abiotic factors. A larger number of grapevine traits are inherited in a complex way and the breeding process usually takes 15 to 20 years. Various methods are used to incorporate useful traits, including the traditional approaches of hybridization, mutation and selection. Biotechnological approaches such as tissue culture, OMIC technologies, new DNA technologies and genome editing provide precision and aim to shorten the breeding cycle in grapevine. The achievements of many breeding programs resulted with numerous new, economically important grapevine varieties which will ensure a new era in the cultivation of this ancient species.

VITIS VINIFERA L., NEW VARIETY, HYBRIDIZATION, SELECTION, BIOTECHNOLOGY TECHNIQUES

PL – 09 Plenary lecture

ISOLATION OF BAMMV/BAYMV BARLEY RESISTANCE GENES ALLOWS THE GENE-EDITING BASED DISEASE MANAGEMENT AND REDEFINITION OF THE GENE ORIGIN

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The viruses of the barley yellow mosaic complex (Barley mild mosaic virus (BaMMV) and Barley yellow mosaic virus (BaYMV) are the most important soil-borne viral pathogens of winter barley. So far, 22 resistance genes against barley yellow mosaic disease have been reported, of which up to now, two recessive resistance genes have been isolated rym4/5 and *rym1/11* and several like *rym13*, *rym15* and r*ymHOR4224* are under the cloning procedure. Although a number of BaMMV resistance genes were identified, resistance of some genes has been broken by new virus strains/isolates. Therefore, mapping and isolation of effective resistance genes is a genuine need for sustainable barley production.

Recently, in order to develop an alternative measure against BaMMV/BaYMV complex we used gene-editing approach to generate new alleles of *rym4/5* and *rym1/11* in winter and spring barley, circumventing the tedious and time-consuming introgression by crossing to current elite lines. We induced knockouts as well as new functional alleles with base substitutions for *rym4/5-HvEIF4E* and *rym1/11-HvPDIL5-1* similar to those that were described to render certain landraces resistant.

During isolation of further two resistance genes *rym13* and *rym15* KASP assays were used for genotyping of the diagnostic set. Alleles of resistant genotypes from the East Asia showed presence of the same alleles. At the same time, presence of same alleles at local landraces MBR530 and MBR532 from Montenegro indicated a simultaneous bi-phyletic origin of both resistance gene. These KASP markers could be used in direct transfer of *rym13* and *rym15* resistance genes in elite barley cultivars.

BARLEY, BAMMV, RESISTANCE GENE ISOLATION, GENE EDITING

PL – 10 Plenary lecture

GENETICS OF EARLY PREGNANCY LOSSES

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Pregnancy loss (PL) is one of the most common complications of pregnancy, affecting approximately 15% of couples trying to conceive. Early pregnancy loss (EPL) occurs before the 12th week of gestation, while recurrent pregnancy loss (RPL) is defined as the loss of two or more pregnancies. Both EPL and RPL present complex challenges in reproductive medicine, causing significant frustration for patients, their families, and healthcare teams. Chromosomal abnormalities, such as trisomies, monosomy, and polyploidy, are the most common causes of EPL. However, up to 50% of patients with RPL have no clearly defined etiology.

This presentation will summarize the known genetic causes of EPL, as well as current approaches and practices in genetic testing for EPL. I will present our study on the incidence and spectrum of chromosomal abnormalities in EPL and their correlation with various clinical characteristics. Additionally, I will discuss association studies conducted on our patient cohort that investigate the role of selected genetic variants in genes related to thrombophilia, angiogenesis, vascularization, and several variants recently identified as strongly associated with RPL in genome-wide association studies (GWAS). Finally, our recent research on the role of rare monogenic disorders as contributing factors to EPL, along with proteomic biomarkers and pathways that could lead to targeted interventions and improved diagnostic tools for managing this condition, will also be presented.

PL – 11 Plenary lecture

NEW INSIGHTS INTO UNIPARENTAL DISOMY

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In 1980, Eric Engel, a cytogeneticist who worked mainly in Switzerland came up with the idea of uniparental disomy (UPD). As it lasted until 1987 until the first proven UPD-case was published, Dorothy Warburton then concluded that UPD might be a rare but not really relevant phenomenon in clinical genetics. Nowadays, thousands of inborn and acquired UPD cases are reported. Disease causing UPD has been linked with activation of recessive gene-mutations and imprinting disorders and mosaic trisomy.

Relevance of UPD, which is seemingly always due to a cytogenetic imbalance / rearrangement, for clinical and tumor genetics is reviewed here. Besides, it is highlighted that it is possible to achieve yet inaccessible insights into early embryogenesis by looking into constitutional UPDs. Accordingly, it is possible to carefully calculate the overall frequency in human population born from an original trisomic embryo as 0.1%, and the rate of successful monosomic rescue as 0.02%. It is also for the first time possible to suggest approximate frequencies of patients affected by imprinting diseases due to UPD, and to narrowed down the timeline when trisomic rescue is taking place to 2 to 7 days for the majority of the cases.

The underlying data is summarized on https://cs-tl.de/DB/CA/UPD/0-Start.html and also put together in Liehr T, All you need to know about uniparental disomy - UPD and imprinting, Epubli, 2024,ISBN 978-3758465581.

PL – 12 Plenary – Closing lecture

rRNA OPERON MULTIPLICITY AS A BACTERIAL GENOME STABILITY INSURANCE POLICY

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The multiplicity of rRNA operons per bacterial genome exceeds the requirements for maximal growth rates under non-stress conditions, but it becomes insufficient to ensure rapid growth restart after ribosome-disrupting stresses. It is commonly assumed that the extended time needed to resume growth after such stressed is determined by how long it takes for cells to synthesize enough ribosomes to produce the proteins necessary for reinitiating growth. However, we showed that prolonged growth recovery primarily stems from elevated mortality rates caused by DNA replication blockage and massive DNA breakage at the sites of the rRNA operons overloaded with RNA polymerases (RNAPs). We have found that mortality rates and growth recovery duration can be mitigated by preventing R-loop formation, enhancing DNA repair capacity and increasing rRNA operon multiplicity. We propose that the lower limit of the rRNA operon multiplicity for a given bacterial species is determined by rRNA copy number, which ensures that individual rRNA operons remain unsaturated by RNAPs during environmental fluctuations. On the other hand, the upper limit of rRNA operon multiplicity is determined by the trade-off between increased capacity to withstand growth perturbations during environmental fluctuations and deleterious consequences of the repartition of RNAPs between rRNA operons and protein coding genes under non-stress conditions.

TOPIC 1

Population genetics and genomics

VII CONGRESS OF THE SERBIAN GENETIC SOCIETY

01 – 01 Invited lecture

B CHROMOSOMES – NATURAL POPULATION STUDY

Jelena Blagojević [1], Saša Malkov [2], Marija Rajičić [1], Ivana Budinski [1], Branka Bajić [1], Milan Miljević [1], Aleksa Rončević [1], Mladen Vujošević [1]

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B chromosomes (Bs) are non-essential genomic elements that have been studied for over a century. They occur in plants, animals, and fungi with varying frequency, depending on the species, populations within a species, and even tissues within an individual. In the last decade, significant progress has been made in understanding their structure and overcoming the long-lasting belief that they are genetically inert.

In natural populations of the yellow-necked mouse, *Apodemus flavicollis*, individuals with B chromosomes are found, on average, in one-third of the population. The frequency within populations tends to remain stable year to year, although it can vary significantly with the seasons. Higher frequencies of Bs have been observed in populations at higher altitudes, suggesting potential adaptive significance. However, there are considerable oscillations in the frequency of individuals with Bs among closely located populations.

To explain this phenomenon, we developed a mathematical model based on our collected data. Various scenarios were tested, the results of which will be presented here.

B CHROMOSOMES, *APODEMUS FLAVICOLLIS*, POPULATION STUDY

01 – 02 Invited lecture

THE GENETIC VARIABILITY OF ROMA POPULATION IN SERBIA

Milica Keckarević Marković [1], Vanja Tanasić [1], Milica Mihajlović Srejić [1], Marija Vuković [1], Miljana Kecmanović [1], Dušan Keckarević [1]

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According to linguistic, cultural, historical and, later, genetic studies, the Roma population comes from Southern Asia, mainly North-Western India. Their migrations from homeland started in 5th century, going through Persia (the Iranian plateau) to the west via Armenia and Anatolia in parallel. The first written record of Roma people in Europe, Balkan Peninsula, dates in 12th century, but the most of them come with the invasion of Ottoman Empire in 14th century.

The genetic structure of Roma people in Europe was shaped by series of founder effects and bottlenecks, and reflects both the ancient and the recent historical events.

In Serbia, Romani groups could be differentiated based on, mostly overlapping, demographic and religious criteria. The results from analysis of autosomal STRs suggest a present substructuring among different groups, with the most abundant gene-flow between Orthodox Roma and cohabiting self-declared Serbian population. Catholic and Muslim Roma have less admixture with the autochthonous Serbian population. Results, also, suggest a substantial barrier among Roma people of different religion. Based on uniparental genetic markers, besides confirming the South Asian origin of Romani people and the traces of migratory routes, results suggest gender-biased gene-flow to Roma populations, with man gene-flow lower than female. As expected, the highest gene-flow from autochthonous population was found to be in Orthodox Roma groups.

Our results suggest that, although there is substructuring of Romani groups in Serbia, Romani people are not completely isolated, but rather admixed, with mostly religious constraints.

AUTOSOMAL STR, MTDNA, Y CROMOSOME, ROMA, POPULATION

01 – 03 Invited lecture

DNA BARCODING AND POPULATION GENETICS OF NON-MODEL PLANT SPECIES: APPROACHES, PROBLEMS AND POSSIBLE SOLUTIONS

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When trying to grasp the molecular background of a plant taxon, the first challenge is its correct identification. However, the traditional taxonomy of plant species is mainly based on morphological characteristics and often provides insufficiently accurate conclusions. This can be overcome by the inclusion of genetic data using the DNA barcoding method, which ensures reliable identification of plant species. Since a universal DNA barcode sequence has not yet been established for plants, four chloroplast regions (*matK*, *trnL-F*, *rbcL* and *psbA-trnH*) have been widely used in combination with genomic *ITS* regions as a part of the DNA barcoding approach. This allows the comparison of the obtained sequences with the reference data and the unambiguous determination of the species as well as an insight into their mutual phylogenetic relationships. At the species level, DNA barcoding can be used to characterize new, unknown species, cryptic species, or to distinguish between two or more species previously classified as one species. High frequency of polyploidy and hybridization among plant taxa sometimes makes it difficult to accurately identify plant species. The development and availability of DNA databases as well as transcriptome sequencing techniques have made it possible to find microsatellite markers within transcriptional sequences (EST-SSR) that can be used to assess variations within and between plant populations. This allows for delving into the population genetic research of plant species that do not represent model organisms.

DNA BARCODING, POLYPLOIDY, HYBRIDIZATION, EST-SSR MARKERS

01 – 04 Oral

GENOME-WIDE PHYLOGENY OF BLIND MOLE RATS

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The blind more rats (BMRs) of the subfamily Spalacinae (genera *Spalax* and *Nannospalax*) are found in a large area surrounding the Black Sea, including Anatolia and Levant and extending to Western Balkans and the Eastern European plain. We have used partial mtDNA and genome-wide ddRAD-seq markers to reconstruct a robust phylogeny across the entire geographic range of BMRs. A novel species delimitation approach applied at the level of the subfamily revealed the presence of the five main MOTUs (molecular operational taxonomic units): (1) all species of large-bodied BMR, i.e. genus *Spalax*, (2) the Palestine BMR (*N. ehrenbergi*), (3-4) two ancient lineages in Anatolia, one of them predating the split of the Lesser BMR (*N. leucodon*) found in Europe and (5) *N. leucodon*. The currently recognized Anatolian BMR (*N. xanthodon*) is therefore clearly not monophyletic: in order to correct this discrepancy, we propose to use the available taxon name *Nannospalax cilicicus* (reinst.stat.) for the separate lineage endemic to central parts of Anatolian peninsula, while keeping the name *N. xanthodon* for the remaining Anatolian populations which are sister to *N. leucodon*. An even more conservative delimitation approach revealed three MOTUs (*Spalax* sp., *N. ehrenbergi* and the remaining Anatolian and European *Nannospalax*). We briefly discuss the utility of the various approaches to taxonomic characterization, especially problematic in the case of BMR. Interestingly, we found that the current geographic ranges of many deeply divergent lineages do not always correspond to the major geographic landforms. The two main emerging motifs are (1) a high degree of relictualism in most lineages, which currently possess small fragmented ranges and high levels of genetic polymorphism, and (2) more recent expansion of a few lineages that currently have large continuous ranges but exhibit low levels of genetic variation.

BLIND MOLE RATS, *SPALAX, NANNOSPALAX,* RAD-SEQ, MTDNA

01 – 05 Oral

THE FIRST GENOME-WIDE CHARACTERIZATION OF GENETIC DIVERSITY AND POPULATION STRUCTURE OF HOVERFLY SPECIES BASED ON DDRAD-SEQ DATA – CASE STUDY ON *MERODON ARMIPES* RONDANI

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Members of the family Syrphidae are recognized as prominent wild pollinators, not only from the perspective of biodiversity conservation, but also for pollination of agricultural crops. Considering the threat of pollinator decline to seriously affect ecosystem function and food security, their conservation has become of utmost importance, entailing the accurate taxa identification in order to establish a long-term monitoring strategy. By providing a comprehensive insight into multiple loci across both nuclear and mitochondrial genomes, genome-wide approaches have great potential to elucidate different processes and distinct evolutionarily significant units. Therefore, here we analyze a panel of genome-wide ddRAD-seq data, with the aim of assessing genetic diversity and clarifying a possible structure within *Merodon armipes*, a widespread species from the *Merodon ruficornis* group, whose range extends from northeastern France, across Central Europe and the Balkans, to the Anatolian Peninsula in the east. Our results revealed a clear separation between Anatolian and European populations, indicating diversification and presence of population with independent evolution. Moreover, a pattern of spatial genetic clustering with signals of admixture in the contact zone has been observed among European populations, whereby specimens from Western Europe and continental area of the Balkan Peninsula show differentiation from populations present on the Apennine Peninsula and the Balkans Adriatic Coast. However, since there are no differences in morphological characteristics between revealed populations, further confirmation should be obtained by morphometric analysis. Considering the lack of consistent and reliable diagnostic markers for the assessment of hoverfly management units, especially within the genus *Merodon* and the *M. ruficornis* group of species, the present study emphasizes the utility of ddRAD-seq as a valuable molecular tool for understanding diversity of this important pollinators group.

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SYRPHIDAE, POLLINATORS, CONSERVATION, SINGLE NUCLEOTIDE POLYMORPHISMS, NGS

01 – 06 Oral

GENETIC VARIABILITY AND POPULATION STRUCTURE OF EUROPEAN ROLLER (*CORACIAS GARRULUS*) FROM SERBIA

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The European Roller (*Coracias garrulus* Linnaeus, 1758) is an insectivorous, long-distance migratory bird that winters in sub-Saharan Africa. Over the past few decades, the number of breeding pairs of rollers has drastically declined across Europe, including Serbia. However, due to conservation measures and the installation of nesting boxes, the population of the European Roller in Serbia has recovered in the last decade. In this study, we used 10 microsatellite loci to assess genetic diversity, structuring and effective population size within the roller population in Serbia. Genetic variability parameters were calculated using Genetix and ARLEQUIN, while relatedness analyses were conducted using ML-RELATE. STRUCTURE and MEMGENE were used to assess the presence of genetic structure and spatial patterns in genetic structuring, respectively. Effective population size was estimated using LDNe software, and the genetic bottleneck effect was tested using Bottleneck software. We detected a moderate level of genetic diversity (HO=0.392) and a slightly increased level of inbreeding and homozygosity (FIS=0.393), with a high level of related individuals. Genetic structuring suggests the presence of three genetic clusters, but without a clear spatial pattern. The estimated effective population size closely corresponds to the current number of breeding pairs recorded based on the monitoring and ringing data. No signs of recent bottlenecks were detected. Our results emphasize the importance of artificial nest boxes for promoting and maintaining population dynamics of European Rollers in Serbia.

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GENETIC VARIABILITY, EUROPEAN ROLLER, MICROSATELLITES, SERBIA

01 – 07 Oral

eDNA METABARCODING OF FLOWERS

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Environmental DNA (eDNA) represents a complex mixture of genomic DNA from many organisms present in environmental samples. The advancements in technologies provided tools to identify the taxa present in an eDNA. eDNA metabarcoding is proved to be a powerful tool for biodiversity assessment and monitoring from various environmental samples. The goal of this study is to assess invertebrate communities linked to flowering plants, and explore local species occurrences and communities. Field work was conducted at one selected site, Glavica (Serbia) across three seasons, spring, summer and autumn. In total, 180 samples were collected. Environmental DNA was extracted using ExtractMe Genomic DNA kit (Blirt), followed by quality and quantity assessment. mtDNA *COI* gene fragment of total length 313bp was amplified. Amplicons were sequenced using Illumina platform, PE 2x250bp. After quality control of the raw reads in FastQC, fastq files were processed with APSCALE pipeline v1.6.3. Clustering and denoising sequences approaches were applied, retained OTUs and ESVs were taxonomically assigned against the BOLD database using BOLDigger and the processing was performed using TaxonTableTools. The larger number of taxa was obtained by denoising approach. Species from following insect orders were detected Hymenoptera, Hemiptera, Coleoptera, Diptera, Lepidoptera, Orthoptera, Thysanoptera, Blattodea and Psocodea, as well as from classes Arachnida, Copepoda, Clitellata, Malacostraca, Collembola, Polychaeta, Gastropoda and Nematoda. Produced data are planned to be further explored for characterization of invertebrate communities across seasons and comparison with faunistic records at the same pilot site.

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EDNA, COI GENE, BIODIVERSITY ASSESSMENT

01 – 08 Oral

Y-CHROMOSOMAL LANDSCAPE IN SERBIAN POPULATION GROUPS ORIGINATING FROM HISTORICALLY AND GEOGRAPHICALLY SIGNIFICANT DISTINCT PARTS OF THE BALKAN PENINSULA

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Historically and geographically significant regions in the Balkan Peninsula inhabited by Serbs, region of Old-Herzegovina and Kosovo and Metohija, were the origin of the largest migration waves towards present-day Serbia that occurred between the 15th and the 18th century. Within this study 1200 Serbian males originating from these three regions were genotyped using the combination of 23 Y-STRs and 39 Y-SNPs in order to distinguish the genetic structure, diversity, haplogroup frequencies and genetic relationship among them. High haplotype diversity and discrimination capacity was observed in all three datasets. Haplogroup I-P37.2 was the most dominant in all three regions, in major subclade I-PH908 followed by subclade I-Z17855. Deeper SNP typing distinguished subclade I-FT14506 as the most frequent in the Kosovo and Metohija dataset, while subclade I-FT16449 was the most frequent in the Old Herzegovina dataset. In the present-day Serbia dataset, more equal occurrence of subclades I-FT14506 (40.2%) and I-FT16449 (34.4%) was detected. Low rate of genetic differentiation was detected between all three datasets, with the lowest level identified between Old Herzegovina and present-day Serbia regions. These results were confirmed, both in the phylogeographic and haplotype sharing analysis within haplogroup I-PH908. Here presented results support the historical thesis that migrations from regions of Old Herzegovina and Kosovo and Metohija had great contribution to the present-day Serbian population genetic structure. Additionally these results give insight into geographic distribution of detected haplogroups I-Z17855, I-Y4460, I-PH908, I-Y5596, I-Y4882, I-FT14506, I-FT16449 and I-A5913, enabling further improvement of the geographic resolution of paternal ancestry inference.

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Y-STR, Y-SNP, SERBIAN POPULATION, MIGRATIONS

01 – 09 Oral

SOIL INVERTEBRATE DIVERSITY SURVEY: THE CHOICE OF eDNA METABARCODING PRIMERS

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The development of the eDNA metabarcoding method has significantly facilitated studying and monitoring of soil invertebrates. This method enables the identification of multiple organisms from an environmental sample by sequencing targeted DNA regions. In the present study, the eDNA metabarcoding method was used for the detection of soil invertebrates in the solonchak soil type and the efficiency of two primer pairs was tested. The soil samples were taken from two agricultural fields (soybean field and maize field) in Rančevo (Serbia). In total, 10 soil samples were collected and DNA extraction was done using DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany). Fragments of the *COI* gene were amplified using two pair of primers (BF2/BR2; mlCOIintF/dgHCO2198) and sequenced using Illumina Miseq. The quality of the raw sequencing reads was checked in FastQC and the fastq files were processed with APSCALE pipeline in order to obtain Operational Taxonomic Units (OTUs) and Exact Sequence Variants (ESVs). Retained OTUs and ESVs were taxonomically assigned using BOLDigger against the BOLD database. Downstream processing of the datasets was performed using TaxonTableTools (TTT). Our results showed that the primer pair mlCOIintF/dgHCO2198 is more efficient in detection of soil invertebrates and resulted in significantly higher number of taxonomically assigned OTUs and ESVs to the expected invertebrate phyla, relative to the primer pair BF2/BR2. This study shows that it is necessary to test the efficiency of different primers to obtain more accurate information about the diversity of the studied group of organisms using the eDNA metabarcoding method.

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SOLONCHAK, ENVIRONMENTAL DNA, SOIL INVERTEBRATES

01 – 10 Poster

HYBRIDISATION OF THE *CANIS* GENUS IN BOSNIA AND HERZEGOVINA

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The (re)colonization of Europe by wolves (*Canis lupus*) and jackals (*Canis aureus*) raises significant ecological and conservation concerns, particularly regarding hybridization. Ongoing studies across Europe are providing an increasing amount of data on these phenomena, with the genus Canis serving as the primary model for understanding them. In Bosnia and Herzegovina, research on hybridization within the genus *Canis* has been limited, despite the country being a central area of the large Dinaric-Balkan wolf population and experiencing rapid growth in the jackal population. Therefore, our study aimed to address these issues by analyzing fifteen microsatellite loci in 131 individuals, including wolves (n=70), jackals (n=48), and free-ranging dogs (n=13). Our results showed: i) a lower genetic diversity in jackals compared to wolves and dogs, ii) a clear differentiation of all three species into separate clusters, with a notable west-east gradient in wolves, and iii) a low degree of hybridization, mainly with dogs. The previously observed range overlap increases the likelihood of future hybridization between jackals and wolves and underscores the need for further studies. Our study is the first to provide insights into the evolving dynamics of the *Canis* genus in Bosnia and Herzegovina, contributing to a broader understanding of their hybridization and coexistence in the wider region.

BOSNIA AND HERZEGOVINA, CANIS GENUS, HYBRIDIZATION, MICROSATELLITES

01 – 11 Poster

UTILIZATION OF DDPCR METHODOLOGY FOR EDNA TARGET SCREENING WITHIN THE JOINT DANUBE SURVEY 5

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Taxon-specific environmental DNA (eDNA) surveillance based on droplet digital PCR (ddPCR) has proven to be a highly sensitive approach for the detection of species with insufficient population status data obtained with traditional monitoring.
Our research aims to pioneer the implementation of this methodology in the Danube River Basin (DRB), as part of the fifth Joint Danube Survey (JDS5) workflow, supported by the International Commission for the Protection of the Danube River (ICPDR). These river expeditions aim to harmonize water monitoring practices across Danube countries, according to the EU Water Framework Directive (WFD).
We will develop novel taxon-specific ddPCR assays for up to 10 species with well-documented ecological and conservation concerns, including endangered sturgeons and ecologically threatening invasive crayfish species. Water samples will be collected along the entire DRB, with the purpose of providing up-to-date information on the state of Danube's biodiversity.
Research results will be compared with the traditional monitoring and metabarcoding datasets obtained by the other JDS5 research groups. Based on their estimated significance, research results will serve as a basis for future regular distribution assessments and planning of conservation measures both in the Danube and in other watercourses inhabited by the species concerned.

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EDNA, DDPCR, DANUBE, BIODIVERSITY, JDS5

01 – 12 Poster

ASSESSING MITOCHONDRIAL GENETIC DIVERSITY OF *MINIOPTERUS SCHREIBERSII* IN SERBIA

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The genetic diversity of the Schreiber's bent-winged bat *Miniopterus schreibersii* has been extensively studied over the years and across its range, using both mitochondrial and nuclear markers. This species has a Mediterranean distribution and is relatively widespread in Europe, where suitable underground roosts exist, including in Serbia. However, in previous studies that showed minimal structuring of populations and identified Anatolia as the only refugium during the last glacial maximum, Serbian samples were often missing.
To fill this gap, we sequenced the fragment of mitochondrial hypervariable region 1 (*HV1*) of *M. schreibersii* from seven localities in Serbia. We aimed to evaluate their genetic relatedness with other populations in Europe and Asia Minor and to determine whether the Balkans have greater genetic diversity than Western Europe, given their proximity to Anatolia.
We identified nine haplotypes in the Serbian samples, six of which were previously unreported, resulting in a haplotype diversity of 0.585. The three haplotypes were shared with bats from Portugal, Greece and Turkey. A single predominant haplotype was found throughout the species' range, indicating a well-connected population. The haplotype network's structure suggested a common origin and a recent population expansion.
These findings improve our understanding of the genetic diversity of *M. schreibersii*, support the notion of a non-structured population and reinforce the hypothesis of an Anatolian origin. This study provides important new data from the Balkans and contributes to a broader genetic picture of this species.

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CHIROPTERA, *MINIOPTERUS SCHREIBERSII*, MTDNA, *HV1*

01 – 13 Poster

THE CURIOUS CASE OF A HYBRID COMMON SWIFT (*APUS APUS*)

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Swifts are highly specialized birds, with three species breeding in Europe and migrating to Africa in winter: the common swift (*Apus apus*), the alpine swift (*Apus melba*), and the pallid swift (*Apus pallidus*). In southern Europe, local breeders include the little swift (*Apus affinis*) and the white-rumped swift (*Apus caffer*). The common swift has a broad breeding range from Spain and Ireland across Europe to Norway and sub-Arctic Russia, extending to China, Siberia, North Africa, and the Middle East, with its migration to Equatorial and sub-Equatorial Africa.
DNA was extracted from the feathers of common swift nestlings in Belgrade, Serbia in 2023 to assess genetic diversity. Genotyping was performed by amplifying and sequencing the mitochondrial *NADH* dehydrogenase subunit 2 (*ND2*) gene and introns of three autosomal genes (locus *12884*, β-fibrinogen intron 7 - *fib7*, and *GAPDH*). The *ND2* sequence confirmed the maternal ancestry of Apus apus. Unexpectedly, the analysis of the *12884* intron sequence pointed to possible hybridization, with two different copies detected: one from *A. apus* and a longer copy suggesting potential hybridization with *A. affinis*, *A. horus*, or *A. pacificus*. The *fib7* and *GAPDH* intron sequences were not informative due to the lack of interspecies differentiation.
Given the species distribution, hybridization most likely involved *A. affinis*, whose breeding range overlaps with *A. apus* in southern Europe, North Africa, and parts of the Middle East. This information gives valuable new insights into the swift population dynamics and interspecies relationships.

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APUS APUS, SWIFTS, GENOTYPING, HYBRIDIZATION

01 – 14 Poster

GENOME-WIDE ASSOCIATION STUDY OF MORPHOMETRIC TRAIT-PELVIC WIDTH OF BUSHA CATTLE FROM SERBIA

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Native breeds are a national asset in every country and their preservation is of great importance. The Busha cattle is one of the two native breeds in Serbia. Difficult calving is a major cause of loss for beef cattle producers. It results in an increased incidence of calf mortality at or near birth, increased cow mortality, and higher veterinary and labor costs. This study aimed to identify significant genetic variants and candidate genes associated with pelvic width (tuber ischii) using a genome-wide association study (GWAS).
36 young cows (2±0.1 years of age) were included in the analysis. The measurement of the pelvic width was carried out on the farm and corresponds to the distance between pin bones (tuber ischii). The blood samples were collected for DNA extraction and genotyped using the Illumina Bovine SNP50 BeadChip. Quality control and association analysis were conducted using PLINK 1.9 software.
Several genetic variants have been detected with association significance of $p<1x10-5$. Based on the vicinity to the identified variants locations, candidate genes were identified, along with their previous associations with cattle traits: TATA-box binding protein Associated Factor 4 (skeletal development and growth), Cyclooxygenase-2 (body size and growth rate, inflammatory response), Utropin (muscle fiber integrity and function) and HHIPL2 (milk quality).
The results of this study could be used for the preservation and sustainable use of Busha breed as an important genetic resource in Serbia.

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GWAS, AUTOCHTHONOUS BREED, BUSHA, MORPHOMETRY

01 – 15 Poster

DIFFERENTIAL EXPRESSION OF HSP70 PROTEIN IN RESPONSE TO COLD SHOCK IN *DROSOPHILA SUBOBSCURA* FROM TWO DIFFERENT ALTITUDES

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Heat shock proteins (Hsp) are crucial for cellular protection under temperature stress conditions, yet their expression dynamics can vary significantly across different biological contexts depending on population history, previous thermal experience, type of thermal stress and even species. Here, we investigated the relative expression levels of Hsp70 protein in response to cold shock in laboratory populations of *Drosophila subobscura* originating from two different latitudes after maintaining them for 12 generations in three laboratory thermal conditions (cold, optimal and warm). Results from our study reveal that females generally exhibit slightly higher Hsp70 expression compared to males following cold shock and that Hsp70 expression varies depending on laboratory rearing temperatures. Interestingly, individuals from the population originating from lower altitude showed a trend of lower expression levels under cold shock, whereas those from the population originating from higher altitude maintained consistent expression levels across treatments. Our findings in *D. subobscura* underscore the complex interplay of genetic background, environmental conditions, and sex in modulating Hsp70 expression, providing insights into adaptive responses to thermal stress at the molecular level.

DROSOPHILA SUBOBSCURA, COLD SHOCK, HSP70 EXPRESSION

01 – 16 Poster

GENETIC DIVERSITY OF HYDATIGERA TAPEWORMS IN EUROPE: MOLECULAR INSIGHTS

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Hydatigera, a revived genus, includes four species: *H. taeniaeformis s.s.*, *H. kamiyai*, *H. parva*, and *H. krepkogorsk*i. These tapeworms mature in felids and viverrids' intestines, with larval stages developing in rodents, and their correct identification requires molecular methods. Understanding their complex life cycles is crucial for public health due to their impact on human and animal health. Given the recent discovery of a cryptic species within this genus and the notable lack of molecular genetic studies, additional research on *Hydatigera* is currently being conducted worldwide. This study aims to assess genetic diversity, distribution, and host suitability of these species. We examined 1.266 small mammals from Serbia and Spain for *Hydatigera* tapeworms and confirmed larval samples by *cox1* and *12S* sequencing. Three species were identified: *H. taeniaeformis s.s.*, *H. kamiyai*, and *H. parva*. The *cox1* sequences of *H. kamiyai* were clustered with European populations and showed significant nucleotide differences (up to 12.9 %) from Asian and African isolates (*H. taeniaeformis s.s.*), indicating different species. *H. kamiyai* showed a high haplotype diversity (Hd = 0.883), in contrast to the low diversity of *H. parva* (Hd = 0.380). The pairwise divergence of *H. parva* from African samples of this species ranged from 1.6 % to 1.9 %. Our study confirms that *H. kamiyai* is a cryptic species within the *H. taeniaeformis s.l.* complex. It is among the few mitochondrial gene-based studies on this complex and the first for *H. parva* in Europe, contributing significantly to the understanding of genetic diversity and host suitability.

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GENETIC, *HYDATIGERA*, RODENTS, TAPEWORMS

01 – 17 Poster

SHOTGUN METAGENOMIC ANALYSIS OF WATER MICROBIAL COMMUNITIES AT SEWAGE DISCHARGE POINT IN THE DANUBE RIVER, NOVI SAD, SERBIA

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Environmental DNA (eDNA) analysis has become a powerful tool for assessing biodiversity and to estimate pollution in various environments, particularly in aquatic ecosystems impacted by industrial and wastewater discharges. Changes in microbial communities in these ecosystems can have serious consequences for human health, including the spread of antimicrobial resistance. This pilot study employed shotgun whole genome sequencing to profile water microbial diversity and detect antimicrobial resistance genes (ARGs) at a sewage discharge point in the Danube River near Novi Sad, Serbia. Total environmental DNA was isolated from collected water samples and sequenced using whole genome shotgun sequencing. Taxonomic analysis revealed a predominance of bacterial taxa with 11 detected classes, among which Clostridia, Gammaproteobacteria, and Bacteroidia were the most relative abundant classes. Five whole genomes were resolved to species level with >80% completeness, most of which belong to fecal and gut microbiota. Functional analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) showed a high number of metabolism-related genes, with carbohydrate metabolism genes and enzymes being the most prevalent, indicating significant sewage pollution. The top 20 ARGs identified by metagenomic sequencing included *vanT*_gene, *vanY_gene*, *vanW_gene*, *msrE*, *mphE*, *adeF*, *tet(W)*, *tet(O)* and *tet(Q)*. These preliminary findings demonstrate the utility of eDNA metagenomic analysis for monitoring microbial diversity, pollution status and antimicrobial resistance in aquatic environments impacted by wastewater discharge, highlighting its potential to mitigate health risks and manage environmental impacts.

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ANTIBIOTIC RESISTANCE GENES, SHOTGUN SEQUENCINQ, SEWAGE

01 – 18 Poster

GENETIC ANALYSIS OF X-CHROMOSOMAL SHORT TANDEM REPEAT (X-STR) ALLELE AND HAPLOTYPE FREQUENCIES IN SERBIAN MALE POPULATION

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Analysis of autosomal STRs is a standard tool in forensic genetics, due to its identification capacity. However, in complex kinship cases, analysis of X-STRs could be used due to the unique structure of the X chromosome and its exclusive method of inheritance.
Analysis of 12 X-STR loci included in the Investigator Argus X-12 kit was performed on a sample of 148 unrelated males from Serbian population aiming to calculate allelic frequencies of the 12 X-STR loci, haplotype frequencies of the four linkage groups and forensic parameters. DNA extracted from buccal swabs was PCR amplified and analyzed by capillary electrophoresis. Statistical analysis and haplotype frequencies for LGs were performed using Arlequin 3.5 software and online tool available at ChrX-STR.org.
The number of alleles in the analyzed loci was found to vary between 5 (DXS7423 and DXS8378) and 22 (DXS10135). The marker DXS10135 was the most informative (PIC 0.927528), while the marker DXS8378 was the least informative (PIC 0.631924). Expected heterozygosities varied from 69.1% to 93.2%. Power of discrimination was calculated to range from 84.5% to 97.9% in females and 69.1% to 93.2% in males. Power of exclusion ranged from 41.4% to 86.1%. The number of detected haplotypes was 124 (LG1), 95 (LG2), 85 (LG3) and 117 (LG4). The most common haplotype was from LG3 with frequency of 4.7%. Linkage disequilibrium was detected within LG3 and LG4.
The results of this study could be used for further population studies and interpretation of forensic casework results.

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X-STR, HAPLOTYPE, LG, POPULATION GENETICS

01 – 19 Poster

TESTING THE EFFECTS OF MOTHERS CURSE HYPOTHESIS ON TWO *DROSOPHILA* SPECIES

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The Mother's Curse hypothesis proposes an accumulation of a male-specific genetic load within the mitochondrial DNA (mtDNA). This is due to selection acting on mtDNA genes only in females because of the uniparental mechanism of mitochondrial inheritance through mothers. Consequently, harmful mutations that affect only males go unnoticed by selection, allowing them to persist and even increase in frequency within the population through genetic drift or even selection if a specific mutation has antagonistic effects between sexes.
While the theoretical framework of the Mother's Curse hypothesis is straightforward, measuring the effects of the Mother's Curse in natural populations has proven challenging, yielding many contradictory results. Most experiments that were aligned with the theory used specially engineered introgression lines that were designed to maximise the effect of male-specific load, minimizing the influence of compensatory nuclear mutations that could mitigate these effects.
Using two model species (*Drosophila subobscura* and *D. obscura*) we conducted life history experiments with sympatric mito-nuclear lines. Measured traits were: desiccation resistance, developmental time, egg-to-adult viability and percentage of males. For each component that showed sex-specific differences, we tested the magnitude of the mitochondrial effects between the sexes to test for signatures of the Mother's Curse. Contrary to theoretical predictions, our results in both model species revealed that mitochondrial variation had a bigger impact on female fitness. These findings suggest a complex interplay between the two genomes and underscore the need for further investigation into the relevance and magnitude of its effects in natural and experimental populations.

MOTHER'S CURSE, *DROSOPHILA*, LIFE HISTORY, POPULATION GENETICS, MTDNA

01 – 20 Poster

MITOCHONDRIAL DNA SEQUENCE VARIABILITY IN WILD BOARS FROM THE NORTHERN SERBIAN PROVINCE OF VOJVODINA

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The wild boar is an opportunistic omnivore and one of the most hunted game species. Historically, the Balkan region served as a refuge for wild boar during the glacial period. Despite a previous decline in wild boar populations in some parts of Europe, they are currently spreading and have become one of the most common ungulates in Europe. Genetic monitoring is an important part of understanding the impact of population size changes on genetic diversity. The aim of this study was to assess the level of genetic diversity in wild boar from Vojvodina and compare it with previous studies using the same marker. A fragment of the mtDNA control region was amplified and sequenced in 86 individuals from eight localities, while population genetic analyses were conducted using the following packages: BioEdit, DnaSP, ARLEQUIN, PopART and Geneland. Five different haplotypes were identified, with haplotype diversity being 0.673. Nucleotide diversity and the average number of nucleotide differences (k) were 0.003 and 1.159, respectively. Spatial genetic analyses indicated the presence of two clusters, named North and Southwest, with an Fst of 0.216. The observed clustering was further supported by AMOVA analysis showing that 21.58% of the variation was due to differences between clusters. Although Tajima's D and Fu's Fs values were negative in the North population, suggesting population expansion, they were not significant. Compared to the previous results of mtDNA variability in wild boars from Vojvodina, comparable values of genetic diversity parameters were detected, suggesting that the population is stable.

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WILD BOAR, MTDNA CONTROL REGION, VOJVODINA, GENETIC MONITORING

01 – 21 Poster

THE ROLE OF miRNAs AND THEIR CANDIDATE GENES IN THE RESPONSE TO CADMIUM EXPOSURE IN *DROSOPHILA MELANOGASTER*

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Cadmium is a toxic, non-essential heavy metal, present in almost all ecosystems mainly due to various human activities. Being one of the most bioaccumulative metals, it can have numerous negative effects on all living organisms, by disrupting cell homeostasis and triggering oxidative stress. Therefore, biological species may demonstrate a specific response to this environmental challenge. It is presumed that a vast network of genes and regulatory factors might drive that response and that certain miRNAs possibly play a central role.
To test this hypothesis and to determine if the response is population-specific, fruit flies (*D. melanogaster*) were collected at the cadmium-polluted and non-polluted sites. These two fly populations were then reared both on standard *Drosophila* medium and medium enriched with cadmium chloride in laboratory conditions. The 3rd instar larvae were then collected in order to sequence small RNA molecules.
Our first analyses of the small RNA sequencing showed four significantly differentially expressed miRNAs. miR-34 was the only down-regulated miRNA in cadmium-treated groups compared to control groups, while the other three were up-regulated. Two of miR-34 target genes, *thor* and *kul*, are involved in response to environmental stress such as heavy metal pollution, affirming its role in adaptation to cadmium toxicity in *D. melanogaster*. These miRNAs were functionally analysed by available software to determine molecular pathways and candidate genes under their post-transcriptional control.

MIRNA, CADMIUM, *DROSOPHILA MELANOGASTER*

01 – 22 Poster

EFFECTIVENESS OF MOLECULAR BARCODES IN PLANT IDENTIFICATION: A CASE STUDY ON THE GENUS *CENTAURIUM* HILL

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More than 20 years, researchers are working on DNA barcoding, a technique in which specific DNA regions are sequenced to identify species. While animals benefit from a "universal" barcode (cytochrome oxidase I, *COI*), plants require barcodes from all the three plant genomes, which makes species identification more difficult. Although different DNA sequences are used for plant barcoding, there is no consensus on the most efficient regions and multiple markers to be recommended. In our study, we evaluated the discriminatory power of five commonly used barcoding regions (*ITS*, *matK*, *rbcL*, *trnH-psbA*, and *trnL-F*) to identify taxa within the genus *Centaurium* Hill: *Centaurium erythraea* Rafn, *C. tenuiflorum* (Hoffmanns et Link) Fritsch and *C. pulchellum* (Sw.) Druce. Comparison of the obtained sequences with those from the GenBank database revealed that *matK* and *trnL-F* were effective in identifying *Centaurium* taxa, while *rbcL* was insufficiently discriminatory for the analyzed species. The analysis of *trnH-psbA* was particularly difficult due to alignment issues. The *ITS* region was very effective for the identification of taxa within the genus *Centaurium*.
The combination of all markers improved the resolution of the distinction between the studied taxa, especially between *C. tenuiflorum* and *C. pulchellum*, which could not be separated by using chloroplast markers alone. The analysis showed that it is necessary to combine *ITS* with chloroplast markers to identify taxa, as introgression and hybridization events are frequent within the genus.
Finally, it is very important to underline the importance of enriching databases with new sequences, as accurate species identification depends on suitable references

DNA BARCODING, *CENTAURIUM* HILL, ITS, CHLOROPLAST MARKERS, SPECIES IDENTIFICATION

01 – 23 Poster

COMPARISON OF LENGTH AND SEQUENCE VARIATION OF 23 AUTOSOMAL STR MARKERS IN SERBIAN POPULATION

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Massively parallel sequencing (MPS) is a breakthrough methodology that has been moving boundaries in various fields of human genetic research. The sequence variation information and simultaneous analysis of greater number of markers obtained by MPS-based analysis add new dimension STR genotyping and in future might even replace the current gold standard in forensic genetics – CE-based length analysis. The main aim of this study was to compare results for standard forensic and population genetic parameters for microsatellite loci obtained in length- and sequence-based analysis.

In this study we performed two types of analysis on the DNA samples for 304 individuals from Serbian population: CE- based analysis using Investigator® 26plex QS kit (Qiagen, Hilden, Germany) and MPS-based analysis using Precision ID GlobalFiler TM NGS STR panel v2 (Thermo Fisher Scientific Carlsbad, CA, USA).

By comparing CE-based and MPS-based results we obtained 99.01% sample, 99.96% locus and 99.98% allele concordance. Usage of MPS technology led to vast increase in number of alleles per locus due to variation in repeat region sequence, SNPs in flanking regions or both. Two SNPs on D16S539 were found among analyzed samples which have no previous occurrence described in European data. Sequence polymorphism and micro-variant alleles had non negligible effect on all forensic parameters as they led to decrease of MP and increases of Hobs, PE, PD, PIC and TPI.

