BOOK OF ABSTRACTS

6. CONGRESS
OF THE SERBIAN GENETIC SOCIETY

2019 | October 13–17

VRNJAČKA BANJA • SERBIA

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Abstracts of the 6th CONGRESS OF THE SERBIAN GENETIC SOCIETY

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WELCOME TO VI CONGRESS OF THE SERBIAN GENETIC SOCIETY!

Dear colleagues,

Welcome to the 6th 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. Over the past years, the Congress has grown from a national to an international meeting.

The experience of the past meetings motivated our efforts to continue with this series with a clear tendency to strengthen the scientific connections among researchers from different European countries.

The Congress will focus on the most recent advances in genetics and on wide range of topics organized in 9 sessions and two workshops. Many of the presentations will be in lecture-like settings, but we hope that there will also be ample opportunities for informal interaction outside the scheduled sessions.

The successful organization of the Congress has required the talents, dedication and time of many members of the Scientific and Organizing committees and strong support from our sponsors. I hope that you will find the Congress both pleasant and valuable, and also enjoy the cultural and natural beauty of Vrnjacka Banja.

Yours sincerely,

Branka Vasiljevic
President of the Serbian Genetic Society

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Plenary lectures

VI CONGRESS OF THE SERBIAN GENETIC SOCIETY

PL – 01 Plenary lecture

UNTANGLING BIOLOGICAL COMPLEXITY: FROM OMICS DATA TO NEW BIOMEDICAL KNOWLEDGE

Natasa Przulj

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We develop methods for extracting new biomedical knowledge from the wiring patterns of systems-level biomedical omics data. Our new methods uncover the patterns from molecular networks and the multi-scale network organization indicative of biological function, translating the information hidden in the omics data into domain-specific knowledge.
We introduce a versatile data fusion (integration) framework to address key challenges in precision medicine: better patient stratification, prediction of driver genes in cancer, and re-purposing of approved drugs to particular patients and patient groups. Our new methods stem from novel network science approaches coupled with graph-regularized non-negative matrix tri-factorization machine learning methods. We utilize our new methodologies for performing other related tasks, including uncovering new cancer mechanisms and disease re-classification from modern, heterogeneous molecular level data, also inferring new Gene Ontology relationships, and aligning multiple molecular networks.

BIOMEDICAL OMICS DATA, NETWORK SCIENCE METHODS, MACHINE LEARNING, PRECISION MEDICINE

PL – 02 Plenary lecture

GENETIC COUNSELLING IN THE ERA OF GENOMICS MEDICINE

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Medical Genetics is at an exciting transition point partly due to technological advancement. Large genome sequencing projects are being instigated across the world. One example of this in the UK is the 100,000 Genomes Project which has directly impacted on the reorganisation of genomic health care particularly in the laboratory domain. Across Europe as the volume of genomic information and its impact on patients and families is increasing the importance of genetic counselling as an activity is being recognised. In this presentation developments in the UK and Europe will be used to illustrate how genetic counselling as an activity and as a profession is becoming relevant to the whole of genomic health care and all health professionals. This needs a close examination of the action of genetic counselling in order to maximise any benefits to patients and families particularly those with rare inherited Mendelian disorders.

GENOME SEQUENCING, GENETIC COUNSELLING

PL – 03 Plenary lecture

UNDERSTANDING THE ROLE OF SOX STEM CELL GENES IN CANCER AND AGEING

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Ageing and age-related diseases are a major cause of mortality and morbidity. These include neurodegenerative, cancer and frailty. The determinants that influence ageing and the onset of age-associated diseases are multifactorial and recent evidence showed that one of the hallmarks that ageing and cancer share is the activity of adult stem cells.
The members of the SOX family are transcription factors and are well-established regulators of stem cell activity during development, and play relevant roles in adult tissue homeostasis and regeneration. Accumulating ev idence documents that they play important ro les in ageing and age-associated diseases, mainly cancer. In the talk, I will present recent results obtained in the laboratory characterizing the role of Sox2 and Sox9, likely the best-established biomarkers and regulators of stem cell activity, in those processes.

SOX, CANCER, AGEING

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PL – 04 Plenary lecture

SOX GENES: FOR BETTER OR FOR WORSE…

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The SOX genes encode a group of transcription factors that act as key regulators of diverse cellular processes. Members of the SOX gene family show dynamic and diverse expression patterns during development playing important roles ranging from blastocyst formation to differentiation into adult tissues and organs. SOX transcription factors are involved in multiple events from the maintaining of stem cells pluripotency and cell fate decision to driving terminal differentiation of cells into specialized cell types. In addition, during adulthood SOX transcription factors control various physiological processes. Mutations in SOX genes have been associated with severe clinical disorders, while misregulation of their expressions cause a broad range of pathological condition. Accumulating evidence suggests that SOX proteins act as oncogenes and recent evidence points toward pro-proliferative, prosurvival and/or antidifferentiation roles of the SOX proteins.
The results of long-term research of the structure, expression regulation and the function of selected SOX genes will be presented. It will include data obtained by studying the roles of SOX genes in in vitro neural differentiation of pluripotent embryonal carcinoma cells, as well as interaction of SOX transcription factors with signaling pathways active during neurogenesis and oncogenesis. Special focus will be made on ongoing research focused on the roles of SOX genes in promotion of malignant phenotype of cancer cells and maintaining of cancer stem cells. Obtained results provide novel insights into the functions of SOX genes in cancer that will help in designing novels strategies for cancer treatment.

SOX GENES, NEURAL DIFFERENTIATION, SIGNALING PATHWAYS, CANCER STEM CELLS

PL – 05 Plenary lecture

THE EFFECTIVENESS OF COMPREHENSIVE GENOMIC TESTING IN DIAGNOSTICS OF RARE GENOMIC DISEASES

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The phylogenesis of Homo sapiens has been accompanied by numerous challenges along the evolution of health and diseases. By addressing the crucial medical problems, infections and starvation, rare diseases have become one of the leading causes of contemporary morbidity and mortality.
Scientific advances improved the knowledge on rare diseases. About 80% of rare diseases are the result of disruption in the genome. The diagnosis of genetic disorders still remains a challenge, and treatment is still a privilege of the rare. The rare occurrence of genomic rare diseases (RGDs), overlapping of symptoms in different RGDs and the diversity between different diseases are the reasons for “wandering” in the diagnosis. According to the EURORDIS study showed that 40% of people with RGDs get an erroneous diagnosis initially and the average time from early symptoms to confirmatory diagnosis of RGD is about 4.8 years.
RGDs present a major challenge in modern medicine and have been recognized as one of EU’s health priorities. The implementation of emergent technologies and comprehensive genomic testing are imperative in the management of RGDs, but also a great challenge for small countries with professional and financial limitations.
A total number of people suffering from rare diseases in Montenegro is estimated at 35 000, or 350 newborns with RGDs per year. The activities of the Clinical and Medical Genetics Service in Montenegro are focused on RGDs. By combining its own clinical professional resources with the technological capabilities of the diagnostic centers in Europe through intensive international cooperation, the acess to RGDs diagnostics in Montenegro has been improved in past years. Conffirmative diagnosis was achieved in the first act in two thirds of patients (67%), with an average waiting time of less than six months. This lecture presents a model of RGDs management in Montenegro with a review of the most clinically relevant genomic diseases. that have been discovered.

RARE GENOMIC DISEASES IN MONTENEGRO, COMPREHENSIVE GENETIC TESTING

PL – 06 Plenary lecture

PRE-EMPTIVE PHARMACOGENOMICS IN PSYCHIATRY - WHERE DO WE STAND?

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Over the recent years and given the outstanding progress of large scale, high throughput technologies, the field of pharmacogenomics has witnessed formidable and revolutionary research findings. However, the implementation of pharmacogenomics into clinical practice remains limited. During the last few years, there are only a few programs that focus on the implementation of pharmacogenomics into clinical practice, which are mainly carried out in the USA. The Ubiquitous Pharmacogenomics project (U-PGx) is a European, prospective, randomized, controlled clinical study that aims to prove that the pharmacogenomic-guided choice of the drug and the dose will reduce the number and the severity of patients’ adverse drug reactions, by performing pre-emptive genotyping of a panel of clinically relevant pharmacogenomic biomarkers, for which dosing guidelines exist. The upper aim of U-PGx is to demonstrate the clinical utility and importance of pharmacogenomics as well as the reduction of healthcare costs and the improvement of patients’ quality of life.

PHARMACOGENOMICS, IMPLEMENTATION, PSYCHIATRY, COST EFFECTIVENESS, ADVERSE DRUG REACTIONS

PL – 07 Plenary lecture

MARINE ENVIRONMENT AS A SOURCE OF NEW MULTIACTIVITY PIGMENTS AND BIOACTIVE MOLECULES

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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.

NATURAL COMPOUNDS, ANTIMICROBIAL, ANTICANCER

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PL - 08 Plenary lecture

GENETIC PREDICTION OF BIOGEOGRAPHIC ANCESTRY WITHIN EUROPE

Tomasz Grzybowski [1], Marcin Woźniak [1], Boris A. Malyarchuk [2], Miroslava V. Derenko [2], Nataša Kovačević-Grujičić [3], Milena Stevanović [3,4], Krzysztof Rębała [5], Magdalena Spólnicka [6], Wojciech Branicki [6, 7,] Rafał Płoski [8], Magdalena Zubańska [9]

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Over the past decade, there has been growing interest of forensic DNA community in genetic prediction of human biogeographic ancestry. Irrespective of forensic applications, predicting of human ancestry based on DNA analysis might also contribute to better understanding of population history and individual's genealogy. Nevertheless, efficient inferences of ancestry rely upon proper ancestry informative markers (AIMs), capable of differentiating between populations inhabiting the region of interest. This presentation will focus on searching for possible AIMs specific for Europe, with a special emphasis put on the markers relevant to central and eastern parts of the continent. Advantages and disadvantages of prediction based on haploid and autosomal markers will be discussed. A panel of 224 autosomal AIMs, recently developed by the NEXT Consortium and its collaborators will be presented, in terms of their performance in prediction of ancestry within Central and Eastern Europe.

BIOGEOGRAPHIC ANCESTRY, ANCESTRY-INFORMATIVE MARKERS, FORENSICS, POPULATION GENETICS

PL - 09 Plenary lecture

ORIGIN AND EVOLUTION OF VERTEBRATE SEX CHROMOSOMES

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Sexual reproduction remains to be a major paradox of evolutionary biology. Recent progress in genomic biology gives a unique opportunity to address many points concerning sex determination and sex chromosome evolution from the whole genome perspective. Especially intriguing is the fact that such an important and well-coordinated process as sexual development is based on a conserved molecular pathway, but the mechanisms triggering this pathway greatly vary in different lineages. We analyzed evolution of sex chromosomes in different vertebrate groups using the combination of molecular cytogenetic, new generation sequencing and bioinformatics methods. Based on our own and previously published data we propose a hypothesis explaining high level of gonosome conservation in therian mammals. We concentrated on the exceptional mammals with highly unusual sex determination mechanisms, including monotremes and rodents lacking SRY gene. To analyze the diversity of sex determination triggers across various vertebrate lineages we studied representatives of fish, amphibian and reptile species and revealed multiple cases of evolutionary parallelism and convergence, including independent involvement of the same syntenic group in gonosome origin. We postulate a considerable role of segment duplications and repetitive elements in the emergence of novel sex determination triggers.

The study was supported by RSF grant №18-44-04007.

SEX DETERMINATION, TRIGGER GENES, COMPARATIVE GENOMICS, SEX CHROMOSOMES

PL – 10 Plenary lecture

DISCOVERY OF THE HUMAN ZGRF1 DNA REPAIR HELICASE

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The removal of DNA interstrand crosslinks (ICLs) is vital for maintenance of genome integrity. ICL repair is aided by the Fanconi anemia-associated FANCM helicase. In *Saccharomyces cerevisiae* the FANCM orthologue is Mph1. Our lab has previously identified Mte1 (Mph1-associated telomere maintenance protein 1) as a novel interactor of Mph1 in budding yeast. Mte1 stimulates the fork regression and helicase activities of Mph1 while inhibiting Mph1-catalyzed D-loop dissociation. Deletion of MTE1 results in reduced crossover recombination and suppresses the replication stress sensitivity of mph1Δ mutant cells. Mte1-Mph1 is recruited preferentially to telomeres and *mte1*Δ mutants suffer over-extension of telomeres, suggesting that DNA replication stress at telomeres is a substrate for Mte1-Mph1.

ZGRF1 (Zinc Finger GRF-Type Containing 1) is the human orthologue of the Mte1-Mph1 complex. To investigate the roles of ZGRF1, we disrupted the ZGRF1 gene in the HCT116, USO2 and RPE-1 human cell lines using CRISPR-Cas9 genome editing system. ZGRF1-deleted cells exhibit increased sensitivity to the DNA crosslinking agent Mitomycin C (MMC) and the Topoisomerase I inhibitor Camptothecin (CPT). Moreover, treatment with MMC results in increased chromosomal aberrations in the ZGRF1 null cell line, and decreased sister-chromatid exchange is observed following treatment with MMC or CPT. In response to genotoxic stress, the ZGRF1-deleted cells exhibit an increase in co-localization of the FA protein FANCD2 and the DNA double-strand break marker gamma-H2AX, and a failure to protect the nascent strand at stalled replication forks. *In vitro*, purified ZGRF1 exhibits ATP-dependent R- and D-loop displacement activity and 5’ to 3’ helicase activity.

DNA INTERSTRAND CROSSLINK REPAIR, HOMOLOGOUS RECOMBINATION

PL – 11 Plenary lecture

EFFECTS OF THE *Dt2* GENE FOR STEM TERMINATION ON AGRONOMIC AND SEED COMPOSITION TRAITS OF SOYBEAN

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Soybean plant architecture is a key characteristic determining agronomic performance and adaptability to particular environments. Southern (late maturity) soybean cultivars have a determinate stem growth (*dt1/dt1*), in which elongation of the main stem is stopping at the onset of flowering, and a terminal raceme of flowers is developed. In contrast, most northern (early maturity) soybeans have an indeterminate stem growth (*Dt1/Dt1*) with stem elongation and flowering taking place simultaneously producing longer stems with more internodes. Recently, the locus *Dt2* has been characterized causing a terminal flower raceme and semi-determinate stem growth in a *Dt1/Dt1* genetic background. The dominant allele for semi-determinacy (*Dt2*) is a gain-of-function mutation, which might be of relevance to adapt plant architecture particularly in early maturity soybeans. Thus, lines from bi-parental populations segregating for the *Dt2* vs. *dt2* phenotype were evaluated across multiple environments in the east of Austria. Semi-determinate (*Dt2*) stem growth was clearly recognizable at the flowering stage due to the formation of a terminal raceme of flowers. Consequently, semi-determinate stems had larger numbers of pods towards the distal end of the stem but a lower number of total nodes than indeterminate stems. Moreover, semi-determinate stem lines had a 10-16 cm shorter plant height, earlier maturity, lower oil and sucrose content, higher protein content and lower thousand-seed weight than indeterminate lines. Grain yield differences between *Dt2* and *dt2* lines were significant in 4 out of 6 environments, and yield in *Dt2* lines was 5-7% higher than in *dt2* lines. The results indicate drastic effects of the *Dt2/dt2* alleles on shoot development, which has subsequent effects on various other plant characteristics including adaptation of particular environments.