Our results point out MPS as more informative and powerful technology than CE and emphasize the need for further studies which will ultimately lead to its implementation in ordinary forensic casework.

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POPULATION GENETICS, AUTOSOMAL STR

TOPIC 2

Evolutionary and conservation genetics

VII CONGRESS OF THE SERBIAN GENETIC SOCIETY

02 – 01 Invited lecture

POPULATION GENETIC STUDIES OF THE *CANIS* GENUS IN BOSNIA AND HERZEGOVINA: CHALLENGES AND ACHIEVEMENTS

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In Bosnia and Herzegovina, comprehensive monitoring of the *Canis* genus has been conducted over the past few years using a range of field and genetic methods. The main aim of this monitoring was to determine species presence, activity patterns, territorial interactions, and the genetic status of their populations. Key field methods have included the use of photo-traps and drones, which have been essential for assessing species distribution, behavior, and territorial overlap. Genetic analyses have shown high genetic variability in dog and wolf populations, in contrast to the low genetic variability observed in jackals. Analyses of population structure have revealed a west-to-east gradient in wolves, while no significant population structure has been observed in jackals and dogs. Subsequent analyses using single nucleotide polymorphisms (SNPs) have supported the above microsatellite-based results and confirmed the observed patterns of population structure. Ongoing research continues to focus on understanding the territorial overlap between these species, particularly given the current expansion of wolves and jackals in Europe and the potential for hybridization. Our findings to date have revealed a significant increase in overlap between wolf and jackal territories, as well as evidence of hybridization between dogs and wolves. These decade-long studies have been instrumental in identifying key challenges within *Canis* populations and have facilitated the development of targeted management strategies, particularly for wolves, to ensure their long-term survival and ecological balance in the country and region.

BOSNIA AND HERZEGOVINA, *CANIS* GENUS, CONSERVATION, HYBRIDIZATION, POPULATION GENETICS

02 – 02 Invited lecture

HARNESSING GENETIC-BASED PEDIGREES FOR THE CONSERVATION OF THE AUTOCHTHONOUS BALKAN SHEEP BREED (PRAMENKA) AND ITS LOCAL VARIETIES

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Sheep breeding in the Balkan Peninsula has a rich tradition, with the Pramenka breed being the most common sheep breed in the farms until recently. Adapted to diverse landscapes and microclimates, Pramenka diversified into various local populations, each known for unique morphological and biological traits. In Serbia, eight varieties of Pramenka exist: sjenička, svrljiška, pirotska, krivovirska, lipska, karakačanska, bardoka, and vlaška vitoroga, each with distinct features. However, not all varieties meet modern production criteria or bring enough economic benefit for sheep breeders, leading to disparities in their population sizes and conservation statuses since some breeds are more desirable than others. Efforts to preserve and manage these varieties necessitate evaluating their genetic diversity and establishing pedigrees using genetic markers. A genetic tool and database are being developed for this purpose in Serbia, aiming to provide valuable information for adequate flock management.
DNA from 400 animals, averaging 50 per local variety, was extracted and analyzed using 30 STR markers across 23 chromosomes. The detected variability of analyzed loci was used to populate this new database. The accompanying software enables breeders to browse the database and assess the genetic relatedness avoiding inbreeding when selecting animals for breeding.
Focusing on genetic-based pedigrees, this database and software facilitate the effective management of sheep flocks, particularly in conserving breeds and their varieties recognized as important genetic resources. The initiative supports the sustainable preservation of local Pramenka varieties, ensuring their continued contribution to agricultural biodiversity and resilience in the face of changing agricultural practices and environmental conditions.

AUTOCHTHONOUS SHEEP BREED, PRAMENKA, CONSERVATION, GENETIC-BASED PEDIGREES

02 – 03 Oral

HORNY BUT BARREN: MITOCHONDRIAL GENOTYPE ASSOCIATED WITH REDUCED FERTILITY INCREASES MATING SUCCESS RATE IN MALE SEED BEETLES

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Due to non-Mendelian, maternal inheritance, mitochondrial genomes are shaped by natural selection acting only on females. This evolutionary phenomenon results in accumulation of mutations that are detrimental for males, but benign for females, and is known as the Mother's curse. The sex-specific effects of these mitochondrial mutations are expected to occur in traits that are more energy demanding in males, such as gamete production or mating behavior.
Previously, we identified three different mitochondrial genotypes in laboratory populations of the seed beetle (*Acanthoscelides obtectus*) by sequencing all 13 mitochondrial protein-coding genes. One of them, MG3b, which is characterized by six amino-acid substitutions, specifically reduces male reproductive output and decreases the activity of all OXPHOS complexes compared to other, control genotypes (MG1a and MG1d).
In this study, we wanted to evaluate whether the reduced fertility of MG3b males is due to their passive reproductive behaviour. To test this, we expressed MG3b and two control genotypes alongside the same outbred nuclear background and measured the mating success rate, start and duration of copulation of males with specific genotypes when crossed with wild-type females.
Our results show that MG3b males have a significantly higher mating success rate compared to MG1a and MG1d males, while start and duration of copulation are the same for all genotypes. This indicates that the lower reproductive output of MG3b males is likely due to impairments in reproductive tissue caused by decreased OXPHOS function, while the higher mating rate could be a compensatory mechanism.

MOTHER'S CURSE, *ACANTHOSCELIDES OBTECTUS*, REPRODUCTIVE BEHAVIOUR, MITOCHONDRIAL GENOTYPE, MALE INFERTILITY

02 – 04 Oral

HIGH MITOGENOME DIVERSITY OF CAVE LIONS (*PANTHERA SPELAEA* GOLDFUSS, 1810) IN SIBERIA

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To date, about several dozen cave lions of Eurasia have been studied at the mitogenome level. However, the data obtained do not allow us to trace the complete mosaic of the origin and characteristics of the distribution of different haplotypes of this extinct subspecies throughout Siberia in the Late Pleistocene. In our study, we expanded the sample of studied mitogenome sequences of cave lions from the territory of Eastern Siberia, and also added samples from Western Siberia and the Urals. The methodology included mitogenome enrichment of initial genomic libraries, their high-throughput sequencing and bioinformatics analysis of the obtained data, including haplotype and phylogeographic analyses. Based on the phylogenetic reconstructions carried out, we were able to trace patterns of the mitochondrial haplotype geographic distribution of cave lions across the territory of Eurasia in the epoch under consideration: the mitotypes of *Panthera spelaea* of the Far East separated most recently, later some mitotypes of Eastern Siberia and Europe separated, and the most recently formed mitotypes are the haplotypes of the Urals. The topology of the constructed phylogenetic tree indicates waves of cave lion migrations, in many of which Eastern and Western Siberia, due to its location, represented the territory of the greatest genetic admixture of cave lions from different regions of Eurasia, which proves the promise of studying the gene pool of cave lions in this region, combining genetic diversity of adjacent and more distant populations of Eurasia.

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02 – 05 Oral

KARYOTYPING OF A STRICTLY PROTECTED EUROPEAN MAMMAL - THE LESSER BLIND MOLE RAT *NANNOSPLAX LEUCODON* SUPERSPECIES USING NON-LETHAL SAMPLING

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The strictly protected underground rodent, the lesser blind mole rat (BMR) *Nannosplax leucodon* superspecies, is characterised by extreme karyotype changes comprising more than 20 chromosomal forms. Alongside with morphological convergence, many cryptic species have evolved. To describe each of them cytogenetically and investigate karyotype evolution mechanisms, it is crucial to analyse their karyotypes. However, all known BMR cytogenetic protocols required animal sacrifices. Additionally, standard protocols for mammalian fibroblast cells cultivation are not applicable due to the particular growth characteristics of cell cultures associated with BMR cancer resistance. Here, we present a karyotyping method from fibroblast cell cultures obtained from a finger-snip tissue, which enables rapid and safe return of the sampled animal to its original underground system.

One animal from each of the four cryptic species with the diploid chromosomal number 2n=48-56 was sampled for cultivation of fibroblast cells and chromosomal preparation. The composition of the complete medium followed the standard fibroblast protocol, except that more frequent medium changes and additional fetal bovine serum were needed. However, karyotyping protocol was significantly altered compared to other rodent species. The treatment with colchicine, ethidium bromide and the type of hypotonic solution required more modifications. The karyotypes from all four animals were successfully prepared and analysed.

This protocol is suitable for karyotyping without animal sacrifice, i.e. for non-lethal sampling, as well as for in vitro studies on cancer resistance mechanisms in this and related genera.

SPALACIDAE, FIBROBLAST CELL CULTURE, CHROMOSOMAL FORMS, CANCER RESISTANCE, CRYPTIC SPECIES

02 – 06 Oral

PHYLOGENETIC RECONSTRUCTIONS FOR ANCIENT AND RECENT REPRESENTATIVES OF CANIDS (CANIDAE) OF SIBERIA AND TAJIKISTAN TERRITORY BASED ON MTDNA SEQUENCES

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The most numerous species of predatory animals in Siberia are Canidae members. The dhole, *Cuon alpinus*, was also widespread in Eurasia during the Pleistocene. Remains of the *Cuon* and *Vulpes* genera are rare in the excavation sites and the ancient canids of Siberia are poorly genetically characterized. The purpose of the study was to conduct a comparative analysis based on mtDNA sequences data for recent and ancient canids. The ancient samples were collected from the territory of the Omsk Oblast, the Republic of Khakassia, the Republic of Altai, the Irkutsk Oblast, the Krasnoyarsk Krai, the Kemerovo Oblast. Prepared ancient DNA libraries were enriched with target sequences in two rounds. Recent material was obtained from the Novosibirsk Oblast, the Republic of Sakha, and the Russian Far East. Based on the obtained mitochondrial DNA sequences, a phylogenetic analysis was conducted. New haplotypes have been identified for a number of ancient canid individuals. Ancient canids from the Vologodskaya cave and the Ineyskaya cave have been genetically characterized for the first time. The reference sequence coverage of the dhole mitogenome have reached 90.1%, which is the best existing result for an ancient dhole. The bone remains of an animal from the territory of the settlement of Khisarak in Tajikistan, which were identified as a dog by archaeologists, were genetically identified as a fox. In the resulting phylogenetic tree, its haplotype was included in the clade of Eurasian foxes.

Acknowledgements: The study was carried out with support of the Russian Science Foundation grant No. 23-74-10060, https://rscf.ru/project/23-74-10060/.

ANCIENT, *VULPES VULPES*, *CANIS LUPUS*, *CUON ALPINUS*, *VULPES CORSAC*, PHYLOGENETIC RECONSTRUCTION, MTDNA, MITOCHONDRIAL GENOME, FOX, WOLF, DHOLE, CORSAC

02 – 07 Oral

PHYLOGEOGRAPHY OF LATE PLEISTOCENE WOOLLY MAMMOTHS (*MAMMUTHUS PRIMIGENIUS*) FROM GEOGRAPHICALLY ISOLATED AREAS OF SOUTHERN AND EASTERN SIBERIA

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To date, a number of studies have been published on the phylogenetics of the woolly mammoth (*Mammuthus primigenius*). These have ranged from analyses of parts of the mitochondrial genome to studies of complete nuclear genomes. However, the genetic diversity of woolly mammoths living in geographically isolated areas such as the Minusinsk Depression, in Southern Siberia, and the Kotelny Island, in North-East Siberia, is still unknown. This work involved high-throughput sequencing of seventeen woolly mammoth bone sample libraries, two-round enrichment using biotinylated probes of modern *Elephas maximus* mtDNA immobilised on magnetic microspheres, sequencing and subsequent bioinformatic analysis. Phylogenetic reconstructions showed that all the mammoths studied were members of clade I, thus extending their range. A sufficiently high diversity of their gene pool may be indicated by the placement of studied mammoth mitotypes in different clades within clade I. Phylogeographic reconstructions revealed the genetic proximity of mitochondrial lineages of Late Pleistocene mammoths of the Minusinsk. Depression and other regions of Eastern Siberia and their divergence in the time interval between 150 and 100 thousand years ago, indicating active migrations of woolly mammoths across large areas of Eastern Siberia from the late Middle Pleistocene to early Late Pleistocene.

Acknowledgements: The study was supported by the Russian Science Foundation grant No. 23-74-10060, https://rscf.ru/project/23-74-10060/.

ANCIENT DNA, WOOLLY MAMMOTH, PHYLOGEOGRAPHY, MITOCHONDRIAL GENOME

02 – 08 Oral

MECHANISMS OF ASEXUAL REPRODUCTION IN VERTEBRATES

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Asexual reproduction in vertebrates presents a fascinating deviation from the typical sexual reproduction observed in most eukaryote taxa. This mode of reproduction, known as parthenogenesis, allows offspring to be produced without the participation of a male genome. Parthenogenetic mechanisms vary across species and can be obligate or facultative, each with its specific characteristics. Key processes are shared among different vertebrate taxa and include meiotic changes, involving pre-meiotic endoreplication, automixis, and apomixis. Genetic factors, such as parental genome divergence, influence the prevalence and success of asexual reproduction in different species. Understanding these mechanisms provides insight into evolutionary strategies, genetic diversity, and the adaptability of vertebrate species to changing environments.

PARTHENOGENESIS, APOMIXIS, AUTOMIXIS, GENOME ENDODUPLICATION, POLYPLOIDY

02 – 09 Poster

PHYLOGENETIC ANALYSIS OF THE LATE PLEISTOCENE-HOLOCENE REPRESENTATIVES OF SEVERAL SPECIES (BOVIDAE FAMILY) FROM CENTRAL ASIA

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To date, different research groups studied several Bovidae species that lived during the Late Pleistocene and Holocene on the territory of Eurasia on the level of ancient DNA, especially in Europe and the Far East, such as *Bison priscus*, *Bison schoetensacki*, *Bison bonasus*, *Capra sibirica*, and others. Still, species from a vast region of Central Asia hasn't been genetically studied yet while having hundreds of fossils remaining of different Bovidae species.
In our study we extracted and high-throughput sequenced ancient mitochondrial DNA of several Late Pleistocene – Early Holocene Bovidae species, including *Capra sibirica* and *Bison priscus*, dating around 20 thousand years old, and phylogenetically compared their mitochondrial genomes to other ancient and modern samples of the Bovidae family members (GenBank database). The methodology also included target mitochondrial genomic libraries enrichment based on hybridization.
Based on the phylogenetic analysis we carried out, the haplotype of *Capra sibirica* from Khakassia region (Late Pleistocene) hadn't been later presented in Central Asian *Capra sibirica* populations (from the Late Pleistocene up to the Early Holocene), samples of which were obtained in modern Kyrgyzstan and analyzed in our research as well.
Phylogenetic analysis of the Bison priscus sample from Irkutsk region showed close maternal relationship to some representatives of the populations that lived in northern Central Asia and the population of northeastern Eurasia, a representative of which was previously discovered in Chukotka (haplogroup B), as well as two Late Pleistocene bisons found in northeastern China (haplogroup C), which reflects the migration history of these haplotypes.

ADNA, MITOCHONDRION, PHYLOGENETIC RECONSTRUCTION, *CAPRA SIBIRICA*, *BISON PRISCUS*

02 – 10 Poster

ORIGIN OF THE B CHROMOSOMES IN TWO MAMMALIAN SPECIES *NYCTALUS LEISLERI* AND *APODEMUS FLAVICOLLIS*

Marija Rajičić [1], Anastasia A. Proskurjakova [2], Branka Bajić [1], Ivana Budinski [1], Milan Miljević [1], Aleksa Rončević [1], Svetlana A. Romanenko[2], Jelena Blagojević [1]

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B chromosomes (Bs) are supplementary, non-essential chromosomes found in addition to the standard karyotype (A chromosomes) in various species. However, their origin, structure, and evolutionary patterns remain largely unknown. This study compares the genetic content, origin, and evolutionary pathways of Bs in two distinct mammalian species: the Leisler's bat *Nyctalus leisleri* and the yellow-necked wood mouse, *Apodemus flavicollis*.
In *N. leisleri*, dot-like Bs were present in all analysed specimens in Serbia (2n = 44 + 1-5 Bs). Small acrocentrics or dot-like Bs were present in all studied populations of *A. flavicollis* in Serbia (2n = 48 + 1-5 Bs), but with different frequencies (0.11-0.67). For both species, chromosomes were prepared from primary fibroblast cell cultures obtained from plagiopatagium skin punches for bats and small tail pieces for mice. This approach is safe and non-invasive for both taxa. Advanced techniques such as microdissection and Fluorescence In Situ Hybridization (FISH), along with B chromosome-specific DNA probes, provided insights into the genetic content of Bs in each species.
In *N. leisleri*, the distribution of signals in joint hybridizations of different B probes suggests different origins or evolutionary pathways for Bs, leading to varying genetic compositions. In *A. flavicollis*, Bs had a consistent genetic structure across individuals and populations, which indicates a unique common origin and/or similar evolutionary pattern for Bs in this species.
This study enhances our understanding of the complexity and evolution of B chromosomes in different species, shedding light on their genetic diversity and potential functional significance.

B CHROMOSOMES, *NYCTALUS LEISLERI*, *APODEMUS FLAVICOLLIS*

02 – 09 Poster

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ADNA, MITOCHONDRION, PHYLOGENETIC RECONSTRUCTION, *CAPRA SIBIRICA*, *BISON PRISCUS*

02 – 10 Poster

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This study enhances our understanding of the complexity and evolution of B chromosomes in different species, shedding light on their genetic diversity and potential functional significance.

B CHROMOSOMES, *NYCTALUS LEISLERI*, *APODEMUS FLAVICOLLIS*

TOPIC 3

Microbial genetics

VII CONGRESS OF THE SERBIAN GENETIC SOCIETY

03 – 01 Invited lecture

ENVIRONMENTAL MICROORGANISMS AND THEIR POPULATIONS AS BIOTECHNOLOGICAL SOLUTION TO PLASTIC POLLUTION

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Global research efforts to develop biotechnological solutions for plastic waste management, based on environmental microbes and their consortia, are underway. Multipronged approach, combining different strategies such as metagenomics, functional screening, directed evolution and metabolic engineering of degrading microbes, represents a step forward for achieving a circular economy for plastics.

Despite a proposed overlap between microbial enzymatic capacity to degrade lignocellulosic biomass and plastics, bioprospecting efforts to identify microorganisms able to catabolize both types of substrates remain limited. Studies focusing on environmental niches that are not readily accessible for sampling, representing underexplored reservoirs of enzymatic activities with potential biotechnological applications, are also relatively scarce.

Screening of 1000 environmental isolates, from water and soil environments that are not typically included in bioprospecting efforts and from laboratory evolved natural and synthetic consortia, for degradation of a range of plastic and lignocellulosic substrates and metataxonomic profiling of selected screened environments, as well as the assessment of their overall functional potential and future directions will be presented.

Acknowledgements: This work was supported by the EU H2020 Research and Innovation Programe (grant agreement No. 870292, BioICEP) and by the Ministry of Science, Innovation and Technological Development of the Republic of Serbia (agreement No. 451-03-66/2024-03/200042).

PLASTICS, LIGNOCELLULOSE, ENVIRONMENTAL ISOLATES, MICROBIAL CONSORTIA, METATAXONOMIC PROFILING

03 – 02 Invited lecture

THE FUNCTION OF THE TYPE III SECRETION SYSTEM IN PLANT-BENEFICIAL BACTERIA

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The type III secretion system (T3SS) is a molecular syringe used by various Gram-negative bacteria to inject effector proteins directly into host cells in order to modulate cellular processes. While the T3SS is well characterised in pathogenic bacteria, where it helps to evade host immune responses, its role in plant-beneficial bacteria is poorly understood. This talk will address the function of T3SS in plant-beneficial bacteria, focusing on recent findings linking T3SS activity to improved sugar beet growth and increased resistance to pathogens. We have characterised two isolates, *Pseudomonas marginalis* OL141 and *Pseudomonas grimontii* ORh26, and demonstrated a correlation between T3SS expression and positive responses to plant growth in sugar beet. Our studies suggest that the presence of T3SS in these beneficial bacteria promotes leaf formation, resulting in greater leaf mass and overall higher plant growth. In addition, T3SS appears to increase plant resistance to infection by *Pseudomonas syringae*, suggesting a dual role in growth promotion and pathogen defence. In addition, we have established a database of T3SS effector genes that are exclusive to beneficial *Pseudomonas*. Future research aims to decipher the mechanisms underlying T3SS-mediated effects on plant gene expression and focus on how these changes affect plant hormone production. By studying the specific T3SS effectors and their targets in plant cells, we aim to uncover the pathways that are modulated by T3SS and contribute to improved plant health. Understanding these interactions will pave the way for innovative agricultural practises that utilise beneficial plant-microbe interactions to improve plant productivity and resilience.

TYPE III SECRETION SYSTEM, PSEUDOMONAS, SUGAR BEET, PLANT GROWTH PROMOTION, PLANT IMMUNITY

03 – 03 Invited lecture

UNVEILING THE GENOMIC LANDSCAPE OF *PSEUDOMONAS SYRINGAE*: EXPLORING T3SS EFFECTOR DIVERSITY AND PHYLOGENOMIC STATUS OF STRAINS PATHOGENIC ON SUGAR BEET

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The *Pseudomonas syringae* species complex represents a heterogeneous facet of pseudomonads with an immense plant host range and habitat within and beyond agro-ecosystems. Despite the increasing number of disease reports on sugar beet worldwide in recent years, information on the genomic characteristics, phylogenomic status and pathogenic strategies of *P. syringae* strains on sugar beet is still lacking. Recently, we have shown the great diversity in pathogenic properties of *P. syringae* strains on sugar beet and hypothesized that the repertoire of virulence factors is responsible for this great diversity. In this context, we compared the genomes of 100 strains from the entire *P. syringae* species complex, focusing on determining the phylogenomic status of strains pathogenic on sugar beet and the spectrum of their T3SS effectors. Although *P. syringae* strains pathogenic on sugar beet were considered to have a small effector repertoire, we detected genes for a considerably broad spectrum of effectors, especially in strains with high virulence. In addition, we discovered a putative novel T3SS effector protein family for the entire *P. syringae* species complex, located outside of the pathogenic islands on the genome. Overall, the results showed that (i) all *P. syringae* strains pathogenic on sugar beet belong to phylogroup 2, (ii) T3SS effector repertoire of *P. syringae* pathogenic on sugar beet is broad-spectrum, and (iii) even though *P. syringae* is the bacterium with the most described T3SS effectors, there are still novel and undiscovered effectors that could orchestrate the pathogenic strategy of this pathogen.

PSEUDOMONAS SYRINGAE, PHYLOGENOMICS, T3SS EFFECTORS, DIVERSITY, SUGAR BEET

03 – 04 Oral

IDENTIFICATION OF CELLULAR FACTORS IN *USTILAGO MAYDIS* INVOLVED IN THE CELL'S RESPONSE TO CYTOTOXIC AND GENOTOXIC STRESS

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Throughout their lifespan, microorganisms encounter various environmental changes. Some changes are beneficial, while others have negative impacts on their survival and reproduction. To adapt to these negative changes, microorganisms have developed different strategies. We have discovered that unicellular basidiomycete Ustilago maydis can restore population abundance after devastating stress by utilizing biomolecules freed from dying cells. We named this repopulation strategy "Repopulation upon shattering" (RUS). However, utilizing these nutrient-rich biomolecules is challenging as they also exhibit toxic effects on cells. Therefore, U. maydis needs to employ a wide variety of cellular factors to utilize these nutrients successfully. Our research has revealed that some of these factors also play important roles in genome stability. We have identified 5 cellular factors (RGS6, RGS9, RGS22, RGS24, RGS26) that are crucial for the mechanisms of RUS and genome integrity. RGS6 refers to nonmuscle myosin heavy chain IIa, RGS9 is the DNA repair exonuclease REC1, RGS22 is the ubiquitin specific protease 7, RGS24 is the receptor for activated kinase 1 (RACK1), and RGS26 is the ubiquitin specific protease 8. Mutations in any of these genes lead to the inability of mutant cells to recover from oxidative stress and to repair DNA lesions caused by genotoxic agents such as MMS, DEB, HU, and UV radiation.

USTILAGO MAYDIS, CELLULAR FACTORS, OXIDATIVE STRESS, GENOME STABILITY

03 – 05 Oral

GENOME ANALYSIS OF *B. VELEZENSIS* SS- 38.4 REVEALED THE GENETIC BASIS OF ITS BIOCONTROL ACTIVITY

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Bacillus spp. are regarded as one of the most successful bacterial biocontrol agents. In this study, we investigated the phylogenetic status of *Bacillus* sp. strain SS-38.4 in comparison to other proven biocontrol and non-biocontrol *Bacillus* strains. Our aim was to understand its biocontrol mechanisms, focusing on its repertoire of genetic traits related to plant growth promotion, biocontrol, and environmental adaptation.
Genomic DNA from SS-38.4 was extracted and sequenced using the PacBio Sequel II system. De novo assembly and error correction were performed with Canu and Racon. Genome annotation was carried out using PGAP and RAST. The phylogenetic analysis involved 50 representative Bacillus spp. genomes, and comparative genomics was conducted using the EDGAR pipeline.
The SS-38.4 genome, comprising two contigs with a total size of 4,007,389 bp and 56.7% GC content, included 4,219 genes. Phylogenetic analysis confirmed SS-38.4 as *B. velezensis*, closely related to biocontrol strain *B. velezensis* FZB42. Comparative genomics identified 78.1% of the core genome and 97 unique genes, indicating high similarity within the species. The genome revealed a wide spectrum of genes responsible for siderophore production, biofilm formation, motility, and antimicrobial compounds, alongside plant-growth-promoting traits. These genes provide SS-38.4 with competitive advantages in nutrient acquisition and plant colonization, indirectly affecting phytopathogens and directly killing them through antimicrobial compounds, highlighting its strong biocontrol potential.
The genome analysis of SS-38.4 elucidates its biocontrol potential through plant growth promotion, environmental adaptation, and antimicrobial activity. This genetic insight supports its use in sustainable agriculture for managing plant pathogens and enhancing crop growth.

Acknowledgements: This work was supported by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia, grant No. 451-03-65/2024-03/200178 and 451-03-66/2024-03/200178

BACILLUS SP, BIOCONTROL, PLANT GROWTH PROMOTION, COLONISATION

03 – 06 Oral

EXPLORING THE BIOCONTROL POTENTIAL: INVESTIGATING HCN-PRODUCING *PSEUDOMONAS* STRAINS AGAINST PLANT PATHOGENS

Aleksandra Mesaroš [1, 2], Nikola Grujić [3], Marija Nedeljković [1, 2], Željko Savković [1], Iva Atanasković [1, 2], Slaviša Stanković [1, 2], Jelena Lozo [1, 2]

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Hydrogen cyanide (HCN) is known as an effective biological control agent produced by certain bacterial strains, that works by disrupting cellular respiration and leads to the suppression of the target organisms. This study aimed to investigate the biocontrol potential of two HCN-producing *Pseudomonas* strains against various plant pathogens.
Two HCN-producing *Pseudomonas strains – Pseudomonas putida* A32 and *Pseudomonas atacamensis* A238 were detected and identified in a collection of bacteria isolated from pepper plants. We confirmed the presence of *hcnB* genes by PCR and generated *hcnB* insertion mutants. The biocontrol potential of the wild type and mutants was tested in a split-section Petri dish experiment against five fungal (genera *Macrophomina*, *Fusarium*, *Botrytis*, *Alternaria* and *Sclerotinia*) and eight bacterial (genera *Xanthomonas*, *Pseudomonas* and *Clavibacter*) pathogens of pepper plants and two plant-parasitic nematodes – *Anguina tritici* and *Globodera pallida*.
The two HCN producers A238 showed antagonistic effects against bacteria, fungi, and nematodes in a split-section Petri dish experiment, which were absent in the mutants, with A32 being more effective. These results indicate that HCN production by the tested strains is involved in preventing the growth of pathogens and plant-parasitic nematodes.
The construction of *hcnB* mutants confirmed that the antagonistic activity of the two strains against different plant pathogens was a consequence of their HCN synthesis. These results suggest that HCN-producing strains are promising biocontrol agents for sustainable agriculture.

Acknowledgements: This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant numbers 451-03-65/2024-03/200178 and 451-03-66/2024-03/200178.

BIOLOGICAL CONTROL; VOLATILE COMPOUNDS; MUTANT CONSTRUCTION; PLANT BENEFICIAL STRAINS; SUSTAINABILITY

03 – 07 Oral

BACTERIAL DIVERSITY AND PHYLOGENETIC RELATIONSHIPS IN THE PHYLLOSPHERE AND RHIZOSPHERE OF ORGANIC AND NON-ORGANIC GRAPEVINE

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Grapevine is one of the most widely cultivated fruit crops of great economic value. Grapevine microbiota can influence its productivity and wine quality, and is affected by biotic and abiotic factors. Chemical treatments common in viticulture can be detrimental to the environment and the ecosystem, causing eradication of pathogens but also beneficial microorganisms, that can increase the productivity and offer protection by improving nutrient acquisition, stimulating growth and suppressing diseases.

The aim of the study was to compare the bacterial diversity in the phyllosphere and rhizosphere of grapevine (cv. Prokupac) from organic and herbicide-treated production at three time points, and determine phylogenetics of the most abundant bacterial taxa of grapevine.

Short-read metabarcoding analysis was performed via Illumina NovaSeq PE250 using 16S rRNA (V3-V4) primers. Taxa annotation and alpha diversity was performed using QUIIME2 software. Phylogenetic relationship of the 100 most abundant genera was assessed using the Neighbor-Joining method.

The ten most dominant phyla in phyllosphere and rhizosphere of grapevine were Actinobacteria, Proteobacteria, Firmicutes, Bacteroidota, Acidobacteroidota, Verrucomicrobota, Chloroflexi, Crenarchaeota, Gemmatimonadota and Myxococcota. Alpha diversity was higher in the rhizosphere than phyllosphere in all three seasons. The phyllosphere was characterized by close phylogenetic relationship between the most abundant genera (Proteus and Methylobacterium) from both organic and herbicide-treated grapevine. The most abundant genera from the rhizosphere belonged to the phylogeneticaly divergent phyla (Bacteroidota and Actinobacteria). Altogether, results showed high diversity of the grapevine bacterial communities, contributing to the characterization of the overall microbial biodiversity which could support grapevine health and productivity.

METABARCODING, BACTERIAL COMMUNITIES, PHYLOGENY, *VITIS VINIFERA*

03 – 08 Oral

SARS-COV-2 CHARACTERISTICS AND DETECTION, FACTS AND/OR MISCONCEPTIONS

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged at the end of 2019 and caused COVID-19 pandemic. So, reliable and accurate testing for SARS-CoV-2 was the principal prerequisite for preventing the spread of virus. Real Time RT-PCR unquestionably represent the most reliable, rapid and sensitive method for detection of SARS-CoV-2 RNA. However, there are numerous different assays, protocols, instruments and analysis methods in use without certified standards, standardized RNA extraction and reporting procedures. In practice, the reliability of RT-qPCR results depends on a number of parameters that include sample collection and processing, method of RNA extraction, choice of assay, etc. Here we present comparative analyses of the efficiency and sensitivity of 10 different amplification assays, as well as the relevance of manual RNA extractions compared to automatic one. Our results revealed that manual viral RNA extraction and amplification assays targeting three viral genes should be a method of choice for high sensitivity. Interestingly, RT-qPCR was exclusively used as qualitative diagnostic test for SARSCoV-2. Why, it is a quantitative method? We think that the ideal testing regimen should involve reliable and meaningful quantitative reporting of SARS-CoV-2 viral load. Now, a key question arises, why the medical community has never agreed on a cut-off value (viral load) for a positive result that would have clinical significance? Can we identify cut-off value for SARS-CoV-2 viral load that generates massive microthrombosis throughout the body, seen by our pathologist?

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SARS-COV-2; RT-QPCR; VIRAL LOAD

03 – 09 Poster

Effects of *Lactobacillus salivarius* on AKT/mTOR and Notch Pathway Gene Expression in Dysplastic Oral Keratinocytes

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Disruption of oral homeostasis leading to dysbiosis may promote diseases, including oral cancer. This study aims to elucidate the modulatory effects of *Lactobacillus salivarius* on dysplastic oral keratinocytes (DOK) over various co-culture periods. DOK were co-cultured with *L. salivarius* for early (2 and 4 hours) and late (6 and 12 hours) time points, with keratinocytes cultured without *L. salivarius* serving as the control.

DOK and *L. salivarius* were cultivated separately under standard conditions. The bacterial suspension was prepared and optimized for co-culture with DOK cells at MOI 100 at various time points (2, 4, 6, and 12 hours). RNA was extracted from the co-cultures at the end of each time point. The relative expression of key molecules in the AKT/mTOR and Notch signaling pathways (*PIK3CA*, *DUSP15*, *AKT2*, *mTOR*, *MAPK14*, *Notch 1*, *Jagged 1*, *Hey 1*, *COX2*, *ALOX5*, *VEGF*, *CyclinD*) was analyzed using qPCR.

Significant changes in gene expression, particularly the upregulation of *PIK3CA*, *MAPK14*, and *ALOX5* in late co-cultures, suggest a modulatory effect over time. Conversely, the downregulation of *JAGGED1* and *COX2* in late co-cultures indicates potential inhibitory interactions.

Lactobacillus salivarius influences the expression of key genes in the AKT/mTOR and Notch signaling pathways in DOKs. Significant changes suggest a modulatory effect over time, but longer co-culture periods are necessary to fully elucidate the complex interactions and long-term effects on these signaling pathways in oral carcinogenesis.

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LACTOBACILLUS SALIVARIUS, CO-CULTURE, DYSPLASTIC ORAL KERATINOCYTES

03 – 10 Poster

THE INVOLVEMENT OF T3SS POSITIVE SUGAR BEET SYMBIONT, *PSEUDOMONAS MARGINALIS* OL141, IN PLANT GROWTH STIMULATION AND PATHOGEN RESISTANCE

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The interplay between plants and microorganisms in agricultural ecosystems offers opportunities to improve crop productivity and resilience in the context of climate change and increasing pathogen challenges. This study addresses the functional dynamics of the type 3 secretion system (T3SS) of non-pathogenic *Pseudomonas* strains associated with sugar beet (*Beta vulgaris* L.). From the collection consisted of plant-beneficial *Pseudomonas* strains isolated from sugar beet T3SS-positive isolates were identified by detecting conserved sctRST genes of the T3SS operon. Genetic diversity and T3SS expression profiles were characterized and we also generated a sctRST insertion mutant. The expression of the system was tested by qPCR. The plant growth-promoting activity and induction of systemic resistance were tested on sugar beet of the variety Heston. Our results show that T3SS is widely distributed with different T3SS gene sequences and expression patterns. In particular, T3SS functionality was detected in one isolate *P. marginalis* OL141. In planta experiments with this isolate showed the central role of T3SS in stimulating sugar beet growth and improving resistance to *Pseudomonas syringae* pv. *aptata* infection. Our study has provided convincing evidence that deletion of T3SS in non-pathogenic *Pseudomonas* abolishes ISR, abrogates the growth-promoting effect and is inducible for T3SS expression upon exposure to sugar beet extract. This will contribute to the understanding and development of a wide range of implications for sustainable agriculture and global food security.

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TYPE 3 SECRETION SYSTEM, *PSEUDOMONAS*, SUGAR BEET, PLANT-MICROBE INTERACTIONS, BIOSTIMULATION

03 – 11 Poster

CANDIDA ALBICANS INFLUENCE ON NOTCH SIGNALING PATHWAY IN PERI-IMPLANT LESIONS

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Candida albicans (Ca) is an opportunistic inhabitant of the oral cavity that typically lives as a saprophytic microorganism but can cause severe oral infections under certain conditions. Peri-implant biological complications, such as peri-implantitis and peri-implant mucositis, are driven by oral biofilm microbiota that activate different signaling pathways involved in the host's immune response. The Notch signaling pathway is one of these and plays a crucial role in remodeling peri-implant bone tissue.

This study aimed to assess the prevalence of Ca and correlate it with the relative expression levels (REL) of *Notch 1*, *Notch 2*, *Jagged 1*, *Hes 1*, and *Hey 1* genes in patients with diseased and healthy dental implant sites.

A total of 102 patients were divided into peri-implantitis (PI), peri-implant mucositis (PM), and healthy implant (HI) groups. Peri-implant crevicular fluid was sampled from each patient, and both Ca and Notch molecules were analyzed using a quantitative real-time polymerase chain reaction method.

The Ca levels did not differ significantly among groups. However, negative correlations were observed between Ca and *Hey 1* REL in the PI group ($\rho = -0.565$; $p=0.001$), and the *Notch 1* gene in the HI group ($\rho=-0.397$; $p=0.030$).

These results indicate that increased expression of the *Notch 1* gene, a known bone promoter, maintains low levels of Ca in healthy implants, while decreased expression of the *Hey 1* gene, the Notch signaling pathway regulator, causes disruption and may lead to peri-implant disease development and progression.

CANDIDA ALBICANS, NOTCH SIGNALING PATHWAY, PERI-IMPLANTITIS, PERI-IMPLANT MUCOSITIS

03 – 12 Poster

PHYLOGENETIC ANALYSIS AND DETECTION OF A HIGHLY VIRULENT HAPLOTYPE AMONG *PSEUDOMONAS SYRINGAE* ISOLATES FROM THE DANUBE-TISA-DANUBE CANAL NETWORK

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Pseudomonas syringae (Psy) is a widespread complex of plant pathogenic bacterial species categorized into 23 clades across 13 phylogroups. Among the various genetic lineages, the dominant haplotype DD.1, identified based on a partial sequence of the citrate synthase (*cts*) housekeeping gene, is particularly common in disease outbreaks and aquatic environments and includes potentially highly virulent strains.

In Serbia, the diversity of Psy strains beyond agricultural contexts remains underexplored. This study aimed to elucidate the phylogenetic diversity of Psy isolates from the Danube-Tisa-Danube (DTD) canal network, an important source for crop irrigation, and to ascertain the presence of the DD.1 haplotype.

A partial sequence of the *cts* gene of 42 isolates was amplified and sequenced. Phylogenetic relationships were analyzed by constructing a phylogenetic tree using the neighbor-joining method in Mega 11 software.

The phylogenetic analysis revealed seven clades within five phylogroups, indicating significant strain diversity within the DTD canal network. The isolates predominantly belonged to phylogroup 2 (36%), followed by phylogroup 1 (31%), 12 (26%), 7 (5%), and 13 (2%). Comparison with the cts sequence of DD.1 confirmed that three isolates from our collection matched this highly virulent haplotype. The first detection of the DD.1 haplotype in the DTD canal network suggests a potential threat to irrigated crops. Investigating the Psy population in irrigation sources is an important step in crop risk management.

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PSEUDOMONAS SYRINGAE, PHYLOGENY, DIVERSITY, CITRATE-SYNTHASE

03 – 13 Poster

LABORATORY EVOLVED MICROBIAL CONSORTIUM TOWARDS ARABINOXYLAN CAN DEGRADE POLYURETHANE

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Biodegradation of hydrocarbon based lignocellulose and plastics is often more efficient by natural and synthetic microbial consortia than by individual microorganisms, due to their extended enzymatic repertoire. Despite structural similarities of some plastics and plant-derived polymers and a proposed overlapping microbial degradation activity, the potential for the plastics degradation by lignocellulose-degrading microbial consortia still remains underexplored.

Synthetic consortia (SC) combining 35 environmental *Streptomyces* isolates with confirmed lignocelullolytic activities (SC0) was subjected to 3 rounds of enrichment on insoluble wheat arabinoxylan (AXYL) as sole carbon source. Reduction in biomass and morphological diversity was observed through rounds of enrichment for SC1 to SC3. In SC2 and SC3, only yellow (Y) and white (W) colonies could be observed. These were identified as *Ochrobacterium*, a genera with lignolytic and cellulolytic activity and *Stenotrophomonas*, a genera known for their high hydrolytic activity and likely represent a background contamination enriched on AXYL.

SC1 demonstrated multiple clearance zones on Impranil SD, a model compound for degradation of polyurethanes. Eight selected isolates from SD clearance zones were identified as *Stenotrophomonas* (3), *Pseudomonas* (3), *Streptomyces* and *Lysinibacillus*. Isolates grew on all 8 tested polyester-based plastic and lignocellulosic substrates. Four isolates produced clearance zones on carboxymethyl cellulose, while SD degradation was confirmed for 5 isolates. Isolates with simultaneous plastolytic and lignolytic capabilities may be of special biotechnological interest and should be further explored.

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PLASTICS, LIGNOCELLULOSE, MICROBIAL CONSORTIA, 16S RDNA SEQUENCING

03 – 14 Poster

THE INFLUENCE OF THE PRESENCE OF PERIODONTOPATHOGENS ON THE EXPRESSION OF IL-1B IN GINGIVAL CREVICULAR FLUID

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Fixed prosthetic restorations (crowns or bridges) are made with the aim of rehabilitating apart of or the entire tooth row, and thus becoming its integral part that is fully integrated into the ecosystem of the oral cavity. Tooth preparation is the procedure of removing hard dental tissue in order to provide space for an artificial crown, forming the finish line. It represents the transition from the prepared to the unprepared part of the tooth. The finish line position in relation to the gingival margin can be supra, equi or subgingival.

To examine the influence of the presence of periodontopathogens on the expression of the IL-1β in the gingival fluid after tooth preparation with equigingival and subgingival finish line position. This study included 20 patients with an indication for a PFM crown on an upper canine. IL-1β concentration was determined using an ELISA kit (Cloud-Clone Corp., CCC, USA), detection range 15.6 pg/mL–1,000 pg/mL, sensitivity 5.8 pg/ml. Gingival crevicular fluid samples were examined for the presence of four bacteria: *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Tannerella forsythia*, *Porphyromonas gingivalis* by polymerase chain reaction (PCR) analysis.

Although the higher concentration values of the IL-1β were found in patients with a positive PCR findings of the examined periodontopathogens (both in patients with equigingival and subgingival finish line position), no statistical significance was found using tests for comparing parameter values between independent samples.

It can be concluded that there could be a connection between the presence of the investigated periodontopathogens and the IL-1β concentration values, however, the lack of statistical significance in this study may be caused by the small sample size.

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TOOTH PREPARATION, PERIODONTOPATHOGENS, BACTERIA, FINISH LINE POSITION, IL-1B

03 – 15 Poster

EARLY BACTERIAL COLONIZATION ON CAD/CAM DENTAL MATERIALS

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Bacterial adhesion on dental restorative materials, prostheses, and various oral appliances poses a significant risk for the development of secondary caries, periodontal disease, peri-implantitis, denture stomatitis, and other conditions. Thus, reducing bacterial colonization on different dental materials is a key milestone in contemporary dental material science. Despite being introduced in dentistry fifty years ago, computer-aided design and computer-aided manufacturing (CAD/CAM) technology continues to play a significant role in modern dentistry. The aim of this study was to investigate bacterial adhesion on five different CAD/CAM-derived dental materials: resin-based composite (RBC), polymethyl methacrylate (PMMA), zirconia glazed (ZG), cobalt-chromium (CoCr) alloy, and polyetheretherketone (PEEK). The study included ten healthy volunteers who were instructed to carry individually made intraoral splints embedded with five different CAD/CAM-prepared discs (50 in total). After 24 hours of intraoral exposure, discs were removed from the splints, briefly soaked in saline and then subjected to bacterial DNA isolation procedure. Bacterial load on each CAD/CAM disc was quantified by real-time polymerase chain reaction. Collected data were analyzed on SPSS v25.0 (IBM SPSS, Chicago, IL, USA) statistical package and presented as median±IQR. PEEK specimens exhibited the highest amount of adhered bacterial biofilm (33.25±40.12×1013), while RBC specimens had the least bacterial accumulation compared to all other materials (2.66±7.33×1013). Bacterial load on RBC was significantly lower than on ZG (p=0,039) and PEEK (p=0.004). In addition, cobalt-chromium alloy collected fewer bacteria than PEEK (p=0.015). The results of this study suggest that CAD/CAM RBC is advantageous for use in oral environment compared to other tested materials.

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CAD/CAM, DENTAL MATERIALS, QPCR

03 – 16 Poster

SUPPRESSORS OF BLM-DEFICIENCY IDENTIFY FOUR NOVEL PROTEINS AFFECTING DNA REPAIR AND HOMOLOGOUS RECOMBINATION IN *USTILAGO MAYDIS*

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The maintenance of genome integrity is a fundamental cellular process and it's highly conserved among all domains of life. Homologous recombination (HR) is one of the DNA repair mechanisms and it is essential for the error-free repair of DNA double strand breaks, which are the most deleterious lesions. Mechanisms of HR are mostly studied in yeast which, unlike higher eukaryotes employs RAD52 as a HR mediator, instead of BRCA2.

The focus of our research is to uncover novel cellular factors that regulate HR, by isolating suppressors of blm in *Ustilago maydis*, a unicellular phytopathogen, which is extremely resistant to radiation and has DNA repair system similar to that in human, with highly conserved *BRCA2* (named *Brh2*).

We have identified 4 novel factors of unknown functions (named Rec3, Zdr1, Bls9 and Bls2), and 4 known factors – Rad51, Dna2, Mph and paraplegin. Mutations in each of these genes suppress hydroxyurea sensitivity of blm.

Three of these four novel proteins are involved in DNA Repair, and play critical roles in mitotic and/or meiotic recombination. Rec3 is a member of the family of Rad51 ATPases, Zdr1 is Cys2-His2 zinc finger protein, whereas Bls9 and Bls2 are completely uncharacterized proteins. Deletion of Rec3, Zdr1 and Bls2 can also suppress HU-sensitivity of Δgen1, and Δmus81 mutants, but loss of Bls9 does not rescue HU-sensitivity of Δgen1.

These novel factors can provide insights into HR regulation, interactions among HR participants and relations to other cellular processes.

DNA REPAIR. BRCA2, BLM, REPLICATION STRESS

03 – 17 Poster

IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF PLANT-GROWTH PROMOTING BACTERIA ISOLATED FROM SOILS AND ROOTS OF RED RASPBERRY (*RUBUS IDEAUS* L.)

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Red raspberry (*Rubus ideaus* L.) is a perennial plant with significant nutritional value and commercially important for Serbia. Plants recruit beneficial microorganisms in the rhizosphere and roots, forming a complex system called holobiont. These beneficial microorganisms contribute to plant-growth promotion (PGP) through various mechanisms, thereby enhancing plant productivity. This study aims to identify and elucidate phylogenetic relationships between PGP bacteria isolated from the roots and rhizosphere of organic and pesticides-treated red raspberries. Recently, 5 highly efficient PGPB strains from organic and 9 from pesticide-treated raspberries have been isolated and characterized. The partial sequence of the *16s rRNA* gene was amplified, while sequences were edited using the FinchTV program and identified via the BLASTn algorithm. A phylogenetic tree was constructed using neighbor-joining method in MEGA XI software. Initial identification revealed strains of *Pseudomonas*, *Variovorax paradoxus*, *Paraburkholderia gardini*, *Bacillus*, *Streptomyces*, *Brachybacterium*, *Cryocola* sp. and *Leifsonia lichenia*. Phylogenetic analysis revealed two main branches. One branch comprised a group with *Pseudomonas* species and a cluster containing *Variovorax paradoxus* and *Paraburkholderia gardini*. The other primary branch included a group of *Bacillus* species and a sub-branch containing three distinct groups: *Streptomyces* sp., *Brachybacterium* sp. and a cluster with *Cryocola* sp. and *Leifsonia lichenia*. The results indicate that in the rhizosphere of chemically-treated red raspberry, *Bacillus* species are predominant, while the organic rhizosphere exhibits a greater diversity of bacterial communities. Additionally, PGPB from both organic and pesticide-treated roots belong to the same phylogenetic group, suggesting a close phylogenetic relationship between communities regardless of treatment type.

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PHYLOGENETIC ANALYSIS, PLANT-GROWTH PROMOTION, RED RASPBERRY, SUSTAINABLE AGRICULTURE

03 – 18 Poster

THE EFFECT OF THERMAL INACTIVATION ON THE IDENTIFICATION OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS-2 (SARS-COV-2) BY RT-QPCR: AN EXPERIENCE FROM THE UNIVERSITY CLINICAL CENTER OF VOJVODINA

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Handling Coronavirus Diseases 2019 (COVID-19) patient samples poses a risk of exposure to infectious particles for healthcare staff. Therefore, the World Health Organization proposed guidelines for specimen handling, which lacked consideration of heat inactivation as an additional protective measure. This study, based on research at the University Clinical Center of Vojvodina, aims to assess the impact of heat inactivation (56 °C/30 minutes) on obtaining positive results and the detectability of three SARS-CoV-2 genes in nasopharyngeal (NP) and oropharyngeal (OP) swabs from patients with pronounced COVID-19 symptoms.

The study involved 200 specimens from adult patients with suspected COVID-19 symptoms. One NP/OP swab was taken from each patient and aliquoted into two tubes, with one batch of each pair subjected to heat inactivation while the second batch was held at 4 °C. Genetic material from all samples was isolated and tested using the GeneFinder RT-PCR SARS-CoV-2 test for RNA-dependent RNA polymerase (RdRp), nucleocapsid (N), and envelope (E) genes.

Out of 200 samples, untreated and inactivated groups showed 111 and 100 positives, respectively, with no statistically significant impact on positive results. Differences in gene detectability were observed in 9 weakly positive samples. Significant mean cycle threshold value differences were found for all gene groups, but only for the E gene in weakly positive samples. Correlation analysis indicated a good correlation for N and E genes and a moderate correlation for others.

As the research showed, the 56°C/30 minute heat inactivation protocol shows no significant impact on false-negative results but influences detectability, although insufficient to cause errors.

Acknowledgements: We are thankful to our patients who contributed to the accomplishment of this study.

COVID-19; RT-QPCR; SARS-COV-2; HEAT INACTIVATION

03 – 19 Poster

GENERATING KNOCKOUT MUTANT IN THE GALU GENE OF CLINICAL ISOLATE *ACINETOBACTER BAUMANNII* 10593

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One of the most important virulence factors of *Acinetobacter baumannii*, an opportunistic pathogen that is one of the most significant causes of nosocomial infections worldwide, is the production of a capsule on the cell surface. This structure presumably protects bacteria against antimicrobial agents, disinfectants, desiccation, and the host immune system. Genes encoding proteins involved in the biosynthesis and export of capsular polysaccharides are localised in the capsule locus (KL). The construction of gene deletion mutants is an important technique to evaluate the functional identification of genes in bacteria. This study describes the generation of knockout mutant in the *galU* gene, which is involved in the biosynthesis of capsular simple sugars. PCR amplicons of the upstream and downstream regions of the target gene were cloned into a PCR cloning vector pJET and in the next step subcloned into a pEMGT plasmid, which is suicidal in *A. baumannii*. The generated constructs were introduced into the selected isolate of *A. baumannii* by triple conjugation and candidates were checked on a selective medium and by PCR method. To induce the second step of recombination, a pSW-Apr plasmid was introduced by conjugation into the candidates. The construction of the mutants was confirmed by monitoring growth on a selective medium and finally by PCR method.
The capsular polysaccharides of *A. baumannii* require further investigation as potential targets for novel antimicrobial therapeutics. In this work, gene deletion mutants were constructed that can be used to study the contribution of the capsule to the virulence of *A. baumannii*.

ACINETOBACTER BAUMANNII, CAPSULAR POLYSACCHARIDES, GALU GENE

03 – 20 Poster

ANALYSIS OF THE PRESENCE OF *E. FAECALIS* IN FRESHWATER SPONGES FROM SERBIAN RIVERS

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Given their filter-feeding nature, sponges can process up to 100 liters of water per day, potentially accumulating pollutants and bacteria. This study evaluates the potential of freshwater sponges as bioindicators for water quality by examining the presence of the bacterium Enterococcus faecalis in DNA samples extracted from sponges collected across Serbian rivers. This research is part of the preparations for the Joint Danube Survey 2025, aimed at enhancing water quality monitoring practices with innovative methods. Our objective was to determine if freshwater sponges serve as concentrated reservoirs of bacteria, providing a convenient model for water quality assessment.
DNA was extracted from 60 sponge specimens using the Sherlock AX kit from A&A Biotechnology, followed by PCR amplification with the KAPA2G Robust HotStart ReadyMix PCR Kit, supplemented with DMSO. Primers specific to the *28s* ribosomal RNA sequence of *E. faecalis* were employed, with a known *E. faecalis* strain serving as the positive control.
The presence of the target sequence was confirmed via agarose gel electrophoresis. Interestingly, none of the 60 sponge DNA samples, analyzed in the present study, contained *E. faecalis* DNA, a finding that might point either to the lack of this specific bacterial species in the examined water bodies, or the lack of retention of these bacteria by freshwater sponges.
Future studies should include larger samples and more bacterial species, in order to obtain conclusive data that would allow the integration of sponge analysis into a broader water quality monitoring.

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FRESHWATER SPONGES, *ENTEROCOCCUS FAECAELIS*, BIOINDICATOR

03 – 21 Poster

GLOBAL DNA METHYLATION AS A PREDICTOR OF SARS-COV-2 DISEASE PROGRESSION AND MORTALITY

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DNA methylation has been implicated in various disease processes, including viral infections. Though its effect on SARS-CoV-2 susceptibility is uncertain, its impact on disease severity and outcomes warrants investigation. This study aims to explore the association between global genome methylation and SARS-CoV-2 infection.

This observational study involved 237 patients with the SARS-CoV-2 Omicron strain and 130 uninfected controls from healthcare institutions in Kragujevac, Serbia. Clinical data were collected from medical records, and whole blood samples were obtained at recruitment. Global genome methylation was assessed using HPLC-PDA analysis. DNA samples were hydrolyzed to nucleosides, with standard curves generated for analysis.

Global genome methylation levels were consistent across sex and age and SARS-CoV-2 infection status. However, lower methylation degree increased likelihood of moderate to severe disease about 5-fold and critical disease up to 7-fold compared to mild cases. Lower methylation was also associated with a 17-fold higher risk for any active lesions on chest X-rays, particularly interstitial changes. Patients with lower global methylation had up to a 3-fold higher mortality risk during hospitalization. Global methylation showed weak correlations with granulocytes, and weak negative correlations with monocytes and lymphocytes.

Global DNA methylation levels are not linked to SARS-CoV-2 susceptibility but are associated with disease severity and outcome, with lower methylation increasing the risk of severe and fatal disease.

GLOBAL DNA METHYLATION; SEVERITY; OUTCOME; SARS-COV-2; COVID-19

03 – 22 Poster

ASSESSING THE TOXIGENIC POTENTIAL OF THE CYANOBACTERIAL STRAIN *OSCILLATORIA NIGRO-VIRIDIS* Z1 USING GENOMICS TOOLS

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In this study, bioinformatics tools were used to assess the toxigenic capability of the cyanobacterial strain *Oscillatoria nigro-viridis* Z1 by identifying gene clusters responsible for cyanotoxin production. Additionally, the cytotoxic and neurotoxic potential of the extracts was assessed in an in vitro biotest.

The strain's genome was sequenced and analyzed to investigate its metabolic profile and identify biosynthetic clusters for known toxic compounds. A hybrid sequencing approach, combining Oxford Nanopore and Illumina reads, facilitated a more accurate and complete genome assembly. Genome annotation and detection of gene clusters for the biosynthesis of secondary metabolites were performed using Prokka and antiSMASH software. The in vitro testing of the strain's cytotoxic potential involved analyzing the effect of different concentrations of the extract (4, 100, 400 i 2000 µg mL-1) on the viability of human neuroblastoma cells (SH-SY5Y) using the tetrazolium colorimetric test (MTT).

The results of the genomic analyses indicated the presence of 15 biosynthetic clusters, including a cluster involved in producing cylindrospermopsin, a potent alkaloid cytotoxin, and bioactive peptides like nostophycin, nostopeptolide, and tenuecyclamide A. As for the cytotoxicity testing, significant effects were recorded only at the highest tested concentration (CI%=48.80%; $p \leq 0.05$), while the calculated IC10 (24h) concentration was 49.58 µg mL-1. After prolonged exposure (72h), the highest concentration of the extract displayed similar cytotoxicity, while at lower doses prolonged exposure resulted in reduced cytotoxicity in the tested cells.

The findings of this study highlight the importance of utilizing bioinformatics tools in the assessment and characterization of cyanobacterial toxicity.

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CYANOBACTERIA, GENOME SEQUENCING, CYTOTOXICITY, IN VITRO BIOASSAY

TOPIC 4

Medical genetics

VII CONGRESS OF THE SERBIAN GENETIC SOCIETY

04 – 01 Invited lecture

PHARMACOGENOMICS AS A BASIS FOR PERSONALIZED MEDICINE

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Even with the same diagnosis, for many diseases, treatment should not be the same for each patient. A need for optimizing therapies based on patient's unique clinical features and genetic background is recognized in almost every patient. Pharmacogenomics is a basis for personalized medicine. It studies the human response to drugs determined by the unique DNA signature in the genes responsible for the metabolism of a particular drug. Knowing pharmacogenomics markers before the therapy administration could help apply therapy protocols that fit to individual patients according to their genetic background. In that way patients receive adequate therapy (the right drug, the right dose at the right time) with optimal management of the drug efficacy and avoidance of adverse drug reactions. Population pharmacogenomics research has pointed out that pharmacogenomics markers are population-specific, with frequencies varying across different ethnic groups. Guidelines for the use of pharmacogenomics tests, the interpretation of results and drug dosage recommendations according to pharmacogenomics marker identified in a patient, are issued, curated and updated by relevant agencies and consortia. Awareness about the importance of the application of pharmacogenomics testing is rising in the scientific community as well as in the general population. The immense development and application of new generation sequencing technologies has opened up the possibility for genome-scale research in pharmacogenomics filed and speed up the translation of knowledge and its implementation from bed to bedside. With the support of bioinformatics and artificial intelligence tools a door for using pharmacogenomics in personalized medicine are wide open.

PHARMACOGENOMICS, PERSONALIZED MEDICINE, POPULATION PHARMACOGENOMICS

04 – 02 Invited lecture

SOMATIC INSTABILITY OF REPEAT EXPANSIONS DRIVES PROGRESSION IN MYOTONIC DYSTROPHY TYPE 1

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Myotonic dystrophy type 1 (DM1) is an incurable disease caused by unstable expansions of CTG repeats in the *DMPK* gene. It is characterized by multisystemic symptoms whose severity and course vary greatly from patient to patient and can be partially explained by the size of the repeat expansion. We have shown that the somatic instability of DM1 mutation, which leads to an increase in expansion size over time, contributes directly to the disease progression.
Our longitudinal study of patients with pure expansions has revealed that somatic instability drives the progression of skeletal muscle symptoms. Patients with a change in MIRS score (a measure of skeletal muscle impairment) and MRC score (a measure of muscle strength) showed a greater increase in modal expansion size than patients without a change in scores. The increase in modal expansion size was a significant predictor of change in MIRS and MRC scores. Further evidence comes from our study of patients with interrupted expansions, who showed a lower degree of somatic instability and a smaller increase in modal expansion size over time compared with patients with pure expansions. Individual-specific factors, which in our study design were primarily repeat interruptions, contributed to a later age at onset by stabilizing expansions in somatic cells.
Our research has provided firm evidence of slower disease progression in patients with less pronounced somatic instability, highlighting somatic instability as a critical therapeutic target for repeat expansion disorders.

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REPEAT EXPANSIONS, SOMATIC INSTABILITY, DISEASE PROGRESSION, MYOTONIC DYSTROPHY, REPEAT EXPANSION DISORDERS

04 – 03 Invited lecture

DIAGNOSTIC YIELD OF WHOLE GENOME SEQUENCING IN A TERTIARY PEDIATRIC REFERRAL CENTRE: A 5-YEAR RETROSPECTIVE STUDY

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The Clinical genetic service at the University Children's Hospital in Belgrade has started to employ NGS-based techniques since November 2014. Initially, we started with solo (patient only) clinical exome sequencing (Mendeliome), followed by solo and trio (patient + parents) whole-exome sequencing, and finally, for those having convincing genetic phenotype and negative exome results - genome-sequencing.
We analyzed data of pediatric and adult patients who: I) visited genetic outpatient clinic at the Clinical genetic service, University Children's Hospital, from 2019 to 2023, II) were referred for diagnostic exome sequencing (+/- other tests) and diagnosed as negative, and III) were referred for diagnostic whole genome sequencing. All patients were suspected to have monogenic disorder.
In total, 112 patients were tested using whole genome sequencing.
So far, a small number of studies have investigated WGS diagnostic yields reporting additional rates between 7 to 34%. These additional diagnostic rates were attributed to CNV detection, improved coverage of difficult-to-sequence regions, and identification of pathogenic variants in non-coding regions. Our study is aimed to assess WGS diagnostic rate and type of detected variants in our WES-negative Mendelian disorder cohort. The results will be compared to literature data and might contribute to better understanding of clinical relevance of diagnostic WGS.

Acknowledgements: We thank the patients, their families, physicians, biologists, nurses and laboratory technicians in our and external centers for their participation in this study and production and processing of genome sequencing data.

WHOLE GENOME SEQUENCING; DIAGNOSTIC RATE; MONOGENIC DISORDER

04 – 04 Invited lecture

COPY NUMBER VARIATIONS IN CHILDREN WITH AUTISM SPECTRUM DISORDERS

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Autism spectrum disorders (ASD) are complex and genetically heterogeneous. The frequency of ASD is as high as 1:59 children with male to female ratio close to 4:1. Copy number variants (CNVs) are important contributors to the pathogenesis of ASD. There is a great necessity for genetic testing in children with ASD since the obtained results can influence the further course of therapy and clinical monitoring of patients.
Study included 232 children with ASD, referred to the Institute of Human Genetics by clinical geneticists. There were 175 boys (75.4%) and 57 girls (24.6%), from 2 to 17 years of age. The array-CGH procedure was performed using Agilent SurePrint G3 Human 8×60K CGH or 4x180K CGH+SNP microarray.
Clinically significant CNVs (pathogenic and likely pathogenic) were detected in 28 patients (12%). Detection rate was significantly higher in girls - 19.3% than boys – 9.7% (p=0.05). Six (20.6%) of the clinically significant CNVs were inherited from the healthy parent and 11 (38%) were recognized as recurrent. Also, detection rate was significantly higher in the group of children with syndromic form of ASD (p=0.016). Although not statistically significant, it was noticeable that duplications led to the less severe phenotype than deletions. Duplications were more frequent in boys, while girls with clinically significant CNVs had more severe phenotype. Chromosomal microarray proves to be a useful diagnostic tool for evaluation of ASD, especially in the syndromic forms of ASD. There is a significant difference in detection rate of clinically significant variants between boys and girls.

MICROARRAY, AUTISM, CNVS

04 – 05 Invited lecture

GENETICS OF NEURODEGENERATIVE DISORDERS

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The neurodegenerative disorders encompass conditions that, although clinically different, have overlapping pathological mechanisms leading to progressive neuronal loss and accumulation of misfolded proteins. The majority of those disorders originate from a complex interaction between aging, genetics and the environment. Despite the significant number of identified genetic variants associated with neurodegenerative diseases, the role of genetic factors in neurodegeneration is not completely understood.

The fifteen years of our genetic research of Alzheimer's diseases (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) revealed numerous specificities of Serbian patients. Two of our genetic studies of AD, although with different methodological approaches, showed that the variant frequencies were within what was expected given previous reports. Nevertheless, the novel variants have been identified in *PSEN1*, as well as the variants in more than single gene in the same patient. In PD patients, the second most common *GBA* variant, D409H, was found only in [D409H;H255Q] allele, which is in concordance with high frequency of that complex allele in Balkan patients. Additionally, two heterozygous variants, D380V and N392S, have been described as novel variants, while the L444P variant alone was underrepresented compared to other studies. Differences in genetic determinants were also found in ALS, as the L144S variant in *SOD1* is the most common variant in our ALS patients due to the founder effect.

These genetic differences are highlighting the importance of establishing region specific genetic databases in order to create accurate guidelines for genetic testing strategies of neurodegenerative disorders.

NEURODEGENERATIVE DISORDERS; ALZHEIMER'S DISEASES (AD); PARKINSON'S DISEASE (PD); AMYOTROPHIC LATERAL SCLEROSIS (ALS); FRONTOTEMPORAL DEMENTIA (FTD)

04 – 06 Invited lecture

CLINICAL MANIFESTATIONS AND INNOVATIVE TREATMENT OPTIONS FOR INHERITED FORMS OF HYPOPHOSPHATEMIA

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Hypophosphatemia includes acute and chronic disorders and refers to the presence of serum phosphate concentrations lower than age-appropriate reference values. Among all forms of hyperphosphatemia, hereditary forms of hypophosphatemia are distinguished as a separate group. One of them is hypophosphatemic rickets which is also a heterogeneous group of disorders. X-linked hypophosphatemic rickets are the most common inheritable form of rickets (80%), which is caused by *PHEX* gene mutation (Xp22.1). There are also three other forms, but less often, autosomal dominant hypophosphatemic rickets caused by a mutation in *FGF23* (12p13.32); autosomal recessive hypophosphatemic rickets caused by a mutation in *DMP1* (4q22.1), *ENPP1* (6q23.2), *FAM20C* (7p22.3) or *SLC34A3* (9q34.3) and X-linked recessive hypophosphatemic rickets caused by a mutation in *CLCN5* (Xp11.23).

In persons who suffer from the most common form, X-linked hypophosphatemic rickets, there is a high level of fibroblast growth factor - FGF23, which causes hyperphosphaturia and leads to hypophosphatemia and clinical manifestations such as weakened bones. Clinical manifestations may vary in severity and include short stature, genu varum or genu valgum that impaired mobility, abnormal head shape due to craniosynostosis, muscle weakness, bone pain, fatigue and dental abscesses due to impaired mineralization of enamel and dentine.

Careful clinical and laboratory examination, and imaging, with detection of mutations, are crucial in establishing a definite diagnosis which is necessary for medical management and to differentiate which forms of disease is possible to treat, except conventional treatment, with specific treatment that includes a recombinant human IgG1 monoclonal antibody to FGF23, that leads to increase in phosphate levels in the blood with more phosphorus absorbed by the bones and teeth due to a reduction in phosphate renal wasting.

HYPOPHOSPHATEMIA; FAMILIAL HYPOPHOSPHATEMIC RICKETS; HYPOPHOSPHATEMIA, X-LINKED DOMINANT; VITAMIN D-RESISTANT RICKETS, X-LINKED

04 – 07 Invited lecture

PRENATAL DIAGNOSTICS OF FETAL ANOMALIES- STANDARDS OF CARE AND FUTURE PERSPECTIVES

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Congenital anomalies affect 2-4% of pregnancies, with significant impact on fetal mortality and morbidity. Various types of genetic changes can cause development of anomalies; the most significant are chromosomal aberrations, copy number variations and single gene disorders. Genetic testing of the fetus is recommended whenever congenital malformations are detected by ultrasound examination. With the application of cytogenetics and molecular cytogenetics techniques, the cause of anomalies can be established in about 35% of cases. The implementation of affordable and fast next generation sequencing technologies has led to revolutionary developments in the field of fetal medicine, with gene panels or whole exome sequencing (WES) most commonly used in routine diagnostics. The diagnostic yield of WES in prenatal diagnosis depends on the type and number of congenital anomalies and ranges from 6-80%. Still, with the currently applied standards of care, a significant proportion of cases remains without genetically confirmed diagnosis. In postnatal setting, use of novel omics technologies such as optical genome mapping, RNA sequencing, whole genome sequencing (WGS), long-read sequencing and metabolomics facilitates diagnostic odyssey in rare disease patients. Due to specificity of prenatal diagnostics such as difficulties in fetal phenotyping or inability to perform functional testing, their use in this field is currently restricted to research setting only. However, the development of new methods has led to a significant improvement in prenatal medicine, allowing for individualized and personalized genetic counseling regarding potential treatment modalities, risks for recurrence in subsequent pregnancies, and informed decisions about the further course of pregnancy.

PRENATAL DIAGNOSIS, FETAL ANOMALIES, NEXT GENERATION SEQUENCING, MULTIOMICS

04 – 08 Invited lecture

GENETIC TESTING IN PEDIATRIC HEMATO-ONCOLOGICAL DISEASES

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Genetic testing represents a fundamental step in diagnosis of hemato-oncological diseases. Numerous clinical trials worldwide, since 1988, accomplished data that provided latest WHO classification of these diseases as mainly based on genetic factors. Definition of genetic changes became necessary for precise diagnosis, risk stratification, prognosis, treatment and follow-up. Modern testing approach includes simultaneous application of cytogenetic, molecular cytogenetic (FISH) and molecular genetic methods (PCR, microarray, NGS).
Laboratory of Medical Genetics at the Mother and Child Health Care Institute of Serbia "Dr. Vukan Čupić" (IMD), stands out as one of the leading national laboratories, considering genetic testing in hemato-oncology cases in children (age ≤18). Since 2022, all analyses are carried out within the Cabinet for Genetics of Hemato-oncological Diseases, separated laboratory section. Testing list includes: karyotyping of bone marrow cells (since 1982, for IMD patients), FISH for recurrent aberration in neuroblastoma and leukemias (since 1998, for all patient from R Serbia and R Srpska), RNA RT-PCR analyses for recurrent fusion genes in leukemia according to ALLIC BFM protocol and neuroblastoma mRNA marker, tyrosine hydroxylase (since 2007, in all patients from R Serbia and R Srpska) and monitoring of chimerism after bone marrow transplantation using STR-PCR analyses (since 2012). Procedures are provided due to current European guidelines and quality control shames in every-day collaboration with hematologist and pathologist.
The aim of this lecture would be to present the strategy of genetic testing in children with hemato-oncological disorders, importance of teamwork and necessity of novel techniques (microarray, NGS) for more adequate treatment of patients.

GENETICS, TESTING, HEMATO-ONCOLOGICAL DISEASES, CHILDREN

04 – 09 Invited lecture

ESTABLISHMENT OF A MODEL SYSTEM FOR STUDYING NEURODEVELOPMENTAL DISORDERS USING INDUCED PLURIPOTENT STEM CELLS DERIVED FROM PATIENTS WITH 22Q11.2 DELETION SYNDROME

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22q11.2 Deletion Syndrome is the most common microdeletion syndrome in humans. It is associated with elevated risk for neurodevelopmental psychiatric disorders and thus represents a powerful genetics-first approach to delineate molecular mechanisms underlying these disorders. Although many animal models mimic human diseases, only limited success has been achieved in revealing molecular mechanisms underlying human brain diseases. Our goal was to establish patient-specific induced pluripotent stem cells (iPSCs) since they represent a powerful tool for establishing in vitro models of disorders.

Peripheral blood mononuclear cells of control subjects and patients with 22q11.2 microdeletion were reprogrammed using CytoTune™-iPS 2.0 Sendai Reprogramming Kit. Generated iPSC cell lines were characterized by analyzing their morphology, pluripotency, genomic integrity and the ability to differentiate into three germ layers. iPSCs were differentiated into neural progenitor cells and neurons using Dual-SMAD inhibition method and 3D cerebral organoids in order to analyze neural differentiation in patient-specific background. RNA sequencing was performed to determine differentially expressed gene sets and dysregulated pathways in neural cells derived from patients with 22q11.2 microdeletion.

We successfully generated iPSC lines from patients with 22q11.2 microdeletion and healthy individuals, characterized them and differentiated into neural progenitors, neurons and cerebral organoids. List of differentially expressed genes was determined. Generated patient-specific iPSCs represent a powerful model system for studying molecular mechanisms underlying NDDs.

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INDUCED PLURIPOTENT STEM CELLS, 22Q11.2 MICRODELETION, NEURODEVELOPMENTAL DISORDERS

04 – 10 Invited lecture

FERROPTOSIS-RELATED PROCESSES GENE EXPRESSION AND GENE VARIANTS: RELEVANCE FOR DISEASE SEVERITY IN PATIENTS WITH MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) involving progressive neurodegeneration. Impairment of the ferroptosis related processes lead to iron overload, lipid peroxidation, oxidative damage and mitochondrial dysfunction, molecular mechanisms involved in various neurodegenerative diseases. Comperhensive genetic regulatin of ferroptosis as orchestrated process in MS have not been investigated in humans, yet. Recently, through FerroReg project we determined the expression pattern/signature of a comprehensive set of 138 ferroptosis-related genes and (miRNAs) in highly homogenous groups of patients with distinctive MS phenotypes, mild relapse-remitting (RR) and severe secondary progressive (SP) MS in peripheral blood mononuclear cells (PBMC), using RNAseq methodology. The panel included genes with role in lipid oxidative metabolism, antioxidant defense and iron metabolism, as well as their related main transcriptional regulators. We have established networks of all ferroptosis-related differentialy expressed genes and miRNAs with regard to disease severity. Further, we performed excessive data mining to prioritize and select single nucleotide polymorphisms (SNPs) that are potential genetic regulators of the relevant ferroptosis related genes and processes with regard to their proposed functionality using publicly available data bases, literature sources, and experimentally confirmed influence on enzyme function. Selected SNPs will be analysed in association with disease severity and with molecular components/indicators of the main ferroptosis related processes, taking into account environmental and demographic factors. Experimentally confirmed functional annotations will enable design of SNP panel of the most relevant genetic variants for regulation of ferroptosis key steps and with regard to the MS severity.

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FERROPTOSIS, RNASEQ, MIRNA, SNP, MULTIPLE SCLEROSIS

04 – 11 Invited lecture

SYNERGISTIC TARGETING OF CD44+ CANCER STEM CELLS IN ORAL SQUAMOUS CELL CARCINOMA THROUGH MIRNA-21 SILENCING AND BET INHIBITION

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Oral squamous cell carcinoma (OSCC) represents one of the most prevalent malignancies globally, often characterized by aggressive behavior. It has been established that a small side cell population -the cancer stem cells (CSCs) play a pivotal role in OSCC recurrences, metastases and resistance to therapy. MicroRNA-21 (miRNA-21) is known to enhance chemoresistance and is associated with poor prognosis in various cancers. This study investigates the effect of miRNA-21 silencing on CSCs treated with the bromodomain and extra-terminal (BET) inhibitor JQ1. We isolated CSCs using their specific marker CD44 from OSCC cultures, silenced miRNA-21, and treated these cells with JQ1. Our findings demonstrate that miRNA-21 silencing significantly enhances the anti-tumor effects of JQ1 on CD44+ CSCs, leading to increased cell death through apoptosis, reduced invasion, and altered cell cycle dynamics. This synergistic effect was further explained through the regulation of apoptosis-related genes (*BAX, CASP3*) and the WNT signaling pathway. These results suggest that combining miRNA-21 silencing with BET inhibition could be a promising therapeutic strategy for targeting CSCs in oral cancer, potentially overcoming resistance and improving clinical outcomes.

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ORAL CANCER, CANCER STEM CELLS, MIRNA-21, BET INHIBITOR, QPCR

04 – 12 Oral

EFFECT OF FREEZE-DRIED LUNG TISSUES (LFDT) ON MODULATION OF GENE EXPRESSION PROMOTING AN AGGRESSIVE PHENOTYPE IN NON-SMALL CELL LUNG CANCER (NSCLC)

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Lung cancer is one of the leading causes of death worldwide, and alternative therapeutic strategies are needed to improve tumor response. In past, bioactive molecules from different tissues and organs have shown potential therapeutic benefits, but additional studies are needed in this research field. Here, we characterized and investigated the biological role of bioactive factors in lyophilized freeze-dried lung pig tissues (LFDT) in inducing gene modulation affecting cancer progression. We used NSCLC H1975 cells treated with LFDT at concentrations of 0.1 mg/ml, 0.25 mg/ml, and 0.5 mg/ml. To detect the expression of genes involved in cell migration and EMT (Epithelial-Mesenchymal Transition), we performed RT-PCR. Cell cycle analysis, western blotting, and mitochondrial membrane potential (MMP) assays were performed to assess cell proliferation and cell death activation.
In H1975 cells, LFDT at 0.25 mg/ml and 0.5 mg/ml reduced cell viability by 50% and 75%, respectively. LFDT treatment also reduced the proliferation ability of cancer cells, as shown by cyclin D1 levels, cell cycle analysis and clonogenic assay. In addition, MMP assay also demonstrated an increased cell death in treated cells. These findings align with RT-PCR results, which showed downregulation of EMT genes *vimentin* and *N-cadherin*. Interestingly, the EMT transcription factor (TF) *Twist* was also significantly reduced in LFDT treated H1975 cells.
Our results showed that bioactive factors contained in LFDT can modulate EMT genes and TFs, thus determining a less aggressive phenotypic signature in H1975 cells and causing increased cancer cell death and toxicity.

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LFDT, EMT, NSCLC, MMP, TFs,

04 – 13 Oral

POLYMORPHISMS OF HLA-A, -B, -DRB1 ALLELES AND HAPLOTYPES IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Polymorphism represents the normal diversity of the hereditary basis of man. Within the study of the role of genetic factors in the origin and development of polygenic diseases, a large number of candidate genes are being investigated. Among them are the genes of the human leukocyte antigen system (HLA), the most polymorphic gene locus in humans, which is considered to be a predisposing factor in the development of acute myeloid leukemia (AML).
To determine the polymorphism of HLA alleles and haplotypes in patients with AML, as well as the potential difference in the frequency of the obtained alleles in patients with AML compared to the available frequencies in the healthy population.
442 patients were included in the retrospective analysis. DNA samples were isolated by manual method (Qiagen) and automatic methods (Maxwell, SaMag). HLA typing was done using sequence-specific oligonucleotide probes (PCR-SSO, One Lambda) on a Luminex device to prove the group of HLA alleles, and sequence-specific primers (PCR-SSP, Olerup) were used to prove individual alleles. Haplotypes were obtained by segregation based on HLA typing of available closest family members for 114 patients. Chi-square test was used to prove statistical significance.
The most common alleles (AF>10%) are: HLA-A*02:01 (28%), HLA-A*01:01 (14%), HLA-A*03:01 (14%), HLA -A*24:02 (12%), HLA-B*51:01 (11%), HLA-DRB1*11:04 (13%) and HLA-DRB1*16:01 (12%). The most common haplotype in AML is HLA-A*01:01-B*08:01-DRB1*03:01 (4%), followed by HLA-A*02:01-B*51:01-DRB1*11: 04 (2.2%) and HLA-A*33:01-B*14:02-DRB1*01:02 (2.2%). Statistically significant differences in the distribution of alleles compared to the healthy population ($p<0.05$) were demonstrated in: HLA-A*03:01 ($p=0.04$), HLA-DRB1*13:02 ($p=0.03$), HLA-DRB1*01:02 ($p=0.005$) and HLA-DRB1*11:04 ($p=0.003$).
The HLA polymorphisms found in patients with AML may be a predisposing factor in the development of AML, which needs to be confirmed by further studies on a larger number of patients.

POLYMORPHISM, AML (ACUTE MYELOID LEUKEMIA), HLA TYPING

04 – 14 Oral

MUTATIONS IN *JAK2*, *CALR*, *MPL* AND *NPM1* GENES IN PRIMARY MYELOFIBROSIS

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Primary myelofibrosis (PMF) is a myeloproliferative neoplasm (MPN), a clonal hematopoietic stem cell disorder characterized by the proliferation of predominantly abnormal megakaryocytes and granulocytes. Mutations in *JAK2* (Janus kinase 2), *CALR* (calreticulin) and *MPL* (myeloproliferative leukemia virus oncogene) genes predispose to PMF.
JAK2 V617F, *MPL* W515L, *MPL* W515K, *CALR* type I and II, *NPM1* type A, B and D, and *FLT3* ITD mutation testing was performed on whole blood samples of 19 patients with PMF, using the real-time PCR technique for *JAK2*, *MPL* and *NPM1* mutations and gel electrophoresis for *CALR* and *FLT3* mutations.
JAK2 V617F mutation was detected in 11 (58,89%), *MPL* W515L in 1 (5,26%), *MPL* W515K in 1 (5,26%), *CALR* type I mutation was found in 3 (15,79%) and *CALR* type II in 1 (5,26%) of 19 samples. Neither the *FLT3* ITD, nor any of the analyzed *NPM1* mutations were present in the patients.
JAK2, *MPL* and *CALR* gene mutations frequently occur in PMF patients. Identifying the specific mutation is essential for evaluating the risk of severe disease or complications and tailoring personalized treatment plans.

JAK2, MYELOFIBROSIS, *MPL*, CALRETICULIN, PCR

04 – 15 Oral

GENETIC EVALUATION OF 203 SERBIAN PATIENTS WITH SKELETAL DYSPLASIA

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Genetic skeletal dysplasia are heterogeneous diseases characterized by bone and cartilage growth abnormalities. The most recent revision of the classification of genetic skeletal disorders from 2023 contains 771 diseases divided into 41 groups.
The goal of the study was to determine the distribution by classification groups for Serbian patients and perform genetic evaluation of patients with skeletal dysplasia admitted to our hospital in the last eight years.
We conducted a retrospective analysis of 203 probands: in 168 next generation sequencing was performed and in 35 gene/mutation targeted testing was done.
Next generation sequencing showed a high detection rate of the causative gene variant(s) of 60.71% (102/168). Additionally, 5.95% (10/168) of a non-diagnostic patient report had gene variant(s) of unknown significance. In the targeted tested group, the result was positive for 77.14% (27/35) of them.
The distribution by groups was, as expected, the highest in the most frequent groups such as the phenotypic spectrum dysfunction of the *FGFR3*, *FGFR2*, collagen type I and collagen type II genes and a group of overgrowth syndromes including Marfan syndrome. However, some of the patients were diagnosed with rare skeletal dysplasia from different groups. Out of 41 groups, we have at least one diagnosed patient in 27 groups.
We showed that a combination of next generation sequencing and gene/mutation targeted testing could achieve a high diagnostic rate for patients with skeletal dysplasia. Knowledge of the skeletal dysplasia classification group contributes to better monitoring of diagnosed patients and increases our competence aimed at improving diagnostics for new patients.

SKELETAL DYSPLASIA, OSTEOCHONDRODYSPLASIA, CLASSIFICATION OF SKELETAL DYSPLASIA, NEXT GENERATION SEQUENCING, GENE/MUTATION TARGETED TESTING

04 – 16 Oral

PROGNOSTIC SIGNIFICANCE OF ALTERED EXPRESSION OF THE MEMBERS OF PI3K/PTEN/AKT/MTOR PATHWAY AND PD-L1, AR AND EGFR TRANSMEMBRANE PROTEINS IN TNBC PATIENTS

Nasta Tanić [1], Nataša Medić – Milijić [2], Mirjana Prvanović [3], Milica Nedeljković [2], Zorka Milovanović [2], Nejla Ademović [4], Nikola Tanić [4]

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Breast cancer is the most frequent type of cancer and the leading cause of cancer related death in women. The most aggressive breast cancer subtype is Triple-Negative Breast Cancer (TNBC). It is associated with high recurrence rates, high incidence of distant metastases and poor overall survival. TNBC has a higher mitotic index than hormone receptor-positive (HR+) BC implying strong upregulation of growth factor signaling. We hypothesized that the activation of PI3K/PTEN/AKT/mTOR (PPAM) pathway might be a driving force behind TNBC aggressive phenotype, followed by the alterations of some important transmembrane proteins like PD-L1 (Programmed Death-Ligand 1), EGFR (Epidermal Growth Factor Receptor) and Androgen Receptor (AR). To that end we analyzed immunoexpression of these genes in 125 postoperative samples of TNBC patients and correlated obtained results with clinicopathological parameters and survival. Our results imply that these genes should be considered as packages of genes and should be analyzed simultaneously, not individually. Namely, loss of PTEN (low expression) and high expressions of PI3K and mTOR are associated with lymphatic node metastases, advanced pathological prognostic stage, larger tumor size and with poor outcome of TNBC patients. Therefore, we think that PTEN-reduced/PI3K-high/mTOR-high immunoexpression is a high-risk profile for TNBC patients. Further, our result showed that PD-L1 low/AR low/EGFR high immunoexpression, with the support of Ki-67 expression, is a real “high risk” profile for these patients. We believe that these two packages could be a promising formula for better prediction and exploration of better treatment modalities for TNBC patients.

TNBC, PI3K/PTEN/AKT/MTOR PATHWAY, PD-L1, AR, EGFR

04 – 15 Oral

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We showed that a combination of next generation sequencing and gene/mutation targeted testing could achieve a high diagnostic rate for patients with skeletal dysplasia. Knowledge of the skeletal dysplasia classification group contributes to better monitoring of diagnosed patients and increases our competence aimed at improving diagnostics for new patients.

SKELETAL DYSPLASIA, OSTEOCHONDRODYSPLASIA, CLASSIFICATION OF SKELETAL DYSPLASIA, NEXT GENERATION SEQUENCING, GENE/MUTATION TARGETED TESTING

04 – 16 Oral

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TNBC, PI3K/PTEN/AKT/MTOR PATHWAY, PD-L1, AR, EGFR

04 – 19 Oral

APOE GENOTYPE, *ATXN1* AND *ATXN2* REPEATS SIZE IN *C9ORF72* EXPANSION CARRIERS

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The *C9orf72* repeat expansion is known as the most frequent genetic cause of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). However, in *C9orf72* expansion carriers, other genetic factors could impact disease phenotype. From this view point, *APOE4* allele (known risk factor for Alzheimer's disease), as well as of CAG intermediate repeats in *ATXN1* and *ATXN2* genes were studied but results are inconclusive.
The study included 57 patients with *C9orf72* repeat expansion (38 with ALS, 9 FTD, 2 with undetermined dementia, and 8 with ALS/FTD) and 94 healthy controls. The *C9orf72* expansion was detected with repeat-primed PCR, and *ATXN1* and *ATXN2* repeat sizing was done with QF-PCR. For *APOE* genotyping we used real-time PCR. Statistical analysis was done with Fisher's exact test.
In *C9orf72* expansion carriers, *APOE4* was registered in 24.56% of patients, all in heterozygous form. Regarding CAG repeats, 29.82% had an intermediate repeat number in *ATXN1* (5.88% on both alleles) and 7.02% in *ATXN2* (25% on both alleles). In controls, *APOE4* allele was registered in 19.15% cases (11.11% homozygous), 30.85% had an intermediate number of repeats in *ATXN1* (13.79% on both alleles), and 8.51% in *ATXN2* (37.5% on both alleles). There was no significant difference in the frequency of *APOE4*, intermediate repeats in *ATXN1* and *ATXN2* between patients and controls ($p>0.05$).
This is the first report of *APOE4*, *ATXN1*, and *ATXN2* genotypes in our *C9orf72* expansion carriers, and further study is needed to elucidate their role in a larger sample size.