SOYBEAN, *Dt2* STEM TERMINATION, GRAIN YIELD, POD DISTRIBUTION, SEED COMPOSITION

PL – 12 Plenary lecture

GWAS FOR BIOMASS RESPONSES TO WATER WITHHOLDING IN GBS GENOTYPED, FREELY AVAILABLE ACCESSIONS OF MAIZE

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Patterns of seasonal variations in rainfall are changing which affects rain-fed agricultural areas. Water deficit during the early vegetative stages of growth represents a threat as it causes variability in plant development, makes plant susceptible to other stresses and deteriorates the stands. For example, in Osijek, Croatia, located within European Corn Belt, hydrological drought or at least 10-day streak with no rainfall occurred in April when the planting is carried out every of last four consecutive years. Plant's responses to water deficit are reflected in biomass traits which represent the morpho-physiological adjustments of plant to new conditions. The aims of this study were to assess the biomass responses of maize inbred lines with expired plant variety protection (PVP), freely distributed worldwide using the genomewide analysis approach. The 109 maize inbred lines, genotyped by sequencing (GBS), was planted in growth chamber (16/8 day/night, 25°C, 50% RH, 200 µMol/m2/s) in trays with soil in three replicates. Plants in control (C) were watered every two days with, while watering was stopped for 10 days in water withholding (WW) treatment. Fourteen days old plants were harvested and fresh weight (FW), dry weight (DW) and dry matter content (DMC) were measured. Different responses to WW were detected in two genetic subgroups: Stiff Stalk and Non-Stiff Stalk. Large number of QTLs was detected on chromosomes 1, 2, 3, 6, 7 and 9, three of which crossed the Bonferroni threshold and it was shown that genetic regulation of DMC is different than regulation of FW and DW. This was further supported by correlations of rrBLUP marker effects between the traits. Measurements of biomass traits represent fast and reliable indicator of plant's response to water withholding and can be used to effectively screen breeding progenies. Integration of the obtained genetic mapping results into the crop growth models along with other physiological variables will be discussed.

PLANT VARIETY PROTECTION ACT, MAIZE, ASSOCIATION MAPPING, WATER WITHHOLDING

PL – 13 Plenary lecture

HEPATIC 3D I*N VITRO* CELL MODELS FOR GENOTOXICITY ASSESSMENT

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Genetic toxicology plays an essential role within hazard identification and risk assessment during the development of drugs as well as chemicals, cosmetic products, food and feed additives, pesticides, herbicides and others. Throughout the early stage of drug development, a substance's ability to damage DNA through genotoxic mechanisms must be fully investigated. For this purpose, hepatic two-dimensional (2D) cell cultures are used in the first stage of drug development before moving to in vivo experiments. However, 2D models have several limitations as they lack hepatic properties and express low levels of metabolic enzymes. Therefore, it is very important and essential to develop improved *in vitro* cell-based systems that more realistically mimic the in vivo cell behavior and provide more predictive results to *in vivo* conditions. In this respect, three-dimensional (3D) cell culture systems have gained increasing interest in drug discovery and tissue engineering due to their evident advantages in providing more physiologically relevant information and more predictive data for *in vivo* tests compared to 2D cultures. The 3D models have improved cell-cell and cell-matrix interactions and have preserved complex *in vivo* cell phenotypes. Moreover, 3D hepatic models exhibit higher level of liver-specific functions including metabolic enzymes compared to 2D models. Thus, tremendous effort has been put into the development of a variety of 3D models, which hold the promise for applications in drug discovery, cancer cell biology, stem cell research, safety studies and many other cell-based analyses by bridging the traditional 2D monolayer cell cultures and whole-animal systems. The presentation will give an overview of different hepatic 3D cell cultures and their potential application in genetic toxicology, which will be supported by the *in vitro* data obtained on hepatic 3D cell models by application of classical genotoxicity endpoints combined with the toxicogenomic analyses.

HEPATIC 3D CELL MODEL, GENOTOXICITY

PL – 14 Plenary lecture

THE GENETIC BASIS OF CLINAL ADAPTATION IN *DROSOPHILA*

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One of the central goals of evolutionary genetics is to understand how organisms adapt to environmental heterogeneity. A promising approach towards this end is to investigate systematic, gradual phenotypic and genotypic changes along environmental (e.g. climatic) gradients, so-called clines, which are thought to be driven by spatially varying selection. Over the past 7 years we have been using next-generation sequencing, population genetics and laboratory assays to identify and characterize candidate genes and polymorphisms that might contribute to variation in fitness-related (life-history) traits among populations of the vinegar fly, *Drosophila melanogaster*, situated along a latitudinal cline spanning the North American east coast. Our genomic and phenotypic analyses suggest that spatially varying selection i s pervasive a nd a cts o n numerous l oci a nd pathways, w ith many candidates implicated in the physiological regulation of life-history, for example in the insulin / insulin-like growth factor signaling pathway, and exhibiting parallel differentiation along the parallel cline along the Australian east coast. In my talk, I will focus on our recent work on two clinal polymorphisms, namely a large chromosomal inversion polymorphism that harbors an excess of clinal SNPs and a clinally varying allele in the insulin signaling transcription factor foxo. In both cases we have experimental evidence that these polymorphisms make a causative contribution to the observed phenotypic clines of several fitness components.

ADAPTATION, CLINES, LIFE HISTORY, INVERSIONS, INSULIN SIGNALING

PL – 15 Plenary lecture

MOLECULAR SIGNATURES OF CLIMATE ADAPTATION AND RANGE EXPANSIONS IN MEDITERRANEAN CONIFERS

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Understanding the interaction among environments, demography and evolution is essential in the face of impending climate change. Species from the Mediterranean Basin, inhabiting highly heterogeneous environments, are particularly at risk because of the predicted increase in aridity and recent land-use change. In this talk, we review our studies on population genomics and association genetics in maritime and Aleppo pines, two relevant components of Mediterranean landscapes. In maritime pine (*Pinus pinaster* Aiton), 17 SNPs (Single Nucleotide Polymorphisms) were found to be strongly correlated with climate, once population genetic structure was removed from environmental association models. The utility of these SNPs to predict climate maladaptation of forest stands was further tested in a common garden. Furthermore, some of these loci were correlated with both fire-related and drought traits using association genetic approaches. In Aleppo pine (*Pinus halepensis* Miller), we are studying population genetic signatures of range expansions, from refugia in Turkey and Greece towards the large western Mediterranean part of the distribution. This species showed signatures of selection in expanding populations based on drought-response candidate genes. However, new microsatellite and SNP data showed signals of recurrent bottlenecks in the colonized range and 'gene surfing' in the expanding wage of colonization appears now as a reasonable alternative explanation. These studies at large spatial scales are accompanied by research at local scales aiming at detecting the role of micro-environmental variation in creating and maintaining genetic diversity within populations. The combination of approaches and spatial scales provides an integrated view to understand the quantitative genetic and molecular mechanisms responsible for adaptation as well as the drivers of selection (both climatic and ecological) in Mediterranean conifers.

CLIMATE CHANGE, GENETIC ADAPTATION, RANGE EXPANSION, CANDIDATE GENES, MEDITERRANEAN CONIFERS

PL – 16 Plenary lecture

THE PATHOBIOMES OF TWO BACTERIAL DISEASES OF RICE; IDENTIFYING MICROBIAL COOPERATORS AND ANTAGONISTS OF THE PATHOGEN

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Just like what occurs in humans, plants have been recently recognized as meta-organisms possessing a distinct microbiome. Plant health is thought to heavily depend also on its microbiome and signaling among microorganisms is crucial for the establishment of the microbial community. Understanding how bacteria undergo interspecies and interkingdom signaling in the microbiome will be a big challenge for future studies. We are studying the plant microbiome at the site of infection of these two rice diseases as this could reveal potential commensal/resident bacteria that can communicate and cooperate with the pathogen: the concept of monostrain/monospecies infections is changing as different studies are beginning to indicate interactions between pathogens and the residential microbiota. In addition, comparing rice microbiomes of healthy and infected rice from the same location could provide insight on the potential role of microbiome in plant resistance towards bacterial pathogens increasing the opportunity to isolate and characterize natural biocontrol agents against the pathogens considered. These microbiome approaches will provide avenues for the design of microbial solutions for a more sustainable agriculture.

MICROBIOME, SIGNALLING, BACTERIA

PL – 17 Plenary lecture

PHEROMONE INDUCED CELL CYCLE ARREST IN *USTILAGO MAYDIS*

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Cell cycle synchronization before cell fusion during mating in simple eukaryotes used to occur by arresting the cells at G1. However, in the phytopathogenic fungus *Ustilago maydis* the arrest takes place at G2. This exception is linked to the distinct developmental steps that mating triggers in this fungus, which final outcome is the infection of corn plants. Here we describe that activation of the pheromone response cascade in *U. maydis* resulted in inhibition of the nuclear transport of Cdc25 by targeting its importin, Kap123. This cytoplasmic retention of Cdc25 leads to its degradation, which seems important to ensure the maintenance of the G2 cell cycle arrest once cell fusion takes place, leading to the formation of the infective filament. In this way, the pre-mating cell cycle arrest is linked to the subsequent steps required for the proper establishment of the infection. Disabling this connection resulted in the inability of the cells to infect corn plants.

FUNGAL PATHOGENICITY, *USTILAGO MAYDIS*, CELL CYCLE, PHEROMONE RESPONSE

PL – 18 Plenary lecture

FROM PHYLOGENETICS TO PHYLOGENOMICS, BETWEEN BACTERIAL STRAINS AND THE TREE OF LIFE

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With the amount and velocity of genomic data currently available, it became clear that no single gene, or even carefully selected gene sets, can provide a single evolutionary tree with confidence. We can therefore appreciate that apparently conflicting phylogenetic signals are not noise, but in fact reflect real biological phenomena — like duplications, lateral transfer, or ancestral polymorphisms. Phylogenomic models explicitly recognise this variation in evolutionary histories across the genome, allowing us to make most use of sequenced genomes. In this talk we will have an overview of popular models, as well as of emerging methodologies that can handle whole genomes from hundreds or thousands of samples. We will also discuss favorite approaches in different fields, where the genomic sets may resemble closer a “tall” (fewer information from many samples) or a “wide” (fewer samples, with more information) data category.

PHYLOGENOMICS, SUPERTREE, GENE FAMILIES, ALIGNMENT, TREE OF LIFE

PL – 19 Plenary - Closing lecture

EXPLORING THE MECHANISMS OF HAEMATOPOIETIC LINEAGE PROGRESSION AT THE SINGLE-CELL LEVEL

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The haematopoietic stem cell (HSC) is responsible for generating more than 10 different blood cell types. This diversity in the lineage output of HSCs is traditionally presented as a stepwise progression of distinct populations of cells along a hierarchical differentiation tree. However, the data used to explain the molecular basis of lineage differentiation were derived from populations of cells isolated based on cell surface markers. An inherent problem with this approach is that the presence of specific cell surface markers does not directly reflect the transcriptional state of a cell. Here we used a marker-free approach to computationally reconstruct the blood lineage tree in zebrafish and order cells along their differentiation trajectory, based on their global transcriptional differences. Within the population of transcriptionally similar cells our analysis revealed considerable cell-to-cell differences in their probability to transition to another, committed state. This suggested that although global transcriptional changes before and after the branching point were continuous, the probability of a cell progressing to any of the committed states was determined only by a subset of highly relevant genes. Once cell fate decision was executed, the progression of cells along the continuum is characterised by suppression of genes involved in ribosomal biogenesis and increased expression of lineage specific genes. I will also discuss our more recent work on human foetal blood development. We characterised the mutational profile and clonal dynamics of human foetal HSCs, by performing whole genome sequencing on 271 single HSC-derived colonies from a 18 week-post-conception human foetus. Initial analyses showed that human foetal HSPCs acquire on average 38 mutations and that there is no significant clustering of cell types and organ of origin. Thus, there is a high level of intermixing between the different haematopoietic organs during development.

HAEMATOPOIESIS, STEM CELLS, SINGLE-CELL RNA-SEQ

SESSION 1

Human omics variation

VI CONGRESS OF THE SERBIAN GENETIC SOCIETY

01 – 01 Invited lecture

NEXT-GENERATION GENOMICS OF RARE DISEASES: ON THE ROAD TO NEW DIAGNOSTICS AND INNOVATIVE THERAPIES

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Majority of rare diseases (RD) are genetic diseases (80%). Therefore, the identification of specific gene defect in each patient is important. Next generation sequencing (NGS) methodology has enabled shortening the diagnostic odyssey of patients with RD, leading to improved treatment and successful genetic counseling.
We have analyzed over 200 RD patients using Clinical-Exome Sequencing TruSightOne Gene Panel (Illumina). VariantStudio and various in silico software tools were used for bioinformatics analysis. Filtration and prioritization of variants were performed according to "in-house" designed pipelines and virtual gene panels.
NGS studies enabled diagnosis of more than 50 different diseases (hematological, metabolic, endocrinological, pulmonary, immunological, orthopedic, dermatological, ophthalmological, cardiological, epileptic encephalopathies etc.). It was particularly important for genetically heterogeneous diseases, such as glycogen storage diseases, branched-chain organic acidurias, primary ciliary dyskinesia, MODY or mitochondriopathies. Moreover, different diseases with overlapping clinical manifestations were accurately diagnosed. In our studies mutation detection rate reached 80-100%. Our work enabled the establishment of RD biobank collections containing DNA, RNA, mononuclear cells and tissue samples from over 2000 patients affected with RD. Furthermore, novel variants in DNAI1, MUT, PAH, PCCB, SLC37A4, SPAG16 and SPAG17 genes were functionally characterized in adequate in vitro systems such as immortalized patients' fibroblasts or Crisper/Cas9 edited cell lines.
Also, we used TruSeq-Amplicon Cancer Panel to analyze different childhood and adult rare hematological malignancies. Our association studies revealed new diagnostic, prognostic and pharmacogenomic markers, resulting in recommendations for personalized therapeutic modalities in accordance with genomic profile of the patients.

RARE DISEASES, NEXT GENERATION SEQUENCING, PERSONALIZED MEDICINE

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01 – 02 Invited lecture

COMPLEXITIES OF VARIANT ANNOTATION USING NGS FOR DETECTING HEREDITARY BREAST AND OVARIAN CANCER PREDISPOSITION

Ana Krivokuca

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Besides many challenges that exist in determining an individual's genome or exome in the NGS era, downstream interpretation of genetic variations and their association with diverse molecular and disease phenotypes have become more challenging than ever. Variant annotation is the process of assigning functional information to sequence variants. There are many types of information that could be associated with variants such as level of sequence conservation, the effect on the protein structure and function or clinical importance of the variant. From the clinical perspective the most fundamental level of variant annotation is categorizing each variant based on its effect on the specific disease. Complex computational approaches that integrate annotations using structure and functional interactions should be implemented for the rapid and reliable interpretation of the coding sequence variations. Bioinformatics market nowadays presents a growing number of tools for the prediction, annotation and visualization of genetic variations. Despite the availability of variant prioritization tools, identifying clinically actionable variants remains a challenge. Complex bioinformatics structure is needed for: organizing separate databases for different types of data, the use of remote servers to store and access data and software programs and organization of infrastructure for data sharing and security of genomic data. Integrated approach that combines multiple tools for the prediction, annotation and visualization of functional variants should be implemented. Another major challenge is the lack of standards for generating NGS data bioinformatics processing, data storage, and clinical decision support. The demands in the clinical genetics are even higher since adequate variant interpretation provides deeper insights into the genetic bases of familial diseases and a better understanding of the biological processes underlying disease phenotypes such as cancers.