Acknowledgments: This study was supported by Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Agreement no: 451-03-66/2024-03/200110)

APOE, ATXN1, ATXN2, C9ORF72

04 – 20 Oral

GENETIC SPECTRUM OF NOONAN SYNDROME IN PEDIATRIC POPULATION

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Noonan syndrome (NS) is an autosomal dominant hereditary disorder with a prevalence of 1:1000 to 1:2500 live births. First described in 1963 by pediatric cardiologist Jacqueline Noonan, this syndrome is classified among RASopathies which arise from pathogenic variants in genes disrupting RAS/MAPK signaling pathway. The most commonly affected genes in NS are PTPN11, SOS1, RAF1, KRAS, and NRAS genes. The genetic diversity results in a spectrum of clinical manifestations. The aim of this study is to explore genetic and clinical heterogeneity of NS.
The patients included were monitored from 2010 to 2024 at the Mother and Child Health Care Institute of Serbia "Dr Vukan Cupic". Diagnosis was established on basis of suggestive clinical findings while definitive diagnosis was confirmed through whole exome sequencing (WES) or next-generation sequencing (NGS) panels for RASopathies.
We present the clinical features and genetic findings of 15 patients with NS with median age at diagnosis of 8 years. Distribution of patients in regard to gender was balanced (f:m - 8:7). The causative variants were found in the PTPN11, RAF1, SOS1, and LZTR1 genes, most frequently in PTPN11. Typical facial dysmorphism was universally present, while developmental delay was noted in 20%. The most common cardiac manifestations of NS in our group was hypertrophic cardiomiopathy (53.3 %) and pulmonary artery stenosis (33.3%).
Identification of causative genetic variants in patients with Noonan syndrome through NGS methodology provides insight into the pathomechanism thereby providing prognostic information and potential therapeutic targets.

NOONAN SYNDROME, RASOPATHIES, HYPERTROPHIC CARDIOMYOPATHY

04 – 21 Oral

GENOMIC TESTING FOR CONGENITAL ANOMALIES IN FETUSES: A COMPREHENSIVE APPROACH

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Congenital anomalies pose significant public health and epidemiological challenges, affecting approximately 2-3% of liveborn infants and 20% of stillborn infants. The etiology of these anomalies is multifaceted and can result from various genetic mechanisms. In our study, we systematically analysed a sample of 204 consecutive fetuses following pregnancy termination to assess the contribution of chromosomal abnormalities, copy number variations, and monogenic causes to isolated and multiple congenital anomalies.
In assessing chromosomal anomalies, we employed classic karyotyping and/or QF-PCR. Molecular karyotyping was utilized to detect copy number variations. In cases where results were normal, whole exome sequencing was subsequently performed.
Notably, aneuploidies and major structural chromosomal abnormalities were detected in 40.2% of fetuses, while pathogenic or likely pathogenic copy number variations were observed in 6.8%. Additionally, in 48 fetuses, after excluding chromosomal anomalies, whole exome sequencing yielded diagnostic insights in 27.1% of cases.
These findings underscore the importance of incorporating systematic genomic testing into the diagnostic approach for congenital anomalies.

FETUSES, CONGENITAL ANOMALIES, KARYOTYPING, WHOLE EXOME SEQUENCING, ANEUPLOIDIES, COPY NUMBER VARIATIONS

04 – 22 Oral

PARENTAL DECISIONS TO TERMINATE PREGNANCY IN THE PRESENCE OF CHROMOSOMAL ABNORMALITIES: THE SLOVENE EXPERIENCE

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The objective was to investigate the rates of termination of pregnancy (TOP) for fetal chromosomal abnormalities and factors related to such parental decisions in Slovenia.
Retrospective analysis using the information system of University Medical Center Ljubljana was performed. The study investigated the pregnancy outcomes and parental decisions in frequent fetal chromosomal abnormalities identified by prenatal diagnostics for the period from 2010 to 2020.
Among 294 couples with established fetal chromosomal abnormalities, 273 (92,9%) decided to terminate a pregnancy. Couples with more severe forms of chromosomal abnormalities (trisomy 13, trisomy 18, and trisomy 21) terminated pregnancy more frequently than couples with milder (XXY, 45,X0, XXX, and XYY syndromes) chromosomal abnormalities (96,7% vs. 45,5%).
In couples in whom a more severe form of chromosomal abnormality was found in the fetus, the number of preterm infants was statistically significantly related to the decision, while in couples with a milder chromosomal abnormality, the age of the pregnant woman was identified as the factor related to the decision to terminate the pregnancy.
In Slovenia, there is a relatively high percentage of couples who decide to terminate a pregnancy following the identification of chromosomal abnormalities, particularly milder ones. We estimate that improved education could contribute to more informed and autonomous decision-making by couples.

PRENATAL DIAGNOSIS, CHROMOSOMAL ABERRATIONS, PARENTAL DECISIONS

04 – 23 Oral

EPIGENETIC SIGNATURES AS BIOMARKERS OF ENVIRONMENTAL EXPOSURE

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Genomic and exposome factors contribute significantly to individual health risks. The exposome encompasses the totality of environmental exposures; however, vague and self-reported methods frequently compromise its data reliability and comprehensiveness. We hypothesize that the methylome could be a biomarker for environmental exposure, providing a more objective and quantifiable measure.

We aimed to conduct a systematic review of studies investigating the potential effects of environmental factors, including alcohol, smoking, heavy metals, and air pollution, on methylation patterns. Following PRISMA guidelines, we conducted an advanced search in the PubMed database and identified 29 relevant studies. These studies analyzed methylation patterns, utilizing epigenome-wide association studies and Illumina arrays.

For alcohol exposure, we compared three studies with a total of 17,191 participants. We discovered 5,298 differentially methylated CpG sites annotated to 3,079 genes. A Venn diagram identified 107 CpG sites and 141 genes that overlapped among all three studies. We included 13 studies with 17,079 participants for smoking exposure, identifying 2,585 differentially methylated CpG sites and 1,613 genes. An upset plot detected two overlapping CpG sites among ten and four overlapping genes across 12 studies. For heavy metals, we included six studies with 2,063 participants; for air pollution, we included seven studies with 12,100 participants. Despite identifying differentially methylated CpG sites and associated genes, the upset plot did not confirm matching CpG sites and genes between studies for each exposure.

This study identified common epigenetic signatures for alcohol and smoking exposures, suggesting the potential role of the methylome in evaluating the human exposome.

EPIGENETICS, BIOMARKERS, HUMAN EXPOSOME

04 – 24 Poster

THE ROLE OF SOX2 AND SOX9 TRANSCRIPTION FACTORS IN THE REACTIVATION-RELATED FUNCTIONAL PROPERTIES OF NT2/D1-DERIVED ASTROCYTES

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Astrocytes are the main homeostatic cells in the central nervous system. During reactive astrogliosis, these cells transform from quiescent into a reactive state by reacquiring some of the precursor properties. This process, activated in response to different pathological conditions of the brain, serves as a compensatory response that modulates tissue damage and recovery. SOX2 and SOX9 transcription factors have important roles during gliogenesis. These transcription factors are down-regulated in mature astrocytes and re-expressed in reactive astrocytes, however, their roles in these cells are still not fully elucidated. We focused our study on reactivation-related functional properties of astrocytes mediated by these proteins. Initial screening of SOX2 and SOX9 expression after sensorimotor cortex ablation injury in rats was performed using immunohistochemistry. Further gain-of-function studies were done in vitro using astrocytes derived from the NT2/D1 cell line (NT2/A). Our results revealed the direct involvement of SOX2 in the reacquisition of proliferation in mature NT2/A, while SOX9 overexpression increased migratory potential and glutamate uptake in these cells. Our results imply that modulation of SOX gene expression may change the functional properties of astrocytes, which holds promise for the discovery of potential therapeutic targets in the development of novel strategies for neural tissue regeneration and recovery.

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REACTIVE ASTROCYTES; SOX GENES; NT2/D1 CELL LINE; GLUTAMATE UPTAKE; ASTROGLIOSIS

04 – 25 Poster

NT2/D1 EARLY NEURAL PROGENITORS IN 3D ALGINATE MICROFIBERS AS A MODEL SYSTEM FOR SCREENING THE EFFECT OF BIOACTIVE COMPOUNDS

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The NT2/D1 embryonal carcinoma cells represent a well-established in vitro model of human neurogenesis. Alginate fibers' platform, a 3D cell culture system, is a biocompatible, structurally supportive environment for neurogenesis suitable for investigating the effects of bioactive compounds on neurodevelopment.
Using manual extrusion NT2/D1 cells were immobilized in alginate microfibers and neural differentiation was induced by 48h of induction with retinoic acid (RA). Initiation of neural differentiation in the 3D model was evaluated by assessment of morphological features, cell viability, apoptosis and expression of pluripotency factors and early neural markers. Using the same methodology, we investigate potential effects of major components of energy drinks, caffeine and taurine, on the NT2/D1 early neural progenitors.
We established the alginate microfibers as a 3D model system for in vitro neural differentiation of immobilized NT2/D1 cells and evaluated the effects of caffeine and taurine on the early stages of neural differentiation of NT2/D1 cells. Upon RA induction NT2/D1 neural progenitors retained viability and proliferative capacity and showed decreased expression of pluripotency markers SOX2, OCT4, and NANOG and increased expression of early neural markers SOX3, PAX6, and miR219. We also revealed the effects of caffeine and taurine on the viability, proliferation and expression of SOX2 and PAX6 of the NT2/D1 neural progenitors.
Neural differentiation of NT2/D1 cells immobilized within alginate microfibers represents a promising 3D model for studying the effects of bioactive compounds on human neurogenesis.

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NT2/D1 CELL LINE; NEURAL DIFFERENTIATION; ALGINATE FIBERS; 3D MODEL SYSTEM

04 – 26 Poster

CRISPR-CAS9 GENERATED CELL LINES FOR MYOTONIC DYSTROPHY TYPE 1 MODELING

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Expansions of short tandem repeats cause over 50 neurological disorders (e.g. Huntington's disease, myotonic dystrophies). Myotonic dystrophy type 1 (DM1) is a progressive multisystemic disorder caused by a CTG repeat expansion in the 3′ untranslated region of the DMPK gene. Cell models play a pivotal role in studying different aspects of the molecular mechanisms underlying this disease. Although patient-derived DM1 cells overcome the problem of cloning the repeats, they are genetically and epigenetically heterogeneous with differing responses to experimental manipulations. To address this, we used CRISPR-Cas9 genome editing to develop isogenic DM1 cell lines with different expansion sizes and genetically matched control cells.
Plasmids containing CTG repeats were seamlessly cloned and integrated into donor vectors for CRISPR-Cas9 knock-in experiments. Five donor vectors were developed, each containing different repeat lengths (10, 34, 48, 58 and 73 CTG repeats), genomic DMPK regions as homology arms, and a Puromycin resistance gene for selection. Guide RNAs were designed and cloned into the Cas9/gRNA vector, and the knock-in procedure involved co-transfection of Cas9/gRNA and donor vectors. The human HEK293 cell line was selected as the paternal cell line. PCR analysis confirmed site-specific integration with an average efficiency of 33.8%, validated by Sanger sequencing.
Developed DM1 cell-line models will complement the patient-derived cell models allowing to isolate the repeat expansion effect of interest and minimizing the potential for genetic and epigenetic variability. The controlled genetic background, precision in genetic modifications, and phenotypic consistency of the isogenic DM1 lines will enable detailed and reproducible studies.

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CRISPR-CAS9, REPEAT EXPANSIONS, ISOGENIC CELL LINE, MYTONIC DYSTROPHY TYPE 1

04 – 27 Poster

EVALUATION OF ANTHROPOGENETIC, ANTHROPOMETRIC AND BIOCHEMICAL PARAMETERS AS RISK FACTORS IN CARDIOVASCULAR DISEASES

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Cardiovascular disease (CVD) remains the most common cause of death worldwide. The aim of our study was to analyse homozygous-recessive characteristics (HRCs), biochemical (concentration of glucose, cholesterol, triglyceride), and anthropometric parameters (body mass index BMI, circumference waist to height ratio WHtR, waist to hip ratio WHR) in the CVD patients and in the control sample. We analysed presence, distribution, and individual variability of 20 selected HRCs in 273 persons (159 CVD patients, and 114 controls). The results showed a significant difference in the individual variation of HRCs comparing the patients and controls (χ^2=110.6; p<0.001). Significant differences were found in the frequency of 7 out of 20 analyzed HRCs. The average value of HRCs in patients sample was significantly higher, in comparison to the controls (6.69 ± 1.57 vs. 5.15 ± 1.93; p<0.001). The significantly higher concentration of biochemical parameters and BMI (p<0.001) was observed in CVD patients compared to controls. Binary logistic regression analysis confirmed that patients with 5.5 or more HRCs had a 1.67 times higher risk to get sick (OR=1.670; CI =1.420-1.963; p<0.0005). The multiple linear regression analysis of tested variables (age, smoking, HRCs, biochemical and anthropometric parameters) showed that only HRCs and age have a significant impact on the onset of the CVDs. The decision tree model (AUC=0.8673), uses various combinations of simultaneously occurring HRCs including biochemical and anthropometric parameters. Our results show that the HRC test, with continuous monitoring of biochemical and anthropometric parameters, could be used as a screening in recognizing predisposition for CVD.

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ANTHROPOMETRIC PARAMETERS, BIOCHEMICAL PARAMETERS, CARDIOVASCULAR DISEASE, HOMOZYGOUS-RECESSIVE CHARACTERISTICS, GENETIC PREDISPOSITION

04 – 28 Poster

PPARD GENE POLYMORPHISM RS2016520 IN COLORECTAL CANCER PATIENTS

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The potential role of *PPAR* genes polymorphisms as diagnostic and prognostic biomarkers in cancer has recently attracted growing interest. PPARδ is a ligand-activated transcription factor that belongs to the nuclear hormone receptor superfamily and is involved in regulation of lipid and glucose metabolism. PPARδ promotes differentiation and suppresses cell proliferation. Higher expression of PPARD has been associated with favorable survival in rectal cancer patients. However, upregulation of PPARD expression/activity acts as a defense against nutritional deprivation and energy stress, improving cancer cell survival and cancer progression. Polymorphism rs2016520 of *PPARD* gene is located in its 5'UTR region. Minor C allele has been associated with increased level of mRNA.

Our study included 188 patients with CRC, 113 men (60.1%) and 75 (39.9%) women, mean age 62,23±12.06 years. Data about tumor location, histological differentiation, the presence of lymph node metastases, distant metastases and stage of the disease were obtained. Genotypes for *PPARD* (rs2016520) polymorphisms were detected by Real-time PCR using standardized genotyping assays.

Multiple logistic regression analysis, with sex and age of the patients and histological differentiation as covariates, has shown that in patients with rectal cancer who were carriers of rs2016520 C allele (TC or CC genotype) distant metastases were significantly more frequent than in carriers of TT genotype (p=0.034; OR=4.17, 95% CI 1.125-22.008). There were no statistically significant associations with other analyzed clinicopathological characteristics.

Our findings point to significant association between *PPARD* rs2016520 polymorphism and the occurrence of distant metastases in rectal cancer patients.

COLORECTAL CANCER, *PPARD*, GENE POLYMORPHISM, RS2016520

04 – 29 Poster

THE IMPACT OF THE DURATION OF HIV INFECTION ON TELOMERE LENGTH

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Non-chronological biological ageing caused by accelerated cellular senescence in human immunodeficiency virus (HIV)-patients became an important issue. Biomarker that predicts the health status of HIV-infected patients and bridges the gap between chronological and biological age is the length of telomeres, protective structures at the ends of chromosomes. Peripheral blood mononuclear cells represent the current physiological state of the organism very well and their telomere length generally reflects the state of telomere maintenance in other tissues of the same individual. Factors in the cellular environment, i.e. the presence of HIV may contribute to telomere shortening.

The aim of this study was to determine whether the duration of HIV infection affects telomere length.

This cross-sectional study was conducted in 176 HIV-infected Caucasian male patients who had been receiving cART for at least 6 months. All patients were ≥18 years old and virally suppressed. At the time of the study, none of the patients had any other chronic or acute infection. Telomere length (RTL) was determined by quantitative polymerase chain reaction from mononuclear cells, relative to a unique reference sequence.

The mean age of patients (42.70 ± 13.18 years) had no effect on RTL ($p = 0.904$). The mean RTL for the entire group of patients was 2.50 ± 1.87. The Kendall correlation test showed no influence of the duration of HIV-infection (92.42 ± 72.22 months) on the length of the patients' telomeres ($p = 0.220$).

In summary, the duration of HIV infection does not impact telomere length.

AGING, HIV INFECTION, TELOMERE

04 – 30 Poster

ENDOGENOUS IFNB1 MRNA LEVEL ASSOCIATES WITH THE COURSE OF MULTIPLE SCLEROSIS

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Genetic variant rs2275888 C/T from eQTL in the 3-Hydroxyacyl-CoA Dehydratase 4 (*HACD4*) gene, displays a stimulus-specific genetic effect on endogenous interferon beta-1 (*IFNβ1*) expression in monocytes after Lipopolysaccharides treatment. IFNβ1 is a type I interferon that plays a crucial role in the immune response, particularly in modulating inflammation and antiviral defense mechanisms. Variations in genes related to the immune system, including those involved in interferon signaling pathways, may contribute to multiple sclerosis (MS) susceptibility. We aimed to investigate association of genetic variant rs2275888 and *IFNβ1* mRNA expression in monocytes of peripheral blood with MS progression. We were analyzed 100 patients (36 males) for genetic and 30 MS patients (10 males) for gene expression. Quantitative PCR with Applied Byosistems TaqMan© technology was used for genotyping and expression analysis. PPIA was used as endogenous control for the expression analysis. Statistica 8 software was used for statistical analysis.

IFNβ1 mRNA relative level was significantly higher among patients in remission (n=25) compared to relapse patients (n=5), T-test p=0.045. Patients with rs2275888 CC genotype had significantly lower level of *IFNβ1* mRNA, compared to the patients with T allele bearing genotypes, T-test p=0.050.

IFNβ1 plays critical roles in the pathogenesis of MS, exerting complex and context-dependent effects on immune responses, CNS inflammation, and neurodegenerative processes. Understanding the molecular mechanisms underlying IFNβ1 signaling can provide insights into disease pathophysiology.

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IFNB1, GENE EXPRESSION, *HACD4*, RS2275888, MULTIPLE SCLEROSIS DISEASE COURSE

04 – 31 Poster

COMPARISON OF *ADARB1* AND *TPH2* VARIANTS BETWEEN SLOVENIAN AND SERBIAN CASES ON THE CONTINUUM OF SUICIDAL BEHAVIOR

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Completed suicide and a 20 times more frequent attempted suicide are on the continuum of suicidal behavior. Genetic background of suicidal behavior predominantly implicated serotonergic system genes including *TPH2*, encoding tryptophan hydroxylase 2, a rate-limiting serotonin synthetizing enzyme in the brain. *TPH2* pre-mRNAs undergo adenosine-to-inosine RNA editing by ADAR and ADARB1 enzymes, which fine-tunes enzyme function, and *TPH2* rs4290270 variant has been shown to affect its own transcript alternative splicing and editing. *TPH2* rs4290270 in combination with childhood general traumas, and main effects of *ADARB1* rs9983925 and rs4819035 variants have been previously associated with suicide attempt in psychiatric patients from Serbian population. Our current aim was to compare our abovementioned findings with completed suicide in Slovenian population.
ADARB1 rs9983925 and rs4819035, and *TPH2* rs4290270 variants were genotyped for 333 suicide completers (305 using violent and 28 using non-violent methods), and 357 non-suicidal autopsy controls from Slovenia. Genotypic data for 165 suicide attempters from Serbia was taken from our previous study.
ADARB1 rs4819035 GT and GG carriers had an increased risk for non-violent completed suicide compared to controls (P(χ2/adjusted) =0.012/0.012), and compared to violent completed suicide (P(χ2/adjusted) =0.021/0.049). *TPH2* rs4290270 AA genotype marginally increased the risk for completed suicide compared to controls (P(χ2/adjusted) =0.038/0.086), mostly due to violent completed suicide when compared to controls as well (P(χ2/adjusted) =0.054/0.115), and for completed suicide compared to attempted suicide (P(χ2/adjusted) =0.052/0.052).
ADARB1 and *TPH2* variants differentiated genetic backgrounds between attempted and completed suicide, as well as between violent and non-violent methods of completed suicide.
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SUICIDAL BEHAVIOR, *ADARB1*, *TPH2*, RS4819035, RS4290270

04 – 32 Poster

METHYLATION OF CCG VARIANT REPEATS IS ASSOCIATED WITH HETEROGENEOUS METHYLATION OF CPG SITES SURROUNDING DMPK EXPANSION IN MYOTONIC DYSTROPHY TYPE 1 PATIENTS

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Myotonic dystrophy type 1 (DM1) is a monogenic disease with huge clinical variability. We showed that variant repeats act as individual-specific genetic modifiers which delay age-at-onset in DM1 patients by stabilizing CTG expansions within the *DMPK* gene in somatic cells. Since CCG variant repeats were most commonly detected, our aim was to investigate whether they were associated with methylation in the repeat region and surrounding CpG sites, similarly to GC-rich repeats causing other repeat-expansion disorders.
To examine the methylation of CCG repeats, we designed methyl-specific repeat-primed PCR on bisulfite-converted genomic DNA and repeat-primed PCR on genomic DNA digested by methyl-sensitive SsiI enzyme. Methylation of surrounding CpG sites was assessed by targeted Illumina and Oxford Nanopore bisulfite sequencing. The study included 18 patients from 11 families who carried *DMPK* expansions with different patterns of CCG variant repeats.
We discovered that variant CCG repeats were heterogeneously methylated in all patients. In addition, CpG sites upstream and downstream of repeat tract showed heterogenic methylation. Importantly, we observed that the extent of methylation depends on quantity and patterns of CCG repeats, suggesting that methylation is initiated on CCG repeats and spreads locally to the surrounding CpG sites.
Our discovery of methylation of variant CCG repeats opens questions about the role of epigenetic mechanisms in stabilization of *DMPK* locus and their clinical relevance.

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MYOTONIC DYSTROPHY TYPE 1, REPEAT EXPANSIONS, DNA METHYLATION, VARIANT REPEATS

04 – 33 Poster

ASSOCIATION OF CYP2E1 RSAI AND TNF-A PROMOTER VARIANTS WITH ONSET OF ALCOHOL-RELATED LIVER CIRRHOSIS

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Only a minority of excess alcohol drinkers develop alcohol-related liver cirrhosis (ALC). Chronic alcohol consumption elevates CYP2E1 activity, leading to increased levels of cell-deleterious reactive oxygen species (ROS), and stimulates Kupffer cells to secrete proinflammatory cytokine tumor necrosis factor-α (*TNF-α*). These factors sensitize hepatocytes and lead to chronic liver injury and cirrhosis. Our study aimed to estimate the association of *CYP2E1* RsaI and *TNF-α* promotor variants (-238G>A and -308G>A) with ALC susceptibility.
A total of 118 patients with ALC and 131 sex- and age-matched healthy controls were clinically examined and genetically tested. DNA was extracted from peripheral blood lymphocytes and genotyping was performed using PCR-RFLP for each variant. The polygenic risk score (PRS) based on these three SNPs was computed, and binary logistic regression was used to obtain odds ratios.
Carriers of the *CYP2E1* c2 allele had 2.89 times higher risk of developing the disease (OR=2.89, 95% CI=1.30-6.41; p=0.009). Concerning the *TNF-α* -238G>A variant, a significant association between A allele carriers and the risk of ALC (OR=2.36, 95% CI=1.15-4.83; p=0.019) was observed. No significant differences were found in either the genotype or allelic frequencies of the − 308 *TNF-α* variant (p=0.463). Patients with ALC had a higher PRS than controls (0.079 vs. 0.030; p=0.0027).
The -238 *TNF-α* –A and *CYP2E1* c2 alleles were associated with a higher risk of ALC. Further development of PRS (inclusion of more variants) will enhance the identification of at-risk patients.

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ALCOHOL RELATED LIVER CIRRHOSIS, CYP2E1, TNF-A PROMOTER VARIANTS, POLYGENIC RISK SCORE

04 – 34 Poster

ANALYSIS OF COPY NUMBER VARIATIONS IN PATIENTS WITH MICROCEPHALY

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Microcephaly is a malformation characterized by an occipitofrontal head circumference that falls below the third percentile or two standard deviations from the mean considering sex, age, and ethnicity. Numerous factors can contribute to the development of microcephaly, and the presence of copy number variations (CNVs) in the genome facilitates the understanding of the complex etiopathogenesis of microcephaly.
Our research aims to assess the diagnostic contribution of molecular karyotyping in children with microcephaly and to analyze the phenotype of those with clinically significant CNVs (csCNVs) in the genome.
110 children, 49 (44.5%) boys and 61 (55.5%) girls, aged 1 month to 18 years, were included in this study. The research included children who were referred for molecular karyotyping due to the presence of microcephaly.
Using the molecular karyotyping method, csCNVs were detected in 23 patients. The csCNV detection rate was 20.9%. Diagnosis was made in ten patients with known microdeletion or microduplication syndrome. One aneuploidy was detected: tetrasomy of the X chromosome. Additionally, a mosaic form of ring chromosome 18 (45% of cells) was found, along with 19 microdeletions (212 kb to 17.9 Mb) and ten microduplications (398 kb to 4.1 Mb) in size. Children were significantly more likely to have csCNV if microcephaly was combined with congenital heart defects (p=0.04), facial dysmorphism (p=0.023), or intrauterine growth retardation (p=0.04).
Our study showed that facial dysmorphia, intrauterine growth retardation and congenital heart defects are predictors of the presence of pathogenic CNVs patients with microcephaly.

VARIATION IN THE NUMBER OF COPIES; MOLECULAR KARYOTYPING; MICROCEPHALY

04 – 35 Poster

EXPRESSION OF PROINFLAMATORY CYTOKINES IN GESTATIONAL HYPERTENSION

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Gestational hypertension (GH) represents one of the most common pregnancy-induced complications. It was rarely investigated apart from other hypertensive disorders of pregnancy, therefore there is a lack of knowledge about molecular mechanisms underlying this condition, including its inflammatory aspects. The aim of the study was to determine whether there is a differential expression of proinflammatory cytokines (*IL-1β, TNF-α, IL-6* and *IL-17*) in women with gestational hypertension compared to healthy pregnant women.
The study included 64 pregnant women divided into two groups: 30 GH patients and 34 controls (CG). Quantitative real-time PCR was used to determine cytokine mRNA expression levels, previously isolated from leukocytes.
TNF-α expression levels were significantly higher in the GH group than in controls (P=0.030). There were no significant differences in *IL-1β, IL-6* and *IL-17* gene expression levels between examined groups. *TNF-α* was also significantly positively correlated with *IL-1β* (r=0.445, P=0.014) and *IL-17* (r=0.616, P<0.001). ROC curve analysis showed insufficient predictive and discriminatory ability of this cytokine to discriminate between GH and healthy pregnant women.
Our study showed that *TNF-α* expression is higher in women with gestational hypertension than in controls, thus giving a contribution to a better understanding of GH pathogenesis. The findings may also help in improving GH patients' management. However, *TNF-α* expression cannot be used as a biomarker for this condition.

GESTATIONAL HYPERTENSION, IL-1B, TNF-A, IL-6, IL-17

04 – 36 Poster

LNCRNA *PVT1* GENE VARIANT RS4410871 AFFECTS PARAMETERS ASSOCIATED WITH HIGHER RISK OF HEART FAILURE IN PATIENTS WITH FIRST ACUTE MYOCARDIAL INFARCTION

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Myocardial infarction (MI) is one of the most common causes of heart failure (HF). LncRNA Plasmacytoma Variant Translocation 1 (*PVT1*) has been increased in myocardial hypertrophy, fibrosis and chronic HF. The aim of this study was to investigate the association between clinical parameters commonly linked to a higher risk of heart failure and the *PVT1* gene variant rs4410871, which was previously associated with coronary artery disease.
The study included 100 patients with the first acute MI. Rs4410871 C>T was genotyped using Applied Biosystems TaqMan® technology. Statistical analyses were performed using Statistica 8 software. Left ventricular end-systolic volume (LVESV) was indexed to body surface area (BSA). LVESVi values >31 mL/m2 in men and >27 mL/m2 in women were defined as dilatation. Left ventricular hypertrophy (LVH) was defined as a left ventricular mass, indexed to BSA, >115 g/m2 in men and >95 g/m2 in women.
We have found that genotypes bearing T allele under the dominant model (CC vs. CT + TT) were significantly more frequent in the MI patients with dilated LVESVi, compared to the patients without dilatation (55% vs. 45%, p=0.04). The same model showed trend in association with LVH (56% vs. 44%, p=0.06).
Our preliminary results suggest that *PVT1* rs4410871 is associated with the higher risk of HF development after the first MI. Further research is warranted.

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MOLECULAR GENETICS, COMPLEX DISEASES, CARDIOVASCULAR DISEASES

04 – 37 Poster

COMPREHENSIVE GENETIC STUDY IDENTIFIES DIFFERENT SUSCEPTIBILITY FACTORS FOR EARLY- AND LATE-ONSET ACETYLCHOLINE POSITIVE MYASTHENIA GRAVIS PATIENTS FROM SERBIA

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Anti-acetylcholine receptor-positive myasthenia gravis (AChR-MG) is a multifactorial autoimmune disease. Genome-wide association studies (GWAS) have identified susceptibility loci, including *CTLA-4*, *CHRNA1/B1*, and *TNFRSF11A*, indicating that early-onset and late-onset AChR-MG have distinct genetic backgrounds. Subsequent fine-mapping of *CTLA-4* identified rs231735 and rs231770 as potential causal variants, while transcriptome-wide association study pinpointed *CHRNB1* rs4151121 as causal variant.

We performed bioinformatic fine-mapping to select the *CHRNA1* and *TNFRSF11A* causal variants using data from GWAS summary statistics, 1000 genome and RegulomeDB. Alongside fine-maped *CHRNA1* rs35274388 and *TNFRSF11A* rs4574025 and rs4369774 (PIP > 0.92 and 0.75, respectively), *CTLA-4* rs231735/rs231770 and *CHRNB1* rs4151121 were genotyped using allelic discrimination assays. We investigated the association with early- and late-onset MG in 519 AChR-MG patients and 519 sex- and age-matched controls in the Serbian population.

We found that *CTLA-4* rs231735 genotype TT decreased, while rs231735-rs231770 haplotype G-C increased the risk of early-onset MG (OR=0.548, 95% CI=0.339-0.888, p=0.014, p10e6-permutation<0.05 and OR=1.360, p=0.027, p10e6-permutation<0.05, respectively). On the other hand, *CHRNB1* rs4151121 genotypes GG and AG increased the risk of late-onset MG (OR=1.420, 95% CI=1.025-1.965, p10e6 permutation<0.05). Furthermore, *TNFRSF11A* rs4574025 genotype CC and rs4369774 genotype AA increased the risk for late-onset MG (OR=1.504, 95% CI=1.016-2.228, p=0.041, p10e6 permutation=<0.05 for both variants). We observed a borderline association of *CHRNA1* rs35274388 with AChR-MG (OR=1.478, 95% CI=1.009-2.166, P = 0.044, p10e6-permutation=0.06). Our study suggests that *CTLA-4* rs231735 and rs231770 may be genetic susceptibility factors for early-onset, while *CHRNB1* rs4151121 and *TNFRSF11A* rs4574025 and rs4369774 could be risk factors for late-onset AChR-MG in the Serbian population.

MYASTHENIA GRAVIS, BIOINFORMATICS FINE-MAPPING FOLLOWED BY A GENETIC ASSOCIATION STUDY, *CTLA-4*, *CHRNA1/B1*, *TNFRSF11A*

04 – 38 Poster

APPLICATION OF PHARMACOGENETICS

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The pharmacokinetics of drugs: 5-fluorouracil, capecitabine, irinotecan, mercaptopurine, and azathioprine are proven to be significantly affected by genetic variants in genes *DPYD*, *UGT1A1*, and *TPMT*. Several genetic variants affect the activity of dihydropyrimidine dehydrogenase (DPYD), involved in pyrimidine degradation, as well as the activity of UDP-glucuronosyltransferase (UGT), responsible for the glucuronidation of irinotecan. More than 30 genetic variants reduce the activity of the thiopurine-metabolizing enzyme thiopurine methyltransferase (TPMT).

DPYD variants c.1236G>A, c.1679T>G, c.1905+1G>A, and c.2846A>T were genotyped in colorectal cancer patients, before 5-fluorouracil treatment using PCR with reverse hybridization. (TA)n repeat polymorphism of *UGT1A1* gene promoter was analyzed before irinotecan therapy in colorectal cancer, using real-time PCR. The analysis of *TPMT* variants c.238G>C, c.460G>A, and c.719A>G was included before thiopurine treatment in acute lymphoblastic leukemia, and inflammatory bowel disease patients using PCR with reverse hybridization.

Among 23 samples for *DPYD* analysis, one was the carrier of heterozygous variant c.1236G>A (HapB3), and one was the carrier of heterozygous variant c.1905+1G>A. *UGT1A1* (TA)n promoter genotyping was conducted in 65 samples. Twenty-one samples had genotype (TA)6/(TA)6, another 22 (TA)6/(TA)7, and 22 (TA)7/(TA)7. *TPMT* testing in 40 samples showed TPMT*1/*1, one *1/*2, and one *1/*3A or *3B/*3C genotype.

An individual approach based on genetic predispositions is enabled by determining the optimal drug and its dose to achieve the maximum therapeutic response for each patient, by reducing the side effects of therapy, as well as treatment costs to a minimum.

PHARMACOGENETICS

04 – 39 Poster

FAMILIAL ANGELMAN SYNDROME WITH INTRAGENIC DELETION IN THE *UBE3A* GENE

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Severe developmental delay or intellectual disability, major speech impairment, ataxia and behavioral uniqueness with an apparent happy demeanor are the main characteristics of the patients affected with Angelman syndrome (AS). AS results from the lack of expression in the brain of a maternal imprinted *UBE3A* gene mapped within 15q11q13 region. Different mechanisms such as maternal deletion of 15q11q13 region, paternal uniparental disomy, genomic imprinting defects and pathogenic variants in the *UBE3A* gene can cause its functional loss.

Here, we report two sisters with AS due to an intragenic exonic deletion within the *UBE3A* gene, inherited from healthy mother. This variant was identified using multiplex ligation-dependent probe amplification (MLPA) method.

The *UBE3A* intragenic deletions are very rare and therefore often underdiagnosed. In every patient suspected of AS, with no deletion of the whole region 15q11q13, with a normal methylation pattern and also no *UBE3A* pathogenic small variants present, testing for large intragenic deletions in the *UBE3A* gene is highly recommended.

ANGELMAN SYNDROME (AS), *UBE3A*, INTRAGENIC DELETION

04 – 40 Poster

ISCHEMIA AFFECTS THE TERMINAL DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS AND NEURONAL PROGENITORS

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Hypoxia/ischemia underpins a broad range of brain pathologies, including stroke and short-term cardiac arrest that lead to the induction of neural stem cell proliferation and the migration of young neurons to injured areas. However, these processes are insufficient to fully restore neuronal function. This study aimed to investigate the effect of hypoxia-ischemia on the neurogenic potential of human pluripotent stem cells and the terminal differentiation of neuronal progenitors.

NT2/D1 cell line was used as an in vitro model system of human neurogenesis. Ischemic stress was achieved by exposure to glucose deprivation and/or cobalt chloride. To analyze the mRNA expression level of target genes, quantitative RT-PCR was performed. Protein expression was determined by Western blot and immunocytochemistry.

The analysis revealed that ischemia, induced in pluripotent cells or neural progenitors, led to a significant decrease in *SOX* gene expression in these cells and a notably reduced number of terminally differentiated neurons. In contrast, there was an increase in the expression level of miR-21. These insights contribute to the understanding of *SOX* transcription factors and miR-21, as well as their potential interplay in diseases related to ischemia. This positions them as promising candidates for biomarkers and targets in the development of new diagnostic and treatment strategies.

Acknowledgments: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-66/2024-03/200042) and Science Fund of the Republic of Serbia, Serbian Science and Diaspora Collaboration Program: Knowledge Exchange Vouchers, (6436225).

ISCHEMIA, HYPOXIA, PLURIPOTENT STEM CELLS, NEURAL PROGENITORS, NEUROGENESIS

04 – 41 Poster

ASSOCIATION OF GENETIC VARIANT RS3176326 RESIDING IN FERROPTOSIS-RELATED *CDKN1A* WITH MULTIPLE SCLEROSIS SEVERITY

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Recently, we investigated targeted expression of ferroptosis-related genes in relation to multiple sclerosis (MS) severity, as it is proposed to be one of the mechanisms that cause neurodegeneration, hallmark of progressive multiple sclerosis (MS). The upregulation of cyclin dependent kinase inhibitor 1A (*CDKN1A*), a gene responsible for reduction in sensitivity to ferroptosis, was found in the secondary progressive (SP) compared to relapse remitting (RR) MS patients. We aim to investigate genetic variant rs3176326 (G/A), which has been marked by an ENCODE as the most probable functional variant in this gene, in association with SP MS.
The study included a total of 213 MS patients (159 RR and 54 SP) from Serbia. Diagnosis of clinically definite MS was performed according to the revised McDonald criteria. Genotyping has been performed by TaqMan® technology.
We found significantly higher frequency of rare allele bearing genotypes according to dominant model (AG+AA vs. GG) in SP MS compared to RR MS patients (p=0.015). Genotypes bearing rare allele were significantly and independently associated with SP MS with an adjusted OR 2.08 (95% CI 1.10 - 3.95, p=0.02). OR was adjusted for sex and active smoking. There was no significant association with EDSS or MSSS scores (p>0.05).
Our preliminary results suggest possible association of *CDKN1A* rs3176326 with MS severity. Further replication of the results on larger sample size and functional analysis are inevitable.

Acknowledgements: This research was funded by the Science Fund of the Republic of Serbia, grant number #Grant no. 7753406, “Identification and functional characterization of extracellular and intracellular genetic regulators of ferroptosis related processes in multiple sclerosis” - FerroReg

FERROPTOSIS, MULTIPLE SCLEROSIS SEVERITY, *CDKN1A* GENE, RS3176326, P21

04 – 42 Poster

ANALYSIS OF MICRORNA-21 AND MICRORNA-491 EXPRESSION IN SALIVARY GLAND TUMORS

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Salivary gland tumors (SGTs) represent a heterogeneous group of tumors with differences in origin, clinical manifestations and prognosis. Micro RNAs play an important role in the regulation of gene expression and are potential biomarkers for various types of tumors. The aim of this study was to compare the levels of two microRNAs (miR-21 and miR-491) between malignant and benign SGT tissues, as well as normal salivary glands, and to investigate their potential use as biomarkers.
Samples were collected from 67 patients in total: 49 with benign tumors (22 with pleomorphic adenoma and 27 with Wartin's tumor), 8 with malignant tumors and 10 healthy controls. After RNA extraction, quantitative PCR analysis was used to assess miR-21 and miR-491 levels.
MiR-491 levels were higher in benign compared to malignant SGTs, but without reaching statistical significance (p>0.05). No apparent trend and no significant differences for the miR-21 levels were observed between malignant and benign salivary gland tumors (p>0.05), as well as between tumor samples and healthy controls (p>0.05).
The obtained results suggest that miR-21 and miR-491 alone are not reliable biomarkers for the differential diagnosis of SGTs. However, these findings do not exclude the possibility that other molecular markers, or combination of multiple markers, may provide useful diagnostic information. Further studies with larger samples and additional molecular analyses are necessary to better understand the role of miRNAs in salivary gland tumorigenesis and to identify reliable biomarkers.

MICRORNA-21, MICRORNA-491, SALIVARY GLAND TUMORS, GENE EXPRESSION, BIOMARKERS

04 – 41 Poster

ASSOCIATION OF GENETIC VARIANT RS3176326 RESIDING IN FERROPTOSIS-RELATED *CDKN1A* WITH MULTIPLE SCLEROSIS SEVERITY

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Our preliminary results suggest possible association of *CDKN1A* rs3176326 with MS severity. Further replication of the results on larger sample size and functional analysis are inevitable.

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FERROPTOSIS, MULTIPLE SCLEROSIS SEVERITY, *CDKN1A* GENE, RS3176326, P21

04 – 42 Poster

ANALYSIS OF MICRORNA-21 AND MICRORNA-491 EXPRESSION IN SALIVARY GLAND TUMORS

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Salivary gland tumors (SGTs) represent a heterogeneous group of tumors with differences in origin, clinical manifestations and prognosis. Micro RNAs play an important role in the regulation of gene expression and are potential biomarkers for various types of tumors. The aim of this study was to compare the levels of two microRNAs (miR-21 and miR-491) between malignant and benign SGT tissues, as well as normal salivary glands, and to investigate their potential use as biomarkers.
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MiR-491 levels were higher in benign compared to malignant SGTs, but without reaching statistical significance (p>0.05). No apparent trend and no significant differences for the miR-21 levels were observed between malignant and benign salivary gland tumors (p>0.05), as well as between tumor samples and healthy controls (p>0.05).
The obtained results suggest that miR-21 and miR-491 alone are not reliable biomarkers for the differential diagnosis of SGTs. However, these findings do not exclude the possibility that other molecular markers, or combination of multiple markers, may provide useful diagnostic information. Further studies with larger samples and additional molecular analyses are necessary to better understand the role of miRNAs in salivary gland tumorigenesis and to identify reliable biomarkers.

MICRORNA-21, MICRORNA-491, SALIVARY GLAND TUMORS, GENE EXPRESSION, BIOMARKERS

04 – 45 Poster

AURICULOCONDYLAR SYNDROME TYPE 1: A NOVEL *GNAI3* PATHOGENIC VARIANT AND PRENATAL MANIFESTATIONS

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Auriculocondylar syndrome type 1 is an extremely rare autosomal dominant condition caused by pathogenic and likely pathogenic variants in the *GNAI3* gene and characterized by severe craniofacial malformations including micrognathia, mandibular condyle hypoplasia, prominent cheeks, and/or distinctive question mark ears. Besides the aforementioned, the syndrome is also known for varying phenotypic traits and incomplete penetrance. Prenatally, craniofacial abnormalities are followed by polyhydramnios.

A 35-year-old gravida was referred for genetic counseling at 32th gestational weeks due to sonographic anomalies detected in the fetus: micrognathia, polyhydramnios and a single umbilical artery. Amniocentesis was performed. Following DNA isolation, three tests were conducted: QF-PCR for common aneuploidies (Aneufast kit), chromosomal microarray analysis (CMA) (Agilent SurePrint G3 Human CGH, 8×60K), and clinical exome sequencing (Illumina DNA Prep with Enrichment protocol, TruSight One panel). Variants were classified according to the ACMG/AMP guidelines.

QF-PCR and CMA analyses indicated a normal male DNA profile. Clinical exome sequencing revealed a novel missense heterozygous pathogenic variant in the *GNAI3* gene, c.119G>A (NM_006496.4), p.(Gly40Asp). The variant is located in a hotspot region for missense and in-frame variants and two alternative pathogenic and likely pathogenic variants have been identified so far.

According to the literature, only a small number of variants associated with auriculocondylar syndrome type 1 have been reported to date. This case emphasizes that prenatally detected craniofacial malformations (particularly micrognathia), accompanied by polyhydramnios and a single umbilical artery, should prompt consideration of a potential link to auriculocondylar syndrome and indicate the necessity of performing exome sequencing in similar cases.

AURICULOCONDYLAR SYNDROME TYPE 1, *GNAI3*, PRENATAL DIAGNOSIS

04 – 46 Poster

PREDICTIVE SIGNIFICANCE OF RELATIVE TELOMERE LENGTH IN COLORECTAL CARCINOMA

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Colorectal cancer (CRC) is the most common malignancy of the gastrointestinal tract. It is well known that genotoxic agents in the intestinal mucosa can induce genomic instability leading to high proliferative activity due to telomere maintenance. The aim of this study was to determine the telomere length in CRC, healthy mucosa and peripheral blood leukocytes.

Tissue samples (tumor and healthy tissue) and peripheral blood leukocytes were collected from 111 subjects with CRC who had undergone surgery at the University Medical Center Zvezdara. Genomic DNA was isolated and processed for qPCR analysis. Relative telomere length (RTL) was estimated using the ΔΔCq method.

In our study, RTL was higher in tumor (2.34) than in peritumor healthy tissue (1.53) and leukocytes (1.89) with significance ($p<0.001$). There was a strong positive correlation between RTL in tumor and peritumoral tissue ($p=0.000$, $r=0.54$). In addition, a significant negative correlation ($p=0.012$, $r= -0.21$) was observed between RTL in leukocytes and aging. No statistical difference was observed between genders, age groups and TNM staging groups with regard to RTL in tumor tissue.

Longer telomeres found in tumor tissue, especially in higher stages, may be an additional prognostic marker for tumor aggressiveness. The shortest telomeres in healthy mucosa may indicate free surgical margins, i.e the absence of invasive proliferative peritumoral cells. In addition, a shorter RTL in leukocytes could be related to a lower immune response, as no effective lymphocyte infiltration was detected in histopathological tumor samples.

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COLORECTAL CANCER; RELATIVE TELOMERE LENGTH

04 – 47 Poster

INFLUENCE OF EPIDERMAL GROWTH FACTOR RECEPTOR GENE RS1468727 POLYMORPHISM ON NODAL CERVICAL METASTASES APPEARANCE IN THE PATIENTS WITH ORAL SQUAMOUS CELL CARCINOMA

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Genetic aberrations together with environmental factors are known to play an important role in oral squamous cell carcinoma (OSCC). Here we aim to clarify the potential influence of *EGFR* gene polymorphism rs1468727 on nodal cervical metastases appearance in patients with OSCC. Sixty-one OSCC patients were included in the study. The genotype of each patient for rs1468727 polymorphism of the *EGFR* gene was detected using Real-Time PCR. The follow-up period for each patient was 3 years from the date of surgery.
rs1468727 polymorphism of the *EGFR* gene showed a statistically significant difference between patients with nodal cervical metastases and patients without nodal cervical metastases ($\chi 2$= 5.589, df=2, p=0.061, fi=0.249). After 3 years follow up from surgery genotype TT was found in the patients without nodal cervical metastases. Among patients who had nodal cervical metastases 56 % had CC genotype, 44 % were heterozygous CT and genotype TT was not determined.
rs1468727 polymorphism of the *EGFR* gene homozygote (genotype TT) showed statistically significant influence on nodal metastases appearance in patients with OSCC in the period of 3 years from the surgery date. A person with genotype TT is more likely not to have nodal cervical metastases. To further confirm the predictive value of this polymorphism, a study with a larger sample size would contribute, as well as collecting additional information on other polymorphisms of the *EGFR* gene and a broader panel of oncogenes.

ORAL SQUAMOUS CELL CARCINOMA; EPIDERMAL GROWTH FACTOR RECEPTOR; POLYMORPHISMS

04 – 48 Poster

IMPLEMENTATION OF ALL-IC BFM 2022 PROTOCOL IN THE ROUTINELY DIAGNOSTICS OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA IN SERBIA

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Acute lymphoblastic leukemia (ALL) represents about 30% of all pediatric cancers and 80% of all pediatric leukemias. Although this hematological disease is very diverse and complex, using certain underlying genetic aberrations in routine diagnostic testing is standard for risk stratification and prognosis. Following the updated ALL-IC BFM 2022 guidelines, it is mandatory for ALL patients to be tested for both favorable genetic risk features (hyperdiploidy and *ETV6*/*RUNX1* gene fusion) and unfavorable genetic risk features (hypodiploidy, *KMT2A* gene rearrangements, *iAMP21* and *BCR-ABL1* and *TCF3-HLF* fusion genes).
This study aimed to show the significance of implementing the ALL-IC BFM 2022 protocol in the group of ALL pediatric patients referred to the Laboratory of Medical Genetics (Mother and Child Health Care Institute of Serbia `Dr Vukan Cupic`) for genetic testing at diagnosis.
From September 2023 to May 2024, 13 patients were tested using cytogenetics, FISH, and RT PCR according to the guidelines. Results showed hyperdiploidy in 7/13 patients and hypodiploidy in 1/13 patients. *ETV6*/*RUNX1* was detected in 3/13 patients, while *KMT2A-AFF1* was detected in 1/13 patients. No patients were *BCR-ABL1* or *TCF-HLF* positive, while 1/13 was *iAMP21* positive.
The authors will discuss the benefits of the ALL-IC BFM 2022 testing strategy in routine laboratory diagnostics including more precise risk stratification and therapy adjustments for the newly diagnosed ALL patients.

PEDIATRIC ALL, ALL-IC BFM 2022, ALL RISK STRATIFICATION

04 – 49 Poster

GENETIC TESTING AS SECOND TIER OF NEWBORN SCREENING FOR CYSTIC FIBROSIS IN SERBIA – TWO YEARS' EXPERIENCE

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The national newborn screening (NBS) for cystic fibrosis (CF) officially started in central Serbia on 01.04.2022. The initial algorithm included a two-stage protocol, with immunoreactive trypsin (IRT) as the primary test and DNA testing for the most common *CFTR* variants as the second tier of screening.

This study included infants born in central Serbia from 01.04.2022.-21.05.2024. A total of 347 samples of dried blood spots initially being positive for IRT, were further tested for the presence of the most common 50 *CFTR* variants (Yourgene® Cystic Fibrosis Base kit, Yourgene Health, UK). Genetic analyses were done using DNA samples extracted directly from dried blood spots with QIAamp® DNA Micro Kit (Qiagen Group, Netherlands).

In the group of 347 samples, ten (10/347, 2.88%) had two CF-causing pathogenic variants identified: eight newborns had genotype F508del/F508del and two were compound heterozygotes–one with F508del/2789+5G>A and one with R347P/G542* genotype. A total of 32 (32/347, 9.22%) heterozygous carriers were detected, with the following pathogenic *CFTR* variants: F508del (24 samples), G542* (2 samples), D1152H (2 samples), and one each with 621+1G>A, R117C, R347P, and G551D variants. In eight cases (8/347, 2.3%) the screening was not completed due to an inadequate initial sampling, so analyses were finished from repeated peripheral blood samples. All families with positive NBS test were invited to visit the Pulmonology Department, for sweat chloride testing and further clinical evaluation.

Results of two years' experience showed that the implementation of NBS in central Serbia is successful, the applied strategy is justified, and will have important clinical benefits.

CYSTIC FIBROSIS, NEWBORN SCREENING, DRIED BLOOD SPOTS, *CFTR* GENE

04 – 50 Poster

PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS WITH 22Q11.2 MICRODELETION: A MODEL SYSTEM FOR INVESTIGATING NEURODEVELOPMENTAL DISORDER

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Neurodevelopmental disorders (NDDs) like autism spectrum disorders, intellectual disability and schizophrenia represent considerable public health challenges. Molecular pathways underlying NDDs remain largely unidentified. Syndrome associated with a heightened risk of NDDs is 22q11.2 Deletion Syndrome (22q11.2DS), caused by microdeletion 22q11.2. In 98% of cases the microdeletion is 1.5Mb or 3Mb in length. This study is focused on a cohort of patients with 1.5Mb microdeletion, aiming to establish model system for investigation of NDDs.

Peripheral blood mononuclear cells from patients with 1.5 Mb microdeletion and healthy individuals were reprogrammed using CytoTuneTM-iPS2.0 Sendai Reprogramming Kit. Generated induced pluripotent stem cells (iPSCs) were genotyped in order to identify if any additional pathogenic CNVs exist. Pluripotency of the iPSCs was assessed through RT-PCR analysis. STEMdiff Trilineage Differentiation Kit was used to validate the ability of iPSCs to differentiate into the cells of three germ layers: ectoderm, mesoderm, and endoderm.

iPSCs were generated from three patients with a 1.5Mb microdeletion and two healthy individuals. They expressed pluripotency markers and differentiated into cells of three germ layers. Genotyping identified additional CNVs in some of the iPSC lines.

iPSC lines from 22q11.2DS patients and healthy controls were successfully established and they provide a valuable platform for studying NDDs.

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NEURODEVELOPMENTAL DISORDERS, IPSCS, 22Q11.2DS

04 – 51 Poster

EXPRESSION OF OSTEOGENIC MARKERS AFTER OSTEOINDUCTION OF DENTAL STEM CELLS IN THE PRESENCE OF CALCIUM SILICATE AND NOVEL CALCIUM ALUMINATE-BASED DENTAL CEMENTS

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The apical papilla is a soft tissue located at the apical portion of developing dental root. This tissue mostly consists of early progenitor cells, called stem cells. Stem cells from apical papilla (SCAP) are in the spotlight of modern regenerative therapy due to their numerous advantages over other stem cell sources. In the presence of endodontic dental cements, SCAP could initiate osteo-differentiation and bone regeneration, thus having an important role in the success of therapeutic procedures.
The aim of the study was to assess and compare the influence of calcium silicate and calcium aluminate-based dental cements on the osteo-differentiation of SCAP.
Materials and methods: The study included pure calcium aluminate cement (CA), CA with the addition of zirconium dioxide (CA+ZrO2), as well as calcium silicate cements - mineral trioxide aggregate (MTA) and Portland cement (PC). The cements were individually mixed and molded into half-disc-shaped samples that were placed in wells seeded with SCAP. Osteogenic differentiation medium (ODM) was added to each well, and SCAPs cultured with and without ODM were used as a control. After 21 days, staining with Alizarin Red S was performed to confirm osteo-differentiation. The gene expression of osteo-differentiation markers was analyzed by the qPCR method.
Two crucial markers of early osteogenic differentiation were analyzed. Both the *ALPL* gene, encoding alkaline phosphatase, and the *BGLAP* gene encoding osteocalcin, showed significant overexpression in the presence of MTA and CA+ZrO2 compared to other groups ($p<0.05$).
Given their osteoinductive characteristics, novel CA cements could represent the cements of choice in regenerative endodontic therapy in the future, although further research is needed.

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STEM CELLS, APICAL PAPILLA, OSTEOGENIC DIFFERENTIATION, DENTAL CEMENTS

04 – 52 Poster

CHARACTERIZATION OF INDUCED PLURIPOTENT STEM CELLS FROM PATIENTS WITH 22Q11.2 DUPLICATION SYNDROME

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The 22q11.2 Duplication Syndrome (22q11.2DupS), caused by a microduplication in the q11.2 region of chromosome 22, is associated with a high risk of developing neurodevelopmental disorders (NDDs). Autism spectrum disorder (ASD) occurs in 14-25% of individuals with 22q11.2DupS, making it one of the genetic syndromes with the highest prevalence of ASD. Conversely, in individuals affected by schizophrenia, 22q11.2DupS occurs less frequently than in the general population, suggesting that this microduplication could have a protective effect against schizophrenia.
Peripheral blood mononuclear cells from 22q11.2DupS patients and healthy controls were reprogrammed into induced pluripotent stem cells (iPSCs) using CytoTuneTM-iPSC2.0 Sendai Reprogramming Kit. Genotyping of iPSCs was performed to identify potential additional pathogenic CNVs. Pluripotency markers' expression was analyzed by RT-PCR. The ability of iPSCs to differentiate into the cells of three germ layers was tested using STEMdiff Trilineage Differentiation Kit.
Successful establishment of iPSCs from three 22q11.2DupS patients, their mothers that carry the microdeletion, and three healthy individuals was confirmed by expression of pluripotency markers. Generated iPSCs have the ability to differentiate into cells of all three germ layers. Additional CNVs in some of the iPSC lines were revealed by genotyping.
iPSCs from 22q11.2DupS patients, their carrier mothers, and healthy controls were generated representing a valuable model for studying the molecular mechanisms underlying NDDs.

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22Q11.2 DUPLICATION SYNDROME; NEURODEVELOPMENTAL DISORDERS; IPSCS; FAMILIAL CASES

04 – 53 Poster

GENETIC SPECTRUM OF PRIMARY DYSLIPIDEMIAS IN CHILDREN - SINGLE CENTER EXPERIENCE

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Primary dyslipidemias are heterogenous metabolic disorders caused by pathogenic genetic variants. Over 100 genes have been identified that impact lipid metabolism, with familial hypercholesterolemia being the most common form, occurring in the general population with a frequency of 1:200–1:500. Dyslipidemias are considered as one of the most significant risk factors for atherosclerotic cardiovascular diseases. Detection of primary dyslipidemia in pediatric population is of crucial importance in preventing lifelong cardiovascular complitations. Four patients in this series were followed from 2012 to 2024 at the Mother and Child Health Care Institute of Serbia "Dr Vukan Cupic". Three patients were identified on the basis of persistent abnormality in lipid profile with definitive diagnosis established by next generation sequencing (NGS) methodology. One patient was diagnosed with hyperalphalipoproteinemia on the basis of incidental NGS finding.

This study presents the clinical features and genetic findings of four pediatric patients with primary dyslipidemias. The causative variants were detected in the *LDLR*, *APOA5*, *LIPA* and *CETP* genes, resulting respectively in familial hypercholesterolemia, familial hypertriglyceridemia, lysosomal acid lipase (LAL) deficiency and hyperalphalipoproteinemia. Diagnosis of LAL deficiency prompted the administration of enzyme replacement therapy. Incidentally found variant in CETP gene is associated with benign hyperalphalipoproteinemia in a child tested due to sensorineural deafness.

Identification of genetic variants in pediatric patients with primary dyslipidemias provides valuable insights into the pathogenesis of particular lipid metabolism disorders. More importantly it enables precise pharmacologic treatment and nutritional management, and provides opportunity for selective familial screening.

PRIMARY DYSLIPIDEMIA, PEDIATRICS, TREATMENT

04 – 54 Poster

HSA-MIR-675-5P IS A POTENTIAL DIAGNOSTIC, BUT NOT PROGNOSTIC BIOMARKER IN ORAL CANCER

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Current pathology endeavors to apply molecular pathological approaches for the sensitive identification of oral cancer. As there are no specific biomarkers used in clinical practice, their identification is an unmet need. The H19-derived miRNA, hsa-miR-675-5p, is generally deregulated in various cancers, but has not been investigated in this cancer yet. The aim of this study was to uncover the diagnostic and prognostic potential of this miRNA in oral cancer.

The study included 35 patients with oral cancer who had undergone surgery. Tumor and surrounding non-tumor tissue were collected. After RNA isolation from the biological samples and cDNA synthesis, the relative expression of hsa-miR-675-5p was measured using the real-time PCR method.

The relative expression of hsa-miR-675-5p was significantly lower in oral cancer tumor than in non-tumor tissues, indicating its tumor suppressive role (p=0.006, Wilcoxon signed rank test). hsa-miR-675-5p has satisfactory diagnostic potential for sensitively discriminating tumor and non-tumor tissues in oral cancer patients (AUC=0.637, 95% CI=0.506-0.768, p=0.047). Patients with low and high hsa-miR-675-5p expression did not differ in overall survival (p=0.090, log-rank test) and therefore cannot be used as a prognostic biomarker. No significant associations were found between expression of either low or high hsa-miR-675-5p in tumor tissue and the demographic and histopathological characteristics of oral cancer patients.

hsa-miR-675-5p has a promising potential to be used as a molecular biomarker for the diagnosis of oral cancer. The current results should be validated in a larger group and functional studies should be performed to fully clarify the role of hsa-miR-675-5p in oral cancer.

ORAL CANCER, HSA-MIR-675-5P, BIOMARKER

04 – 55 Poster

HSA-MIR-18A-5P AND HSA-MIR-135B-5P EXPRESSION ASSOCIATION WITH NEOADJUVANT CHEMORADIOTHERAPY RESPONSE IN LOCALLY ADVANCED RECTAL CANCER PATIENTS

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The first line of therapy for patients with locally advanced rectal cancer (LARC) is neoadjuvant chemoradiotherapy (nCRT), aimed at downsizing and downstaging the tumour before surgery. A complete response to nCRT is achieved in less than 20% of patients, highlighting an urget need to identify novel predictive biomarkers to avoid unnecessary treatments for those who will not benefit from nCRT. Given that hsa-miR-18a-5p and hsa-miR-135b-5p are deregulated in colorectal cancer, the current study investigated their expression as potential biomarkers for predicting response to nCRT.

The study included 19 LARC patients treated with nCRT. RNA was isolated from tumour tissue both before and after nCRT. The relative expression of hsa-miR-18a-5p and hsa-miR-135b-5p, normalised to RNU6B, was determined by qRT-PCR.

Hsa-miR-18a-5p was significantly downregulated in tumour tissue after nCRT compared to tissue before therapy (p=0.001). There were no differences in the expression of hsa-miR-135b-5p before and after therapy (p=0.114). No significant correlation was observed between two studied miRNAs in LARC patients before and after nCRT. Responders and non-responders to nCRT did not differ in expression of analysed miRNAs. Based on ROC analysis, neither hsa-miR-18a-5p (AUC=0.794, 95% CI=0.580-1.000, p=0.184) nor hsa-miR-135b-5p (AUC=0.382, 95% CI=0.132-0.632, p=0.595) were identified as predictive biomarkers.

Hsa-miR-18a-5p and hsa-miR-135b-5p cannot be used to predict nCRT response in LARC patients. The decreased expression of hsa-miR-18a-5p in tumour tissue after nCRT may indicate the therapeutic potential of this miRNA to be altered as a target in LARC patients. Further studies should be conducted in a larger group of patients.

HSA-MIR-18A-5P, HSA-MIR-135B-5P, RECTAL CANCER, THERAPY RESPONSE

04 – 56 Poster

COMPARED EFFECTS OF DOXORUBICIN AND QUERCETIN COMBINED TREATMENT ON 2D AND 3D OSTEOSARCOMA MODEL SYSTEMS

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Osteosarcoma (OS) is an aggressive bone tumour affecting paediatric patients with a 5-year survival rate of 66.5%. Standard treatment consists of surgical resection, chemotherapy, and radiation for unresectable tumours. To improve and streamline current therapies for OS there is a need for new, effective treatments. The aim of this study was to establish OS three-dimensional (3D) model able to reproduce key cancer features based on alginate microbeads with immobilized human osteosarcoma cells (SAOS-2), as well as to examine of effects of combined doxorubicin and quercetin treatment in two-dimensional (2D) and 3D models.

We successfully immobilized SAOS-2 cell in alginate microbeads (~ 1mm in diameter) and cultivated them up to 21 days. The results of treatment showed that quercetin enhanced doxorubicin's effect on viability in 2D conditions, while this effect missing in 3D model.

However, analysis of the expression of genes associated with poor prognosis showed that combined treatment in 3D model decreased the expression of pluripotency genes (*SOX2*, *Nanog*), OS marker (*SOX9*) and resistance-related gene (*ABCB1*) compared to doxorubicin treatment in 3D model. These results implying that combined treatment could potentially improve the effectiveness of doxorubicin in osteosarcoma treatment.

Hence, further research is needed to understand the response in the 3D models upon combined treatment that could be beneficial for OS treatment.

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OSTEOSARCOMA, ALGINATE MICROBEADS, ANTICANCER TREATMENT

04 – 57 Poster

MICRORNA-21 AS A KEY REGULATOR OF CANCER STEM CELLS PROPERTIES IN OSCC

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MicroRNAs (miRNAs) are small non-coding RNAs that play crucial roles in regulating gene expression and have been implicated in various processes, including cancer progression. MiRNA-21 is notably overexpressed in oral squamous cell carcinoma (OSCC). Cancer stem cells (CSCs) are a small subpopulation of cancer cells which have been identified as key players in tumor recurrence and resistance to conventional treatments. Similarly to normal stem cells, CSCs express *OCT4*, *SOX2*, and *NANOG* genes which encode crucial transcription factors involved in the regulation of cellular fate, influencing self-renewal, differentiation, and tumorigenic potential. The Wnt signaling pathway also plays a pivotal role in the regulation of stemness, resistance to therapy, and ability to promote tumor growth and metastasis.
Our research aimed to elucidate the impact of miR-21 inhibition on the stemness of CSCs
We isolated CSCs (CD44+) from SCC-25 cell line using MACS system (Miltenyi Biotec, CA, USA). CD44+ cells were seeded into 6-well plates (1 × 106 per well) and grown to 80 % confluence. Cells were transfected with MiRNA inhibitors according to manufacturer's instruction. The effects of miR-21 inhibition on miRNA-21, *OCT4*, *SOX2*, and *NANOG* levels, and Wnt/βcatenin signaling pathway were evaluated by qPCR.
The results of our study revealed a significant reduction of *OCT4*, *SOX2* and *NANOG* expression following miR-21 inhibition. Inhibition of miR-21 also led to a decrease in the expression of Wnt/βcatenin signal pathway markers, impairing the self-renewal and tumorigenic capabilities of CSCs.
The downregulation of important signaling pathways upon miR-21 inhibition, suggests that miR-21 plays a crucial role in maintaining the stemness of CSCs in oral cancer.

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ORAL CANCER, CANCER STEM CELLS, MIR-21, *OCT4*, *SOX2*, *NANOG*

04 – 58 Poster

BET INHIBITOR JQ1 INDUCES APOPTOSIS IN CANCER STEM CELLS

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Cancer stem cells (CSCs) are a subset of tumor cells that possess a marked self-renewal ability, and are believed to be responsible for tumor initiation, recurrences, therapy resistance and metastases. Epigenetic regulation is essential for the dynamic control of gene expression and one of the epigenetics mechanisms is histone modification. The Bromodomain and Extra-Terminal Domain (BET) family of proteins play a crucial role in regulating gene transcription through epigenetic interactions between bromodomains and acetylated histones. BET blockade leads to selective repression of the transcription, hence inhibitors of BET (iBETs) are emerging as a novel class of antineoplastic agents.
The aim of the study was to examine whether inhibition of BET proteins can also contribute to the activation of apoptosis in CSCs.
CSCs (CD44+ cells) were isolated from the heterogenous oral squamous cell carcinoma (OSCC) cell culture, using MACS and then seeded into 24-well plates (1 × 105 per well). The next day cells were treated with three inhibitors (IBET 762, IBET 151 and JQ1), at concentration of 10 μM. CD44+ cells treated with cisplatin were positive controls. After 7 days of treatment, Annexin staining for apoptosis detection was performed, as well as RNA isolation, reverse-transcription and qPCR for *CASP3* and *BAX* expression analysis.
The results of 7-day treatment with different antineoplastic agents were as follows: cisplatin-25.45%, IBET 762-12.32%, IBET 151-20.88% and JQ1- 78.68% of apoptotic cells, pointing to a remarkable effect of JQ1. This finding was further confirmed by a significantly higher expression of proapoptotic *CASP3* and *BAX* genes in JQ1 treated cells compared to cisplatin treated cells.
In conclusion, JQ1 seems to be a strong apoptosis activator, besides being transcription inhibitor of oral CSCs.

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CANCER STEM CELL, EPIGENETIC REGULATION, JQ1, IBET

04 – 59 Poster

MMPs AND TIMPs EXPRESSION PROFILES MAY ASSIST IN DIFFERENTIAL DIAGNOSIS OF SALIVARY GLAND TUMORS

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Salivary gland tumors (SGTs) represent a somewhat rare, yet highly heterogenous group of pathologies. The aim of the present study was to analyze the differences in relative expression levels of matrix metalloproteinases (*MMPs*) and their tissue inhibitors (*TIMPs*) between various benign SGTs, malignant SGTs and normal salivary gland tissues (NSG), and their potential as diagnostic and prognostic biomarkers of SGTs. Relative gene expression levels of *MMP2*, *MMP7*, *MMP9*, *TIMP1* and *TIMP2* were assessed in 50 benign SGTs (23 pleomorphic adenomas-PA and 27 Warthin tumors-WT), eight malignant SGTs and ten NSGs by means of reverse transcription real time polymerase chain reaction.
MMP9 relative gene expression was significantly higher in benign tumors compared to NSGs (P=0.036). Higher expression of *MMP2* was observed in PA compared to WT (P=0.001), while a significant overexpression of *MMP9* was observed in WT compared to NSG (P=0.016). The changes of *MMP* gene expression should also be considered in the context of their ratio to *TIMP* gene expression. Higher *MMP9* to *TIMP1* ratio was observed in WT compared to PA (P=0.037) and also compared to NSG (P=0.037). *MMP2* to *TIMP2* ratio was higher in PA than in WT (P=0.021), but not in NSG.
In conclusion, this study highlights significant variations in the expression levels of *MMPs* and *TIMPs* in SGTs, suggesting their potential role as diagnostic and prognostic biomarkers. These findings underscore the importance of *MMP* and *TIMP* expression profiles in the pathogenesis, differential diagnosis and potential clinical management of salivary gland tumors.

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SALIVARY GLAND TUMORS, BIOMARKERS, GENE EXPRESSION, MATRIX METALLOPROTEINASE, TISSUE INHIBITORS OF MATRIX METALLOPROTEINASES

04 – 60 Poster

BIOCOMPATIBLE TYPE I COLLAGEN-COATED DENTAL IMPLANT SURFACES: IN VITRO OSTEOINDUCTION POTENTIAL ON STEM CELLS FROM THE APICAL PAPILLA (SCAPs)

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Effective osteogenesis and integration are primary objectives for ensuring the long-term implant success. This work focused on enhancing implant surface characteristics with a collagen coating to potentially improve osseointegration.
The cytotoxicity (MTT at 24, 48, 72h, and 7, 14, 21 days) of native (COL N) and hydrolyzed (COL I) forms of horse tendon-derived type I collagen (Typeone Biomaterials S.r.l.) at different concentrations (0.1, 1, 2 mg/cm^3) was evaluated on stem cells from the apical papilla (SCAPs) to select the most biocompatible collagen coating for implant surfaces. Subsequently, osteogenic differentiation was induced and studied through bone matrix deposition (ARS), ALP activity, and gene expression of osteogenic markers (*BMP4*, *ALP*, *RUNX2*, *OCN*) at 7, 14, and 21 days. All analyses following SCAPs viability were exclusively performed on COL N, which exhibited superior characteristics compared to COL I. The same examinations were conducted on uncoated and COL N-coated titanium implant discs (Implacil De Bortoli), featuring various macro- and micro-roughness levels: machined (M), sand-blasted and dual acid-etched (S), S with linear roughness (L), and S with waved roughness (W).
The ALP activity and bone deposition were significantly higher on COL N2, W discs, and COL N2-covered W discs across all experimental times. In the latter condition, SCAPs demonstrated significantly up-regulated expression of all assessed osteogenic markers.
In conclusion, COL N2-covered W surfaces demonstrated exceptional biocompatibility and a high osteoinductive effect. These preliminary findings regarding collagen-coated implant surfaces may lay the groundwork for advantages associated with promoting continuous progress in biocompatible dental implants.

COLLAGEN, DENTAL IMPLANTS, OSSEOINTEGRATION, ORAL REGENERATION

04 – 61 Poster

AKT AND COX-2 GENE EXPRESSION IN PATIENTS WITH ORAL SQUAMOUS CELL CARCINOMA

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Different genetic and epigenetic modifications are described as risk factors for oral squamous cell carcinoma (OSCC) formation. These modifications may also lead to transition from oral potentially malignant disorders (OPMDs) to OSCCs. AKT (Protein kinase B) gene stimulates cell proliferation, differentiation and affects cell survival. Cyclooxygenase 2 (COX-2) modulates cell differentiation and apoptosis and promotes angiogenesis. The aim of this study was to assess gene expression patterns of *AKT* and *COX-2* in patients with OPMDs and OSCCs.
64 patients were included in the study – 33 with OSCCs and 31 with OPMDs. Surgical biopsy was performed and tissue samples were stored in RNAlater solution. After RNA extraction from tissue samples, quantitative real time PCR (qPCR) was performed to assess the relative gene expression of *AKT* and *COX-2*.
A statistically significant difference was observed in *AKT* and *COX-2* expression between OSCCs and OPMDs patients (p=0.003 and p=0.006, respectively).
AKT and *COX-2* genes were overexpressed in OSCC patients compared to OPMD patients. This may indicate the mentioned genes' role in OSCC development. Further examinations on larger patients' groups are required to confirm obtained results.

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AKT, *COX-2*, ORAL SQUAMOUS CELL CARCINOMA, ORAL POTENTIALLY MALIGNANT DISORDERS

04 – 62 Poster

GENE EXPRESSION PATTERN OF ENDOTHELIAL-RELATED MARKERS IN ECTOPIC OSTEOGENIC IMPLANTS BASED ON BIOLOGICAL TRIAD

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Adipose tissue is an abundant source of stem cells that can be used in cell-based bone tissue engineering (BTE). Adipose-derived mesenchymal stem cells (ADSCs) can be applied directly after the isolation of adipose tissue or cultivated in vitro, with or without induction of differentiation toward particular cell line. Vascularization is of essential importance for bone repair and regeneration. Therefore, the aim of this research was to examine gene expression pattern of endothelial-related markers in ectopic osteogenic implants that were composed based on biological triad principle: ADSCs, platelet-rich plasma (PRP) as a source of regulatory signals and bone substitute biomaterial (BSB) as a carrier. ADSCs were isolated from epididymal fat pads taken from male Balb/c mice and expanded in standard proliferative medium up to the twelfth day after the third passage. At the twelfth day after the third passage, the cells were used for the construction of implants that, besides uninduced ADSCs, contained PRP and BSB. The other type of implants contained PRP and BSB (control). After three different implantation periods, the implants were explanted and relative gene expression levels of endothelial-related markers *Flt1*, *Vcam1* and *Egr1* were determined using quantitative Real Time PCR method. The gene expression patterns of all examined endothelial-related markers shows that expression of these genes was significantly higher in implants containing uninduced ADSCs, PRP and BSB in comparison with the implants composed of PRP and BSB. Therefore, the enrichment of ectopic osteogenic implants with uninduced ADSCs represent one of the possible strategies in cell-based BTE.

ENDOTHELIAL-RELATED GENE MARKERS, ADIPOSE-DERIVED MESENCHYMAL STEM CELLS, PLATELET-RICH PLASMA, BONE MINERAL MATRIX, ECTOPIC OSTEOGENIC IMPLANTS

04 – 63 Poster

DETERMINATION OF THE MOST COMMON NON-DRIVER MUTATIONS IN MYELOPROLIFERATIVE NEOPLASMS

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The presence of more than one high molecular risk mutation gives shorter overall survival in patients with myeloproliferative neoplasm (MPN). We hypothesized that progression of MPN can be predicted by the quantity and specific combination of driver and non-driver mutations. Regarding driver mutations determined by Sanger sequencing of granulocyte DNA, *JAK2*-V617F was found in 98.2% of 91 patients with polycythemia vera (age 60.1±10.3), 56.1% in 66 patients with essential thrombocythemia (51±13.7) and 63.9% of 61 patients with primary myelofibrosis (PMF, 60.8±14.3), while *CALR* mutations were found in 24.2% of ET and 26.2% of PMF, *MPL* mutations in 1.5% of ET and 3.3% of PMF. The somatic mutations were evaluated by next generation sequencing (NGS) of 84 suspected genes in de novo 186 MPN patients since 2007. Out of 460 non-artifact variants, 352 variants have an allele frequency of 25% or more, 157 are driver mutations and 17 are somatic mutations. Tet methylcytosine dioxygenase 2 (*TET2*), additional sex combs-like 1 (*ASXL1*) and DNA methyltransferase 3 alpha (*DNMT3A*) are the three most common non-driver mutations. The most common germline mutations are CCAAT enhancer binding protein alpha (*CEBPA*), FAT atypical Cadherin 4 (*FAT4*), DNA polymerase subunit gamma (*POLG*) and *TET1* differentiated by buccal swab or root hair. The 6.8 % of examined MPN patients had progression into PMF and 23.1 % have died due to co-morbidities (mostly cardiovascular and cancers). The most frequent non-driver mutations are *ASXL1*, *TET1* and tumor protein p53 in deceased patients, especially *ASXL1* in patients with progression to PMF.

MYELOPROLIFERATIVE NEOPLASM, PROGRESSION, DRIVER MUTATIONS, NON-DRIVER MUTATIONS, SOMATIC MUTATIONS

04 – 64 Poster

DETERMINATION OF THE MOST RECENT COMMON ANCESTOR

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We traced the paternal ancestry of 11 families in the Balkan region sharing the common R1b-M269 haplogroup and the specific allele 20.2 at the Y-STR locus DYS448 to determine their the most recent common ancestor (tMRCA). According to the DYS439 locus, the families were separated into 2 groups: the first A-D (13 repeats) and the second E-K (12 repeats) by PCR amplification of 23 Y-STR loci. The first group showed high similarity (1 locus difference), while the second group was separated into 3 subgroups: E-F, G-I (1 locus) and J-K (4 loci). Using the NevGen tMRCA calculator for the PPY23 marker format, we determined a range of 7 meiosis (generation / 25 years) for 1 locus, 14 meiosis for 2 loci, 21/22 meiosis for 3 loci, and 29 meiosis for 4 loci differences. Consequently, the closest families were A-B, B-C and A-D. We performed whole-genome sequencing to observe STR and SNP similarities between families A, B, D and G. Using the NevGen tMRCA calculator for the 111 Y-STR markers format, we determined generation difference of family A with families: B (12±3), D (19±4) and G (48±10). Using Y-full SNP analyses, we found that families A and B had tMRCA born in 1674 (CI95%: 1150-1800), while families A, B and D had tMRCA born in 908 (CI95%: 350-1400) and finally A, B, D and G families had tMRCA born in 711 (CI95%: 150-1200). Records from Ottoman and Austrian archives confirmed that families A and B had tMRCA born in 1720.

THE MOST RECENT COMMON ANCESTOR, Y-CHROMOSOME, SINGLE TANDEM REPEAT (STR), SINGLE NUCLEOTIDE POLYMORPHISM (SNP)

04 – 65 Poster

QUALITY OF DNA MOLECULES ISOLATED FROM DIFFERENT BLOOD SAMPLES

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The aim of this study was to evaluate the purity, concentration and integrity of DNA obtained from blood samples stored in various conditions. In total 32 blood samples were taken during medico-legal autopsies from healthy persons who died from a violent death. The samples were divided into four groups: blood from which DNA was isolated immediately after autopsy, blood stored at +4°C for 45 days, blood stored at -20°C for 45 days and blood swabs stored at room temperature for 45 days after autopsy. DNA molecules were isolated using a commercial kit, while spectrophotometric analysis, PCR of *hTERT* gene, and agarose gel electrophoresis were employed for the quantitative and qualitative assessment of the samples.

The results indicate that DNA isolated from blood immediately after autopsy has the lowest protein content and the highest DNA concentration, whereas blood swabs have the lowest purity and concentration. The best-preserved DNA integrity was found in blood samples from which DNA was isolated immediately after autopsy and from blood swabs.

In conclusion DNA isolated from blood taken immediately after autopsy is the best choice for forensic genetics analyses. If fresh blood is unavailable, blood swabs and blood frozen at -20°C can serve as good alternatives for molecular analysis. It is recommended to promptly isolate DNA from blood stored at +4°C or transfer it to -20°C for optimal results. This study underscores the importance of optimizing DNA collection, storage, and isolation methods in forensic genetics, contributing to the accuracy and reliability of forensic analyses.

FORENSIC GENETICS, DNA CONCENTRATION, DNA PURITY, DNA INTEGRITY, STORAGE CONDITION

TOPIC 5

Challenges of genotoxicology in the 21st century

VII CONGRESS OF THE SERBIAN GENETIC SOCIETY

05 – 01 Invited lecture

POTENTIAL OF *GENTIANA LUTEA* EXTRACTS TO REDUCE DNA DAMAGE INDUCED BY FOOD MUTAGENS AND UV RADIATION

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Exposure to numerous genotoxins can lead to development of various diseases. Given the fact that occurrence of mutagens in thermally processed protein-rich foods cannot be avoided, it is important to find a way to reduce their genotoxicity. Furthermore, one of the most common physical genotoxin in nature, ultraviolet radiation (UV), can also lead to genomic instability. In recent years intensive studies have been focused on plants, with the aim of finding new, genoprotective phyto-antimutagens. *Gentiana lutea* is an important medicinal plant, grown on plantations for commercial use. Additionally, it could be cultivated *in vitro*. In this study, plant material from plantation and *in vitro* conditions was used to prepare methanolic and 50% ethanolic-aqueous root and leaf/shoot extracts, which were used for antigenotoxicity testing against food mutagens (IQ and PhIP) and UV radiation. Antioxidant activity of the extracts was also screened in order to analyze one of the possible underlying mechanisms of antigenotoxicity. The results obtained in alkaline comet assay on human hepatoma cells (HepG2) indicated strong genoprotective potential, with reduction of IQ- and PhIP-induced DNA damage up to 78%. The same assay on normal fetal lung fibroblasts (MRC-5) and human melanoma cells (Hs 294T) pointed out strong UV-protective effect of all the extracts (inhibition up to 78%). Higher antioxidative activity was recorded for leaf/shoot extracts. Selected extracts up-regulated the expression of *Nrf2* gene and successfully contributed to protection against glutathione depletion in HepG2 cells. Results obtained encourage further analysis with the possibility of obtaining potential dietary supplements and UV-protective agents.

ANTIGENOTOXICITY, ANTIOXIDANT ACTIVITY, FOOD MUTAGENS, *GENTIANA LUTEA*, UV RADIATION

05 – 02 Invited lecture

INCREASED PRIMARY DNA DAMAGE CAUSED BY THYROID HORMONE IN VARIOUS PHASES OF TYPE 2 DIABETES MELLITUS

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Diabetes mellitus is a major health concern, especially in developed countries. In the last few decades type 2 diabetes mellitus (T2DM) became epidemic. It is well established that patients with T2DM have increased oxidative stress which cause damage to various biomolecules including DNA. Thyroid hormone increases the aerobic metabolism and reactive oxygen species production. Therefore, it is not surprising that thyroid hormone influences the development of T2DM and its outcome. Thus, the aim of this investigation was to examine whether peripheral blood mononuclear cells (PBMCs) from healthy persons exhibit less DNA damage (in the alkaline Comet assay) resulting from triiodothyronine (T3) treatment (0.1, 1 and 10 μM) in comparison to PBMCs from obese, prediabetic and diabetic patients. Untreated cells were the negative, and 100 μM H_2O_2 the positive control. In addition, the levels of certain parameters of oxidative stress (thiobarbituric acid reactive substances - TBARS, catalase) and lactate dehydrogenase (LDH) were monitored. PBMCs from prediabetic and diabetic patients exhibited heightened sensitivity to T3 resulting in elevated levels of DNA damage, inhibition of catalase, and the increase in TBARS and LDH. PBMCs from obese patients reacted in the same manner, except for DNA damage. In conclusion, increased propensity to oxidative stress and DNA damage is present in early phases of T2DM, even before the diagnosis is established. This research may contribute to a better understanding of the role of T3 in the development and progression of T2DM and help in the development of novel therapeutic approaches aimed to avoid diabetes complications.

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DIABETES, DNA DAMAGE, OXIDATIVE STRESS, PBMC, THYROID HORMONE

05 – 03 Invited lecture

NEW INSIGHTS INTO MOLECULAR COMPLEXITY OF GENOME PROTECTION IN *USTILAGO MAYDIS*

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The maintenance of genome integrity is one of the most fundamental cellular processes that ensures genome stability by employing molecular factors that are mostly conserved in all domains of life. Our research focuses on identification of novel cellular components of the molecular machineries underlying preservation of genome integrity concentrating our focus on homologous recombination (HR)-mediated repair of double strand brakes. For this purpose, we exploit a model system *Ustilago maydis*. Several facts make this microorganism suitable for this type of research: it is extremely radiation resistant with HR at the base of this resistance; 30% of its genes are of unknown function which opens possibility that some of these are dedicated to modulation/regulation of HR and DNA repair; it is a BRCA2 organism showing some surprising features in common with the higher eukaryotes' (including humans') HR system. Besides, *U. maydis* possesses an impressive capacity to recover from massive damage i.e., to reconstitute devastated cell populations by recycling biomolecules released from dead cells. Importantly, we have found that this substrate may be mutagenic which opens up a methodological approach to identify new genes involved in both processes-repopulation and genome protection.

Through different approaches we have identified and partially characterized a number of cellular factors responsible for maintenance of genome integrity. Two are previously known HR factors, while majority are completely uncharacterized genes. Notably, we have identified novel components of HR machinery, as well as components of cytoskeleton, endosomes, chromatin remodeling complexes, transcription regulation whose absence leads to genome instability.