CLINICAL SIGNIFICANCE, HEREDITARY CANCER, NGS, VARIANT ANNOTATION

01 – 03 Invited lecture

NEXT GENERATON SEQUENCING IN NEURODEGENERATIVE DISORDERS: OUR EXPERIENCE

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Next generation sequencing (NGS) transforms today's biomedicine allowing huge progress in each field of human and medical genetics. Different NGS strategies could be used for whole genome or whole exome sequencing, or for analysis of selected gene panels such as "clinical exome" panel. This versatile panel contains coding regions of about 5000 clinically relevant genes and could be used in testing of neurological diseases too. Neurodegeneration is an umbrella term for a range of conditions which primarily affect the neurons in the human brain and could have different causes and phenotypic expression. We used "clinical exome" panel on Illumina MiSeq NGS platform for genetic testing of 30 unrelated cases with different neurodegenerative disorders. Bioinformatic analysis was done by Illumina's Variant Studio and other software tools. In 6 cases we have detected disease causing mutations, all confirmed by Sanger sequencing. Affected genes and associated clinical phenotypes were: PSEN1 / early onset Alzheimer disease, SETX / spinocerebellar ataxia, PANK2 / panthotenate kinase associated neurodegeneration, TUBB4 / dystonia, DCTN1 / cerebellar degeneration, PDGFB / Fahr syndrome. In one case with complex phenotype we expected POLG variant responsible for Parkinsonism, but only MYH14 mutation associated with distal myopathy was found. In the remaining 23 cases "clinical exome" analysis did not help in detecting the cause of disease. Mutation detection rate in our group of patients is within hoped-for range and could be improved by more stringent selection criteria. Our results confirm advantages and limitations of NGS methodology and "clinical exome" panel in genetic testing of neurodegenerative diseases.

NGS, NEURODEGENERATIVE DISORDERS, CLINICAL EXOME

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01 – 04 Invited lecture

GENOMICS-BASED CLINICAL DISCOVERIES: A 5-YEAR EXPERIENCE IN A TERTIARY PEDIATRIC REFERRAL CENTRE

Goran Čuturilo [1, 2], Jelena Ruml-Stojanović [1], Marija Mijović [1], Aleksandra Miletić [1], Brankica Dimitrijević [1], Hristina Janeski [1]

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Recently, new technologies have made it possible to obtain a huge number of molecular data for each patient. Genomics aims to characterize as many genes as possible. There are many motivations for conducting (gen)omics research. In medicine, one common reason is to establish diagnosis, and, ultimately, to obtain a comprehensive understanding of a gene function and related disorders.
Clinical genetic service at the University Children's Hospital started to employ modern techniques for wide genome/exome assessment from November 2014. For multiple-gene assessment we started with solo (patient only) Mendeliome sequencing, followed by subsequent introducing of solo whole-exome sequencing, trio (patient + parents) whole-exome sequencing, and finally genome-sequencing. In terms of chromosomes, traditional and low-resolution analysis of a karyotype has been replaced with chromosomal microarray techniques.
We present the data for pediatric and adult patients who were first assessed in our genetic outpatient clinic, and then referred for diagnostic Mendeliome/exome/genome sequencing, from November 2014 to September 2019. Wide variety of disease categories are included. We will present distribution of frequencies and diagnostic rates, as well as impact on diagnosis for different disease categories. We will also present discovery of new genes implicated in occurrence of known disorders (e.g. RRAS2 and Noonan syndrome, TUBGCP5 and primary microcephaly, DNAH9 and laterality defects) and results of additional characterization of known causative genes (e.g. PTPN11, RAF1, PITX2). In addition we will present first results of molecular characterization of several groups of genetic disorders, such as epileptic encephalopathies, skeletal dysplasia, intellectual disability and congenital heart disorders, conducted by professionals from our Centre.

GENOMICS, PATIENTS, EXOME SEQUENCING, GENOME SEQUENCING, GENE CHARACTERIZATION

01 – 05 Oral

MOLECULAR PROFILING OF COLORECTAL CANCER USING NEXT GENERATION SEQUENCING

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Cancer is a dynamic disease and during the development of which is expected that every tumor has a different molecular signature. Tumor heterogeneity leads to drug resistance and therefore detection of these mutations is essential for development of effective therapies. Next Generation Sequencing (NGS) is establishing itself as currently a most potent instrumental method of analysis for this, as it has the power of whole genome sequencing, whole exome sequencing and cDNA (on a RNA template) sequencing, it can separate germline from somatic mutations and has high sensitivity in detecting low frequency heterogenic mutations. Targeted NGS panels cover clinically significant hotspots and are much less time consuming and cost effective than PCR-based techniques. Additionally, NGS can be performed on a small amount of DNA or even fragmented DNA which is very important since pathologist have to work with formaldehyde fixation, small sized samples and variable tumor cell content. For this study 36 samples of colorectal adenocarcinoma were obtained from different medical centers and analyzed by multiparallel sequencing. Ion AmpliSeq™ Colon and Lung Cancer Research Panel v2, which was used in this study, allows detection of hotspot mutations on 22 genes in a single reaction.
This study has an aim to report somatic mutations that have correlation with known therapeutics and prognosis as well as novel mutations and mutations reported only in literature. Furthermore, it is further hoped to escalate this study with clinical feedback. Threshold for mutation reporting has been set at 3%. The NGS analysis revealed 23 unique mutations in 36 colorectal carcinoma patients.

NGS, COLORECTAL CANCER, TARGETED THERAPY

01 – 06 Oral

IMPORTANCE OF NEXT GENERATION SEQUENCING FOR DETECTION OF NOVEL PHARMACOGENOMIC MARKERS IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood. Therapy causes side effects in 75% of patients and 1-3% of patients have a lethal outcome as a consequence of treatment. Since treatment improvements of pediatric ALL have not been achieved by introduction of novel drugs, but by avoiding the adverse effects of the drugs, pharmacogenomics has become essential in pediatric ALL treatment. A leukemia patient has two genomes: constitutional and leukemia genome. Variants in the constitutional genome are responsible for the efficacy of the drugs and the side effects, while somatic mutations in leukemia clone are responsible for the resistance to drugs. Next generation sequencing (NGS) has made breakthroughs in pharmacogenomics by discovering novel genetic markers which could be candidates for targeted therapy and predictors of efficacy and toxicity of drugs.
We used NGS analysis to discover novel potential pharmacogenomic markers in pediatric ALL. DNA samples from bone marrow of 17 pediatric ALL patients were analyzed for somatic mutations using NGS TruSeq Amplicon–Cancer Panel (Ilumina). DNA samples from blood of 100 individuals were analyzed for germinative mutations using the NGS platform TruSightOne (Ilumina). For prediction of effects of variants, we used software tools SIFT, PolyPhen-2 and PROVEAN. For protein modeling we used STRUM method and i-TASSER server.
In the NGS study of somatic mutations in pediatric ALL, 9 novel variants have been identified. Bioinformatic analysis has shown that STK11 c.1023G>T and ERBB2 c.2341C>T are candidates for molecular targeted therapy. In the exome sequencing study, according to the in-house virtual panel for GC response markers and the prediction algorithms, 3 new potential markers in pharmacogenes related to GC response have been identified, ABCB1 c.947A>G, NCOA3 rs138733364 and TBX21 rs14059812. Pharmacogenenomic profiling of each pediatric ALL patient is indispensable for efficient therapy design.

ACUTE LYMPHOBLASTIC LEUKEMIA, PEDIATRIC, PHARMACOGENOMICS, NEXT GENERATION SEQUENCING

01 – 07 Oral

GENOMIC PROFILING AS A TOOL FOR DIFFERENTIAL DIAGNOSIS OF PATIENTS WITH PEDIATRIC LUNG DISEASES AND DISCOVERY OF NOVEL DISEASE-CAUSING GENES AND GENETIC VARIANTS

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Primary ciliary dyskinesia (PCD) is a rare disorder that affects lungs, reproductive organs and the internal organs laterality. The disease is inherited in autosomal recessive or X-linked manner. PCD is clinically and genetically heterogeneous disorder with overlapping symptoms with other pediatric lung diseases. The aim of the study was genomic profiling of suspected PCD patients in order to establish the genetic background of PCD in our patients, to confirm clinical diagnosis, and to design a strategy for differential diagnosis of PCD patients among patients with similar clinical presentation.
Using Clinical-Exome Sequencing Panel, we analysed 93 genes related to PCD and other pediatric lung diseases in a cohort of 21 Serbian patients with clinically suspected PCD.
Analysis of obtained results revealed genetic variants in CCDC39, CCDC40, DNAI1, DNAL1, DNAH5, DNAH11 and LRRC6 genes, and pointed and confirmed SPAG16 and SPAG17 as novel PCD disease causing genes. Twenty variants in these genes were pathogenic, of which fourteen (70 %) were novel. The PCD diagnosis was established in 54.55 % of patients. Analysis of genes related to individual symptoms of PCD, revealed 6 pathogenic variants in ABCA3, CFTR, MUC2, SCNN1A, and SLC26A9 genes, of which 5 (83.33%) were novel. This enabled the diagnosis for additional 28.57% patients.
The analysis of extended list of genes enables mutation detection rate of 95.45% (21/22 patients), while the rate of established diagnosis reached 81.82% (18/22 patients).

This work was funded by the MESTD, Republic of Serbia (grant no. III 41004).

PCD, NGS, GENOMIC STRATEGY, NOVEL GENES AND GENETIC VARIANTS

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01 – 08 Oral

GENOMIC PROFILING OF GLYCOGEN STORAGE DISEASES: FROM NGS METHOD TO THE CRISPR/CAS9 TECHNOLOGY

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Glycogen storage diseases (GSD) are inherited disorders of glycogen synthesis or degradation, which primarily affect the liver, kidneys and intestinal mucosa. Since the consequences of these disorders are serious and irreversible, molecular-genetic testing is essential for precise diagnosis and optimal medical treatment. We analyzed 41 patients with clinical suspicion of GSD and 75 control subjects from Serbia using Sanger and next-generation sequencing (NGS). Pathogenicity of novel variants was determined based on expressional, computational analysis and patient's phenotype. The genomic profiling of analyzed patients revealed 5 patients with GSD Ia and 31 patients with GSD Ib. Using the NGS method we identified patients with GSD III, VI, IXa, cholesteryl ester storage disease and Shwachman-Diamond syndrome. In SLC37A4 gene of GSD Ib patients we detected 4 novel variants: p.Gly83Glu, p.Gly135Asp, p.Pro191Ser and p.Ser263Glyfs*33. The CRISPR/Cas9 knockin method was used to introduce p.Gly83Glu variant in SLC37A4 gene of HEK293 cell line in order to establish the new in vitro model system for the GSD Ib and to functionaly characterize this variant. Computational, expression analysis and clinical presentation in patients confirmed the pathogenic effect of the novel variants (p.Gly83Glu and p.Ser263Glyfs*33) in SLC37A4 gene. Comprehensive molecular-genetic analysis of patients with clinical suspicion of GSD from Serbia achieved 100% mutation detection rate, allowing early application of proper therapy specific to the patient's genotype. The results of this work supported the usefulness of NGS for correct diagnostics of GSD and differential diagnosis in patients with overlapping phenotypes. Furthermore, we established a novel human kidney cell model for GSD Ib which lacks SLC37A4 expression and therefore could be a useful tool to study the pathogenesis of the GSD Ib and to test the molecular therapeutics designed to correct metabolic abnormalities related to the GSD Ib.

GLYCOGEN STORAGE DISEASES, NGS, CRISPR/CAS9 GENE KNOCKIN, SLC37A4

01 – 09 Poster

PPARγ rs3856806 POLYMORPHISM AND ITS ASSOCIATION WITH BMI, FASTING GLUCOSE LEVELS AND LIPID PROFILE IN SERBIAN ADOLESCENTS

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Background: Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear hormone receptor and ligand-activated transcription factor. PPARγ gene is mainly expressed in adipose tissue where it has a key role in regulation of adipocyte differentiation, lipid metabolism, regulation of insulin resistance and blood glucose levels. Studies have shown that polymorphism within PPARγ gene rs3856806 (C161T, His447His), may be associated with obesity, insulin sensitivity, type 2 diabetes and dyslipidemia. The objective of the study was to investigate the association of rs3856806 with body mass index (BMI), fasting glucose levels and lipid profile in Serbian adolescents.
Methods: The study included 274 randomly selected healthy adolescents of both genders, 139 (50.36%) boys and 137 (49.64%) girls, 14-15 years of age. Data including age, gender, height, weight, lipid profile and fasting glucose were recorded. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (RFLP) method.
Results: No association of this polymorphism was found with BMI and lipid profile. Also, results did not reach statistically significant difference in terms of fasting glucose level on the whole sample size, p=0.066. However, when divided by gender, statistically significant difference was observed among girls p=0.013. Carriers of T allele had significantly lower fasting glucose levels comparing to carriers of CC genotype. To confirm these results multiple linear regression analysis was performed, using BMI as covariate and statistically significant difference was confirmed among girls (β=-0.185, p=0.033).
Conclusion: This polymorphism could be associated with fasting glucose level among girls, thus further research with larger sample size would be of great interest to validate these results.

PPARγ, rs3856806, LIPID PROFILE, BMI, FASTING GLUCOSE LEVEL

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01 – 10 Poster

MATURITY ONSET DIABETES OF THE YOUNG (MODY): THE IMPORTANCE OF COMBINED NGS AND MLPA GENETIC TESTING

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Maturity onset diabetes of the young (MODY) is a rare form of diabetes characterized by an onset of hyperglycaemia before 25 years of age, autosomal dominant inheritance and in some cases insulin independence. MODY is caused by changes in one of the genes important for function, regulation and development of pancreatic β-cells, glucose sensing or interaction with insulin. Thus far, variants of 13 genes, most frequently HNF1A, GCK, HNF4A and HNF1B, have been associated with 13 different MODY subtypes. Being clinically and genetically heterogeneous, MODY is often misdiagnosed as type 1 or type 2 diabetes, leading to inadequate therapy.
The aim of this study was to genetically characterize clinically suspected MODY patients, evaluate the relative frequency of MODY subtypes and to determine the type of variants in MODY genes of Serbian pediatric patients.
Twenty-nine unrelated pediatric patients were analyzed using TruSight One panel for next-generation sequencing (NGS) and multiplex ligation-dependent probe amplification (MLPA) assay. Usage of these two genetic tests enabled detection of both single nucleotide variants and large deletions, characteristic for some MODY subtypes.
Variants in MODY genes were identified in 22 out of 29 patients (75.9%). Most of these variants were located in the GCK gene, followed by variants in HNF1B. The rest of the variants were found in the NEUROD1 and HNF1A genes. We identified one novel variant in the GCK gene: c.596T>C, p.Val199Ala. The applied genetic tests excluded the suspected diagnosis of MODY in two patients and revealed variants in other genes possibly associated with the patient's clinical phenotype.
The combined NGS and MLPA-based genetic tests used in this study present a comprehensive approach for genetic characterization of patients with suspected MODY diabetes and provide a successful differential diagnosis of MODY subtypes, important for the right therapy, prognosis, and genetic counseling.

MODY, NGS, MLPA, DIFFERENTIAL DIAGNOSIS

01 – 11 Poster

THE ASSOCIATION OF LEPTIN (LEP) rs7799039 VARIANT WITH LEP mRNA EXPRESSION IN RELAPSING-REMITTING MULTIPLE SCLEROSIS

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Introduction: Leptin (LEP), a cytokine-like hormone, acts in the pathogenesis of multiple sclerosis (MS) by disturbing Th1/Th2 balance. By affecting the transcription of LEP, LEP gene variant rs7799039 may be involved in MS. Therefore, in this study, we have investigated the association of LEP rs7799039 variant with LEP mRNA levels in peripheral blood mononuclear cells (PBMC) of patients with relapsing-remitting MS (RRMS) and controls.
Material and methods: The study included 61 RRMS patients (age (mean±std.dev.)=40,1±8,4 years, sex ratio (female/male)=1,2) and 60 control subjects (age (mean±std.dev.)=37,3±8,7 years, sex ratio (female/male)=1,4). Whole blood samples were collected for genomic DNA extraction, while total RNA was extracted from PBMC samples. Genotyping and relative gene expression analysis were performed using TaqMan® technology.
Results: There was no significant difference in frequency of rs7799039 A rare allele between MS patients and controls (44,9% and 42,7% in patients and controls, respectively). Patients had significantly higher LEP mRNA levels compared to controls (fold change=1,4, Mann-Whitney U test, p=0,01). Among patients, carriers of the rare rs7799039 A allele had significantly higher LEP mRNA expression than GG homozygote (fold change=1,3, Mann-Whitney U test, by dominant model (AA+GA vs. GG), p=0,01). The association remained significant after correction for age and gender (OR=2,3, 95%CI=1,2-4,4, p=0,01). In controls, there was no significant difference in LEP mRNA levels with regard to rs7799039 genotypes.
Conclusions: The present study indicates a significant role of LEP in RRMS pathophisiology. The LEP mRNA expression was associated with RRMS and affected by LEP rs7799039, leading to its increase in carriers of rare allele. Validation of the current findings needs to be done in a larger study group.

LEPTIN, MULTIPLE SCLEROSIS, EXPRESSION, GENE VARIANT

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01 – 12 Poster

THE EFFECTS OF ApoB Thr71Ile AND ApoE 112/158 GENE POLYMORPHISMS ON PARAMETERS OF LIPID METABOLISM IN THE SERBIAN OVERWEIGHT AND OBESE ADOLESCENTS

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Genetics plays an important role in pathogenesis of the obesity, along with behavioral and environmental factors. Polymorphisms of apolipoprotein B (apoB) and apolipoprotein E (apoE) genes could have impact on serum lipid profile. Aim of this study was to determine the effect of apoB Thr71Ile and apoE 112/158 polymorphisms on serum lipid levels in overweight and obese adolescents. 91 overweight and obese adolescents were genotyped for selected polymorphisms using PCR and RFLP analysis. We formed three groups for apoE genotype: E2 (e2/e2 and e2/e3), E3 (e3/e3) and E4 (e3/e4 and e4/e4). Serum lipid levels (total cholesterol - TH, LDL, HDL, triglycerides) and apolipoproteins (apo A-I, apo A-II and apoB) were also determined. There were no statistically significant differences between apoB Thr71Ile genotype groups and TH, HDL, LDL and triglyceride levels in adolescents. Boys with rare apoB Thr71Ile TT genotype had significantly higher triglyceride levels (p=0.017). Adolescents with CC genotype had significantly lower apoA-II (p=0.004). Multiple regression analysis confirmed statistically significant influence apoB Thr71Ile genotype on apoA-II levels with gender as covariate. Statistically significant differences between three apoE genotype groups and TH, HDL, LDL and triglyceride levels were not observed. TH was significantly higher in boys with E2 compared to E3 and E4 genotypes (p=0.013). Girls with E4 genotype had trend to have higher TH (p=0.057). Adolescents carrying E4 allele had higher apoB levels (p=0.049). Also, girls carrying E4 allele had the highest values of apoA-II (p=0.051) and apoB (p=0.033). Boys with E2 allele had statistically higher apoA-I levels (p=0.011). Statistically significant influence apoE genotype on apoA-I, apoA-II and apoB levels was not confirmed by multiple regression analysis with gender as covariate. Our results suggest that apoB and apoE genotypes may influence lipid metabolism in overweight and obese adolescents.

ApoB Thr71Ile GENE POLYMORPHISM, ApoE 112/158 GENE POLYMORPHISM, OBESITY, LIPID METABOLISM

SESSION 2

Medical genetics

VI CONGRESS OF THE SERBIAN GENETIC SOCIETY

02 – 01 Invited lecture

INTERACTIONS OF GENETIC AND ENVIRONMETAL FACTORS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Chronic obstructive pulmonary disease (COPD) is featured by abnormal inflammatory response of the lungs to toxic particles or gases, such as cigarette smoke. High heterogeneity and complex etiology of the disease have made the genetic components and the mechanisms of COPD pathogenesis difficult to unravel. Although recent advances in genetic technologies have enabled revelation of various genetic factors as a cause of the disease, a huge portion of estimated COPD heritability is still unknown. However, the ”missing heritability” of COPD is thought to be hidden in unidentified interactions of genetic and environmental factors. The aim of our research was to identify interactions between genes involved in xenobiotic metabolism, antioxidative defense, matrix remodeling, inflammation and vascular function, tagged by functional variants, and cigarette smoking in COPD using a case-control model. Functional effects of gene-gene and gene-environment interactions were assessed by measuring the levels of marker of systemic oxidative stress urinary 8-oxo-7,8-dihydro-2’-deoxyguanosine (8-oxodG), the relation with biochemical pathways or relevant cell culture model. We identified interactions of GSTM1-GSTP1 ($p=0.029$) and CYP1A1-GSTM1-EPHX ($p=0.025$) in COPD and cumulative effect of risk variants on the levels of 8-oxodG ($p=0.026$) in patients. Further, interactions of eNOS-ACE ($p=0.041$) and eNOS-smoking ($p=0.013$) are revealed in COPD and related to protective markers e.g. caveolin. Furthermore, gene-environment interaction of MMP9-smoking is detected in COPD occurrence ($p=0.005$) and severity ($p=0.001$), showing higher expression of MMP9 risk variant in response to cigarette smoke extract ($p<0.05$) using macrophage cell line. Our research is the first to reveal several interactions of genetic and environmental components as a possible cause of lung damage contributing to “missing heritability” of COPD and ultimately improving our understanding of the mechanisms of COPD pathogenesis.

COPD, COMPLEX DISEASE, GENE-GENE INTERACTION, GENE-ENVIRONMENT INTERACTION

02 – 02 Invited lecture

NEW INSIGHTS INTO REGULATORY GENETIC MAPS IN COMPLEX DISEASES: FROM ‘GENE DESERTS’ TO SUPER-ENHANCERS

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Recent progress in the understanding of genetic architecture of complex diseases, e.g. cardiometabolic and immune diseases, has been made by application of integration strategies. Large scale studies, encompassing more than 100000 individuals, imputation and bioinformatic approaches, as well as expression quantitative trait loci (eQTL) analyses have been applied to detect novel genetic variants in order to improve estimation of complex diseases heritability. Up to date, approximately 28% of heritability in coronary artery disease has been explained using the genome-wide association approach. Majority of novel detected genetic loci are in the non-coding DNA regions, thus the efforts toward identification of their role are highlighted in the current research. Herein we will discuss the mapping strategies, algorithms and data-bases, which were used to identify potential regulatory role of genetic variants, predominantly non-coding variants. We will focus on the mapping of regulatory elements, such as enhancers and super-enhancers that are enriched by the risk variants for certain complex diseases. Such variants frequently perturb transcription factor binding and regulate distant gene expression, due to three-dimensional genome organization. Distinguishing the causal disease variants from others being in high linkage disequilibrium with the causal ones is a step forward toward an invaluable precision medicine approach. The population of Serbia is usually underrepresented in the large-scale genetic studies thus, we analyzed several proposed regulatory/causal genetic variants and expression of corresponding genes in patients and controls from Serbia. The abovementioned approaches and strategies will be described guided by the analysis of investigated set of genetic variants in 9p21 locus, and PHACTR, IL2-RA, IKZF3 and IFI30 genes.

GENETIC VARIANTS, REGULATORY, COMPLEX DISEASE, HUMAN

02 – 03 Invited lecture

CLINICAL AND GENETIC ASPECTS OF OVERGROWTH SPECTRUM SYNDROMES

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Overgrowth spectrum syndromes are heterogeneous group of diseases that are characterized by excessive tissue development with manifestations often overlapping each other. It could be generalized or segmental, with prenatal or postnatal onset, with or without intelectual impairment, hereditary or non-hereditary condition, associated or not associated with increased risk for tumors and vascular malformations. Most of them are rare and have a genetic basis, others are rare disorders and have uncertain etiology. Some of them are recognized as a part of PROS disorders that can be caused due to mutations in the somatic and germline activating pathways in PI3K/AKT/mTOR signaling, which underlie the mechanism of heterogeneous segmental overgrowth phenotypes. Careful clinical examination, imaging, and histopathology, with detection of mutations, are crucial in establishing definite diagnosis. It is not always possible to detect mutations, even in cases where it exists, due to low-level somatic mosaicism. These patients should be monitored for other possible associated complications such as vascular malformations and skeletal abnormalities.
In conclusion, overgrowth syndromes are important to diagnose, not just for accurate genetic counseling, but also for knowledge surrounding neoplasm surveillance and prognosis. Further screening of the genes encoding overgrowth spectrum diseases will improve molecular diagnosis and genetic counseling. Although genotype–phenotype correlation for some overgrowth syndromes is not sufficiently understood, the correlation of genetic discoveries and molecular pathways that may contribute to the clinical phenotypic expression is also important, as may lead to potential therapeutic strategies.

PIK3CA-RELATED OVERGROWTH SPECTRUM (PROS); SOMATIC MOSAICISM;
GENOTYPE-PHENOTYPE ASSOCIATION; GENETIC COUNSELING

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02 – 04 Invited lecture

THE IMPORTANCE OF CORRECTLY REPORTING DIAGNOSTIC GENETIC TEST RESULTS

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The increasing use of genetic analyzes in routine medical practice has led to a need for improving writing and interpretation of results. This worldwide trend was also recognized by the Serbian Medical Genetics community. For that purpose we have reviewed the recommendations of various associations such as ECA (European Cytogeneticists Association), GenQA (Genomics Quality Assessment), ESHG (European Society of Human Genetics), ACGS (Association for Clinical Genetic Science), ACMG (The American College of Medical Genetics and Genomics), CF Network etc.
Every laboratory has their policies and protocols of reporting the outcome of genetic investigations to the patient. But, the report has to fulfill some requirements that are common to all. The main request is correct and appropriate nomenclature, followed by a clear written description of the genetic finding (normal or abnormal), the name of any associated syndrome/disease/prognosis, clinical interpretation, assessment/calculation of risk/recurrence, referral for genetic counselling, the basis of the test, further tests and/or information, authorizers of reports and references within reports.
The aim of this lecture is to present educational resources for medical geneticists and other clinicians in order to help them provide good quality medical services.

DIAGNOSTIC GENETIC TESTING, REPORT, INTERPRETATION

02 – 05 Oral

PHARMACOGENOMIC PROFILING OF SERBIAN PATIENTS WITH PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Pharmacogenomics is one of the cornerstones of personalized medicine. Treatment of pediatric acute lymphoblastic leukemia (ALL) based upon the incorporation of the principles of pharmacogenomics significantly improves the outcome of the disease. Standard treatment options for pediatric ALL encompass cytotoxic agents grouped into so called therapeutic phases, namely remission induction, consolidation, intensification and maintenance phase.
We performed PCR-based and Sanger sequencing analyses, and next-generation sequencing in retrospective study to investigate variants in genes important for metabolizing corticosteroid drugs used in the induction phase and 6-mercaptopurine and methotrexate drugs used in the maintenance phase in 120 Serbian pediatric ALL patients and 100 healthy controls.
Variants in NR3C1, GSTP11 and ABCB1 genes have been associated with the response to glucocorticoid treatment in children with ALL. Genetic variants in TPMT (both coding and promoter regions), ITPA, ABCC4 and ABCB1 genes have been shown as predictors of 6-mercaptopurine induced toxicity. Also, we have identified genetic variants in DHFR (promoter region), TYMS, MTHFR and SLC19A1 genes related to side effects of methotrexate use. Association study revealed potential new pharmacogenetics markers that have to be validated in prospective studies as well as in different populations.
True understanding of processes leading to disease development and mechanisms of treatment efficacy and toxicity, as well as gaining new knowledge from big data obtained in large omics studies and validation studies from various populations, could be the way to implement personalized treatment to each patient. Research efforts have to be focused on data analysis and designing prediction model using machine learning algorithms. Bioinformatics tools and implementation of artificial intelligence are expected to open the door wide for personalized medicine in clinical practice of childhood ALL.

PHARMACOGENOMICS, PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA (ALL), PERSONALIZED MEDICINE

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02 – 06 Oral

CYP450 FUNCTIONAL VARIANTS PANEL FOR POSTMORTEM GENETIC SCREENING IN SERBIAN POPULATION

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Introduction: In forensic medicine, molecular autopsy is a set of molecular techniques employed to determine the cause and manner of death. In suspicious drug intoxication, functional variants of cytochrome P 450 (CYP) genes, involved in phase I of drug metabolism, are investigated. Out of 5 CYP gene families, the metabolism of 70% of commonly used drugs is affected by families 1, 2 and 3. According to metabolism affection, CYP alleles can be categorized as fully functional, with decreased function, and non-functional. CYP alleles are inferred as haplotypic combination of sequence variants, and in diploid combination are used to classify metabolic phenotype into four groups: poor, intermediate, extensive, and ultra-rapid metabolizers. There is a high variability in these CYP variants both at within and between populations, and therefore pharmacogenetic tests designed for one population are not equally informative in others.
Materials and Methods: DNA samples from 500 unrelated people, originated from 5 regions (North Serbia, West Serbia, East and South Serbia, Central Serbia, and region of Belgrade) were collected. TaqMan drug metabolism assays were used for genotyping of SNVs in CYP2C9 (rs1799853 and rs1057910), CYP2C19 (rs4244285 and rs12248560), CYP2D6 (rs3892097, rs1065852, rs28371725 and rs28371706), and CYP3A4 (rs2740574). In CYP1A1 gene variants m1 and m2 (rs4646903, rs1048943) were genotyped with RFLP.
Results: From 11 tested SNVs, 9 had frequencies higher than 5%, while one (rs28371706 in CYP2D6) was monomorphic and one (CYP3A4, rs2740574) had low frequency (2.2%). The most significant contributor to poor metabolizer phenotype for CYP2D6, involved in clearance of many opioids, is *4 allele defined by rs3892097.
Conclusion: Proposed test of 9 SNVs, which define the most common alleles with decreased or null function in four CYP genes (1A1, 2C9, 2C19, 2D6), could be used as screening panel in post mortem analysis.