USTILAGO MAYDIS, HOMOLOGOUS RECOMBINATION, DNA REPAIR, BRCA2

05 – 04 Invited lecture

HOW THE SELECTION OF ORGANISMS, APPROACHES, AND ASSAYS IN BIOMONITORING AFFECTS THE EVALUATION OF GENOTOXIC POTENTIAL IN AQUATIC ENVIRONMENTS

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According to our historical data and extensive experience in the field of aquatic eco/genotoxicology, the design of experimental setups in environmental studies is crucial for accurately interpreting field conditions and their effects on biota. The choice of organisms, approaches, and assays significantly impacts the evaluation of genotoxic potential in aquatic environments. Where possible, in situ and ex situ studies should be conducted in parallel, utilizing both prokaryotic and eukaryotic models. This includes relevant cell lines, laboratory animals, and animals collected from natural habitats, while adhering to the 3Rs principle (Replacement, Reduction, and Refinement). Water samples analyzed under in vitro conditions show significant effects at highly polluted sites or in concentrated samples. For example, the prokaryotic SOS/umuC test on *Salmonella typhimurium* TA1535/pSK1002 demonstrates lower sensitivity compared to eukaryotic cell models. Among animal models, zebrafish embryos have shown high sensitivity when testing concentrated water samples, while animals collected from the field exhibited greater sensitivity. When using animals from the field, it is essential to consider their ecological preferences, lifestyle (sedentary or mobile), feeding habits (filter-feeding, herbivorous, or predatory), and whether they are bottom dwellers or live in the middle or upper parts of the water column. Our studies indicate that comet and micronucleus assays are sensitive enough to distinguish polluted sites and have high potential for early warning monitoring, as DNA damage is an early response to environmental pollution. Thus, careful selection of organisms, approaches, and assays is essential for accurately evaluating genotoxic potential in aquatic environments.

FRESHWATER, BIOASSAY, AQUATIC ORGANISM, GENOTOXICITY

05 – 05 Invited lecture

UNLOCKING THE POTENTIAL: EXPLORING COMPLEMENTARITY OF MULTIPLE BIOMARKERS IN FISH TISSUES FOR UNDERSTANDING AQUATIC ECOSYSTEM POLLUTION

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Although freshwater ecosystems provide many ecosystem services, human activities have significantly degraded them. To assess impact on fish, biomonitoring and multibiomarker approach are recommended.
This study investigated three sites in the Republic of Serbia exposed to different types and levels of pollution using the European chub (*Squalius cephalus*). The first site, the Kruščica reservoir, is a drinking water source. The second site on the Ibar River is exposed to treated municipal wastewater. The third site on the Pek River is polluted with untreated municipal and mining waste. The municipal wastewater burden was determined by the numbers of *E. coli* in water. In fish, the concentrations of metals and metalloids, oxidative stress parameters, genotoxic response and histopathological alterations were monitored. All responses were linked using the Integrated Biomarker Response (IBR).
The highest values of oxidative stress parameters and metal pollution index (MPI) were observed in the gills at the Ibar River and in the liver at the Pek River. The comet assay on erythrocytes revealed significant differences among all three sites, indicating the lowest level of DNA damage at Kruščica and the highest at Pek. Micronucleus test showed significant differences only between the least polluted Kruščica and the most polluted Pek. Gills' histopathological analysis revealed significantly higher values in fish from the Pek River compared to the other two sites.
The study emphasises the reliability of IBR to rank sites based on pollution load. Genotoxicity biomarkers play a crucial role in bridging biomarkers at different levels of biological organisation.

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AQUATIC POLLUTION, EUROPEAN CHUB, ECOGENOTOXICOLOGY, INTEGRATED BIOMARKER RESPONSE

05 – 06 Oral

ASSESSMENT OF THYMOL AND *AGARICUS BISPORUS* EXTRACT GENOTOXIC AND ANTIGENOTOXIC EFFECTS ON A CONTINUOUS HONEY BEE CELL LINE

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Positive health benefits to both humans and animals, including honey bees, of bioactive compounds such as natural essential oils and mushroom's extracts have been demonstrated. In this study, the honey bee (*Apis mellifera* L.) continuous cell line AmE-711 was used to assess the genotoxic and antigenotoxic potential of three concentrations of thymol (0.01, 0.1, and 1 mg/mL) and *Agaricus bisporus* extract (0.1, 0.2, and 0.4 g/mL) using the alkaline Comet assay. Cells in positive control were treated with 100 µM H_2O_2 and negative control cells were left untreated. In order to test the antigenotoxic effect, all tested concentrations of thymol and *A. bisporus* extract were co-incubated with H_2O_2. The Trypan blue exclusion test was used to verify the level of thymol and the *A. bisporus* extract cytotoxicity. *A. bisporus* extract did not exhibit genotoxic potential at any of the tested concentrations, as well as thymol at the lowest concentration. On the other side, higher concentrations of thymol increased DNA damage in AmE-711 honey bee cells. All tested concentrations of thymol did not exhibit any antigenotoxic effect. Moreover, thymol increased the H_2O_2-induced DNA migration. However, all concentrations of *A. bisporus* extract demonstrated antigenotoxic effects against H_2O_2-induced DNA damage. While *A. bisporus* extract is not genotoxic to honey bee cells, and has promising antigenotoxic properties, the obtained results indicate that thymol exhibits genotoxic effects on cultured honey bee cells, suggesting that it should be applied carefully in beekeeping practice to avoid potential negative effects on honey bees.

THYMOL, *AGARICUS BISPORUS*, AME-711, GENOTOXICITY, ANTIGENOTOXICITY

05 – 07 Oral

FRANGULA ALNUS MILL AS A SOURCE OF POTENTIAL CHEMOTHERAPEUTICS EFFECTIVE AGAINST COLORECTAL CARCINOMA

Stefana Vuletić [1], Marina Bekić [2], Sergej Tomić [2], Biljana Nikolić [1], Stefana Cvetković [1], Tea Ganić [1], Dragana Mitić-Ćulafić [1]

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Phenomenon of drug resistance in cancer is multifaceted since the cancer is leading cause of mortality worldwide. According to the high heterogeneity among tumors, finding the appropriate strategy to overcome drug resistance is a challenge. Bearing in mind traditional use of plants, their diversity in secondary metabolites as well as its fewer side effects they seem as excellent choice. Therefore, the aim of the study was to investigate anticancer activity of *Frangula alnus* ethyl-acetate extract (FA) and its dominant constituent emodin (E), as well as their potential mechanism of action on colorectal carcinoma cell line (HCT116) and normal fetal fibroblasts (MRC-5). Chemical analysis of FA (LC-MS/MS) revealed that extract is rich in phenolic and flavonoid compounds, with emodin as dominant one. Cytotoxicity was examined by MTT test and both FA and E reduced HCT116 cell viability (up to 88%). Flow cytometer analysis demonstrated that FA and E led to G1 phase arrest and slight accumulation of cells in the G2/M phase. In addition, annexinV-FITC/7AAD dying showed that FA and E decreased cell viability and triggered apoptosis in both cell lines. Further investigation of underlying mechanism through monitoring the fluorescence of JC-10 dye, demonstrated that both substances affected mitochondrial membrane potential of both tested cell lines. FA and E also showed strong genotoxic potential in comet assay. Based on the obtained results, it could be concluded that both FA and E have significant anticancer activity against colorectal carcinoma, but notable selectivity was not observed.

FRANGULA ALNUS, EMODIN, CELL CYCLE, APOPTOSIS, COMET

05 – 08 Oral

EVALUATION OF CYTOTOXIC, GENOTOXIC AND ROS-MEDIATED OXIDATIVE STRESS CAUSED BY NANOCOMPOSITE MATERIAL BASED ON RESVERATROL AND SELENIUM NANOPARTICLES

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Nanobiotechnology has recently given rise to numerous possibilities in the bioengineering. The nanoparticle formulations, especially those based on the natural compounds, have been shown to have greatly enhanced biological effects. On the other hand, the widespread use of nanoparticle-based materials is often not properly monitored, especially regarding possible genotoxic effects. Here, we present the effects of nanocomposite (ResSeNPs) material as well as its' constituents, resveratrol nanobelt-like particles (ResNPs) and selenium nanoparticles (SeNPs), on human MRC-5 and A549 cells. Resveratrol is a highly promising biologically active compound, and selenium is an essential micronutrient. The results of several cell-based assays showed combined effects (synergism, antagonism or additive) of nanocomposite components. In the MTT test, SeNPs were significantly less cytotoxic than ResNPs, furthermore, significantly lower cytotoxicity of the ResSeNPs was observed in the same assay. This effect was observed in both cell lines, especially in MRC-5. On the other hand, ResNPs neutralized the genotoxic effects of SeNPs, as shown by the comet assay in both cell lines. The superoxide anion production, as determined by the NBT test, was enhanced by the SeNPs, but this enhancement was attenuated by ResNPs in MRC-5 cell line, although the effect was highly different in A549. Antagonism was observed also in the catalase assay, in both MRC-5 and A549. All of this confirmed the necessity of thorough testing of nanomaterials-based composites but also the benefits of using more than one active substance.

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NANOTOXICITY, GENOTOXICITY, NANOCOMPOSITES, SELENIUM, RESVERATROL

05 – 09 Poster

EVALUATION OF CYTOTOXIC, GENOTOXIC AND ANTIOXIDANT PROPERTIES OF *AGRIMONIA EUPATORIA* AQUEOUS-ETHANOLIC EXTRACT

Tamara Anđić, Biljana Nikolić, Dragana Mitić-Ćulafić, Stefana Vuletić, Tea Ganić, Ana Ignjatijević, Ana Džamić, Lazar Žarković, Stefana Cvetković

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Cancer is a leading cause of mortality worldwide, with lung cancer being the most diagnosed in recent decades. Treatment is complicated due to the selectivity of chemotherapeutics and cancer cell resistance. Furthermore, oxidative stress significantly contributes to carcinogenesis, causing genetic and epigenetic changes. It is known that natural anticancer drugs, working through multiple mechanisms, can be effective against resistant cells. Therefore, plants with antioxidant properties represent a promising source of bioactive compounds with potential application in cancer therapy. Taking into account all the above mentioned, the aim of this study was to examine content of polyphenols, antioxidant, cytotoxic and genotoxic effect of *Agrimonia eupatoria* 70% aqueous-ethanolic extract. By applying colorimetric assays, total phenolics (354,2 ± 2,1 mg GAE/g DE) and flavonoids (43,3 ± 0,6 mg QH/g DE) were determined. The assessment of antioxidant potential revealed good antioxidant activity with EC50 being 15,3 µg/mL in DPPH assay and 3,4 mg/mL in ferrous ions chelating assay. Further on, cytotoxicity was evaluated by MTT assay on normal fetal fibroblast (MRC-5) and lung adenocarcinoma (A549) cell lines, revealing that extract exhibited higher cytotoxicity towards A549 cell line (IC50 values detected at 1,5 mg/mL and 0,8 mg/mL for MRC-5 and A549, respectively). In the alkaline comet assay, greater genotoxicity of extract towards A549 cell line (tail intensity of 18,6%) was detected, compared to MRC-5. Based on the results, *A. eupatoria* extract emerges as a promising candidate for new lung cancer therapy, though further investigation into their underlying mechanisms is necessary.

AGRIMONIA EUPATORIA, CYTOTOXICITY, GENOTOXICITY, ANTIOXIDANT ACTIVITY

05 – 10 Poster

GENTIANA LUTEA L. ROOT EXTRACT GENOPROTECTIVE EFFECTS AGAINST HYDROXYUREA-INDUCED DAMAGE OF HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Hydroxyurea (HU) is a chemotherapeutic agent used to treat variety of hematological malignancies. However, it can cause late adverse effects, such as secondary malignancies, due to disrupting DNA synthesis, generating reactive oxidative species (ROS), and suppressing DNA repair mechanisms. The use of protective agents that do not affect the antiproliferative potential of HU, but alleviate its genotoxicity on normal cells is important for adverse effects prevention. Among natural plant products, used for the mitigation of chemotherapeutics-induced genotoxicity, *Gentiana lutea* root extract (GRE) has shown promising potential. This study aimes to elucidate the genoprotective potential of GRE on HU-induced genotoxicity on human peripheral blood mononuclear cells (PBMC). PBMCs were treated with 100 µM of HU for 24 hours, and a parallel set of cultures were pretreated with increasing concentrations of GRE (0.25, 0.5, 1 and 2 mg/mL) for 24 hours. Genotoxic and cytotoxic effects of treatments were assessed by cytokinesis-block micronucleus assay (CBMN), by calculating the micronuclei incidence and cytokinesis-block proliferation index (CBPI). To assess GRE effects on the oxidative status of all treatments, pro-oxidant/anti-oxidant (PAB) assay and reduced glutathione (GSH) levels were monitored. The results showed that GRE pre-treatment significantly decreased the incidence of HU-induced micronuclei at all tested concentrations, without significant alteration of the proliferation index. GRE also reduced PAB levels, and increased GSH levels concentration-dependently, significantly at the concentrations of 1 and 2 mg/mL compared to HU treatment. These results suggest that GRE protects PBMC against HU-induced genotoxic and oxidative damage, by activation of antioxidative defense mechanisms.

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HYDROXYUREA, *GENTIANA LUTEA* ROOT EXTRACT, GENOPROTECTIVE, PAB ASSAY, GSH

05 – 11 Poster

CYTOTOXIC AND GENOTOXIC ACTIVITY OF CINNAMON ESSENTIAL OIL AND ITS EMULSION

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Cancer represents the leading cause of high mortality rate worldwide. Due to its persistence, alternative approaches to find new therapeutic agents are being explored for decades. Plants are natural source of various secondary metabolites able to express wide specter of biological activities. Cinnamon has been used in folk medicine over the centuries and its essential oil has been studied as a potential anticancer agent. Although it has good potential, it also has disadvantages such as volatility, instability and insolubility which is why emulsion was synthesized in order to overcome these problems. Therefore, aim of study was to investigate anticancer activity of cinnamon essential oil (EO) and its emulsion (EM). In order to define cytotoxic effect of cinnamon EO and EM on lung adenocarcinoma (A549) and normal cell line, fetal fibroblast (MRC-5) MTT assay was applied, followed with light microscopy to evaluate their effect on cell structure and morphology. Further, level of DNA damage was evaluated, using alkaline comet assay on both cell lines. Production of superoxide anions was estimated by colorimetric NBT assay in order to potentially define mechanism of action of EO and EM. IC50 values in MTT assay were lower on A549 cell line then on MRC-5. Further, none of the tested treatments expressed genotoxic potential on both cell lines. Trend of total share of superoxide anions on MRC-5 cell line was decreasing, while on A549 was increasing. These results are encouraging for further investigation on cinnamon EO and EM to be used as a potential cancer therapy.

CINNAMON ESSENTIAL OIL, EMULSION, CYTOTOXICITY, GENOTOXICITY

05 – 12 Poster

CYTOTOXIC AND GENOTOXIC POTENTIAL OF PULMONARIA OFFICINALIS *ETHANOLIC LEAF EXTRACT*

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Pulmonaria officinalis, commonly known as lungwort, is traditionally used in herbal medicine, primarily for respiratory ailments, including bronchitis, asthma, and coughs, due to its anti-inflammatory and soothing properties. Despite its traditional health benefits, specific studies on its cytotoxic and genotoxic effects are limited. As we continue to explore natural compounds for therapeutic uses, evaluating the safety and biological activity of plant extracts is crucial. This study aimed to explore the chemical composition of *P. officinalis* 70% aqueous ethanolic extract and evaluate its cytotoxic and genotoxic activities to provide scientific support for its traditional use. Chemical analysis using GCxGC-MS revealed that the most abundant compounds were phytol and paromomycin, along with other terpenoids, alcohols, fatty acids, polyketides and alkaloids. The cytotoxic effect of the tested extract on normal fetal fibroblast (MRC-5) and lung adenocarcinoma (A549) cell lines, evaluated using the MTT assay, showed a reduction in cell viability ranging from 22% to 40%. Additionally, we investigated genotoxicity using the alkaline comet assay on the above-mentioned cell lines. The results revealed greater genotoxicity of the lungwort extract towards A549 cells at all tested concentrations in a dose-dependent manner, suggesting that this genotoxicity may contribute to its cytotoxic effects. The obtained results support further investigation and evaluation of potential applications for the *P. officinalis* extract and its pure bioactive constituents.

PULMONARIA OFFICINALIS, CYTOTOXICITY, GENOTOXICITY, COMET ASSAY, MTT

05 – 13 Poster

TOXICITY ASSESSMENT OF POLYCYCLIC AROMATIC HYDROCARBONS AND THEIR DEGRADATION PRODUCTS

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Polycyclic Aromatic Hydrocarbons (PAHs) are environmental pollutants known for their carcinogenic and mutagenic properties. Due to their persistence and bioaccumulative nature, PAHs pose a significant risk to human health. Extensive research has focused on developing new methods for their safe removal from various media. Electrochemical oxidation is a new, non-invasive method for the PAHs degradation into potentially less toxic compounds. This study investigates the toxic effects of increasing concentrations of PAHs (PAH-Mix 14) and products generated through their electrochemical oxidation (treatment duration 0-60 min) to evaluate their impact on human health and explore the efficacy of electrooxidation as a remediation strategy. Genotoxicity, cytostasis/cytotoxicity, and oxidative stress parameters (the level of malondialdehyde and activity of catalase) in response to PAH exposure were assessed using human peripheral blood cells as a model system. Genotoxicity and cytotoxicity were assessed using a cytokinesis-block micronucleus assay. The results demonstrate that, before electrochemical oxidation, PAHs induced a significant increase in micronuclei incidence ($p<0.01$), reduced cell proliferation capacity ($p<0.001$), and induced oxidative stress in a concentration-dependent manner. Products generated at different time points during the electrochemical oxidation of PAHs induced less toxic effects than parental compounds but remained more toxic than untreated control, indicating persistent cellular damage and oxidative stress. These findings suggest that the observed cytotoxicity is influenced by the inherent toxicity of PAH mixtures and the formation of oxidation by-products during electrochemical treatment. Understanding these dynamics is crucial for assessing the health risks associated with PAH exposure and optimizing remediation strategies to minimize toxicological effects.

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POLYCYCLIC AROMATIC HYDROCARBONS, ELECTROCHEMICAL OXIDATION, DEGRADATION PRODUCTS, GENOTOXICITY, CYTOTOXICITY

05 – 14 Poster

IN VIVO GENOTOXIC AND DNA PROTECTIVE POTENTIAL OF SELECTED PYRAZOL-CHROMENO[2,3-D]PYRIMIDINE DERIVATIVE

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Heterocyclic compounds, notably pyrazole, chromeno, and pyrimidine derivatives, are crucial in medicinal chemistry because of their broad biological activities. Pyrazole derivatives exhibit anti-inflammatory, antipyretic, analgesic, antimicrobial, and antiviral properties. Chromeno compounds, found in natural products like alkaloids, tocopherols, flavones, and anthocyanins, have similar properties. Pyrimidines are vital in biological processes as nucleic acid components and are used in various drugs (risperidone, fluorouracil, rosuvastatin), including thyroid medications and leukemia treatments. Accordingly, the compound 5-(5-methyl-3-oxo-2,3-dihydro-1H-pyrazol-4-yl)-1,5-dihydro-2H-chromeno[2,3-d]pyrimidine-2,4(3H)-dione was synthesized via a three-component reaction involving pyrazolone, barbituric acid, and salicylaldehyde. Its structure was confirmed by NMR and IR spectroscopy. The genotoxic and potential protective effects of this compound against ethyl methanesulphonate-induced DNA damage were evaluated by the alkaline comet assay on the third instar larvae of *Drosophila melanogaster*. The mentioned compound in the concentration of 1.25 mg/mL of a standard *Drosophila* food was not genotoxic and also significantly decreased the level of DNA damage induced by ethyl methanesulphonate with a percentage reduction of 75.54. Furthermore, at 2.5 and 5 mg/mL of a standard *Drosophila* food compound demonstrated a low level of genotoxicity with significantly lower total comet scores than those observed in the positive control group. At the same concentrations, the tested compound reduced the level of total comet scores with a percentage reduction of 61.46 and 54.5, demonstrating a protective effect on DNA damage induced by ethyl methanesulphonate. Selected pyrazol-chromeno[2,3-d]pyrimidine derivative may represent a potential source of the pharmacologically active compound due to its DNA protective effect and absence of genotoxicity at lower concentrations.

PYRAZOL-CHROMENO[2,3-D]PYRIMIDINE DERIVATIVE, COMET ASSAY, GENOTOXICITY, DNA PROTECTION

05 – 15 Poster

AG@ICG NPS-A NEW TOOL IN THE CANCER DIAGNOSTIC

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Noble metal nanoparticles (NPs) have been extensively studied due to their unique optical, chemical, and physical properties. They have found many applications in different areas, including nano-medicine, biosensing, and drug delivery. To explore their potential to be used in diagnostics and/or therapy, Ag NPs were synthesized via a green method using L-methionine amino acid and functionalized with Indocyanine green dye (ICG). The resulting nanoparticles are characterized by nearly spherical Ag cores with ICG shells (Ag@ICG NPs). Atomic force microscopy (AFM), fluorescence microscopy, and Raman spectroscopy (RS) were utilized to track morphological and molecular changes in human peripheral blood lymphocytes, MRC5, and HeLa cell lines treated with Ag@ICG nanoparticles. The genotoxicity and cytotoxicity of the Ag@ICG NPs were assessed in a concentration-dependent manner in lymphocytes using the cytokinesis-block micronucleus assay.
Preliminary AFM and RS findings reveal the presence of nanoparticles only within the HeLa cells and indicate that cells maintain a smooth membrane after Ag@ICG treatment. Fluorescence microscopy confirmed the presence of Ag@ICG NPs in the cell cytoplasm and/or nuclei. Genotoxicity assessment indicated that lower concentrations of Ag@ICG NPs did not significantly affect micronuclei incidence compared to the control. In addition, the tested concentrations exhibited mild cell growth inhibition while maintaining cell viability above 90% relative to the control. These findings suggest that Ag@ICG NPs at low and medium concentrations demonstrate non-genotoxic and non-cytotoxic effects, thereby supporting their potential for further biomedical research and applications. Further studies are warranted to explore molecular interactions between nanoparticles and specific cells or subcellular compartments.

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AG@ICG NANOPARTICLES, CANCER DIAGNOSTIC, GENOTOXICITY, CYTOTOXICITY

05 – 16 Poster

OXIDATIVE DNA DAMAGE INDUCED BY AQUEOUS EXTRACT OF *SALVIA PRATENSIS* L. AND ITS GREEN SYNTHESIZED SILVER NANOPARTICLES IN HUMAN LYMPHOCYTES *IN VITRO*

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Nanoparticles have attracted much attention in recent years, but the knowledge regarding their mode of action and potential effect on health remains insufficient. The study aimed to investigate the genotoxic potential of green synthesized silver nanoparticles (AgNPs) and *Salvia pratensis* L. aerial parts aqueous extract used for AgNPs synthesis. Synthesized AgNPs (diameter between 40 and 70 nm) are composed of silver and compounds identified in the extract. The total phenolic and flavonoid content in extract was determined using spectrophotometric methods, while the level of DNA damage (expressed as genetic damage index - GDI) of extract and AgNPs was evaluated through comet assay. The human peripheral blood lymphocytes (PBLs) were treated with four concentrations (25, 50, 75, and 100 µg/mL) of extract or AgNPs. Total phenolic and flavonoid content of the extract was 239.66 ± 2.86 GAE/g and 36.40 ± 1.14 QE/g, respectively. The extract dose-dependent ($r = 0.848$, $p = 0.000$) and significantly increased the GDI in three higher concentrations (from 0.38 ± 0.01 to 0.50 ± 0.01), compared with untreated cells (0.25 ± 0.01). AgNPs also increased the GDI (from 0.44 ± 0.02 to 0.71 ± 0.02) in PBLs. A strong positive correlation between AgNPs concentrations and GDI ($r=0.976$, $p= 0.000$) was obtained. *S. pratensis* extract and AgNPs induced oxidative DNA damage on PBLs in vitro, thus in concentrations above 50 µg/mL they represent a potential risk to human health.
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GENOTOXICITY, NANOPARTICLES, *SALVIA PRATENSIS*, COMET ASSAY

05 – 17 Poster

NANOPARTICLE-INDUCED DNA DAMAGE IN AQUATIC ECOSYSTEMS: A COMET ASSAY STUDY

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The increasing production of nanomaterials has led to their extensive accumulation in the environment, particularly metal-oxide nanoparticles (NPs) like titanium dioxide (TiO_2), cerium dioxide (CeO_2), and magnetite (Fe_3O_4). Aquatic ecosystems are especially vulnerable to NP pollution, necessitating biomarkers for early detection of toxic effects. This study uses a comet assay to detect the genotoxicity of sublethal concentrations of these metal-oxide nanoparticles on *Chironomus riparius*. Larvae treated with 20 mM H_2O_2 for 1 hour served as the positive control. Ten larvae from each sample were homogenized, and the homogenate underwent centrifugation, cell treatment, cell embedding in agarose, electrophoresis, staining, visualization, scoring, and data analysis. Staining was done with 20 µL of GelGreen (Biotium) and examined under a fluorescence microscope at 400x magnification, using specific filters. TriTek CometScore Pro 2.0.0.38 Software was used to score 50 nuclei per slide (150 per treatment group), with tail intensity (TI%) indicating DNA damage. The results showed significantly higher DNA damage in groups treated with CeO_2 and Fe_3O_4 NPs, while nano-TiO2 showed no significant difference from the control group. Studies on the genotoxicity of TiO_2 NPs are conflicting, with some showing significant damage and others showing none, aligning with our findings. TiO2 NPs might initiate DNA damage, but repair mechanisms could restore DNA, resulting in the detection of intact DNA in the comet assay.

NANOPARTICLES, CHIRONOMIDS, COMET ASSAY, ECOTOXICOLOGY

05 – 18 Poster

EVALUATION OF QUERCETIN TREATMENT ON DNA DAMAGE IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS *IN SILICO* AND *IN VITRO*

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Quercetin, a plant-derived bioflavonoid, displays antitumor properties against a variety of solid tumors, but its effect on normal primary human cells is not enough investigated. Since the elimination of the cancer cells could be due to treatment-induced DNA damage, we applied in silico VEGA-QSAR analysis, followed by experimental in vitro estimation of quercetin genotoxic potential by comet assay, to assess its effect on the healthy human peripheral blood mononuclear cells (PBMCs). Additionally, to test if quercetin treatment impacts intracellular reactive oxygen species (ROS) levels and causes cell death, we analyzed prooxidative/antioxidative balance (PAB) levels. All tests were performed after 72-hour quercetin treatment with previously determined non-cytotoxic concentrations (5, 10, and 20 µg/mL) on PBMCs. According to an in silico study, quercetin is classified as a carcinogen in 3/4 carcinogenicity models and as mutagenic in 2/3 mutagenicity models. Based on the micronucleus and chromosomal aberration test predictions, its genotoxic impact is described as active. Our experimental results showed a concentration-dependent increase in DNA damage and a shift to the prooxidative state. The obtained results indicate that in vitro quercetin treatment, which does not affect cell viability, could affect the oxidative state of exposed cells and induce DNA damage in primary human cells that could lead to carcinogenesis. The potential use of quercetin in prevention and treatment of cancer could potentially rely on a higher toxicity impact on cancer than on healthy cells. The use of quercetin must be applied with caution concerning concentration to achieve the optimum outcomes.

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QUERCETIN, GENOTOXIC, VEGA-QSAR, COMET ASSAY

05 – 19 Poster

METFORMIN: *IN VITRO* ANTIOXIDANT ACTIVITY AND *IN SILICO* PREDICTION OF ANTIGENOTOXICITY IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Metformin is used for the regulation of blood sugar levels in patients with type 2 diabetes mellitus, and in addition, its anti-cancer, immunoregulatory, and anti-aging effects were also reported. However, many people misuse metformin in weight-loss treatment. To test how metformin could affect peripheral blood mononuclear cells (PBMCs), which are exposed to the drug through therapy *in vivo*, we initially ran *in silico* analyses of its cancerogenic/anticancerogenic activity, chromosomal aberration, and micronucleus activity. Modern tools enable a quick and informative analysis of metformin's activity, marking it as a potential antigenotoxic agent by numerous VEGA-QSAR models. Further experiments were then used to analyze the viability and oxidative parameters upon 10, 20, and 50 μM 24-hour metformin treatments. The number of cells was determined by the trypan blue dye exclusion assay, while in PBMC lysates, levels of reduced glutathione (GSH) and prooxidative/antioxidative balance (PAB) were additionally analyzed. Our results indicated that the highest treatment concentration lowered the number of viable cells, increased levels of GSH, and shifted PAB to an antioxidative status. Reactive oxygen species are essential molecules for cellular functioning since they play a critical role in numerous physiological processes at low concentrations, including cellular signaling, gene expression, and immune response control. Although metformin's antioxidant effect could be beneficial to patients with type 2 diabetes, awareness must be raised that misuse of this drug could disturb the redox homeostasis of healthy cells.

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METFORMIN, GLUTATHIONE, ANTIGENOTOXIC, ANTIOXIDATIVE

05 – 20 Poster

EFFECTS OF EARLY AND DELAYED X-RAY EXPOSURES ON DNA DAMAGE IN MESENCHYMAL STEM CELLS

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Techniques based on X-ray phase-contrast (XPC), have shown great potential for a number of biomedical applications due to their ability to provide soft tissue contrast through their reliance on alternative X-ray properties (refraction, scatter). It is shown that XPC can be used to image biomaterials and soft tissue. Exposure of cells and biomaterials to X-rays even at diagnostic doses may influence the final outcome. Essential to the development of this technique and its routine use is an understanding the potential radiation damage to the cells and tissue that may result from routine imaging. The overall aim of the study was to investigate the effects of early (immediately after irradiation) and delayed (24 hours after irradiation) X-ray exposures generated by low (15 mGy) and intermediate (150 mGy and 1.5 Gy) irradiation on mesenchymal stem cells (MSCs) using polychromatic X-ray imaging. We used the alkaline Comet assay to quantify DNA damage. Our data showed that 15 mGy did not increase the number of DNA-damaged cells after irradiation, while 150 mGy and 1.5 Gy caused a significant increase ($p<0.01$) in DNA-damaged cells compared to the control. It is noteworthy that 24 h after irradiation, DNA damage at intermediate doses returned to control levels, indicating that MSCs provide significant protection at the XPC irradiation dose studied. The current work provides additional evidence for the use of MSCs in the clinical setting and emphasizes the need to investigate the effects of low and intermediate doses of X-ray PC irradiation in the field of tissue engineering.

X-RAY PHASE-CONTRAST (XPC), DNA DAMAGE, MESENCHYMAL STEM CELLS

TOPIC 6

New breeding technologies and perspectives

VII CONGRESS OF THE SERBIAN GENETIC SOCIETY

06 – 01 Invited lecture

PHENO_MAIZE PROJECT: UAV-DRIVEN HIGH-THROUGHPUT PHENOTYPING FOR ENHANCED MAIZE BREEDING

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Assessing and measuring plants across various environments in plant breeding is crucial for enhancing genetic gains but is also resource-intensive and prone to bias. High-throughput field phenotyping (HTFP) enables the study of numerous genotypes across multiple environments and time points. The PHENO_MaizE project aims to explore the use of drone-based HTFP in temperate hybrid maize breeding programs. The plan involves imaging approximately 200 maize inbred lines and their test crosses with an elite tester multiple times during the season in three different environments in Serbia (in 2025 and 2026) using a drone equipped with an RGB sensor. The image data will be utilized to determine if digitally derived traits can replace manual measurements and to develop prediction models for several key agronomic traits. Moreover, different training populations, including inbreds and/or test crosses, will be used to predict traits of interest in four scenarios that are highly relevant to breeders. In this presentation, we will elaborate on the project concept and provide an update on our main activities in the first year, highlighting the challenges encountered so far.

Acknowledgements: This research was supported by the Science Fund of the Republic of Serbia, Grant No 6672, High-throughput field phenotyping in temperate maize hybrid breeding: how can phenomics improve speed and accuracy of selection? – PHENO_MaizE.

GRAIN YIELD PREDICTION, HIGH-THROUGHPUT PHENOTYPING, MAIZE BREEDING, UAV

06 – 02 Invited lecture

GENOMICS-ASSISTED BREEDING FOR CROP IMPROVEMENT

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Genomics-assisted breeding is a revolutionary approach to crop improvement, using advanced genomic tools and technologies to accelerate and improve the efficacy of breeding programs. Combining novel methodologies and applications of genomics with traditional breeding for crop improvement aims to address global agricultural and food security challenges. In this way, breeders can identify and select desirable traits more effectively, accelerating the development of high-yielding, disease-resistant, climate-resilient, adaptive and stable crop varieties. Technological advancement in the area of genomics and the development of genomic resources have made genomics-assisted breeding a cost-effective and time-saving method. The use of high-throughput sequencing technologies, genome-wide association studies (GWAS), genomic selection (GS) and genome editing (GE) enables the identification of genetic markers linked to agronomically important traits, facilitating marker-assisted selection (MAS), enabling breeders to predict the performance of breeding lines more accurately and offering the capacity to enhance breeding efficiency. Case studies will illustrate the practical applications of genomics-assisted breeding in cereal, legume, and oilseed species at the Institute of Field and Vegetable Crops (Novi Sad, Serbia), demonstrating benefits such as improved yield and increased resilience to biotic and abiotic stresses. Moreover, the challenges and future directions in this field, including the need for more extensive genomic resources, the integration of multi-omics data and speed breeding, and ethical considerations will be addressed.

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BREEDING, GENOMICS, MARKER-ASSISTED SELECTION, GENOMIC SELECTION, GENOME EDITING

06 – 03 Invited lecture

PREVIOUS ACHIEVEMENTS AND TRENDS IN FODDER CROPS BREEDING

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The breeding of fodder crops began in Serbia in 1930s. Together with other Serbian institutions that have made a significant contribution to the development of the entire research area, the Institute for forage crops Kruševac has been active in the process of selection and breeding of all commercially recognized forage species for decades. Over the past decades, 39 varieties of fodder species, 22 grasses, and 17 legumes were selected in Kruševac. The most common approach for creating varieties was phenotypic recurrent selection. In contrast to the traditional approach, which relies on phenotyping tools, modern breeding includes genotyping tools as well as software for data collection and processing, making the breeding process faster but more complex and challenging. The Institute currently conducts two simultaneous breeding programs of English ryegrass and alfalfa using molecular and biochemical methods. English ryegrass is selected to produce drought-tolerant variants, whereas alfalfa research is focused on developing genotypes that are resistant to the acidity of the soil. Furthermore, as members of the European research community, we conduct very complex pre breeding research on red clover, faba bean, birdsfoot trefoil, and sainfoin, in which a large number of genotypes are tested throughout Europe and the selection process is aimed at selecting genotypes that have the potential to be applied in a large geographical areas, known as the mega environments. A holistic approach to fodder species breeding, as well as the use of novel technologies, provide a promising outlook for the successful breeding of this group of plants.

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FORAGE CROPS, IKBKS, BREEDING PERSPECTIVES, GRASSES, LEGUMES

06 – 04 Invited lecture

CONTEMPORARY BREEDING OF TOMATOES

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Tomatoes represent one of the most economically significant agricultural crops at the beginning of the 21st century. The total production of tomatoes in the world is around 186 million tons, with a value of 195 billion dollars. Such a high value of production justifies high investments in tomato breeding, as well as a creation of a large number of hybrids and varieties. Conventional methods of breeding are based on the creation of new genetic variability through hybridization, phenotypic evaluation, and selection of the obtained hybrids or selection of inbred, pure lines in order to create new varieties. In breeding programs, the emphasis is placed on the yield and quality of the fruits. The application of modern methods of breeding, should improve the resistance of tomatoes to biotic and abiotic factors of the environment, especially in conditions of pronounced climate change. In recent decades, innovative breeding methods and techniques have included marker-assisted selection, induction of double haploids, application of the male sterility system in the production of hybrid seeds, and high-throughput genotyping (SNP array or genotyping-by-sequencing) in order to determine QTLs/genes and associated SNP markers. Genome editing and genomic selection technologies appear as the last step, which should provide increase of efficiency and speed up the tomato breeding process itself.

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TOMATO, BREEDING, HYBRID, CULTIVARS, WILD RELATIVES

06 – 05 Invited lecture

PERSPECTIVES OF MILK PRODUCTION IN THE REPUBLIC OF SERBIA USING MODERN METHODS OF COW SELECTION AND BREEDING

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Cow milk accounts for approximately 97% of the total milk production in the Republic of Serbia. The focus of this paper is on the milk production of Simmental cows, which are produced in the territory of central Serbia, where this breed is the most represented with over 75% of the total number of cattle, which is not surprising considering that the Herd book for this breed was established in 1935. However, the achieved results are not in accordance with the tradition and popularity of the breed. Namely, the results of the average milk production in the last 10 years for cows that are under the control of breeding organizations show a positive trend, however, the milk production is at a very low level. The average annual milk yield per cow in 2014 was only 4741 kg of milk, while in 2023 it was around 5100 kg.

The progress in milk production so far has been achieved to the greatest extent by the application of artificial insemination of cows with the semen of high-quality bulls. In recent years, sex-determined semen has been used to a greater extent. The evaluation of the breeding value of bulls in the progeny test is performed using mixed statistical models (BLUP sire model and BLUP model of the individual animal, i.e., BLUP-AM).

In order to improve the productive capacity of the herd, as well as to solve challenges such as low heritability traits, gender-limited traits, traits that are difficult to quantify, etc., it is necessary for the wide use of modern selection methods (genomic selection, MAS selection, genome sequencing, genetic modification, etc.) in the population of dairy cattle in the Republic of Serbia to become the standard.

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MILK PRODUCTION, SIMMENTAL BREED, MODERN SELECTION METHODS

06 – 06 Invited lecture

INFLUENCE OF AGRO-ECOLOGICAL CONDITIONS AND GENOTYPES ON YIELD OF EARLY POTATOES IN WESTERN SERBIA

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Agricultural producers in Serbia do not make enough use of favorable agro-ecological conditions for the production of early potatoes, which represent a good opportunity for generating profit. Early potatoes for harvesting arrive early in the spring, when there is not yet a large selection of other vegetable plants and, as a rule, they always have a good price. The agrotechnical importance of growing early potatoes is reflected in the crop rotation, because it enables two harvests a year.

The aim of the research was to determine the influence of the year and genotype on the early yield of potato tubers in the conditions of western Serbia. The experiment was carried out during 2021 and 2022, at the site of KO Bogatić. The objects of the research were three potato varieties: one early (Colomba) and two medium early (Esmee and Memphis). The field experiment was carried out as a two-factorial split-plot method in four replications.

Analysis of the productive characteristics determined that the genotype (factor G) had a highly significant ($r<0.01$) effect on the number of tubers per plant and the yield of early potato tubers, while it had a significant effect on the mean tuber weight ($r<0.05$). At variety Colomba in both years of testing was determined a significantly higher yield of early potato tubers, compared to the other two varieties. Based on the testing of three potato genotypes over a two-year period in the conditions of western Serbia and the achieved yield of early potatoes, we can recommend early variety Colomba for cultivation.

MEAN TUBER WEIGHT, EARLY POTATO

06 – 07 Invited lecture

CONSERVATION OF GENETIC RESOURCES OF HERBACEOUS PEONIES IN SERBIA THROUGH VARIOUS PROPAGATION METHODS

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Serbia is the natural habitat for four herbaceous peony species: *P. peregrina*, *P. tenuifolia*, *P. daurica*, and *P. banatica*. Although these medicinal and long-lived species are valuable, they are endangered in their natural habitats. These habitats are threatened due to climate change and significantly diminishing due to human activities. One of the reasons for their endangerment is their poor regeneration from seeds due to dormancy, low germination rates, and long germination periods (up to two years). Poor germination is also due to an underdeveloped embryo that needs to grow inside the mature seed before it can sprout. Another significant problem is the rapid loss of seed viability, which falls below 50% within the first year if not stored under optimal conditions.

To preserve genetic diversity, it is necessary to propagate peonies from seeds. The impact of various pretreatments (imbibition, GA3, temperatures) is significant in accelerating, synchronizing, and increasing germination rates. Seed germination begins with imbibition which the duration varies according to the length of the period from harvesting to sowing, and among species: 1 day for *P. tenuifolia*; 1-2 days for *P. daurica* and *P. banatica*, and 2-3 days are needed for *P. peregrina*. After imbibition, seeds need to be exposed to higher temperatures (15-23°C) for 3-6 months to break hypocotyl dormancy, during which time the root reaches a length greater than 3 cm. Following this, low temperatures (3-6°C) are necessary to break epicotyl dormancy and produce seedlings. Low temperatures are also needed to awaken winter buds in mature plants for flowering and seed production.

PAEONIA PEREGRINA; PAEONIA TENUIFOLIA; PAEONIA DAURICA; P. BANATICA; PRETREATMENTS

06 – 08 Invited lecture

CONSERVATION OF THE GENE POOL OF WOODY SPECIES IN BELGRADE'S URBAN FORESTS

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Urban forests are the basis of green infrastructure and an important resource for every city. They are composed of different forest tree species, each of which has specific genes that determine phenotypic characteristics, growth dynamics, and physiological responses to various provocations, allowing trees to adapt and survive in changing climatic conditions. All this points to the importance of genetic information, which has also received increasing attention in urban forests in recent years. The paper presents the results of research conducted in Belgrade's urban forests: "Great War Island", "Košutnjak", "Kosmaj" and "Zvezdara Forest", with the aim of identification, assessment of variability, conservation, and sustainable use of the gene pool, especially of rare and endangered tree species. On the "Great War Island" these are *Populus nigra* L., *Populus alba* L., *Salix alba* L. and *Ulmus leavis* Pall., whose populations consist of old trees in poor health without natural rejuvenation. In the "Košutnjak", "Kosmaj" and "Zvezdara Forest", a significant gene pool is represented by autochthonous oaks: *Quercus pubescens* Willd., *Quercus frainetto* Ten., *Quercus robur* L., *Quercus petraea* Liebl., and *Quercus cerris* L., which represent the remnants of former primary communities whose succession is ongoing. In addition to the oaks, there is a significant gene pool of forest fruit trees such as *Prunus avium* L., *Pyrus pyraster* (L.) Burgsd., *Sorbus domestica* L., and *Sorbus torminalis* (L.) Crantz, which are species at risk in the forest fund of the Republic of Serbia and contribute significantly to the biodiversity of these areas.