MOLECULAR AUTOPSY, PHARMACOGENETICS, CYTOCHROME P450, FORENSICS

02 – 07 Oral

THE IMPLEMENTATION OF ARRAY-CGH TECHNOLOGY IN CLINICAL POSTNATAL TESTING IN SERBIAN POPULATION

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Array Comparative Genomic Hybridization (CGH) is a method for molecular karyotyping in individuals with birth defects, dysmorphic features, intellectual disabilities and autism spectrum disorders. We performed array-CGH analysis of Serbian patients with intellectual disability and/or congenital malformation. Blood samples were collected from 83 patients and their parents when possible. DNA was extracted and investigated using the array-CGH analysis with 60 K (n = 72) and 180 K (n = 8) slides. Detected copy number variations (CNVs) were evaluated for the pathogenicity in databases of benign and pathogenic changes (ISCA, DGV and DECIPHER).
Twenty nine CNVs were identified in 21 (25.3%) patients, 14/47 (29.8%) males and 7/36 (19.4%) females. We considered 15 of these to be clinically significant, reaching a diagnosis rate of 18.1%. Detected CNVs were pathogenic in 10 (34.5%) cases, likely pathogenic in 5 (17.2%) cases, with uncertain clinical relevance in 12 (41.4%) cases and likely benign in 2 (6.9%) cases. We observed 12/29 (41.4%) deletions which varied in size from 15 kb to 5.9 Mb. The frequencies of duplications are 17/29 (58.6%) and varied from 240 kb to 9.726 Mb in size. In 3/21 (14.3%) patients, the rearrangement involved a sex chromosome. Sixteen patients had one chromosomal aberration, while 2 or 3 concomitant abnormalities were detected in 4 and 1 patients, respectively. Seven patients had CNVs related to known syndromes (7/21, 33.3%). Six of 29 CNVs (20.1%) were > 5Mb in size. Detected CNVs are mostly small rearrangements below the resolution of karyotype analysis. Main cause of high number of observed CNVs with uncertain clinical relevance is unavailability of paternal samples for analysis. High detection rate of CNVs using an array-CGH analysis confirmed importance of implementation of array-CGH analysis for Serbian patients.

ARRAY-CGH; CONGENITAL MALFORMATION; INTELLECTUAL DISABILITY; SERBIAN PATIENTS

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02 – 08 Oral

ASSOCIATION OF THE MMP-2 rs2285053 CC AND TIMP-2 rs2277698 CT GENOTYPES COMBINATION WITH BONE EROSION IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: For the decades methotrexate (MTX) is the first choice drug in the therapy of rheumatoid arthritis (RA). Despite numerous studies on genes coding for proteins targeted by MTX, including several from our group, patient's response to the treatment remains unpredictable. Matrix metalloproteinase 2 (MMP-2) is over expressed in synovium of RA patients, and is involved in pannus expansion, cartilage damage and bone erosion. Tissue inhibitor of metalloproteinase 2 (TIMP-2) gene codes for the inhibitor of the MMP-2 protein. Recently it has been shown, on rat model, that MTX causes significant decrease in MMP-2 mRNA levels.
Material and methods: Study included 122 patients with RA, diagnosed according to the ACR 1987 revised classification criteria and treated at the Institute of Rheumatology, University of Belgrade. All patients were on the MTX monotherapy for at least 6 months. According to the EULAR response criteria, patients were classified as MTX therapy responders (good or moderate) and non responders. Genotypes of the MMP-2 gene rs2285053 polymorphism were detected by polymerase chain reaction-restriction fragment length polymorphism method and of the TIMP-2 gene rs2277698 polymorphism by TaqMan assay.
Results: According to EULAR response criteria, 107 (87.7%) patients were responders, 19 (15.6%) good, 88 (72.1%) moderate. Genotype CC was detected in 79 (75.2%) and genotype CT in 26 (24.8%) among 105 patients analyzed for MMP-2 rs2285053 gene polymorphism. Among 109 genotypized patients, 88 (80.7%) harboured CC, and 21 (19.3%) CT rs2277698 TIMP2 genotype. Adverse effects were present in 30 (24.6%) patients. Significantly higher frequency of bone erosions has been found in the patients with rs2285053CC and rs2277698 CT genotypes combination (p=0.048, RR 2.54, 95% CI 0.98-6.59).
Conclusion: In RA patients on the MTX monotherapy rs2285053 CC and rs2277698 CT genotypes combination could be predictive factor for the bone erosion.

ARTHRITIS, METHOTREXATE, MMP-2, GENE POLYMORPHISM

02 – 09 Oral

HYPERPHENYLALANINEMIA: FROM COMPLEX GENETICS TO COMPLEX PHENOTYPES

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Hyperphenylalaninemia (HPA) is the most frequent inborn error of amino acid metabolism and it is characterized by an increased level of blood phenylalanine. Excessive phenylalanine has toxic effect on brain development, and leads to severe and irreversible mental retardation of a patient. In 98% of all cases, HPA is caused by mutations in phenylalanine hydroxylase (PAH) gene, known as phenylketonuria (PKU). In other 2%, HPA results from deficiency of PAH enzyme cofactor, tetrahydrobiopterin (BH4). BH4 deficiency results from mutations in genes responsible either for BH4 biosynthesis (PTS and GCH1), or BH4 metabolism (DHPR and PCBD). To ensure an accurate differential diagnosis of HPA in a patient and to introduce adequate treatment, an appropriate genetic diagnosis is necessary. By combining Sanger sequencing, MLPA and next generation sequencing (NGS), using our virtual gene panel, we reached mutation detection rate of 99,34% for HPA in Serbia. We detected 29 known and 1 novel mutation, p.Gln226Lys PAH, which we functionally characterized as a disease causing mutation.
Although variants in a single gene are the main determinant of PKU phenotype, it is not always possible to predict the phenotype based only on PAH genotype. Genetic factors which attribute to the development of final PKU phenotype are still poorly understood. In the research which aimed to identify new modifier genes of cognitive PKU phenotype, complete genome sequence of 10 untreated PKU patients (from 6 families) with normal cognitive development was analyzed using NGS. After bioinformatics analysis, variats in SHANK genes were selected as possible modifiers which contributed to the atypical phenotype in our patients. Computational analysis showed that SHANK proteins encoded by identified variant genes could gain new characteristics related to synaptic transmission, and could have a protective modifier effect on development of cognitive dysfunction in PKU patients.

HYPERPHENYLALANINEMIA; MODIFIER; MUTATION DETECTION; PHENYLKETONURIA

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02 – 10 Oral

GLUTATHIONE-S-TRANSFERASES POLYMORPHISMS COUPLED WITH INCREASED EXPRESSION OF PROINFLAMMATORY CYTOKINES PLAY A ROLE IN THE DEVELOPMENT OF PREECLAMPSIA IN SERBIAN WOMEN

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Preeclampsia is a pregnancy related hypertensive disorder and one of the leading causes of maternal and neonatal morbidity and mortality, as well as a risk factor for cardiovascular diseases later in life. Its onset is generally attributed to genetic, immunological and environmental factors. Oxidative stress as a putative cause of endothelial dysfunction, could arise as the result of poor placental perfusion, coupled with increased systemic inflammatory response. This study aimed to investigate glutathione-S-transferase M1 (GSTM1) and T1 (GSTT1) gene polymorphisms and the expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) in preeclampsia and uncomplicated pregnancy.
DNA and RNA were extracted from leukocytes of fifty women with preeclampsia and fifty healthy pregnant women. Gene polymorphisms were analyzed by PCR, while cytokine relative gene expression was analyzed by means of real-time PCR.
GSTM1 deletion alone, without GSTT1 deletion, increased the risk for preeclampsia development, while deletion of GSTT1 without deletion of GSTM1 increased the risk for early preeclampsia. Relative expression of TNF-α was significantly higher in preeclampsia compared to controls ($P = 0.006$), while IL-1β was significantly overexpressed in late preeclampsia compared to the controls ($P = 0.007$).
In conclusion, homozygous deletion of GSTM1 represents a risk factor for preeclampsia development, while homozygous GSTT1 deletion favors early preeclampsia. Preeclampsia is also associated with increased expression of pro-inflammatory cytokines, predominantly TNF-α and IL-1β.

PREECLAMPSIA, PRO-INFLAMATORY CITOKINES, GLUTATHIONE-S-TRANSFERASE, GENE POLYMORPHISM, RELATIVE GENE EXPRESSION

02 – 11 Oral

PREIMPLANTATION GENETIC SCREENING: PREVENTION OF ANEUPLOIDIES BEFORE IMPLANTATION

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Preimplantation genetic diagnosis is prenatal diagnosis on embryos obtained after in vitro fertilization process and before embryotransfer. Preimplantation genetic diagnosis is performed to reveal monogenic disorders already known in family and preimplantation genetic screening is performed to reveal aneuploidies – usually not seen in family before. Preimplantation genetic screening and preimplantation genetic diagnosis are extra stages of the procedure that patients can choose if they are worried that on factors that might prevent them from conceiving without complications. Basic goal of preimplantation genetic screening procedure is to increase in vitro fertilization success rate and decrease number of *in vitro* fertilization attempts in women older than 35 years and pairs with previous unsuccessful in *vitro* fertilization attempts. The main features of complete procedure to gain success are screening all chromosomes, cryopreservation of blastocysts and single embryo transfer.

PREIMPLANTATION GENETIC SCREENING; GENETIC COUNSELING

02 – 12 Oral

VARIANT REPEATS WITHIN DMPK EXPANSIONS AS GENETIC MODIFIER OF DM1 PHENOTYPE

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Myotonic dystrophy type 1 (DM1) is caused by expansion of CTG repeats in the DMPK gene. The number of repeats is the major determinant of clinical presentation but cannot explain solely a huge phenotypic variability seen in DM1. Small percent of patients carry variant repeats scattered among CTG repeats, which are emerging as modifiers of DM1 phenotype. We detected variant repeats at the 3' end of expansions in 9 out of 243 DM1 patients (3,7%). One expansion with a de novo CTC variant repeat has been described for the first time, while the rest contained CCG repeats presented as individual repeats, CCGCTG hexamers, or short/long CCG blocks. The analysis of 20 intergenerational transmissions of variant expansions (four from our study) showed more frequent stable transmissions or contractions, independently of the sex of transmitting parent. The somatic instability of variant expansions, characterized by single-molecule small-pool PCR in blood and buccal swab samples, showed characteristics similar to pure expansions: tissue specificity and bias toward further expansions. However, mathematical modeling demonstrated that variant expansions were characterized by lower level of somatic instability, accompanied by lower expansion size increment in blood cells over time. Some patients with variant expansions were characterized by atypical symptoms and relatively later age at onset than expected based on expansion size. Mathematical modeling demonstrated that individual specific differences in the level of somatic instability had greater impact on age at onset in patients with variant expansions compared to those with pure expansions.
Our results provide evidence that different types and patterns of variant repeats contribute to the stability of DMPK expansions in somatic and germ cells. Moreover, they contribute to the later age at onset by mechanism related to the stabilization of DMPK locus in somatic cells, emphasizing their clinical relevance.

CTG EXPANSION, DMPK, GENETIC MODIFIERS, MYOTONIC DYSTROPHY 1, VARIANT REPEATS

02 – 13 Oral

TYROSINE KINASE DOMAIN MUTATIONS OF bcr-abl GENE IN THE PATIENTS WITH CHRONIC MYELOID LEUKEMIA RESISTANT TO FIRST LINE THERAPY

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Chronic myeloid leukemia (CML) is a clonal disease of a pluripotent, hematopoetic, stem cell, characterized by the bcr-abl chimeric gene. Tyrosine kinase (TK) product of this gene has elevated activity, which lies in the center of pathogenesis of CML. TK inhibitors are first line therapy for CML. TK domain mutations, of bcr-abl gene, are one of the well known reasons for TK inhibitors therapy resistance.
The aim of this study was to analyze the presence of the most frequent mutations: T315I, F317L, Y253H, E255V/K and F359V, with a known influence on further therapy options choice. We analyzed peripheral blood samples, of 12 CML patients (pts), with acquired resistance to the first generation of TK inhibitors. We used standard methods for: RNA isolation by the Trizol® reagent, Multiscribe® Reverse transcriptase kit, for cDNA synthesis, and competitive allele specific real-time PCR method analysis, with TaqMan mutations assays.
Three of 12 (25%) analyzed (25%) pts, were positive for those mutations. We detected F317L in one pt, E255K in the second pt and F359V in third pt.
Mutations presence analysis, in bcr-abl TK domain, is important diagnostic and prognostic tool, for further treatment options and clinical decisions making. This is a step forward to personalized medicine for CML patients.

CML, BCR-ABL, MUTATIONS, TK INHIBITORS

02 – 14 Poster

ANALYSIS OF ASSOCIATION OF POTENTIALLY FUNCTIONAL GENETIC VARIANTS WITHIN GENES ENCODING miR-34b/c, miR-378 AND miR-143/145 WITH PROSTATE CANCER IN SERBIAN POPULATION

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MiRNA-associated genetic variants occurring in regulatory regions can affect the efficiency of transcription and potentially modify pri-miRNA or pre-miRNA processing. Since miRNA-based mechanisms are shown to be involved the pathogenesis of prostate cancer (PCa), the aim of the present study was to evaluate the effect of rs4938723, rs1076064 and rs4705343 occurring in regulatory regions of miR-34b/c, miR-143/145 and miR-378, respectively, on PCa risk and progression in Serbian population. We examined a total of 1060 subjects. 350 peripheral blood samples were obtained from patients with PCa and 354 samples from patients with benign prostatic hyperplasia (BPH). The control group comprised 356 healthy volunteers. Genotyping of rs4938723, rs1076064 and rs4705343 was performed by using Taqman® SNP Genotyping Assays. Allele C of rs4705342 was found to increase the risk of PCa (P=0.031 for codominant model, P=0.0088 for recessive model). Rs1076064 minor allele G was found to associate with serum PSA score, as well as with primary PCa stage and disease aggressiveness. For rs4938723 minor allele C was shown to be associated with the lower stage disease (Pdom=0.0046; OR=0.36, 95%CI 0.17-0.76) in T2 vs. T1 comparison. Rs4705342 was identified as PCa susceptibility variant in Serbian population, while for rs1076064 and rs4938723 association with PCa progression parameters was found.

PROSTATE CANCER, MIRNA, ASSOCIATION STUDY

02 – 15 Poster

SMN1 COPY NUMBER AS A MODIFYING FACTOR OF SURVIVAL IN SERBIAN PATIENTS WITH SPORADIC AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a devastating motor neurone disease. The majority of cases are apparently sporadic (SALS) with variants in susceptibility genes or sometimes in the high-risk ALS genes. Two ALS susceptibility genes are SMN1, whose functional loss causes spinal muscular atrophy (SMA), and a nearly identical SMN2 gene which modulates SMA severity.
This study expanded previous association studies of the SMN1 and SMN2 genes, and SALS to other genes residing in the same genomic region, i.e. 5q13.2 segmental duplication, such as SERF1 and NAIP, and assessed whether their copy number variations (CNVs) were associated with susceptibility to SALS and its severity in patients from Serbia. MLPA was used to determine SMN1, SMN2, SERF1 and NAIP CNVs in a clinically well-characterised group of 153 Serbian SALS patients and 153 controls. Ten patients (6.54%) carried the SOD1 p.Leu145Phe mutation, whose high frequency is likely due to a founder effect, and nine patients (5.88%) carried the GGGGCC repeat expansion in the C9orf72 gene. Individual association between SMN1, SMN2, SERF1 or NAIP CNVs and SALS susceptibility or survival was not found. Survival curves based on the multivariable Cox regression analysis showed that three SMN1 copies, lower ALSFRS-R score at the time of diagnosis, faster decline of the ALSFRS-R score over time and shorter diagnostic delay result in shorter survival of Serbian SALS patients. Therefore, clinical variables might be complemented with SMN1 copy number in prediction of survival in Serbian SALS patients.

SURVIVAL MOTOR NEURON, AMYOTROPHIC LATERAL SCLEROSIS, MOTOR NEURON DISEASE, H4F5, NAIP

02 – 16 Poster

ASSOCIATION STUDIES OF GENETIC VARIANT 10842262 WITH IDIOPATIC MALE INFERTILITY IN SERBIAN AND NORTH MACEDONIAN MAN

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Recent genome-wide association study performed in the Han Chinese population identified significant associations between non-obstructive azoospermia (NOA) risk and three single-nucleotide variants (rs12097821, rs2477686, and rs10842262). Additionally, case-control study conducted in the Japanese population showed an association between rs10842262 and NOA, suggesting that the 12p12.1 genomic region was a possible risk factor for idiopathic male infertility. Therefore, we cerried out two case-control studies, to further validation the potential association between this susceptibility loci and male infertility risk in Serbian and North Macedonian men. The first study included 208 Serbian males with idiopathic sterility and 223 controls, while the other study in the North Macedonian population involved 105 cases of idiopathic sterility and 118 normal controls. In the Serbian population we found statistical significance of the rs10842262 with the decreased risk of male infertility, according to overdominant and codominant genetic models, even though the genotype frequencies in controls deviated from Hardy-Weinberg equilibrium. Therewith, rs10842262 displayed statistical significance trend with idiopathic male infertility in the North Macedonian population, according to dominant genetic models. Our study supported previos evidence about association for the rs10842262 in SOX5 gene with male infertility risk.

RS10842262, MALE INFERTILITY, CASE-CONTROL STUDY

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02 – 17 Poster

FAMILIAL MELANOMA IN POPULATION OF SERBIA: FREQUENCY, CHARACTERISTICS AND CDKN2A MUTATION STATUS

Nemanja Ivković [1], Stevo Jovandić [1], Jovana Broćić [1], Aleksandra Petković Ćurčin [1], Lidija Kandolf Sekulović [2], Bojana Cikota-Aleksić [1]

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Melanoma is a malignant disease with rapidly growing incidence, especially among Caucasoid population. According to the literature, 5-12% of patients have hereditary predispositions for melanoma, and around 40% of these cases are associated with mutations in high-penetrability gene CDKN2A.
We collected data about behavioral and phenotypic melanoma risk factors, clinicopathological characteristics and occurrence of melanoma and/or other malignant diseases in families, for 564 patients with earlier diagnosed melanoma. Two defined groups for statistical analysis were: familial melanoma for patients with at least one first- or second-degree relative that has or has had melanoma, and sporadic melanoma. Among melanoma patients with two or more relatives with diagnosed melanoma or pancreatic cancer, the Sanger sequencing method was used to determine mutation in promoter, exons 1α, 2, 3 and intron 2 of CDKN2A gene.
Familial melanoma was determined for 37 patients (6,6%) while 572 patients (93,4%) had sporadic melanoma, with almost equal sexual distribution. Median age in familial melanoma group was 53 years and 58 years in sporadic group ($p>0.05$). Analysis showed a statistical significance of eye color ($p=0,009$) and number of lifetime sunburns ($p=0,01$) for two analyzed groups. Sequencing analysis for 6 samples and comparison to NM_000077 referent sequence show SNPs: c.116G>A in promoter of all 6 samples, c.442G>A in exon 2 of 1 sample and c.806G>C in exon 3 of 4 samples.
Our results on frequency of familial melanoma correspond to literature finding and previous population observations in other countries. Younger age at the time of diagnosis, lifetime history of sunburns and fair eye color were found more frequently in familial melanoma group. According to our preliminary research, found SNPs c.116G>A and c.806G>C are common variants with no reported clinical significance and one rare variant c.442G>A is reported as benign.

FAMILIAL MELANOMA, FREQUENCY, CHARACTERISTICS, CDKN2A

02 – 18 Poster

CDK4 CODON 24 MUTATION STATUS IN FAMILIAL MELANOMA PATIENTS: SINGLE CENTER STUDY

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Familial melanoma constitutes 5-12% of all melanoma. Association with CDKN2A and CDK4 genes is found in 45% of familial melanoma patients and mutation in this genes increase familial melanoma risk by 40% and 20% respectively. CDK4 gene, located on 12q14.1, codes for cyclin-dependent kinase, a protein which is a member of Ser/Thr kinases family. Protein kinases participate in cell cycle regulation. Changes in their function cause abnormalities in cell cycle regulation, resulting in neoplastic transformation.
Our study included 49 patients - 24 male (48,9%) and 25 (51.1%) female patients, average age 51.3 years, mediana 53, from Melanoma outpatient clinic, Military Medical Academy, Belgrade, Serbia who had been surveyed in order to gather information about the existence of melanoma and/or pancreatic cancer within their first or second degree relatives.
Hotspot mutation in CDK4 gene (R24H and R24C) were analyzed on 7500 Real Time PCR System (Applied Biosystems, USA) using TaqMan assays (rs104894340 and rs11547328).
The results for all of 49 familial melanoma patients did not indicate presence of mutation on the abovementioned position and all patients had wild type genotype for rs11547328 (GG) and rs104894340 (TT).
Conclusion: Those findings proves the absence of CDK4 mutations in our patients with melanoma in family.

CDK4, FAMILIAL MELANOMA, HOTSPOT MUTATION

02 – 19 Poster

KRIT1 GENE ANALYSIS IN SERBIAN PATIENTS WITH FAMILIAL CEREBRAL CAVERNOUS MALFORMATION

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Familial cerebral cavernous malformation (FCCM) is characterised by vascular malformations in the brain and spinal cord comprising closely clustered, enlarged capillary channels - caverns. It is an autosomal dominantly inherited disease clinically expressed with seizures, nonspecific headaches, cerebral haemorrhage. There are at least three known CCM genes in human genome, and mutations in CCM1/KRIT1 gene account for up to 50% of all FCCM cases. This study evaluated two Serbian families and one sporadic patient, whose clinical picture meets the FCCM criteria. Genetic testing involved direct sequencing of coding region of the KRIT1 gene by Sanger sequencing. The analysis was performed on ABI 3500 genetic analyzer. Detected variants were characterized using theoretical predictions of pathogenicity (MutationTaster, Polyphen, SIFT, Human Splice Finder). Furthermore, GnomAD (Genome Aggregation Database) was employed as source of variant frequencies in worldwide populations, GERP++ rejected substation scores as a source of evolutionary sequence conservation and finally, literature and databases (ClinVar, HGMD, LOVD) were searched as a source of disease association of identified variants. Genetic testing revealed two novel variants in the KRIT1 gene that present likely diagnostic findings. Heterozygous splice site variant (NM_194456.1;c.1819-1G>T) was detected in one FCCM family and described as likely pathogenic. In the other FCCM family as well as in sporadic patient heterozygous synonymous variant (NM_194456.1;c.2025G>A;p.K675K) was observed and described as variant of uncertain significance favoring pathogenicity. Both variants were found in exon 18 suggesting it as a possible mutation prone KRIT1 exon in our population. These results signify the importance of KRIT1 sequencing as a standard genetic test in FCCM. Our findings contribute in better characterization of FCCM in Serbian population and facilitate future studies into genotype-phenotype correlations.

KRIT1, FCCM, GENETIC TESTING

02 – 20 Poster

SPECTRUM OF MUTATIONS IN PRESENILIN 1 GENE IN PATIENTS WITH EARLY ONSET ALZHEIMER DISEASE

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Introduction: Early onset Alzheimer disease (EOAD) is defined as dementia which cognitive and behavioral simptoms appear before age of 65. In familiar casses, dominant mutations in presenilin 1 (PSEN1) gene represent the most common genetic cause. PSEN 1 gene encodes the transmembrane protein - presenilin 1, one of core proteins in gamma-secretase complex plaing important role in generation of amyloid beta protein. Aim of this study was to analyse the spectrum of PSEN1 mutations in cohort of patients with clinical confirmed EOAD including cognitive and behavioral disturbances.
Methods: we have analysed 46 patients with EOAD at Neurology Clinic of Clinical Center of Serbia. After DNA extraction from peripheral blood samples, the whole coding sequence of PSEN1 gene was analyzed by Sanger sequencing method using ABI 3500 genetic analyzer.
Results: genetic analysis detected four patients with PSEN1 mutations, hetrozygous in all ceses. Two patients had substitutions in exon 5: c.416T>C (p.Met139Thr) and c.476A>G (p.Tyr159Cys). Substitution in exon 8: c.806G>A (Arg269His) has been detected in another two unrelated patients. According to PSEN1 variants databeses, Tyr159Cys is novel change, while other two variants have been described previously. According to insilico prediction softwers and guidelines for variant classifications all three PSEN1 mutations are considered patogenic. Our PSEN1 positive patients have a family history of EOAD. Regarding correlation with phenotype, one of our patients with PSEN1 Arg269Cys change, have rare noncognitive neurological simptoms such as myoclonus, visual and auditory hallucinations. Only three cases with this atypical simptoms were reported in literature.
Conclusion: these results confirms significance of mutations in PSEN1 gene in sporadic and family cases of EOAD. Our findings that novel mutation may cause noncognitive symptoms corresponding to EOAD suggest a need for futher analysis of PSEN1 mutation spectrum in EOAD.

PSEN1, EOAD, SEQUENCING

02 – 21 Poster

ANALYSIS OF THE ASSOCIATION BETWEEN ITPA RS1127354 GENE POLYMORPHISM AND EFFICACY AND TOXICITY OF METHOTREXATE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Methotrexate (MTX), anchor treatment in Rheumatoid arthritis (RA), probably achieves at least part of its antiinflammatory effects through adenosine cycle modulation. Inosine triphosphate pyrophosphatase (ITPA) is one of the enzymes that control the adenosine cycle. Polymorphism rs1127354 leads to enzymes reduced activity. Heterozygotes retain 25% of normal enzyme activity. Previous studies showed contradictory results on the impact of this polymorphism on the therapeutic response to MTX. Our aim was to analyse the influence of ITPA rs1127354 polymorphisms on disease activity and MTX therapy outcome.
This study included 121 RA patients on MTX monotherapy. Each patient was diagnosed according to the ACR 1987 revised classification criteria. Disease activity was estimated using the Disease Activity Score (DAS28). Treatment efficacy was assessed based on DAS28 change after 6 months of treatment according to EULAR response criteria. Adverse effects (AE) were collected during the follow-up period. Presence of bone erosions (BE) was estimated after 6 months of treatment. For genotypisation of rs1127354 polymorphism within ITPA, we used the KASP (Kompetitive Allele Specific PCR) genotyping kit according to manufacturer's instructions and Real-time PCR machine.
Among patients 107 (88.4%) were responders, 14 (11.6%) patients were good responders and 88 (72.7%) patients had a moderate response. During the follow-up period, 78 (64.5%) of our patients developed bone erosions. We registered AE in 30 (24.8%) patients. ITPA rs1127354 A allele carriers had significantly higher erythrocyte sedimentation rate at baseline than patients with CC genotype (83.36±31.53 versus 65.34±24.54; p=0.036). Responders and nonresponders did not differ significantly in genotype or allele frequency. This polymorphism did not influence AE or BE appearance.
ITPA rs1127354 polymorphism did not influence therapy outcome measured in DAS28 score change or AE and BE appearance.

ITPA, METHOTREXATE, RHEUMATOID ARTHRITIS, POLYMORPHISM

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02 – 22 Poster

ANALYSIS OF ATP7B GENE IN SERBIAN PATIENTS WITH WILSON DISEASE: 10 YEARS EXPERIENCE

Ana Marjanović [1], Katarina Jović [1], Valerija Dobričić [1], Marija Branković [1], Milena Janković [1], Aleksandra Tomić [1], Dragana Cvetković [2], Marina Svetel [1], Ivana Novaković [1], Vladimir Kostić [1]

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Wilson disease (WD) is a rare autosomal recessive disorder caused by mutation in the ATP7B gene affecting the copper metabolism. This copper disorder is presented with hepatic, neurologic and psychiatric disturbances. It has been shown that in Serbian WD patients, mutations are predominantly located in exons ATP7B 5, 8, 13, 14 and 15, so standard genetic testing in our laboratory includes direct sequencing of these exons. During the last 10 years total number of 407 patients clinically diagnosed with WD was analyzed by Sanger sequencing using ABI 3500 Genetic analyzer. Among them, 110 patients had bi-allele pathogenic mutations in ATP7B gene confirming WD diagnosis. The largest number of mutations was found in exon 14 (54.09%) followed by mutations in exon 5 and exon 8 (14.54% each), exon 13 (14.09%) and exon 15 (2.73%). The most frequent mutations in our group were p.H1069Q, c.2304_2305 ins C, p.R616Q and p.A1003T, found in 51.82%, 13.64%, 9.55% and 8.64% of the 110 patients, respectively. In addition, 43 patients with clinical picture of WD had only mono-allele mutation in one of the mentioned mutation-prone exons. Further expanded analysis of ATP7B gene in this group included exons 12, 18, 19, 20 and 21, because mutations in these regions were also described in our region. Genetic testing revealed 3 different variants in 4 patients: missense variant p.G1341D in exon 20, nonsense variant p.Q1427X in exon 21 and frameshift variant p.R1459Gfs*2 in exon 21. Variants p.G1341D and p.R1459Gfs*2 were described in the literature among patients from Balkans and classified as disease causing, while p.Q1427X is novel one and also pathogenic. Considering these results sequence analysis of exons 20 and 21 should be included in routine genetic testing of WD in Serbia.