URBAN FORESTS, WOODY SPECIES, GENE POOL, CONSERVATION

06 – 09 Invited lecture

ROOT SPECIFIC GENE EXPRESSIONS CONFER RESISTANCE TO SOIL-BORNE BYMOVIRUS IN WHEAT AND BARLEY

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Wheat yellow mosaic virus is a pathogen transmitted into its host's roots. *Ym1* and *Ym2* genes protect the host from the significant yield losses caused by the virus, but the mechanistic basis of these resistance genes remains poorly understood. Here, it has been shown that *Ym1* and *Ym2* complementary act within the root to perfectly protect the wheat from the virus. To understand the basis of the root specificity of the *Ym2* product, the gene was isolated from bread wheat. The *Ym2* gene (chr. 3B) encodes a NLR protein and it correlated allelic variation with respect to its sequence with the host's disease response. *Ym2* was found to be present in several accessions of *Aegilops sharonensis*. the near-relatives of *Aegilops speltoides* (a close relative of the donor of bread wheat's B genome). Breeding of a novel wheat breeding line expressing both *Ym1* and *Ym2* showed perfect resistance to the virus without any yield penalty. Similar story by barley *rym* genes conferring resistance to Barley yellow mosaic virus at the root system will be reported.

VIRUS RESISTANCE, ROOT-ORIENTED RESISTANCE, NLR, GENE INTROGRESSION, EVOLUTION

06 – 10 Oral

MOLECULAR IDENTIFICATION OF *FUSARIUM GRAMINEARUM* PATHOGEN OF SMALL GRAINS AND MAIZE IN SERBIA

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Fusarium head blight (FHB) ranks among the most pervasive and economically damaging diseases affecting cereals globally. The intensified occurrence of fusariosis on wheat spikes has resulted in significant economic losses worldwide. Beyond economic impacts, FHB is also linked to mycotoxin contamination in maize kernels. The primary causative agent of FHB is *Fusarium graminearum* Schwabe, initially recognized as a single species but now classified within the *Fusarium graminearum* species complex (FGSC). The FGSC is genetically diverse, comprising at least 15 phylogenetically distinct species, including the traditional *F. graminearum*. The aim of this study was to make a molecular identification of *F. graminearum* species. In this research, 8 isolates were selected from the collection of fungi originating from grains of wheat, barley and maize from different localities in Serbia. Total DNA was extracted from mycelium collected from 7-day-old colonies of single-spore isolates grown on PDA using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Identification procedures involved amplification and bidirectional sequencing of transcription elongation factor (*TEF-1α)*, histone H3, and β-tubulin using the primers ef1/ef2, H3-1a/H3-1b, and T1/T22, respectively. The obtained sequences were deposited in GenBank under accession numbers MF974400–MF974408 (*TEF-1α*), MG063784–MG063792 (β-tubulin), and MF999140–MF999148 (histone H3). Sequence analysis was conducted using BLAST, while genetic similarity was assessed using MEGA 6.0 software. Tested isolates shared 99-100% nucleotide identity with *TEF-1α*, histone H3 and β-tubulin compared to most isolates of *F. graminearum* available in the gene bank.

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FUSARIUM HEAD BLIGHT, *FUSARIUM GRAMINEARUM*, *TEF-1A*, HISTONE H3, B-TUBULIN TEF-1

06 – 11 Oral

GLIADIN ALLELE COMPOSITION IN WHEAT GENOTYPE (*TRITICUM AESTIVUM* L.)

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The six gene loci with multiple alleles control the inheritance of gliadin proteins, which are deposited in seed endosperm of wheat. Gliadins have share 30-40% of total fluor proteins. Gliadinas are monomeric molecules of proteins, globular conformation, soluble in 70% ethyl alcohol. The aim of this study is identification of gene allele composition encoding gliadin in wheat genotypes. For study used 10 wheat genotypes and 30 seeds from each genotype were used for the extraction of gliadin by 70% ethanol. The gliadins were separated by acid PAGE electrophoresis (pH=3.1) on 8.33% polyacrylamide gel. Electrophoregrams were used for determining Gli-1 and Gli-2 alleles. The four alleles (a, b, f, m) at the Gli-A1, four alleles (a, b, d, l) at the Gli-B1, three alleles (a, b, k) at the Gli-D1, five alleles (a, b, e, f, g) at the Gli-A2, four alleles (a, b, h, m) at the Gli-B2 and five alleles (a, b, e, k, m) at the Gli-D2 locus were identified. In ten wheat genotypes, at the six gliadin loci were identified 25 different alleles. Identified alleles at each Gli-1, Gli-2 and Glu-1 loci indicate high polymorphisms of gliadin proteins and genetic divergences of analyzed wheat genotypes.

WHEAT, GLIADIN, GENE, ALLELE, GENOTYPE

06 – 12 Oral

GENES INVOLVED IN COLD RESPONSE IN EARLY DEVELOPMENTAL STAGES OF MAIZE

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Ensuring satisfactory yield and crop quality despite poor environmental conditions resulting from climate change is one of the most important goals in modern agriculture. One strategy entails sowing maize in early spring, as it enables the avoidance of drought and high temperatures during the most sensitive development stages (flowering, grain filling). However, this approach results in exposure of maize plants to suboptimal temperatures during early development, necessitating an understanding of their response and adaptation to cold conditions in these stages. The strategy of whole transcriptome sequencing (WTS) was employed using two maize inbred lines – one tolerant to cold, LT and one susceptible, LS, at the five-day-old seedling stage. RNA extraction and sequencing were performed after 6h and 24h of cold treatment (10/8° C). The same was done for control seedlings (25°/20°C). Raw data acquired was then subjected to filtering and quality checks (FastQC; trimmomatic), alignment to the reference genome (STAR), gene expression quantification (HTSeq), differential expression (DE) profiling (edgeR), hierarchical clustering, and enrichment analysis (GO – clusterprofiler, BLAST). RNAseq results show that there is a significant difference in expression between the cold and control conditions in more than 500 genes, at one of the time points. Many of these genes were enriched for abiotic stimulus response (*mbfc1, THI1, per, hsp*). Still, the results show that the maize genotype doesn't seem to have much effect on the response to cold conditions.

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MAIZE, COLD STRESS, RNASEQ, FIVE-DAY-OLD SEEDLINGS, GENE EXPRESSION

06 – 13 Oral

EXPRESSION ANALYSES OF DROUGHT RELATED GENES IN LEAVES AND STEMS IN TWO WILD CHERRY CLONES

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Wild cherry (*Prunus avium* L.) is a tree species that has significance in economics, agroforestry, and the ecology of one country. Revealing biodiversity and the preservation of this species play an important role in scientific research. This species grows in deep, moist soils, so drought notably affects its yield and quality. The effects of drought induced genes by simulated via osmotic stress induced by polyethylene glycol (PEG) in the growing medium were examined on two Wild cherry (*Prunus avium* L.) clones (6A and 8A). The shoot tips were exposed to two PEG concentrations (20 and 50 g L-1) in a growing medium designed for micropropagation with axillary buds. A transcriptomic approach has been used for stress-related genes identification that reveals the different molecular mechanisms of drought stress. We used quantitative real-time PCR (qRT-PCR) to evaluate and confirm DEGs of selected 5 genes (*PaDHQ-SDH*, *PaChlorophyllase*, *PaGRAS17*, *PaP5CS*, *PaDREB2*) that showed strongly divergent expression patterns in leaves and roots that were subjected to drought stress. Besides ubiquitin, β-tubulin and β-actin were used as reference genes to verify the expression results, where β-actin showed the best efficiency. qRT-PCR reactions were performed with triple biological replicates and triple technical replicates. The relative expression of each gene was calculated by the 2-△△Ct formula. Our study provided theoretical support for different molecular mechanisms of drought resistance and differential responses of drought related genes in leaves and stems in two Wild cherry clones.

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PRUNUS AVIUM, DROUGHT RELATED GENES, QRT-PCR, TISSUE CULTURE

06 – 14 Oral

ASSESSMENT OF HULLESS WINTER BARLEY IN THE SERBIAN CLIMATE

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As global consumer trends shift towards healthier and more sustainable food choices, hulless barley's nutritional benefits could align well with these needs. Currently, the production of hulless barley in Europe and in Serbia does not exceed 5% of the total production of barley. Therefore, the breeding of hulless barley has a great potential and represents a promising pathway for agricultural innovation. The aim of this study was to explore genetic diversity present in European gene banks accessions which could be valuable tool for future hulless barley breeding programs. The experimental trial was conducted at Rimski šančevi with 72 winter genotypes using two standards (RGT Planet and Maltesse varieties) set up as an augmented block design. The sowing time was on November 2, 2023, on the 1 m² microplots with 300 viable seeds. During the growing season, the following traits were recorded: emergence date, canopy cover, heading time, lodging before and after flowering, stem height, and spike length. The monitoring of crop growth was estimated by percentage of canopy coverage using Canopeo application during the three most important developmental phases and ranged from 0.75 to 5.03 (in GS11), 35.42 to 85.48 (in GS31) and 49.56 to 93.51 (in GS 59). The earliest genotype (accession 56) started heading on April 1, while the latest was on May 9 (accession 69). Lodging was more expressed after flowering, with only two standards and one genotype (accession 14) showing no lodging at all. The initial findings of Cropdiva`s project indicate that the selected genotypes panel exhibits substantial diversity, forming a strong basis for advancing hull-less barley breeding programs.

NAKED BARLEY, GENETIC DIVERSITY, PHENOTYPING

06 – 15 Oral

BACTERIAL TREATMENT ENHANCES THE GROWTH OF ONE-YEAR-OLD SESSILE OAK (*QUERCUS PETRAEA* (MATT.) LIEBL) SEEDLINGS OF TWO SERBIAN PROVENANCES

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The root rhizosphere is one of the densest habitats on the planet, where significant intimate interactions between plants and their recruited rhizomicrobiota are implemented. Plant genetics impacts community composition. However, this communication is a two-way process regarding microorganisms modulating plants's physiological processes as well, which are eventually manifested in plant vigor, growth, and development.

One-year-old sessile oak seedlings of provenances Đerdap and Goč were treated with *Bacillus* sp. (treatment 1) and *Pseudomonas* sp. (treatment 2) in order to study their effect on the enhancement of seedling growth. Sessile oak seedlings were monitored for one growing season in semi-controlled conditions in the nursery of the Institute of Forestry. At the end of the experiment, seedling height and root collar diameter were measured by a ruler and Vernier's caliper as the standard morphological parameters of seedling growth. The obtained data were analyzed statistically in GraphPad Prism, and ANOVA and Dunnett's test were applied. The results indicate the enhancement of seedlings' growth treated with bacteria. Seedlings of Đerdap provenance treated with treatment 1 were 35% higher, and the ones treated with treatment 2 were 32% higher compared to the control. Seedlings of Goč provenance treated with treatment 1 were 85% higher than control. The effect of bacterial treatments on seedling root collar diameter of both provenances was not statistically significant, although it enhanced their mean values.

The obtained results indicate the potential of bacterial treatments to increase seedling growth, which should be researched comprehensively in the future, especially in the context of biological fertilizer commercialization.

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BACTERIA, SEEDLINGS, SESSILE OAK, PROVENANCES

06 – 16 Oral

CUTTING-EDGE BREEDING TOOLS FOR SOYBEAN YIELD PREDICTION

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Soybean is a crop of major economic and environmental importance. Due to its broad importance, there is a need for developing long-term breeding strategies based on state-of-the-art technologies that involve breeding predictive tools. Genomic prediction (GP), high-throughput phenotyping prediction (HTPP), and Fourier transformed near-infrared reflectance spectroscopy (FT-NIRS) are selected as the three main pillars for predictive models because they reflect certain parts of soybean plant biology. Single-nucleotide polymorphism (SNP) data describes molecular differences between genotypes, while NIR spectra can be considered as surrogates for overall tissue chemical composition and reflect the culmination of physiological processes, and HTPP can detect subtle differences in canopy growth and developmental changes. Implementation of these prediction models through their compilation has the potential for application in a cost-effective manner. Estimating the accuracy and effectiveness of each specific model and describing their interaction is an essential step in understanding the complicated relationships between yield predictors. For that purpose, two sets of field trials are established to simulate the real breeding evaluation process (early and late-stage panels). For prediction model compilations, several strategies are used. The first strategy treats all data sets equally, while the second and third approaches take into account data set hierarchy and the application of different algorithms. Using all the strategies for combining prediction models should provide an answer to whether it is possible to improve the precision and accuracy of soybean phenotype prediction within and between environments as a key indicator for increasing genetic gain.

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PREDICTION TOOLS, YIELD, SOYBEAN

06 – 17 Poster

OPTIMIZING NON-FOOD CROPS THROUGH BREEDING AND APPLIED AGRONOMY PRACTICE FOR CULTIVATION ON MARGINAL LANDS

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This study aims to optimize non-edible crops for cultivation on marginal lands. Modern biotechnology tools are combined with tailored agronomic practices to achieve this goal, focusing on lowinputs and efficient water use. To increase resource use efficiency, the following activities are designed: a) identifying optimized, resource-efficient varieties for marginal lands, b) developing modern biotechnology tools for industrial crops, and c) implementing tailored agronomic practices. Advanced breeding material for specific non-edible industrial crops has been developed in breeding programs through EU research projects (GRACE, MAGIC, OPTIMISC, MultiHemp, SUNLIBB, and FIBRA) and national/multinational projects. Several advanced high yielding genotypes with increased tolerance to different abiotic factors have been evaluated in order to be registered as new varieties/hybrids suitable for marginal lands with low inputs (water / fertilizers). The following crops: hemp, miscanthus, crambe, and castor bean were grown on multi location trials in Greece, Italy, Spain, and Serbia as part of MIDAS project. Optimal agricultural practices (improved varieties/hybrids, different irrigation and fertilization rates, and/or plant densities) were applied in order to evaluate the phenology, biometric and agronomic performances, as well as yield quantity and quality. Advanced breeding tools, including marker assisted selection – MAS, genome editing, and speed breeding, were used to further develop elite material. These advanced tools help produce improved varieties and hybrids to meet the future needs of agricultural production.

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BREEDING, INDUSTRIAL CROPS, MARGINAL LANDS

06 – 18 Poster

EVALUATION OF GENOTYPE × ENVIRONMENT INTERACTION IN MAIZE BASED ON AMMI MODEL

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Grain yield expression is influenced by the genotype (G), the environment (E), as well as the genotype by environment interaction (GEI) which may adversely affect its stability. Hence, in three-year field experiment carried out at Zemun Polje, fifteen maize inbred lines (G1–G15) of FAO 300-700 maturity groups were analyzed by AMMI model, used to identify high yielding and stable maize genotypes. Lines were sown in two plant densities (PD1-30cm and PD2-40cm) and two sowing dates (ten-day interval between each sowing), in two replications, according to Complete Randomized Block Design. Analysis of variance of AMMI-1 calculated per sowing date (R1 and R2) showed that all sources of variation (genotype, treatment – plant density, year and their interaction) significantly affected the grain yield ($p<0.01$). In the R1, it has been shown that the factor Year exhibited the most pronounced effect on grain yield expression (37.78%), while in R2, the most significant effect expressed the factor Genotype (49.63%). The AMMI biplot enabled the identification of maize inbreds with the most pronounced yielding performance in terms of high grain yield: G5 inbred (R1, PD2; i.e. R2, PD1), G7 line (R2, PD2), as well as G8 line (R1, PD2). According to AMMI biplot, the following inbred lines exhibited the best performing yield stability: G4 line (R1, PD2), G5 inbred (R1, PD1/PD2; i.e. R2, PD1), G6 line (R2, PD1) and G7 line (R2, PD2). The obtained results revealed that mid-early G5 and G7 lines could be considered as suitable for growing across wide range of environments.

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GRAIN YIELD, PLANT DENSITY, SOWING DATE, YIELD STABILITY, *ZEA MAYS* L.

06 – 19 Poster

PRELIMINARY STUDY ON CHLOROPHYLL CONTENT DIFFERENCES IN NS SUNFLOWER HYBRIDS AND THEIR IMPACT ON SEED YIELD AND OIL CONTENT

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Chlorophyll content indicates plant health, photosynthetic efficiency, and nutrient status. High chlorophyll contents often correlate with vigorous growth and higher yields, while changes can signal environmental stresses like drought. During drought, chlorophyll content can decrease, reflecting impaired photosynthesis and nutrient uptake, making it a valuable indicator of drought tolerance. This study examines the variations in chlorophyll content across twenty different NS sunflower hybrids and its correlation with seed yield and oil content. Chlorophyll measurements were conducted at four growth stages, from bud to flowering phases within a month during 2022. Each hybrid exposed unique chlorophyll dynamics, reflecting their genetic diversity and adaptability to varying environmental conditions. Statistical analysis revealed no significant correlations between seed yield and chlorophyll content in any of the four growth stages measured. However, negative correlations between oil and chlorophyll content were obtained at all four growth stages, but statistical significance was obtained at the end of budding ($r = -0,443^{*}$) and the beginning of the flowering phase ($r = -0,433^{*}$). In conclusion, the lack of significant correlations between tested traits and chlorophyll content suggests that other factors as well as wider variability of sunflower genotypes is essential.

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SUNFLOWER, CHLOROPHYLL CONTENT, SEED YIELD, OIL CONTENT

06 – 20 Poster

GENETIC EVALUATION IN CAROTENOID CONTENT IN KERNELS OF OPEN-POLLINATED MAIZE VARIETIES

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One of the primary objectives of breeding is to enhance the composition of maize grain. Maize grain is abundant in a diverse range of carotenoids, which are natural pigments for the vibrant yellow, orange, and red tones. These carotenoids provide visual appeal and contribute to the nutritional quality of maize grain and protect the plant from oxidative stress. This work aimed to evaluate the whole kernel carotenoid content of 34 open-pollinated maize varieties with yellow kernels and different types of kernel hardness. High-Performance Liquid Chromatography (HPLC) was employed to quantify and determine the levels of lutein, zeaxanthin ß-carotene within analyzed maize varieties. The detected ranges for lutein were from 1.11 to 13.48 µg g−1, for zeaxanthin from 1.12 to 12.6 µg g−1 and from 0.15 to 2.97 µg g−1 for ß-carotene. The highest value of lutein and ß-carotene was observed in the landrace with yellow dent/semi-dent kernels, while the highest zeaxanthin was measured in the landrace with yellow flint kernels. On the contrary, the lowest value of lutein was observed in the genotype with orange flint kernels, while yellow semi-flint and yellow semi-dent had the lowest value of zeaxanthin and ß-carotene, respectively. The correlation between kernel color and hardness with carotenoid contents did not show the expected strength, suggesting a need for further investigation. Specifically, the variations in carotenoid content among these open-pollinated maize varieties make them especially promising for utilization in further maize breeding improvement programs.

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ZEA MAYS L, HPLC, CAROTENOIDS

06 – 21 Poster

EVALUATION OF MAIZE SILK (*ZEA MAYS* L.) MATURITY STAGE WITH BEST BIOACTIVE PERFORMANCE FOR APPLICATION IN PHARMACY

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Historically, people have consumed maize silk (*Stigma maydis*) as an herbal remedy for a wide range of ailments due to its antioxidant activity, antibacterial, hypoglycemic, anti-cancer, anti-diabetic, anti-adipose, and anti-obesity properties. The yellow, green, pink, and purple-colored silks from four maize genotypes (ZP Exp, ZP 555, ZP 341, and ZP 366) created and grown at the Maize Research Institute Zemun Polje were harvested 5-7 (silking stage, R1) and 25–27 (dough stage, R4) days after emergence. The samples were analyzed for the content of total phenolics, phenolic acids, flavonoids, anthocyanins, proanthocyanidins, macro-mineral elements, and total antioxidant capacity. The phenolic profile was determined on the HPLC system, and spectrophotometric methods were used in the analyses as well. The content of all detected bioactive components was significantly higher in the silking R1 stage compared to the R4 maturity stage, which qualifies these silk extracts as more appropriate for application as a natural source of bioactive compounds. The most prevalent phenolic compounds were 3-O-caffeoylquinic acid and maysin, respectively. The silk extracts obtained from the ZP 341 genotype (R1 stage) contained the highest total phenolic content (10,160.8 mg CGAE/100 g) as well as total flavonoids (6,573.8 mg CE/100 g). The health-promoting properties of maize silk can be attributed to considerable levels of potassium and antioxidant capacity. The findings of this study indicate a potential for the development and advancement of novel pharmaceutical products.

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CORN GENOTYPES, MAIZE SILK, BIOACTIVE COMPOUNDS, ANTIOXIDANTS, PHARMACEUTICAL APPLICATION

06 – 22 Poster

FENOTYPIC VARIABILITY OF STEM HEIGHT IN SERBIAN WHEAT GENOTYPES

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The stem height is biological trait in wheat which is significantly in correlation with lodging and yield. The aim of this study was to identify diversity and similarity for wheat varieties according to stem height variation in 50 genetically divergent Serbian wheat varieties. The experiment was set up as a randomized block design in three replications in two vegetation seasons. Sowing was done manually, by laying the seeds at a distance of 0.1 m in rows 1.0 m long, with a space of 0.20 m between rows, in order to enable the examined plants to fully manifest their traits. For all 50 wheat genotypes stem height variate in range from 41.24 cm in Rana niska to 75.48 cm in Kragujevačka 75 variety, with average of 55.83 cm. In the second vegetation season stem height varied in, 60 plants (20 plants per replicate) were harvested in the full maturity phenology phase for analysis of stem height. The results showed differences among wheat varieties for stem height in both vegetation season and between seasons. In the first vegetation season stem height range from 60,45 cm in Rana niska to 107.75 cm in Kragujevačka 75, with average of 66.61 cm for all 50 varieties. On average the value of stem height in all analysed wheat varieties, was higher in second year than in the first year. Expressed differences of stem height between varieties within both season and between seasons are due to impact of different genetic, and environmental factors.

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WHEAT, GENOTYPE, VARIABILITY, HEIGHT OF STEM.

06 – 23 Poster

MYCOTOXIGENIC *FUSARIUM FUJIKUROI* SPECIES COMPLEX PATHOGENS ON SMALL GRAIN IN SERBIA

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Due to climate changes occurrence of *Fusarium fujikuroi* species complex (FFSC) on small grain has been observed in the last decade in higher frequency. A significant aspect of the FFSC is their ability to synthesize mycotoxins which can have a harmful effect on the health of people and domestic animals, which is reflected in the manifestation of carcinogenic, teratogenic, neurotoxic and other toxic effects. The aim of this study was to examine the virulence of FFSC isolates and their ability to synthesis fumonisins. Total of 50 FFSC isolates originating from 17 localities in Serbia and isolated from 4 plant hosts (wheat, durum wheat, barley and triticale) were collected from MRI Collection of fungi. The virulence of the isolates was tested on durum wheat spikes in a two-year study. The concentrations of synthesized fumonisins were tested using the ELISA method.

Significant difference was recorded between the two years of research, in the sense that in the second year all isolates were more virulent. Regarding the influence of the species on virulence, it was shown that the species *F. verticillioides* is more virulent than the species *F. proliferatum* and *F. subglutinans*, which are equally virulent in both years. The results of the fumonisin synthesis potential indicated the presence of fumonisin in all isolates of the species *F. verticillioides* and *F. proliferatum* in high concentrations. Fumonisin synthesis was recorded only in some isolates of the species *F. subglutinans* while in the other isolates it was not detected.

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FUSARIUM FUJIKUROI SPECIES COMPLEX, MYCOTOXINS, FUMONISINS

06 – 24 Poster

GRAIN QUALITY OF LOCAL FABA BEAN POPULATIONS COLLECTED IN SERBIA

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Faba bean (*Vicia faba* L.) is one of the first legumes to be domesticated in the Near East and is still widely used in the region's culinary traditions. In Serbian agriculture it is not a very important crop, there are only two varieties recognized for animal feed. In the future, faba bean may play a significant role in agriculture due to their ability to grow in climatic circumstances that are less favorable. Furthermore, a large number of research studies suggest that this species has a very high concentration of bioactive compounds. Fifty distinct faba bean populations whose seeds were collected across Serbia were the subject of research. Most of accessions had large seeds (major type), whereas fewer accessions included medium or smaller type seeds.

Dry matter content, crude protein content, crude fat content, mineral content, and crude cellulose content were all evaluated. Our results indicate that there is a great deal of variability in the material under study, and the high protein values are particularly significant. More than 27% of the crude protein content was found in all accessions. These results indicate that faba beans can be classified as high-protein plant, as more than 20 populations had a protein content higher than 29%. This information will be a valuable resource in the future selection process and will be used to enhance the present range of faba bean varieties available in Serbia. Future studies on the bioactive substances in these local populations would be the next phase.

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FABA BEAN, QUALITY, PROTEIN CONTENT, DRY MATTER CONTENT, NUTRITION

06 – 25 Poster

BREEDING HIGH-YIELDING FODDER PUMPKIN VARIETIES SUITABLE FOR PURE CROPPING

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Traditionally, people have grown fodder pumpkin (*Cucurbita pepo* L.) as an intercropped plant alongside maize, providing an excellent nutritional resource for livestock feed. Over the last 50 years, with the predominant use of herbicides in maize cultivation, this type of production has been compromised, leading to the cultivation of pumpkins as a pure crop. The Forage Crops Institute in Kruševac, in collaboration with the Faculty of Agronomy in Čačak, has initiated the collection of domesticated genotypes of fodder pumpkin from the territory of Serbia. The goal is twofold: the first direction is selection based on the oil content in the seeds, and the second is the favorable yield of flesh and seeds intended for livestock feed. This research is conducted at the Forage Crops Institute in Kruševac. The diversity of 21 collected genotypes has been examined, and significant differences were found between the genotypes regarding the analyzed morphological traits (fruit mass, number of fruits per plant, ratio of pericarp mass to seed cavity mass, etc.). The determined difference at the level of $P \leq 0.05$ indicates significant genetic variability between the genotypes. According to the morphological traits, the genotypes were divided into 5 clusters, with genotypes from Vojvodina (Nova Crnja) - G18 and Golija (Ivanjica) - G2 standing out. The breeding material, selected using the pedigree method, is currently in the F3 generation, and lines have been formed that are promising for obtaining a variety with high nutritional potential and suitable for pure cropping based on their morphological traits.

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CUCURBITA PEPO L, COLLECTED GENOTYPES, CLUSTER ANALYSIS, PEDIGREE SELECTION

06 – 26 Poster

VARIABILITY OF SUGAR CONTENT IN SWEET CORN (*ZEA MAYS* L. SACCHARATA)

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Sweet corn is a maize variant with a natural recessive mutation in genes governing sugar-to-starch conversion within the endosperm of the maize kernel, which provides it with elevated sugar content. It is cultivated as a vegetable for human consumption, both fresh and processed, primarily through canning and freezing. It is harvested and consumed in the milky stage of endosperm development, due to the high sugar content before transitioning into starch. High concentration of simple sugars such as glucose and fructose and disaccharide sucrose provides characteristic sweet flavor of sweet corn. In this paper we analyzed the sugar content in 15 experimental sweet corn hybrids. Hybrids were sown in three different sowing dates. The content of sucrose as the dominant sugar was the highest and amounted from 69.96 – 76.49% of the total sugars. The content of glucose ranged from 10.50 – 10.84%, while the content of fructose was from 2.44 – 3.34%, out of total sugar content. The content of sucrose was the highest in first sowing date, while the content of other two was higher in the second and the third. Significant differences were found among analyzed hybrids and between sowing dates. This indicates that sugar content in sweet corn is both genotypic and environmental influenced.

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SWEET CORN, SUGAR CONTENT, SUCROSE, FRUCTOSE, GLUCOSE

06 – 27 Poster

GENETIC VARIABILITY OF SWEET MAIZE KERNEL PHYTOCHEMICALS

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Sweet maize (*Zea mays* L. saccharata) is widely used in human nutrition due to its sweetness and aroma. The increased demand and production of sweet maize require more attention for profiling the nutritional quality of the kernel. This study aimed to evaluate the phytochemicals content (carotenoids, vitamin E, free phenolic acids, and soluble sugars) in the kernel of four sweet maize hybrids. Linear regression analysis was used to determine the correlation between the maize yield and phytochemicals content. Obtained results showed the positive correlation between yield and total vitamin E content in hybrids ZP1 ($r = 0.73$; $p \leq 0.05$), ZP2 ($r = 0.89$; $p \leq 0.05$), and ZP3 ($r = 0.82$; $p \leq 0.05$). A negative correlation between the yield and free phenolic acids content was recorded in hybrids ZP2 ($r = -0.82$; $p \leq 0.05$) and ZP3 ($r = -0.85$; $P \leq 0.01$), while a positive correlation was achieved in hybrid ZP4 ($r = 0.72$; $p \leq 0.05$). A negative correlation between the yield and β- carotene was achieved in hybrid ZP1 ($r = -0.71$; $p \leq 0.05$). Likewise, a negative correlation between yield and total soluble sugars content was observed in hybrid ZP4 ($r = -0.89$; $p \leq 0.05$). This study's results indicate the importance of the linkage between yield, content of bioactive compounds, and genetic background/variability of sweet maize hybrids. Although a relatively small number of hybrids was examined, the obtained data could be useful for developing new high-yielding hybrids with increased content of health-benefit compounds and promoting sweet maize in terms of functional food.

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SWEET CORN, SUGAR CONTENT, SUCROSE, FRUCTOSE, GLUCOSE

06 – 28 Poster

DIFFERENTIAL GENE EXPRESSION IN 5-DAY-OLD MAIZE SEEDLINGS UNDER WATERLOGGING STRESS

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It has been predicted that plant breeding will focus on developing heat-resistant, drought-tolerant, and flood-resistant crops to ensure food security under climate challenges. The on-going climate change can cause sudden heavy rainfalls leading to waterlogging (flooded/ponded/saturated soils), which negatively influences crop growth. The consequence of waterlogging in crop plants is oxygen deficiency (hypoxia) or complete absence (anoxia), which inhibits root respiration, rate of photosynthesis and CO_2 assimilation. Germinating seeds/emerging seedlings are very sensitive to waterlogging, as their level of metabolism is high. Herein, one sensitive and one tolerant maize inbred line grown on Knop nutritive solution were submitted to waterlogging stress by keeping the 5-day-old seedlings 2 cm below the solution surface for 24h, 72h and seven days. RT-qPCR analysis was conducted on the control (optimal saturation) and treated samples in order to determine differential expression of *Zmpdc1* and *Zmpdc3* (involved in metabolism maintenance under anaerobic conditions), *XET* (involved in root aerenchyma development), *ATPSchl* (involved in sulfur metabolism), *FS2chl* and *FS2cyBa* (involved in PSII function) genes. Student's t-test analysis revealed statistically significant differences between the sensitive and the tolerant genotype for *FS2cyBA* after 24 h, *XET* after 72h and seven days, *Zmpdc1* for all three time points and *Zmpdc3* after 24h and 72h, indicating their involvement in waterlogging tolerance. Further on, more in-depth studies will be conducted in order to reveal molecular and genetic data favorable for breeding tolerant maize genotypes.

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DIFFERENTIAL EXPRESSION, GENES, MAIZE, WATERLOGGING

06 – 29 Poster

ADVANTAGES OF MARKER ASSISTED SELECTION IN PLANT BREEDING

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Combined with traditional selection techniques, marker assisted selection (MAS) has become a valuable tool in selection for the traits of interest. In MAS, the results of DNA testing are used to select parents for the next generations. Marker analysis can be done using DNA extracted from leaf tissue of very young plants to identify individuals with desirable alleles early in the breeding cycle. This allows breeders to discard plants without these alleles prior to pollination, reducing the size of the breeding population. The main advantages of MAS are direct selection of target gene with gene-specific markers (foreground selection) and fast recovery of recurrent parent's genome (background selection). Using co-dominant markers allows effective selection of recessive alleles, so no selfing or test crossing is needed to detect the traits controlled by recessive alleles. Both foreground and background selection decrease the number of generations required to create desirable genotype, saving time and accelerating breeding progress. Furthermore, MAS is not affected by environment, which is very helpful for improvement of traits expressed only in favorable conditions, as well as for low-heritability traits that are easily affected by environment. For the traits controlled by multiple genes, individual genes can be selected at the same time and in the same individuals, and thus MAS is particularly suitable for gene pyramiding. At the Maize Research Institute Zemun Polje, the use of integrated conventional and molecular breeding approach aimed at maize quality improvement led to development of high-yielding hybrids with improved nutritional benefit adapted to temperate climate.

MARKER ASSISTED SELECTION, PLANT BREEDING, ADVANTAGES, MOLECULAR MARKERS, MAIZE QUALITY IMPROVEMENT

06 – 30 Poster

VARIABILITY OF CARBOHYDRATE COMPOSITION IN SMALL GRAIN CEREAL GENOTYPES AS A PRECURSOR FOR NEO-FORMED CONTAMINANTS

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Cereal-based products have always been among the most important sources of energy in human nutrition. These products are created using common technological processes that positively impact the nutritional content, texture, flavor, and color development of the end product. However, technological processes are mostly accompanied by a thermal treatment that can result in the formation of processing contaminants such as acrylamide, which may have negative health concerns. In order to determine genetic resources with reduced potential for acrylamide formation, the content of sugars, as reactants of the Maillard reaction, was analysed in different small-grain cereal species. The experimental material consisted of two bread wheat (*Triticum aestivum* L.), two rye (*Secale cereale* L.), and two spelt wheat (*Triticum spelta* L.) genotypes grown in the field at the Maize Research Institute, Belgrade, Serbia, in the 2023 growing season. The wheat genotype Belija had the highest glucose content (0.88%) compared to those of the wheat genotype Savo (0.55%), as well as the rye and spelt genotypes (mean 0.61% and 0.56%, respectively). The fructose content ranged from 0.26 to 0.55, 0.37 to 0.39, and 0.27 to 0.37% in wheat, rye, and spelt samples, respectively. The mean maltose content in the wheat varieties amounted to 1.02%, which was about 45% and 34% higher than that found in the rye and spelt samples. Since then, the ability to reduce sugars to form acrylamide with amino acids has decreased in the following order: glucose > fructose > sucrose > maltose. It could be expected that the spelt genotype Ostro will have a lower potential for acrylamide formation.

CARBOHYDRATES, SMALL GRAIN CEREAL GENOTYPES, VARIABILITY, MAILLARD REACTION, NEO-CONTAMINANTS

06 – 31 Poster

ORGANIC SOYBEAN BREEDING: PARTICIPATORY PLANT BREEDING & FARMER PARTICIPATORY TRIALS

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Participatory Plant Breeding (PPB) is the plant breeding approach that actively involves farmers in the process of selection and breeding of different plant species. Farmer participatory trials (FPTs) are the practical and valuable tool for increasing the introduction of new and improved soybean varieties and space for screening of plant material. Key principles of PPB are genetic diversity, local adaptation, and farmer active participation. Application of this method in practice in organic production has multiple benefits. First, enable to be in line with local environmental conditions and farmer needs. Second, selecting and breeding soybean varieties with identified traits of interest. Third, establish field trials directly with organic farmers. Finally, evaluate the material and collect data prior to making selection decisions. Within FPTs under the framework of PPB, testing of cross-composite populations (CCPs) and varieties was the first step in the direct involvement of organic farmers in the Republic of Serbia. CCPs and up to 10 soybean varieties were tested in up to 5 locations during a two-year period in Serbia (2021 and 2022). Results of grain yield showed big variations among varieties (1-4.5 t/ha), but they were comparable with varieties already produced at farm site. Farmers positively reacted to FPT approach, which was useful for selection of soybean varieties according to the farmers needs.

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SOYBEAN, ORGANIC, CROSS COMPOSITE POPULATIONS

06 – 32 Poster

GENETIC ARCHITECTURE OF SOYBEAN TRAITS FOR ORGANIC PRODUCTION

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Growing demand for varieties appropriate for organic production to better meet the specific needs of value chain participants stimulated the development of targeted soybean breeding programs for organic farming. Important traits for organic soybean production were identified, including yield, thousand seed weight, canopy cover in vegetative and reproductive phases, grain quality (protein and oil content), plant architecture (average internode length, node number, plant height, number of branches) and local adaptation (flowering time, full maturity, reproductive stage length). These traits are complex quantitative traits affected by multiple genes and environmental factors. While a large number of main quantitative trait nucleotides (QTNs) have been reported, less information on QTN-by-environment interactions (QEIs) and epistasis QTN-QTN interaction (QQIs) is available for selected traits. Mapping without QEIs and QQIs result in missing data that are significantly related to phenotypic variation. The present research conducted genome-wide association studies (GWAS) to map genetic basis for selected traits using panel of 182 diverse soybean lines tested in two independent environments (Serbia and Austria), in two years 2020 and 2021. 3VmrMLM method was used to conduct association analysis on 34352 SNPs and phenotypic data. Significant and stable main-effect QTNs, QEIs, and pairs of QQIs were detected, indicating an important role of non-additive effects on all traits. Some of detected QTNs co-localized with previously known loci, while others were novel. Obtained results provide a valuable genomic resources in developing organic-compliant varieties by targeting specific traits and preserving organic standards, providing more efficient and sustainable strategies for organic production.

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GWAS, SNP, MAS, EPISTASIS, QUANTITATIVE TRAITS

06 – 33 Poster

ANALYSIS OF INBRED LINES DERIVED FROM TWO SYNTHETIC POPULATIONS

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In this paper we analyzed inbred lines and their hybrid combinations, heterosis, general and specific combining abilities. Analyzed inbred lines were derived from different cycles of selection of two synthetic populations BSSS and BSCB1. The highest average estimate for number of kernel rows expressed hybrid combination B73 x B84 (16.40), while the lowest was for ZPL2 x B99 (13.53). Considering the GCA/SCA for synthetic BSSS and the parents of elite hybrid being above 1, it could be concluded that additive gene effect is the most significant in the inheritance of kernel row number. The expression of this trait in synthetic population BSCB1, and inbred lines is under the dominant gene effect considering the GCA/SCA ratio below 1. Inbreds B73(C5) and B84(C7) from BSSS synthetic population had significant positive GCA values in both examined years and locations, while from the BSCB1, significant positive values had B90(C7) and B97(C9). Significant positive values of SCA, as well as the highest heterosis expressed hybrid combination B14 x B37 from the BSSS, and the hybrid combination B91 x B97 from the BSCB1 had high and significant SCA.

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MAIZE, INBRED LINES, COMBINING ABILITY, HETEROSIS, KERNEL ROW NUMBER

06 – 34 Poster

RAPID IDENTIFICATION *ASPERGILLUS PARASITICUS* ORIGINATING FROM MAIZE KERNELS

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Maize contamination is a global concern due to its critical role in the food and feed supply chain and its susceptibility to aflatoxin contamination. There is heightened attention on aflatoxins because they reduce agricultural yields, leading to substantial global economic losses, and pose threats to food safety owing to their highly toxic and carcinogenic properties. *Aspergillus parasiticus* exists in both virulent and non-virulent strains, which, under varying climatic conditions, can produce specific aflatoxins, with aflatoxin B1 (AFB1) being the most carcinogenic. Early detection of toxigenic fungi, coupled with accurate species identification, is critical for implementing an effective strategy to minimize fungal growth and mycotoxin production. This approach aims to safeguard maize cultivation and its final products. Because each toxigenic fungal species has its unique mycotoxin profile, accurate species identification is crucial for effective mycotoxin prevention strategies. Identifying species based on morphological characteristics is time-consuming and demands expert taxonomists with specialized knowledge in specific groups of species. In presented work species-specific primers were tested for rapid, sensitive, simultaneous, and PCR-based identification of fungal species. We have verified the presence of *A. parasiticus* using the specific primers (AFLA1/AFLA2) developed by Susca et al. in 2020. In all 20 isolates previously identified using a multidisciplinary approach, the presence of this species was confirmed. Since this species synthesizes aflatoxins, rapid and timely identification is essential.

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ASPERGILLUS PARASITICUS, MAIZE, SPECIFIC PRIMERS, AFLATOXINS

06 – 35 Poster

MAIZE GRAIN YIELD TESTING SYSTEM IN MULTI-LOCATION TRIALS AS ESSENTIAL PART OF THE BREEDING PROCESS

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Field multienvironment yield trials are conducted to enable the selection of the most successful genotypes. This is critical due to the existence of genotype by environment interaction. In general, data can be considered of high quality if it is suitable for its intended use, ie. if it enables the selection of superior genotypes. As it is a very demanding process in terms of the engagement of financial, material and personnel resources, it is of crucial importance to make this process as efficient as possible. In this sense, "data cleaning" is of great importance in order to ensure reliable statistical processing and the main function of the trials themselves, the selection of superior genotypes. It is generally accepted that even up to 30% of the data can be replaced by a calculated value without significant loss of quality. Additional information about potential non-representative data values can be obtained from Box plot analysis. A good researcher spends more than 75% of his time collecting and cleaning data and developing a hypothesis, and only up to 25% on actually processing statistical data and deriving results. The experiments themselves are performed at several levels where each subsequent level differs qualitatively from the previous one in terms of a larger plot, more repetitions, and a larger number of locations, with a smaller reduction in the number of hybrids being tested, providing a more reliable decision.

FIELD OBSERVATIONS, CORN BREEDING, PLOT EVALUATION, VALID RESULT, MULTIENVIRONMENT YIELD TESTING

06 – 36 Poster

ASSESSMENT OF THE VARIABILITY OF BLACK PINE AT THE "KRČANIK" LOCALITY (GOČ MOUNTAIN, SERBIA) AS A BASIS FOR THE IDENTIFICATION, CONSERVATION AND MONITORING OF THE GENE POOL OF VAR. *GOCENSIS*

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Black pine (*Pinus nigra* Arnold) is one of the most widespread conifer species in Europe. The topographic and ecological diversity of the areal, the disjunct distribution, and the species' long history have caused its pronounced morphological variability. Several varieties of black pine have been described in Serbia, including var. *gocensis*, which was first described in 1931 by Petar Đorđević on Goč Mountain. Even then, there were only a few trees of this variety, and they did not rejuvenate naturally. Research conducted ninety years later (2020-2023) shows that the number of trees of this variety has been reduced to a minimum, they are very old (the average age of the population representatives is 300 years) and under constant pressure. Another problem is the phenotypic identification of this variety, which is not clearly defined, making it difficult to identify, which further threatens it. This research aimed to assess the intra-population variability of black pine at the "Krčanik" locality, in the Special Nature Reserve "Goč-Gvozdac" as a basis for the identification, conservation and monitoring of the gene pool of var. *gocensis*. Significant intra-population variability in the morphological characteristics of the bark, needles, and cones was found, which can serve as a basis for distinguishing between the common black pine and var. *gocensis*. Research will continue using anatomical and molecular markers to identify the gene pool of var. *gocensis*. Trees of this variety should be georeferenced and permanently labelled, and concrete activities should be undertaken towards conservation and monitoring of the available gene pool.