WILSON DISEASE, GENETIC ANALYSIS

02 – 23 Poster

TELOMERE LENGTH IN DOWN SYNDROME FETUSES

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Telomeres are nucleoprotein complexes that protect chromosome ends and maintain genome stability. They shorten with each cell division and thus are cellular biomarkers of ageing. Down syndrome (DS) is most common autosomal aneuploidy among live births. Phenotype of Down syndrome is well described, and one of its characteristics is early onset of age related diseases such as menopause or Alzheimer's disease. It has been shown that adult DS patients have shorter telomeres compared to age matched controls, but mechanisms leading to telomere shortening as well as age of onset are still elusive.
The aim of our study was to determine telomere length (TL) in DS fetuses and thus to investigate if their telomeres are constitutively shorter compared to euploid controls.
The study was conducted on cultured amniocytes of nine fetuses with DS and 9 fetuses with normal karyotype. Q-FISH on metaphase spreads with Cy3 marked PNA probe was employed for telomere length determination. Captured images were process in Telo Tel 2 software and results of TL measurement are expressed in relative telomere length units (RTLU). Ten metaphases were analyzed per each sample.
Our result showed no statisticaly significant difference in average TL among DS fetuses and controls (p=0.453). Also, average TL in p and q arms did not differ among tested groups (p=0.517 and p=0.397). No aberrant telomeric structures were noted in DS fetuses.
We found that telomere length in DS fetuses are not constitutively shorter compared to euploid controls. Accelerated telomere shortening due to increased oxidative stress can be possible explanation for short telomeres in adults with Down syndrome.

TELEOMERES, DOWN SYNDROME, AGEING

02 – 24 Poster

FREQUENCY OF THE CYP2B6, ABCB1, UGT1A1*6 GENE POLYMORPHISMS AND HLA-B*57:01 ALLELE IN THE POPULATION OF SERBIA

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The data of the Public Health Institute in Serbia indicate that about 4000 people were infected with HIV from 1984 to 2019. Failure of antiretroviral therapy is a matter of great clinical importance. CYP2B6, ABCB1, UGT1A1*6 and HLA-B*57:01 allele participate in the metabolism of the most important HIV antiviral drugs: Abacavir, Efavirenz, Atazanavir. According to currently available literature, there is a disproportion in the distribution of this polymorphisms. The aim of the study was to simultaneously analyze the above-mentioned plymirphisms in the population of Serbia and to compare the results with previously published data for other ethnic groups.
A group of 104 unrelated, healthy subjects was included in the present study. Determination of polymorphisms performed on Real Time PCR System (Applied Biosystems, USA), using genotyping method.
The incidence of the CYP2B6 genotype is 62% for WT (GG), 29% for Het (GT) and 9% for RH (TT), while the frequency of others coincides with the frequency of the other European populations. The HLA-B*57:01 results are in processing.
Our results could be relevant for further investigation of a disproportion in the distribution of some drugs polymorphisms that are important for HIV epidemics.

HLA-B*57:01, CYP2B6, ABACAVIR, HIV, POLYMORPHISM

02 – 25 Poster

NEXT GENERATION SEQUENCING IN CHILDREN WITH EPILEPTIC ENCEPHALOPATHY: EXPERIENCE OF A SINGLE GENETIC CENTER FROM SERBIA AND RECOMMENDATIONS FOR GENETIC TESTING

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Introduction: Early etiological diagnosis is of paramount importance for epileptic encephalopathy for further monitoring and treatment pediatric patients. Numerous genetic causes of epileptic encephalopathy have been reported.
Materials and Methods: In the last four years at the Department of Medical Genetics at University Children's Hospital in Belgrade we had 44 children with epileptic enecphalopathy, and performed clinical exome sequencing (NGS technology) for all. For most of these patients microarray analysis was performed prior CES. Additionally, we did review of the literature.
Results: Of the 44 patients with epileptic encephalopathy, that were selected based of clear criteria, we detected causative gene variant in 29 patients (65.9%) using clinical exome sequencing. We isolated six groups of patients: neonatal epileptic encephalopathy; early onset infant epileptic encephalopathy including West syndrome and other entities with specific EEG correlate; childhood onset epileptic encephalopathy; drug-resistant epilepsy within non-syndromic intellectual disabilities; inborn eerors of metabolism with seizures and other unclassified epileptic encephalopathies. In addition, ARX c.428_451 dup(24bp) duplication was confirmed in two male patients with negative exome. For 19 out of all 44 patients we also performed chromasomal microarray, which confirmed MECP2 duplication syndrome in two families with male siblings. Also, for four families we performed prenatal genetic testing. There is ongoing genome sequencing for negative patients.
Conclusion: On the basis of a literature review and our results, we propose a strategy in genetic testing for well-selected children with epileptic enecphalopathy. Exome sequencing might be first genetic test, because of high detection rate of causative gene variant. Also, consensus statement from literature is application of high resolution microarray chromosome analysis for this group of patients.

NEXT GENERATION SEQUENCING (NGS), EPILEPTIC ENCEPHALOPATHY, GENETIC TESTING

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02 – 26 Poster

ANALYSIS OF ANTHROPOGENETIC AND ANTHROPOMETRIC MARKERS IN ASSESSING THE RISK OF CARDIOVASCULAR DISEASE

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Cardiovascular disease (CVD) at large remains a critical cause of morbidity and mortality worldwide. The aim of our study was to analyse antropogenetic homozygous-recessive characteristics (HRCs) and anthropometric markers: body mass index (BMI), circumference waist to height ratio (WHtR), waist to hip ratio (WHR), and biochemical parameters (glucose, cholesterol, triglyceride) in the group of CVD patients and in the control sample. This study analysed individual variability of 20 HRCs in a sample of 60 CVD patients (30 males and 30 females), average age 63.52 ± 10.97 and 60 healthy controls (30 males and 30 females), average age 54.95 ± 6.25. Our results showed a significant difference in the individual variation of HRCs between the patients and controls (χ^2=110.22; $p < 0.001$). Statistically significant differences were found in the frequency of 7 out of 20 analyzed morphophysiological traits. The average value of HRCs in group patients was a significantly higher, comparing to the control sample (6.13 ± 1.72 vs. 4.97 ± 1.66; $p < 0.001$). In the CVD group the frequency of HRCs for males was 6.37 ± 1.61, while for females, it was 5.90 ± 1.83. In controls the frequency of HRCs for males was 4.80 ± 1.77, and for females it was 5.13 ± 1.55. This study showed difference in the type of distribution HRCs; in the CVD patients' the most were with 5 to 8, while in control sample were with 3 to 6 recessive traits. The results of multiple linear regression analysis of tested variables (gender, smoking, BMI, WHtR, WHR, glucose, cholesterol, triglyceride, HRCs) showed that only BMI, WHtR and HRCs had significant variability between the CVD patients and controls. Our conclusion is that the increased degree of recessive homozygosity of CVD patients indicate the potential genetic predisposition for CVD and that anthropometric characteristics BMI and WHtR significantly contribute to the development of this predisposition.

ANTHROPOMETRIC MARKERS, HOMOZYGOUS-RECESSIVE CHARACTERISTICS, CARDIOVASCULAR DISEASE, GENETIC PREDISPOSITION

02 – 27 Poster

PRENATAL SCREENING MARKER ALPHA-FETOPROTEIN, BETA HCG AND FREE ESTRIOL SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE EXPECTED VALUE METHOD

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Introduction: Genetic screening on chromosomopathy is performed in the second trimester of pregnancy, by determining the fetoplacental Alpha-fetoprotein (AFP), human chorionic gonadotropin (beta HCG) and unconjugated estriol (freeE3) biomarkers in maternal serum.
The aim of study: Testing the sensitivity, specificity, positive and negative expected values of each marker with the aim of setting a model for prenatal screening readings and interpretations of pathological values.
Methods: AFP, betaHCG and freeE3 biomarkers have been read with IMMULITE 2000 SIEMENS. The processing of data and determination of the risk of trisomy 21 have been done with PRISCA 5 SOFTWARE. Statistical data treatment has been performed on the sample of 340 risk-free pregnant women with respect to age, all with false positive results of prenatal screening in the first trimester of pregnancy. Normal karyotyping of a fetus has been obtained with amniocentesis. Using a sensitivity, specificity analysis, positive expected and negative expected value method.
Results: Sensitivity AFP 0,1538 (probability15,38 %), betaHCG 0,6923 (69,23 %), freeE3 0,3846 (38,46 %). Specificity AFP 0,9534 (probability 95,34 %), betaHCG 0,7669 (76,69 %), freeE3 0,9492 (94,92 %). Positive expected value AFP 0,1538 (probability 15,38 %), betaHCG 0,1406 (14,06 %), freeE3 0,2941 (29,41 %). Negative expected value AFP 0,9534 (probability 95,34 %), betaHCG 0,9784 (97,84 %), freeE3 0,9655 (96,55 %).
Conclusion: The greatest influence on the biochemical risk of trisomy 21 shows marker beta HCG, the sensitivity of 69.23%, the specificity of 76.69% and the negative predictive value of 97.84% which means that its normal value will significantly reduce the biochemical risk of trisomy 21. It is important comparison free beta hCG in the first trimester, with beta-hCG in the second trimester. Reducing the value of beta-hCG in the second trimester may indicate a false positivity screening in the first trimester.

PRENATAL SCREENING, DOWN SYNDROME

02 – 28 Poster

CARRIER RATES OF 7 FOUNDER MUTATIONS ASSOCIATED WITH SINGLE-GENE DISORDERS IN SERBIAN ROMANI POPULATION – A PILOT STUDY

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Introduction: The Roma represent the most widespread ethnic minority in Europe. The string of population bottlenecks and founder effects formed a unique genetic profile of Roma population. High carrier rates for rare monogenic disorders and many founder mutations were described in studies performed in different countries across Europe. In this study, we analyzed carrier status of 39 members of Roma community in Serbia for seven monogenic disorders: HMSNL, HMSNR, CCFDN, LGMD2C, GALK deficiency, CMS, and ARCA3.
Methods: Genomic DNA was isolated from buccal swab samples. Affected regions of genes associated with the diseases (NDRG1, HK1, CTDP1, SGCG, GALK1, CHRNE, and ANO10) were PCR amplified, and then subjected to restriction digestion and agarose gel electrophoresis, except for ANO10, which was subjected to fragment analysis using the genetic analyzer.
Results: For 5 of 7 analyzed diseases, we identified carriers among analyzed samples. No carrier was identified for HMSNL and HMSNR. One carrier was identified for CCFDN and ARCA3, two carriers for LGMD2C, GALK deficiency, and CMS. Also, one person was identified as a carrier of CMS and CCFDN.
Conclusion: More samples should be analyzed in order to assess true carrier rates, but our data confirm the significance of medical genetics in an improvement of the health of socially marginalized Roma population. Carrier-testing programs and community-based education could improve their poor health status, and reduce the occurrence of analyzed monogenic disorders.

ROMANI POPULATION, FOUNDER MUTATIONS, MONOGENIC DISORDERS

02 – 29 Poster

ARCA3 IN SERBIAN ROMANI FAMILY CAUSED BY FOUNDER MUTATION IN ANO10 – A GENETIC APPROACH

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Introduction: Autosomal-recessive cerebellar ataxias (ARCAs) are a heterogeneous group of disorders with different genetic causes. Recently discovered ARCA3 is caused by mutations in ANO10 gene and most frequently associated with c.1150_1151del [p.Leu384fs], founder mutation, originated in Romani population. Although genetic causes of ARCAs are different, ARCA3 could be misdiagnosed as Friedreich ataxia or some other type of ARCA, so genetic analysis should be done in order to assess the right diagnosis and adequate therapy. Based on these facts, in this study, we analyzed 55 individuals: 16 ARCA patients previously subjected and negative for Friedreich ataxia, 38 Serbian Roma with no apparent neurological disease and one Romani woman (53 years old) with an undiagnosed neurological disorder.
Methods: Genomic DNA was isolated from buccal swabs. A region within exon 6 of ANO10, bearing founder mutation, was PCR amplified with fluorescently labeled primers. Fragment analysis was performed using ABI3130 Genetic Analyzer and analyzed using the GeneMapper® software.
Results: Patients negative for Friedreich ataxia were also negative for ANO10 founder mutation. A woman with the undiagnosed neurological disorder was found to be a recessive homozygote for c.1150_1151del, and her sister to be a carrier of the mutation. No other analyzed Roma were found to be the carriers.
Conclusion: Knowing that Romani people are marginalized and often disregarded in terms of social and health care, especially in not so developed countries, one should consider a genetic approach that could potentially facilitate the diagnosis of inherited disorders caused by recessive founder mutations originated in Romani population.

AUTOSOMAL-RECESSIVE CEREBELLAR ATAXIAS (ARCAs), ROMANI POPULATION, ANO10

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02 – 30 Poster

DETECTION OF DNA METHYLATION PROFILES IN MOUSE EMBRYONIC FIBROBLASTS USING FOURIER TRANSFORM-INFRARED SPECTROSCOPY

Ana Sarić [1], Anja Tolić [1], Nevena Grdović [1], Svetlana Dinić [1], Jovana Rajić [1], Miloš Đorđević [1], Marija Đorđević [1], Jelena Arambašić Jovanović [1], Mirjana Mihailović [1], Goran Poznanović [1], Aleksandra Uskoković [1], Melita Vidaković [1], Tanja Dučić [2]

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Epigenetic processes orchestrate the cell type-specific use of the genetic information essential for normal development and for maintaining the overall integrity of the genome. DNA methylation is probably the most extensively studied epigenetic mark and represents the covalent attachment of a methyl group to cytosine that is located next to guanine within the genomic DNA. The alteration of DNA methylation patterns by hyperglycaemia, oxidative stress and inflammation may have potential epigenetic impacts on gene regulation in diabetic individuals. Further research devoted to improve the steps that could be undertaken in the early diagnosis, prevention and treatment of diabetes and its complications is a scientifically and socially significant task. We used the Fourier transform-infrared (FTIR) spectroscopy (ALBA synchrotron, Cerdanyola del valles, Spain) for qualitative spectral analysis of normomethylated DNA from mouse fibroblast cells (NIH3T3) and hypomethylated DNA from PARP-1 knockout mouse fibroblast cells (PARP-/-). We obtained the global information regarding the DNA methylation profiles in mouse fibroblast cells by FTIR spectroscopy that was visualised by immune-fluorescent staining using anti-methyl cytosine (5mC) antibody. Some differences in DNA methylation profiles between examined cell lines were observed in spectral region significant for cytosine (990-1250 nm). The most interesting picks were observed approximately at wavelength: 1240 nm, 1150 nm, 1110 nm and 1010 nm. These results are in the same time a verification of the proof of principle for FTIR based analysis of the differences between normomethylated and hypomethylated genomic DNA which could be set as a potential predictive and diagnostic tool in future. To our knowledge this is a first time that synchrotron-based FTIR micro-spectroscopy is used for detection of the presence of 5mC and changes in the DNA methylation profile in cells.

EPIGENETICS, DNA METHYLATION, FT-IR SPECTROSCOPY

02 – 31 Poster

METABOLIC SYNDROME IN INFLAMMATORY BOWEL DISEASE: ASSOCIATION WITH GENETIC MARKERS OF OBESITY AND INFLAMMATION

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Metabolic syndrome (MS) and inflammatory bowel disease (IBD) share common pathophysiological features including chronic inflammation in visceral adipose tissue. However, the interplay of these two pathologies is still unraveled. This study aimed to analyze MS, obesity and dyslipidemia, in association with IBD occurrence and disease activity markers. For the first time, obesity related FTO rs9939609 genetic variant was examined as potential risk factor of IBD.