PINUS NIGRA VAR. *GOCENSIS* GEORGEV., INTRA-POPULATION VARIABILITY, MORPHOLOGICAL CHARACTERISTICS, SPECIAL NATURE RESERVE "GOČ-GVOZDAC"

06 – 37 Poster

THE NATURAL AGING-RELATED PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN *EX-SITU* CONSERVED MAIZE SEEDS

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The underlying a rich reservoir of diversity maintained in *ex situ* collections – seed gene banks is the lifeblood of plant breeding, making the conservation of the diversity of major crops critical, and mining these collections for useful traits. Therefore, modern seed gene banks management expresses an increasing interest in the storage behavior of seeds. By comparing with the performances of freshly harvested maize seeds (controls), the present study aimed at evaluating physiological and biochemical changes in seeds after six- and 38-year of cold and dry long-term storage conditions (CS) time span. As a result of prolonged CS time span, deterioration occurred, and orthodox maize seeds aged. Namely, decrease in parallel with seed aging was a clear pattern observed regarding seed physiological quality (i.e. germination rate) and initial seedling development. Opposite clear pattern – content increase with seed aging was observed for free phenolic acids (protocatechuic, vanilic, syringic, p-Coumaric and ferulic acid), for both seeds and corresponding seedling shoots. Although with the same pattern of content change, sinapic and cinnamic acids were not detected in seeds, i.e. seedlings shoots, respectively. Correlations significance pointed out that phenolic acids (especially vanilic, p-Coumaric and ferulic acid) of analyzed maize samples had strong impact on germination and overall seedlings performance. Findings of a clear patterns of behavior suggested that the observed physiological and biochemical parameters can serve as viability biomarkers towards a better monitoring of seed natural ageing under ex-situ seed conservation.

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GERMINATION RATE, LONG-TERM COLD STORAGE, PHENOLIC ACIDS, *ZEA MAYS* L

06 – 38 Poster

ASSESSMENT OF SUNFLOWER INBRED LINES FOR RESISTANCE TO *MACROPHOMINA PHASEOLINA* USING AGGRESSIVE AND NON-INVASIVE INOCULATION METHODS

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Macrophomina phaseolina is increasingly threatening sunflower yields in Europe, intensified by climate change. In this study, 12 inbred sunflower lines were tested under two infection conditions: the aggressive toothpick method, with tissue penetration, and the less invasive Unfounded Stem Base Inoculation method (USBI), without penetration. Three parameters were measured: the height of stem sections covered with microsclerotia, seed yield, and the 1000-seed mass. The results revealed distinct differences among the inbred lines regardless of the parameter measured or the infection method used. The toothpick method categorized genotypes into two groups based on seed yield, six groups based on the1000-seed mass, and five groups based on the height of stem sections covered with microsclerotia. Conversely, the USBI method grouped the genotypes into four groups by seed yield, two groups by the 1000-seed mass, and four groups by the height of stem sections covered with microsclerotia. The study found that the toothpick method highlighted greater differences in seed quality, specifically the 1000-seed mass, whereas the USBI method better differentiated the genotypes based on seed yield. This suggests that while both methods are effective in evaluating the impact of *Macrophomina phaseolina*, they may be suited for different aspects of genotype assessment in sunflower breeding programs aimed at improving resistance to charcoal rot.

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CHARCOAL ROT, THOUSAND SEED MASS, TOOTHPICK METHOD, USBI

06 – 39 Poster

GENETIC STRUCTURE AND DIVERSITY OF EX-PVP MAIZE INBRED LINE ASSESSED WITH SNPS MARKERS

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In the US, inbred parents and hybrids are protected by the U.S. Patent/or U.S. Plant Variety Protection Act (PVPA) for a period of 20 years. After the end of the protection period, inbred lines become annually available to be used in public and private breeding programs as a potential source of new germplasm. The scarcity of data on the heterotic origins of ex-PVP inbred lines can often be a limiting factor for their efficient inclusion in breeding programs which highlights the need for the genetic characterization of these inbred lines using molecular markers. A panel was constructed using 61 Ex-PVP inbred lines that were obtained from the NCRPIS station in Ames, Iowa, USA by the Maize Research Institute "Zemun Polje" in the 2019-2023 period. The assessment of the genetic diversity of this panel was done using a Maize 25k Infinium array, which after data filtering, provided 20522 highly informative SNP markers. Using bioinformatical software, the genetic structure and diversity of the panel were analyzed. The genetic distance between Ex-PVP inbred lines was in the range from 0.039 to 0.470, while the average distance value was 0.387. A neighbor-joining cladogram showing two distinct subclusters was constructed using TASSEL. Population structure analysis was done in STRUCTURE and optimally grouped the inbred lines into 4 populations (k=4).

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MAIZE, Ex-PVP, SNPs, INBRED LINE

06 – 40 Poster

GENETIC DIVERSITY WITHIN COLLECTION OF MAIZE INBREDS FROM GENE BANK REVEALED BY SNP MARKERS

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The presence of well-described germplasm collections is crucial for the thorough examination and understanding of genetic and phenotypic diversity within crop species. Hence, a set of 93 maize inbred lines from the Maize Research Institute "Zemun Polje" Gene bank underwent genetic diversity analysis employing an array of 25k SNP markers. After filtering initial marker set by removing SNPs with >10% missing data and <5% minor allele frequency (MAF), 19941 SNP markers were used for downstream analyses. Minor allele frequency varied from 0.005 to 0.49 with an average of 0.30. The observed and expected heterozygosities were at ranges of 0 to 0.48 and 0.01 to 0.50, respectively. Calculated genetic distance was the lowest between L1 and L39 (0.018) and highest between L21 and L35 (0.428). Population structure was revealed applying cluster and PCoA analysis. According to cluster analysis 93 inbreds were clearly separated in two main groups. In one of the two main clusters, the majority of inbreds were grouped into smaller subclusters, while the other cluster was formed by just five inbreds. PCoA analysis showed somewhat different grouping of maize genotypes. Bayesian population structure analysis (STRUCTURE software) revealed the most likely number of clusters at K=3.

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MAIZE, GENETIC RESOURCES, GENETIC DIVERSITY, SNP MARKERS

06 – 41 Poster

VARIABILITY IN THE GRAIN CHEMICAL PROPERTIES OF HYBRID MAIZE (*ZEA MAYS* L.) GENOTYPES AS A SOURCE OF PROSPECTIVE APPLICATIONS

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The objective of this study was to evaluate the effect of year and genotype, as well as their interactions, on the main parameters of the maize grain chemical composition. The plant material encompassed ten commercial maize hybrids from different FAO groups developed at the Maize Research Institute, Zemun Polje (MRIZP). Three-year trials were set at the experimental fields of MRIZP, following a randomized complete block design with two replications. After the harvest, the grain samples were analyzed for starch, protein, oil, and crude fiber content by applying standard analytical methods. Three-year means (% of dry matter) were subjected to analysis of variance (mixed model) and Tukey test for assessing significance of differences between means Analysis of variance showed a predominant influence of the year in total variation of all observed traits (starch: 41.35%***, protein: 41.55%***, crude fiber: 73.84%***) except for the oil content, where the genotype had a high and significant effect (59.22%***). Genotype×year interaction was highly significant for all the observed traits, with the highest value for protein (31.35%***) and the lowest for oil content (19.49%***). The Tukey test singled out hybrid ZP 7777 as significantly superior in terms of starch content (73.23%) compared to other hybrids. The highest protein content was observed in hybrid ZP 6119k (12.49%). For the oil content, a negligible variation was determined among the hybrids, as well as for crude fibers. The study highlighted the significant influence of environmental factors on the content of the investigated nutritional components, except for the oil levels, which proved to be stable over the years and hybrids.

MAIZE HYBRIDS, CHEMICAL COMPOSITION, ANALYSIS OF VARIANCE, GENOTYPE, ENVIRONMENTAL FACTORS

06 – 42 Poster

GENETIC STRUCTURE OF *CASTANEA SATIVA* MILL. POPULATION IN BOSNIA AND HERZEGOVINA REVEALED BY SSR MARKERS

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In this study, a genetic structure assessment of natural populations of sweet chestnut in Bosnia and Herzegovina was conducted. Six nuclear microsatellite markers were chosen to analyze the genetic structure of six natural populations of sweet chestnut (Banja Luka, Bratunac, Buzim, Konjic, Kostajnica, and Prijedor) comprising 287 analyzed individuals. Bayesian analysis using the STRUCTURE program was employed to define the genetic structure of sweet chestnut populations (*Castanea sativa*), based on allele frequencies at SSR markers. According to the ΔK method, the most likely number of genetic clusters (K) in the populations of the studied region was K=2, with a secondary peak at K=6. Results for K=2 indicate that the populations of Banja Luka and Konjic are very similar, while Bratunac, Buzim, Prijedor, and Kostajnica differ from each other. For K=6, each population clearly separates into distinct genetic groups, indicating different origins for each population. Analysis of genome contributions (Q) suggests that the Kostajnica and Buzim populations have mixed origins dominated by certain original populations. These results underscore visible genetic differences within and between sweet chestnut populations, potentially influenced by natural selection and strong anthropogenic factors.

SWEET CHESTNUT, MICROSATELLITE MARKERS, GENETIC CLUSTER, STRUCTURE

06 – 43 Poster

A COMPARISON OF TWO MULTIVARIATE APPROACHES IN ASSESSMENT OF SOYBEAN PHENOTYPIC DIVERSITY

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Cluster analysis and Principal Component Analysis (PCA) are two multivariate techniques commonly used in breeding studies. Although both methods allow visualization of differences between genotypes and identification of potential groups, it is a common dilemma which one is more informative for breeders. Ninety soybean genotypes of different maturity groups (MG 00-III) from Maize Research Institute Zemun Polje soybean collection were evaluated for 8 agronomic traits - seed yield and major yield components, as well as for protein and oil content. Data were subjected to UPGMA cluster analysis based on Euclidean distance matrix, and PC analysis. Grouping pattern obtained with cluster analysis reflected the tendency of genotypes from the same MG to cluster into the same subgroup. Genotypes were divided into two main clusters which corresponded to division into early and mid-season (cluster I) and late varieties (cluster II). PC biplot displayed that large number of genotypes were located close to the origin, being intermediate for majority of the observed traits. Dissemination of genotypes across the quadrangles showed high consistency with the information about MG. Along the PC1, genotypes were distinguished by seed yield, oil content, and yield related traits. The genotypes with high yield and high oil content (MG I and MG II) were on the left side of the biplot, while the genotypes with high protein content and low yield (GZ 00 and 0) were on the right. Along the PC2, genotypes were mostly discriminated by the 100 seed weight. Although both multivariate methods displayed similar pattern of genotypes grouping, PCA has proven to be more informative from the breeder's point of view, revealing groups of genotypes for specific breeding purposes and identifying varieties simultaneously superior for multiple traits.

UPGMA CLUSTERING, PCA, EVALUATION, AGRONOMIC TRAITS

06 – 44 Poster

EXPLORING MAIZE GENETIC DIVERSITY THROUGH EUROPEAN EVALUATION NETWORK – EVA

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The European Cooperative Programme for Plant Genetic Resources (ECPGR) is a collaborative programme on evaluation of existing diversity and structure, aiming at their long-term preservation and increased utilization in breeding. Within the ECPGR, the European Evaluation Network (EVA) is an international project started in 2019, with the goal to introduce valuable traits from PGR into breeding programmes, through public-private partnerships of crop-specific networks. The EVA maize network involves 18 research institutions, gene banks and private breeding companies from nine countries (https://www.ecpgr.org/eva/eva-networks/maize). The joint evaluation was conducted on ~700 maize accessions from European gene banks collections, using high-throughput genotyping, harmonized methods and standard phenotyping protocols. Generated genotypic and phenotypic data (both per se and test-cross performance) obtained from evaluation in multi-location trials under different agro-ecological conditions, will help to increase the knowledge on valuable traits of publicly available maize germplasm. In that respect, the Maize Research Institute Zemun Polje gene bank contributed to EVA maize collection by providing 95 accessions (52 local landraces and 43 introduced populations). The EVA Network approach promotes conservation and utilization of maize GR for development of climate-resilient hybrids, of higher resistance to abiotic/biotic stresses. The future network activities aspire towards extending its geographic scope to explore additional genetic diversity.

GENETIC DIVERSITY, MULTI-LOCATION TRAILS, PHENOTYPING, *ZEA MAYS* L

06 – 45 Poster

IDENTIFICATION OF WHITE WILLOW GENE POOL ON THE GREAT WAR ISLAND AS A BASIS FOR MONITORING AND DYNAMIC CONSERVATION

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Identifying the gene pool at the population level, a specific area, or a species is fundamental for assessing the state and risk level. Subsequently, particular conservation, sustainable use, and monitoring activities are initiated. These activities are especially important for rare and/or endangered species with isolated and relatively small populations, such as white willow in the Great War Island area (Belgrade, Serbia). Field research was carried out to identify the white willow gene pool. Individual trees and representatives of a group of trees were recorded, classified, and georeferenced using the UTMGeoMap mobile application. The spatial arrangement of the georeferenced trees was obtained by overlaying digital field data with a satellite image of the Great War Island terrain and a vector representation of department and section borders from Google Earth Pro. In this way, data on the size of the gene pool and its spatial distribution were collected. For each recorded individual tree or tree marked as representative of the group, the following characteristics were determined: tree height, tree diameter at 1.30 m, trunk length, tree form, crown quality, fullness, stem twisting, and health status. The research found that the white willow gene pool is represented by older trees with significant damage, and there is no recorded natural rejuvenation. These trees are exposed to many factors that lead to genetic erosion. The lack of white willow reintroduction to the Great War Island through regular reforestation further threatens the survival of this species. To preserve and enhance the white willow's ecological adaptability and evolutionary potential on Great War Island, activities have been carried out to dynamically conserve and sustainably use the available gene pool.

GENETIC EROSION, IDENTIFICATION, MONITORING, DYNAMIC CONSERVATION

06 – 46 Poster

SENSORY CHARACTERISTICS OF TRADITIONALLY PREPARED BREAD FROM THE FLOUR OF DIFFERENT SPECIALTY MAIZE GENOTYPES

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Modern maize hybrids which can currently be found on our market are usually very uniform in their nutritional and technological quality and are not able to meet some specific market requirements. The given research was focused on examining the sensory characteristics of traditionally prepared corn bread made from maize flour of five different germplasm type. Four samples were obtained by mill grinding of the following genotypes grain: ZP532b - white maize hybrid; Beli osmak - in situ landrace from Dragačevo improved through three cycles of mass selection; Bosanac - small-grain flint landrace improved through one cycle of recurrent and one cycle of mass selection; ZP614k - popcorn hybrid. White maize flour purchased from a food store was used as a control. Corn bread Proja was made according to the same procedure, based on the traditional recipe. Twenty four trained evaluators participated in sensory characteristics evaluation (colour, smell, taste, fineness of the pores, chewiness). The evaluators along with other 24 respondents participated in the consumer survey. Categorical PC analysis of the obtained results indicated that the colour, taste and smell of the Projas' are defined by the first axis and are in positive correlation with each other, while chewiness and pore fineness are defined by the second axis and are negatively correlated. Correspondence analyses indicated that the control (a commercial sample of maize flour) was rated the worst by the majority of trained evaluators. According to the conducted survey, the Proja made from white eight-row landrace and popcorn flour ranged the first.

CATEGORICAL PCA, CORN BREAD, TRADITIONAL FOOD, *ZEA MAYS* L

06 – 47 Poster

CREATING NEW GENERATION OF SUNFLOWERS FOR FUTURE CHALLENGES

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In recent years, overcoming the negative impact of climate change on the oil crops productivity and creating tolerant genotypes have become one of the strategic goals in agriculture. Climate change has different effects in different agroecological conditions, which requires the creation of different genotypes, adapted to the specific environmental conditions and tolerant to the predominant stresses still having appropriate adaptability and stability. Sunflower breeders are thus using all available resources and tools to increase genetic variability, and mine for desirable traits and genes for creating productive genotypes in stressful conditions. At the Institute of Field and Vegetable Crops, we are currently combining different –omics approaches for extensive characterization of our breeding material for abiotic stress resilience. By exploiting transcriptomics, we will be able to identify desirable tolerance genes, while phenomics will help get more insight into genotype-environment interactions which is essential in crop breeding. Combining different approaches will facilitate tailored breeding for a particular environment and help create new generation sunflower hybrids ready to face new challenges.

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HELIANTHUS ANNUUS L.; CLIMATE CHANGE; RESILIENCE; BREEDING, MULTI-OMICS

06 – 48 Poster

AGRONOMIC APPROACH TARGETING FREE ASPARAGINE AND REDUCING SUGARS IN WHEAT GRAINS AS THE MAIN PRECURSORS IN THE FORMATION OF ACRYLAMIDE IN THERMALLY TREATED CEREAL-BASED FOOD

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Identification of environmental factors, including crop management, that affect acrylamide-forming potential of cereal genotypes is one of the approaches aimed at its reduction in cereal-based food. The main agronomic approach is aimed at reducing levels of its precursors in the grains, free asparagine and reducing sugars. The goal of these researches was the use of sulfur fertilization in varied doses in order to reduce free asparagine content in wheat grains. The effect on the sugars content was also examined. One common/bread wheat genotype Sara and one soft wheat genotype Belija were used in this study. The winter wheat trial was set for the 2022/2023 season and the experimental field consisted of: 1) control plots (no fertilizer application), and 2) fertilized plots with 100kgN/ha, 3) N:S=100:10kg/ha, 4) N:S=100:20kg/ha, 5) N:S=100:40kg/ha. Nitrogen application was observed to elevate free asparagine levels by 15% and 17% in common and soft wheat, respectively. However, our study confirms that the added amount of sulfur in a concentration of 10 kg/ha reduces the content of free asparagine by 6% and 7%, respectively, compared to fertilization with nitrogen, while further increase of sulfur concentration had no effect on free asparagine content reduction. A significant difference in the content of glucose, fructose and sucrose between the investigated genotypes was determined, as well as the dependence of the sugars content on sulfur fertilizer addition. Preliminary findings indicate that a nitrogen to sulfur ratio of 10:1 kg/ha was sufficient to prevent large increases in free asparagine in wheat grains.

WHEAT GENOTYPES, SULFUR FERTILIZATION, FREE ASPARAGINE, ACRYLAMIDE

06 – 49 Poster

DROUGHT TOLERANCE IN SUNFLOWER: TWO PHENOTYPING METHODS

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Drought conditions can be significant constraint is sunflower cropping. Determination of variability in sunflower drought response is necessary step in breeding effort to select tolerant genotypes. Experiments were conducted to compare two approaches aiming to isolate drought stress from other sources of stress, control water availability to plants and to determine impact of drought on plant growth. Two inbred lines, HA-26-PR and IMI-AB-14, were selected based on their response to drought. In the first experiment plants were grown for period of 14 days in rhizotrons and in the second for period of 60 days in pots. Both experiments were placed in growth chamber. Drought conditions in rhizotron experiment were on constant level compared to pot experiment where plants were gradually exposed to drought at one stage of development. Plants were phenotyped during and at the end of rhizotron experiment and only at the end of pot experiment. Growing plant in rhizotrons gave insight in root morphology and growth, but for the limited duration of time and only for early stages of sunflower growth. Pot experiment enables one to monitor plant growth and development over longer time and to impose drought at selected moment making it closer to the drought scenarios in field conditions. Both methods are reliable for drought stress induction and determination of drought impact and can be considered as complementary. Further research will aim the comparison of methods in controlled conditions with field drought experiments.

SUNFLOWER,DROUGHT, TOLERANCE, RHIZOTRON, POT

06 – 50 Poster

BOOSTING INNOVATIVE BREEDING AT IFVCNS – CROPINNO

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Providing new solutions for accelerating climate-smart crops creation has become a number one task in breeding. A project funded by the European Commission under the agreement No. 101059784 “Stepping up scientific excellence and innovation capacity for climate-resilient crop improvement and production” CROPINNO is tackling with this problem. Within the framework of this project, Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia (IFVCNS) is teaming up with reputable European research institutions and Universities for the introduction of new tools in IFVCNS breeding programs. On genetic level, we are exploiting state-of-the-art platforms for analysing sunflower epigenome, namely histone methylation and activities of non-coding RNAs to quest for important epiQTLs that would improve sunflowers quick response to abiotic stress. Moreover, we are introducing new phenotyping tools such as root phenotyping to identify root traits related to abiotic stress response. By combing latest molecular and phenotypic tools we will be able to accelerate creation of climate-resilient sunflower and be ready for what future environmental change brings us.

Acknowledgement: This work is supported by the European Commission through projects CROPINNO, grant number 101059784, and HelEx, grant number 101081974, the Science Fund of the Republic of Serbia, through IDEAS project “Creating climate smart sunflower for future challenges” (SMARTSUN), grant number 7732457, by the Ministry of Science, Technological Development and Innovations of Republic of Serbia, grant number 451-03-66/2024-03/200032, and by the Centre of Excellence for Innovations in Breeding of Climate-Resilient Crops - Climate Crops, Institute of Field and Vegetable Crops, Novi Sad, Serbia, as well as COST Actions RECROP – CA22157 and EPI-CATCH – CA19125.

CLIMATE CHANGE; RESILIENCE; BREEDING, MULTI-OMICS, PHENOTYPING

06 – 51 Poster

EVALUATION OF GENOTYPE-BY-LOCATION INTERACTION IN THE EARLY STAGES OF MAIZE BREEDING

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In the early phases of a maize breeding program, numerous new genotypes are assessed across field trial locations to identify the best performers for advancement to subsequent stages. The best ones are identified to proceed to the next phase so that, in the final stages of the breeding program, a small number of superior hybrids are tested in pre-registration and post-registration field trials across different locations. In 2020, field trials using a partially replicated design were conducted, with each genotype being replicated twice per location. The trials included 176 hybrids derived from crosses between different S2 progenies with an elite inbred tester. These trials aimed to identify top-performing genotypes using a linear mixed model. The factor analytic (FA) model is a powerful statistical tool used in maize breeding for analyzing genotype-by-location (G×L) interactions. The FA model assumes that observed performance of genotypes in different locations can be explained by a few latent factors. In our study, the FA model was employed to address the heterogeneity of genetic variance among locations and the genetic covariance between pairs of locations. Given the high degree of heterogeneity in our trial data, the FA model approach provided precise predictions of G×L effects. The FA model with one latent factor identified as the best-fitting model, explaining approximately 80% of the G×L interaction. The resulting genetic correlation matrix indicated moderate to high positive correlations among the locations. Utilizing the FA(1) model, the most promising genotypes regarding grain yield and yield stability were identified.

MAIZE BREEDING, GRAIN YIELD, YIELD STABILITY, EARLY TESTING, HYBRID × LOCATION INTERACTION, FACTOR ANALYTIC MODEL (FA)

06 – 52 Poster

GENOTYPING OF *FAGUS SYLVATICA* FROM THE PAN-EUROPEAN PROVENANCE TRIAL IN SERBIA

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Beech is one of the most valued and the most studied European tree species. In context of climate change, productivity reduction and mortality increment are expected in beech populations. Pan-European provenance beech trials, which were established from 2007 to 2010, represent an invaluable source for gathering data about most suitable provenances for restoration and afforestation especially in the light of climate change. We genotyped 155 beech individuals from 16 European provenances from the pan-European provenance trial in Debeli Lug, Serbia, using 13 nuclear microsatellites to assess whether the genetic structure reflects the European phytoclimatic regions as an important aspect for seed transfer. Data analysis was performed using GenAlex 6.5 software and the tailored R scripts. A total of 166 alleles were detected, the number of alleles per locus ranged from 5 to 20, and the level of genetic diversity was rather high, He=0.774. The AMOVA results revealed a low level of inter-provenance (4%) and inter-individual (6%) variability. Discriminant analysis of principal components (DAPC) applied at the level of two strata, provenance and region, indicated that genetically close provenances belong to the same phytoclimatic region. Our results, together with previous studies on growth, physiological parameters, estimates of adaptive and productive potential indicators of different provenances represent an important contribution to the formulation of strategies for seed/seedlings transfer across the Europe.

NUCLEAR MICROSATELLITES, GENETIC DIVERSITY, BEECH, CLIMATE CHANGE, PROVENANCE TRIALS

06 – 53 Poster

YIELD POTENTIAL OF MAIZE HYBRIDS AS A FUNCTION OF SOWING DENSITY

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In maize cultivation under the conditions of climate change, various cultivation measures are being implemented to mitigate the consequences. The study aimed to observe yield variability at different sowing densities. The trial was designed as a three-factorial experiment in which the relationship between genotype, sowing density and water regime on the grain yield of maize was observed. In the trial, four hybrids of maturity group FAO400 (H1-H4) were observed, sown at seven sowing densities (G1-G7; 40,000-98,000) under natural water regime and irrigation conditions.

The yield varied between the different hybrids depending on the density and type of irrigation. Hybrid H4 had the highest average yield. This hybrid obtained the highest yield in the density of G6, 12.61 t/ha, and in terms of type of water regime, in irrigation, 11.54 t/ha.

When analyzing the distribution of the frequency of maize grain yields as a function of density, the hybrids in the trials with densities G1, G6 and G7 showed the greatest difference between the minimum and maximum yield values at 7 t/ha, the lowest was G5, 4 t/ha.

Crop density was significant for most hybrids and resulted in a linear increase in yield to density. Since there are different production areas for maize cultivation, the production technique should be adapted to the specific conditions of the climate, soil and other factors of the external environment to make the best use of the potential of the location and genotype.

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MAIZE, DENSITY, SEED, TECHNOLOGY

06 – 54 Poster

ENVIRONMENTAL IMPACTS ON THE NUTRITIONAL QUALITY OF *CUCURBITA* PLANTS: IMPLICATIONS FOR SELECTIVE BREEDING

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Plant breeding programs have traditionally focused on maximizing yield, often placing greater emphasis on quantity rather than on nutritional quality. Recently, there has been an increasing recognition of the importance of food nutritional quality. This has raised the question about the potential role of plant breeding in its improvement.

This multi-year study aimed to evaluate the variability within a breeding collection of *Cucurbita* species (*C. maxima*, *C. moschata*) concerning carotenoid and sugar content, which are key determinants of pumpkin fruit nutritional quality. Additionally, the study investigated how high air temperature stress during the reproductive phase of plant development affects these quality parameters.

Significant differences were observed between plant species, genotypes within these species, and across different growing seasons regarding the content of carotenoids and sugars. Temperature stress led to a reduction in carotenoid content, with *C. moschata* showing a more pronounced effect. In contrast, sugar content was reduced in *C. maxima* and slightly increased in *C. moschata* when compared to temperate seasons.

The variation in carotenoid and sugar content, as well as their response to temperature stress across different genotypes, indicates the potential for breeding Cucurbita plants to improve their fruit nutritional quality.

Acknowledgement: This research was supported by the Science Fund of the Republic of Serbia, #GRANT No 6680, Nutrition-sensitive breeding of Cucurbita plants - NutSens_PumpBreed

CUCURBITA, NUTRITIONAL QUALITY, TEMPERATURE STRESS

06 – 55 Poster

VARIABILITY OF YIELD IN MAIZE SEED PRODUCTION ACROSS GENOTYPE, YEAR AND LOCATION

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Successful maize seed production highly depends on genetic potential of parental inbred lines, selection of suitable micro-climatic conditions, proper location and position of isolation, as well as agronomic management of seed production fields. Differences in these factors can lead to considerable variations in the quantity and quality of produced hybrid seeds. In order to perceive seed yield and stability, the data on seed production of two commercial maize hybrids developed at Maize Research Institute Zemun Polje Belgrade were analyzed. Data were collected from large scale seed production fields (47 cases). Seed yield was recorded in 3 consecutive seasons (2021, 2022 and 2023) and at all production locations (mainly in the province of Vojvodina). Across all years and locations, seed production of hybrid ZP 427 achieved mean value of 3452.7 kg/ha with standard deviation (SD) of 1068.16 while ZP 606 had 2673.88 kg/ha with similar value of SD (1128.65). In years 2021 and 2023, two hybrids performed very similar, achieving mean seed yield of 3179.87 kg/ha and 3149,38 kg/ha, respectively (SD 1080.34 and 999.99). Unfavorable conditions during 2022 led to yield reduction and greater variation across hybrids and locations (2351.28±1323.17 kg/ha). Mean values of main production locations ranged from 2617.21-3415.98 kg/ha. Generally, these values indicate that examined hybrids are very good performing in terms of seed production in most parts of Vojvodina and keep relatively stable performance even in years with high temperatures and water deficit, as was the case in 2022.

MAIZE, SEED YIELD, VARIATION

06 – 56 Poster

DERIVED UAV DATA FOR EVALUATION OF SOYBEAN GERMPLASM GROWN IN DIFFERENT ENVIRONMENTS

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The potential of unmanned aerial vehicles (UAVs) and digital cameras in different areas of agriculture has been greatly exploited in recent years. The new technologies enable fast and non-destructive estimation of plant traits which can be especially useful in screening of breeding material. This study aimed to explore the potential of specific growth parameters derived from the UAV images to evaluate diverse soybean germplasm. During 2020 and 2021, the 206 genotypes were sown in drought simulation environments and the control. In both seasons, trials were photographed multiple times with UAV and multispectral camera. After each flight, plant height (PH), biomass (BM), and canopy cover (CC) of soybean genotypes were calculated from the images and used to construct the growth curves. Derived from the curves, traits such as maximum plant height (PHmax), biomass accumulation rate (BAR), and time required to reach 50% of canopy cover (CC50) were used for the evaluation of soybean varieties. The genotype evaluation was based on the following principles: the PHmax should be between 70 and 90 cm, the lower limit for BAR was 0.01 kg of BM/growing degree day (GDD) while the upper limit for CC50 was 200 GDDs in drought and 150 GDDs in the control. The results showed that two varieties from the control and five from water-deficient environments met the criteria and were selected as superior germplasm. The study indicates that UAV data can be successfully utilized as a new tool for the evaluation of soybean breeding material.

SOYBEAN, UAV, HEIGHT, BIOMASS, CANOPY COVER

06 – 57 Poster

MOLECULAR DIVERSITY ANALYSIS OF LOCAL WHEAT GENETIC RESOURCES FROM SERBIA AND BULGARIA

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Molecular diversity analyses of crop genetic resources contribute to understanding genetic variation, gene discovery, identifying valuable traits for crop improvement, adapting to changing environments, food security and sustainable agriculture. Germplasm in collections and gene banks is insufficiently characterized and consequently largely underutilized in breeding programs. The aim of this study was to assess the molecular diversity of old traditional varieties and local landraces of bread wheat that originate from Serbia and Bulgaria. The 79 wheat accessions, 38 Serbian and 41 Bulgarian, collected within the FAO's Benefit-Sharing Fund project, were analyzed with 47 microsatellites. The total of 402 alleles was detected with an average of 8.55 alleles per locus. The average PIC value was 0.612, while the average gene diversity was 0.643. For all parameters of genetic diversity, the landraces showed higher level of diversity that the varieties, indicating that selection and breeding process lead to the diversity bottleneck. However, this difference can be contributed only to Serbian accessions, since the Bulgarian landraces and varieties differed very little. The gene diversity, number of alleles and PIC were considerably higher among Serbian accessions compared to Bulgarian. The UPGMA clearly separated Serbian and Bulgarian accessions. The landraces and varieties from Serbia formed two distinct groups, but the Bulgarian accessions did not group by type. The results indicate differences in relatedness of the accessions and breeding practices in two countries, contributing to knowledge needed to utilize local wheat genetic resources in breeding climate-resilient varieties.

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DIVERSITY, GENETIC RESOURCES, LANDRACES, MICROSATELLITES, WHEAT

06 – 58 Poster

MAIZE BREEDING AND CLIMATE CHANGE CHALLENGES

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Climate change pose a significant threat to maize production. In the last decade, we have experienced severe drought, floods, supercell storm and a heat dome. As a consequence, in some years the grain yield was dramatically reduced due to lack of precipitations, the grain quality was compromised due to presence of mycotoxins, in some years the yield was lost due to lodging but in some others due to extreme heat and drought. The improved growing solutions are the powerful adaptation measure, but the development of maize hybrids capable to cope with these challenges is of crucial importance. Traditional maize breeding have contributed to significant increase in grain yield, and tolerance to abiotic and biotic stress. We have identified hybrids tolerant to drought and grain mycotoxin accumulation, tolerant to stalk and root lodging caused by high wind speed, and tolerant to extreme heat and drought occurring at the same time. In the years to come, climate change will determine the breeding programs, but some traits, such as grain yield, tolerance to heat and drought, stalk and root quality and tolerance to mycotoxin accumulation will remain at the breeder's top priority. However, longer time needed for conventional hybrid development could be an obstacle to quick response of maize breeders to dynamic environmental changes. Integration and use of new generation technologies will increase the precision, efficiency and speed in development of climate resilient hybrids.

MAIZE, BREEDING, CLIMATE CHANGE, TRAITS

06 – 59 Poster

THE EFFECTS OF TESTER AND LOCAL LANDRACE VARIABILITY ON GRAIN YIELD AND GRAIN COMPONENT TRAITS

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In maize breeding, understanding the relationships between traits is crucial for optimizing yield and improving grain components. This study investigates the impact of different testers and local maize landraces on kernel row number and number of kernels per row in test cross hybrids, and how these traits correlate with grain yield. Three testers L217 (Iowa Dent), L73B013 (BSSS × Iowa Dent), and L255/75-5 (Lancaster) were crossed with 31 local landraces. Trials were conducted at four locations over two years and replication. Significant effects from environment, tester, landrace, and their interactions were observed ($p \leq 0.01$), except for the triple interaction ($p=0.917$). Landrace AN288 had the highest kernel row number (14.99), followed by AN13, AN1509, and AN632. Among testers, L73B013 had the highest kernel row number (13.84), while L217 and L255/75-5 were similar (13.67 and 13.60). The average number of kernels per row was 36.31. Significant differences were found among all factors except for replication and the triple interaction. Landraces AN632 (38.97) and AN1569 (38.82) had the highest kernels per row, differing from AN1346 and AN2006. Significant correlations between traits and grain yield were found. High-performing landraces of kernel row number and number of kernels per row also achieved high yields. The effect of AN288 in crossing with L255/75-5 gives the best grain yield (9,245t/ha). These correlations suggest that AN288, AN632, AN1509 and AN1569 are promising for breeding programs aimed at yield improvement.

Acknowledgments: This research was supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia (Grant No. 451-03-66/2024-03/200040).

MAIZE, LANDRACES, KERNELS TRAITS, TESTCROSS, YIELD

TOPIC 6

Bioinformatics and big data analysis authors index

VII CONGRESS OF THE SERBIAN GENETIC SOCIETY

07 – 01 Oral

A NEW TOOL: VUS NOTIFIER

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There are over 300 million people living with one of over 7,000 identified genetic rare diseases (RD) around the world. Over 80% of RD are genetic, and nowadays comprehensive genetic tests (such as whole-exome sequencing and whole-genome sequencing) become the first-tier test in the process of reaching diagnosis. Accurate and timely diagnosis is necessary for specific and effective treatment of affected people, and can enable their family members to perform prenatal diagnostics and have healthy offspring.
However, interpretation of genetic test is limited by the currently available scientific data and for substantial part of genetic variants detected by genetic test, there is just not enough data to be classified as pathogenic or benign and therefore they are classified as variants of uncertain significance (VUS). In time, new scientific evidence will be gathered and VUS will eventually be re-classified as pathogenic or benign.
To aid in the process of providing latest information, we developed a VUS notifier application. VUS notifier solves the problem of providing notifications regarding VUS. Now, an interested party, whether a patient, family member, doctor, etc., can conveniently receive notifications when new information relevant to a specific VUS appears in genetic databases or scientific publications.

Acknowledgements: This work was supported by SAIGE PoC grant.

RARE DISEASES, GENETIC TESTING, VARIANTS OF UNCERTAIN SIGNIFICANCE

07 – 02 Oral

CODI: CONTRASTIVE DISTANCE REFERENCE-BASED CELL TYPE ANNOTATION FOR SPATIAL TRANSCRIPTOMICS

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In the era of single-cell genomics, deciphering cellular heterogeneity is paramount for understanding complex biological systems. We present CoDi, an innovative tool designed for precise cell-type annotation using reference single-cell datasets. It leverages the power of deep neural networks based on contrastive learning with data augmentation, and advanced distance calculation methods to mitigate the potential batch effects and bring additional robustness. Existing approaches employ mapping of the reference high-quality scRNA gene expressions and cell types, to lower-quality spatial transcriptomics datasets. However, the accuracy and the performance of cell type annotation is not objectively evaluated, and methods lack the capacity to perform on large datasets with sparse data produced by high-resolution technologies like Slide-seq v2 and Stereo-seq. CoDi represents a significant advancement by demonstrating superior performance, and scalability compared to existing solutions on several evaluation metrics. On the retention rate of the marker genes metric, CoDi achieves 5% to 25% higher marker gene retention percentages than the existing tools, and, on accuracy on the downsampled high-quality datasets CoDi archives up to 10% higher accuracy than the existing tools. By harnessing the intrinsic structure of the data, CoDi effectively captures subtle features that characterize distinct cell types, resulting in enhanced annotation accuracy that can detect rare cell types such as neurons in the heart. In summary, CoDi is a valuable tool that contributes to our understanding of cellular heterogeneity and offers insights into the specificity of various cell types within diverse tissue structures.

Source code: https://github.com/STOmics/CoDi

CELL TYPE ANNOTATION, SPATIAL TRANSCRIPTOMICS

07 – 03 Oral

PCR BASED NANOPORE SEQUENCING APPROACH FOR ANALYZING SHORT TANDEM REPEAT EXPANSIONS

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Expansions of short tandem repeats (STRs) are implicated in >60 rare neurological disorders. Unlike any other mutation types, they exhibit dynamic length variations throughout individual's lifetime. Since STR expansions pose challenges for accurate characterization due to lengthy repetitive sequences and high GC content, Nanopore sequencing offers a promising solution with its single-molecule sequencing capabilities. We have developed a comprehensive wet lab and bioinformatic protocol that uses PCR-based enrichment to facilitate Nanopore sequencing of STR expansions

Using the Native Barcoding Kit 24 V14, we multiplexed samples from up to six patients and sequenced them on R10.4.1 Flongle cells and Oxford Nanopore Technologies Mk1C device. Guppy was used as the basecaller and reads were analyzed as strings with repeat sequences. We employed regular expressions to count repeats and detect interruptions, validating results against wet lab data to ensure computational robustness.

Our Nanopore sequencing pipeline accurately determines the length and structure of complex STR expansions in spinocerebellar ataxia type 8 (e.g., (CTA)6(CTG)56-60CCG(CTG)53-56) and myotonic dystrophy type 1 (e.g., (CTG)350-700(CCGCTG)3(CTG)4(CCGCTG)2CTGCCG (CTG)18), surpassing traditional methods like repeat-primed PCR and Southern blot. We achieved reliable allele length distribution for somatically unstable DM1 mutations in expansions carrying up to 400 repeats. Modal allele size and somatic instability degree were accurately estimated with ≥200X coverage.

In conclusion, our developed Nanopore sequencing protocol proves robust for accurately characterizing complex STR expansions implicated in neurological disorders, potentially resolving these challenging regions with greater accuracy

Acknowledgements: This study was supported by the Science Fund of the Republic of Serbia, grant number 7754217, READ-DM1.

STR EXPANSIONS, NANOPORE SEQUENCING, REGULAR EXPRESSIONS, LONG-READ SEQUENCING

07 – 04 Oral

HSLAR MODEL GENE CO-EXPRESSION NETWORK AND COMPARATIVE TRANSCRIPTOMICS REVEAL KEY PATHWAYS IN DM1 PATHOLOGY

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Myotonic dystrophy type 1 (DM1) is a rare, incurable multisystemic disease, characterized by skeletal muscle weakness, atrophy, and myotonia. It is caused by CTG expansion in the 3′ UTR of the DMPK gene, resulting in globally altered RNA metabolism and splicing. The DM1 molecular pathomechanisms are not fully understood and should be further investigated. To address this, comparative transcriptomics with network analysis was applied to the most used DM1 mouse model (HSALR).

We retrieved all publicly available RNA-seq datasets from the mouse model expressing 250 CTG repeats in its skeletal muscles (HSALR). We performed read preprocessing with unified parameters, identified differentially expressed genes (DESeq2), and constructed gene co-expression networks (WGCNA). Modules with strongly co-expressed genes were subjected to enrichment analysis (g:Profiler, SIGORA).

Integration of numerous datasets makes this study the most comprehensive for differential gene expression analysis in HSALR mice. Observed differences were larger in samples from proximal muscles than in samples from distal muscles. WGCNA recovered three modules of strongly co-expressed genes (adjacency>0.5). The turquoise module was enriched for extracellular space and muscle development. The midnight-blue module contained the Mup gene family members. The brown module was associated with innate and adaptive immune responses, highlighting multiple signaling pathways and cytokine secretion.

Identified dysregulated gene expression patterns reflect disease-specific muscle development disruption while highlighting the new paradigm of muscle as an endocrine organ and its importance in immunological processes in DM1. These results can guide towards specific molecules implicated in one of the unexplored pathomechanisms, providing possible new therapeutic targets.

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MYOTONIC DYSTROPHY TYPE 1, DIFFERENTIAL GENE EXPRESSION, TRANSCRIPTOMICS, GENE CO-EXPRESSION NETWORKS, MUSCLE DEVELOPMENT AND IMMUNOLOGY

07 – 05 Oral

COMPREHENSIVE BIOLOGY KNOWLEDGE GRAPH FOR MULTI-OMICS DATA INTERPRETATION

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In recent years, the complexity of biological data has necessitated advanced methods for its interpretation. Biological research and precision medicine rely on interpretation of omics data, and knowledge graph (KG) has emerged as a solution, enabling the integration and analysis of diverse data sources within a structured framework. A KG is a network of entities (nodes), connected by relationships (edges) that encapsulates rich information.

We developed BGI-BIO-KG by integrating data from 57 databases, 23 ontologies, and information from 5.5 milion publications. BGI-BIO-KG encompasses 10299720 nodes and 74745798 edges, ensuring a robust and detailed representation of biological entities and interactions. Extensive integration, along with the incorporation of the most up-to-date versions of each database, sets BGI-BIO-KG apart from existing alternatives. Unlike most tools, BGI-BIO-KG is designed with a user-friendly interface that eliminates the need for programming expertise, and ensures that researchers, educators, and clinicians can effortlessly perform queries in common english language.

We demonstrate the application of BGI-BIO-KG in cell annotation analysis of mouse pancreas scRNA-seq samples. We had equal or better performance than other cell annotation tools (ARI score 0.95). Next, by using BGI-BIO-KG for differential gene expression in macrophages, we could easily distinguish known macrophage markers and propose some upregulated genes as novel markers. Currently, we are developing the use of BGI-BIO-KG in variant effect analysis and GWAS by utilizing graph neural networks.

In conclusion, BGI-BIO-KG is a great resource for academic and industry professionals seeking to enhance analysis and deepen understanding of their omics data.

KNOWLEDGE GRAPH, OMICS DATA, BIG DATA, GENOMICS

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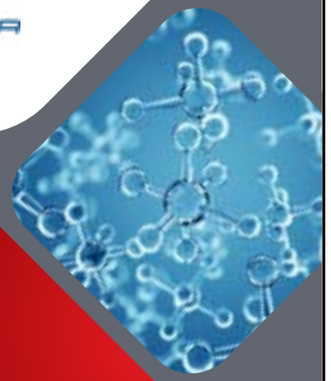

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