The study encompassed 104 IBD patients, of which 50 were diagnosed as Crohn's disease (CD) and 54 as ulcerative colitis (UC), as well as 45 non-IBD controls. Participants' medical records were examined for body mass index (BMI), waist circumference (WC), total cholesterol (TCh), low and high density lipoprotein cholesterols (LDL-C, HDL-C), triglycerides, disease and endoscopic activity scores, C-reactive protein and fecal calprotectin. Expression levels of IL17A, IL17F, IL23A and TLR9 genes were measured in paired non-inflamed and inflamed intestinal biopsies of each patient, collected during the colonoscopy. Additionally, total of 94 CD, 98 UC and 91 control subjects were genotyped for FTO variant rs9939609.

Results showed association of MS with CD, while MS components, central obesity and low HDL-C, were associated with both CD and UC. IBD lipid profile was characterized with significantly decreased TCh and HDL-C, while LDL-C was significantly reduced only in CD. Negative correlations were detected between the level of TCh and Crohn's disease activity index. WC negatively correlated with both IL17A and IL17F mRNA levels in inflamed colonic CD mucosa. We showed that FTO rs9939609 AA genotype carriers had increased risk for CD development.

This study demonstrates that MS, central obesity and altered lipid profile are significant factors involved in IBD pathogenesis. Association of FTO rs9939609 variant with CD could direct further nutrigenomic studies in IBD research.

IBD, CENTRAL OBESITY, DYSLIPIDEMIA, FTO, IL17

02 – 32 Poster

LONG NONCODING RNA GAS5 IN INDUCTION REMISSION PHASE OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA TREATMENT

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Introduction and Aim: Growth arrest specific 5 (GAS5) is a long noncoding RNA that has a role in cell growth arrest and apoptosis. GAS5 also interacts with the glucocorticoid receptor, which makes it a possible pharmacotranscriptomic modulator of glucocorticoid (GC) treatment response. We intended to find correlations between GAS5 expression and GC therapy response in pediatric acute lymphoblastic leukemia (ALL) and elucidate the molecular mechanism of GC effects on the GAS5 signaling pathway.

Material and Method: Peripheral blood mononuclear cells were collected from 29 patients on the day of diagnosis (0), day 15 and day 33 of ALL therapy. GAS5 expression was measured using RT-qPCR. HeLa cells were transfected with GAS5 expression vector and NF-κB DNA-binding activity was analyzed using EMSA.

Results: For each ALL patient, GAS5 expression was higher on day 15 in comparison to its level at diagnosis ($p<0.0005$), but it was still significantly higher than at diagnosis for the majority of patients ($p=0.001$). On day 33, the level of GAS5 expression decreased in comparison with day 15. In patients with a higher number of blasts on day 8, reflecting poor therapy response, higher GAS5 expression on day 0 ($p=0.016$) and a lower ratio of day 15/diagnosis expression levels ($p=0.009$) were detected. Thus, GAS5 expression profile influences GC therapy response in pediatric ALL.

Discussion: The results presented in this study suggest that GAS5 could be a promising pharmacotranscriptomic marker of response to GC treatment of pediatric ALL.

This work has been funded by MESTD, Republic of Serbia (III41004).

GAS5, GLUCOCORTICOIDS, PEDIATRIC ALL, PHARMACOTRANSCRIPTOMICS

02 – 33 Poster

miR-146a GENE VARIANT rs2910164 IS NOT ASSOCIATED WITH THE RISK FOR CORONARY IN-STENT RESTENOSIS IN A GROUP OF SERBIAN PATIENTS

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Coronary in-stent restenosis (ISR) is a complication that occurs in 30% of patients who have undergone percutaneous transluminal coronary angioplasty (PTCA) with stent implantation. The fact that not all patients will develop ISR raises the question of genetic factors' contribution to susceptibility for this condition. Micro RNAs (miRNAs) are small, 22 nt long non-coding RNAs, that regulate gene expression on post-transcriptional level. miR-146a is associated with the risk for coronary heart disease, which has a similar epidemiology as ISR. Variants in miRNA genes can affect the function of mature miRNAs. mir-146a rs2910164 gene variant is located in the seed region of mature miR-146a, key for regulation of target mRNAs.
The aim of this research was to examine if rs2910164 variant is associated with the risk for coronary ISR in a group of Serbian patients.
Samples of peripheral blood were obtained from 61 patients subjected to PTCA with stent implantation, 25 of which had angiographically confirmed in-stent restenosis. DNA was isolated by commercial kit and salting-out method and genotype assessment was done by Real Time PCR with TaqMan genotyping assay.
No association of rs2910164 gene variant with the risk for coronary ISR was found. rs2910164 is also not associated with clinical and angiographical characteristics of patients with ISR. The absence of association can be explained with small study group and low statistical power of the study.
Increasing the study group could assist in better understanding of the role of variant rs2910164 in mir-146a gene in epidemiology of in-stent restenosis.

rs2910164, miR-146a, CORONARY IN-STENT RESTENOSIS

02 – 34 Poster

ASSOCIATION BETWEEN GENOTYPE AND AUDIOLOGICAL FEATURES IN SERBIAN PATIENTS WITH INHERITED SENSORINEURAL HEARING IMPAIRMENT

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Introduction: Hearing impairment (HI) is the most common sensorineural disorder with an incidence of 1/700-1000 newborns. Variants in the GJB2 gene, which encodes connexin 26 protein, are the major cause of autosomal recessive nonsyndromic sensorineural hearing loss (ARNSHL). The degree of HI in patients with detected mutations in GJB2 gene ranges from mild to profound and is generally unprogressive. Aim of this study was to determine possible genotype-phenotype association between audiometric characteristics and detected genotypes in ARNSHL patients from Serbia.
Material and Methods: In 2016. we started with long-term study introducing genetic testing of GJB2 gene in Serbian patients suffering from ARNSHL. DNA analyses were performed in a group of 119 pediatric patients, using PCR-ARMS and PCR-RFLP methods for direct detection of specific mutations and direct sequencing of exon 2 of connexin 26. According to European protocol, audiological analyses were obtained in all patients and showed mild to profound bilateral sensorineural HI.
Results: In this group of patients 11 different variants were found, with c.35delG being the most frequent (26.47%). Five of these variants had truncating effect on connexin 26. Genotype was determined in 31.09% of the probands (37/119) with bi-allelic disease causing GJB2 variants. Fully characterized genotypes showed association with severe (29.73%, 11/37) and profound (59.4%, 22/37) degree of HI. An isolated group of patients (32), being homozygous or compound heterozygous carriers of the c.35delG variant, was most commonly associated with severe (28.12%, 9/32) to profound (59.38%, 19/32) hearing loss.
Conclusion: Results of this study showed that range of HI depends on the effect of the variant on the structure and/or function of connexin 26. Truncating mutations cause more severe phenotype than nontruncating mutations, which is in agreement with published studies worldwide.

HEARING IMPAIRMENT, GJB2 GENE, DNA ANALYSIS, AUDIOLOGICAL FEATURES

02 – 35 Poster

INFLAMMATORY AND APOPTOTIC GENETIC MARKERS ASSOCIATE WITH SPECIFIC CROHN'S DISEASE PHENOTYPES

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Crohn's disease (CD) is a chronic inflammatory disease that can affect different parts of gastrointestinal tract with different phenotypic characteristics influencing disease prognosis and therapeutic strategies. Therefore, defining molecular profiles of different CD subtypes is crucial since there is a lack of knowledge regarding this issue.
The aim of our study was to analyze selected inflammatory and apoptotic markers in inflamed and non-inflamed ileal mucosal samples, as well as within different CD mucosal phenotypes: non-stricturing/non-penetrating (NS/NP), stricturing (S) and penetrating (P).
Twenty CD patients were enrolled in this study. For each patient, paired samples of non-inflamed and inflamed mucosa were collected from ileal part of intestine and used for measuring: cytokine (TNF-α and IL-6) and apoptotic (Bcl2, Bax, Fas and FasL) genes' expression levels by qRT-PCR; the level of binding activity between nuclear proteins and NFκB consensus DNA sequence by EMSA assay.
Results of this study indicated altered molecular patterns in inflamed CD ileal mucosa compared to non-inflamed. Namely, TNF and IL6 showed increased expression and strong mutual correlation; Bcl2 expression was decreased, which, accompanied with positive correlations between TNF, Fas, and FasL, point out to possible altered apoptotic processes. NFκB, which favors survival of the cell, showed negative correlations with pro-apoptotic TNF and Fas. Furthermore, the difference between inflamed NS/NP and S mucosa was also detected. The NFκB-DNA binding activity level was lower while TNF and Bax expression levels were higher in NS/NP compared to S mucosal phenotype, indicated more "apoptotic" profile of NS/NP.
This study highlights the difference between specific CD phenotypes, which could contribute in improvement of current and development of new therapeutic strategies based on more specific molecular stratification of CD patients.

ILEAL CROHN'S DISEASE, MUCOSAL PHENOTYPES, MOLECULAR PROFILING

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02 – 36 Poster

A CASE SERIES OF IDENTIFIED STRUCTURAL CHROMOSOMAL ABNORMALITIES BY HIGH COVERAGE SEQUENCING NON-INVASIVE PRENATAL TESTING

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Objectives: NIPT continues to expend and offer the most advanced testing option. High coverage sequencing NIPT extended the availability of clinically relevant information.
This case series examines some complex positive results and and important clinical and technical points raised.
Methods: Maternal blood samples submitted to Sequenom laboratories for MaterniTGenome testing, were subjected to DNA extraction, library preparation, and whole genome massively parallel sequencing as described by Jensen et al.
Sequencing data were analysed using novel algoritam to detect genome wide structural changes as described by Lefkowits et al.
Results: MaterniTGenome identified loss of chromosome 9 (p24-p23) and gain of chromosome 4 (q32-q35) and suggested unbalanced translocation in anomalous fetus. Amniocentesis confirmed result.
Amniocentesis showed unspecified change on chromosome 7 without precise definition of breaking points. MaterniTGenome identified structural change suggestive of a deletion 5.95 Mb in size in the region q36.2-q36.3 of chromosome 7 in anomalous fetus. Amniocentesis showed mosaic structural change 46,XX/46,XX, del (3) (pter-p14.2).
MaterniTGenome did not identified any change in chromosomal representation. Postanatal karyotype was normal 46XX.
Conclusions: High coverage sequencing NIPT (MaterniTGenome by Sequenom) delivers information regarding chromosome conditions beyond other conventional NIPTs. These results showed 100% of accuracy and may have clinical relevance and may provide further insight into the etiology of fetal structural chromosomal abnormalities.

HIGH COVERAGE SEQUENCING, PRENATAL TESTING

02 – 37 Poster

CYTOGENETIC AND MOLECULAR GENETIC FINDINGS IN PEDIATRIC PATIENTS WITH ACUTE LEUKEMIA AND TRISOMY 21: 10 YEARS OF EXPERIENCE

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Trisomy 21 is aberration commonly found in childhood acute leukemia (AL). It can be present in constitutional karyotype (Down syndrome AL, DS-AL) or acquired in malignant clone (Non-Down syndrome AL, NDS-AL). In DS patients with acute myeloid leukemia (AML) the importance of additional aberrations are still controversial. In DS group with acute lymphoblastic leukemia (ALL) classic cytogenetic aberrations are mostly absent. Patients with acquired trisomy 21 almost exclusively have specific additional aberrations.

Goal of this study was to investigate the presence/absence of additional aberrations in AL patients with trisomy 21.

From november 2009 to may 2019. year in Laboratory of medical genetics at Mother and Child Health Care institute of Serbia, 12 AL patients with trisomy 21 were detected (5 AML and 7 ALL). Chromosomal analysis was done on bone marrow (BM) and peripheral blood lymphocytes (PB) using GTG banding. Detection of recurrent (BCR-ABL, MLL-AF4, TEL-AML, PBX-E2A, PML-RARA, AML1-ETO, CBFB-MYH and FLT3-ITD) and P2RY8-CRLF2 fusion transcripts was performed by RT-PCR and PCR protocols.

In ALL and AML groups all DS patients had 47,XX/XY,+21 karyotype. Two DS-ALL patients (40%) were positive for P2RY8-CRLF2 fusion transcript. Two NDS-ALL patients had additional aberrations: 48,XX,t(5,10)(q13,p11.2)?,-17,+21,+21,+mar(4)/46,XX(6), 48,XY,i(7)(q10),+8,+21(3)/46,XY(17). In DS-AML group additional aberrations were not found. Two of three NDS-AML had aberrant karyotype: 46,XX,inv(2)(p21q11),-7,+21(9)/45,XX,inv(2)(p21q11),-7(8)/46,XX(6) and 48,XY,+21,+mar(2)/46,XY(35).

Our results showed that acquired trisomy 21 is mostly present together with other specific aberrations. Recurrent genetic changes were not detected in DS patients. Aberrant expression of CRLF2 was found in 40% of DS-ALL cases. Although our results are in line with published data, larger study is needed to define the significance of trisomy 21 in these patients.

TRISOMY 21, ALL, AML

02 – 38 Poster

FREQUENCY OF SIL-TAL1 FUSION GENE AND CHROMOSOMAL ABERRATION IN T-ALL PEDIATRIC PATIENTS FROM SERBIA

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Acute lymphoblastic leukemia (ALL) is the most common cancer in children comprising T-cell fenotype in 10-25%. Fusion gene SIL-TAL1, product of deletion of chromosome 1p32, is the most frequent aberration in pediatric T-ALL, seen in about 15% of patients. However, its prognostic implication remains controversial.

Aim of this study was to determine the freqency of SIL-TAL1 fusion gene in T-ALL patients from Serbia, and to define presence of other chromosomal aberrations.

From May 2010. to May 2019. bone marrow (BM) samples of 523 patients with first diagnose ALL, were analysed in Laboratory of medical genetics at Mother and Child Health Care Institute "Dr Vukan Cupic". In all T-ALL samples reverse transcriptase polymerase chain reaction (RT-PCR) assay was used to identify the SIL-TAL1 fusion gene, and for standard fusion transcript specific for ALL t(9;22), t(1;19), t(4;11), t(12;21). Cytogenetic analyses were done on BM samples of 22 patients using conventional and GTG banding methods.

Of 523 new cases of ALL patients, 49 were with diagnose of T-ALL at first presentation (9,3%). Among them 16.3% (8/49) were positive for SIL-TAL1 rearrangement (SIL-TAL1+). None of T-ALL patient was positive for other fusion transcript specific for ALL. Results of cytogenetic studies showed that 11 patients had aberrant karyotypes. From SIL-TAL1+ patients, one had aberrant karyotipe: 48,XX,der17?+mar1+mar2(2)/47,XX,der17?+ mar(10)/46,XX(27). In group of SIL-TAL1 negative patients 10 had aberrant karyotypes: 46,XY,t(5;14)(q35;q11); 46,XY,t(1;14)(p32;q11), 46,XX,t(8,12)(q21?;q13?)(6)/46,XX(16); 46,XY,del(9)(p13)(22); 35-44Hr(18)/46Hr(2), 45Hr(6)/46,XY(11); 48 Hr(5), 92Hr(10). Two had aberrant complex karyotypes with 46 chromosomes without G banding.

The results of this study confirmed that the frequency of T-ALL and the frequency of SIL-TAL1 fusion gene in patients correspond to published data. Two aberrant karyotypes, including region 14q11, are specific for T-ALL.

T-ALL, ABERATION, SIL-TAL1

02 – 39 Poster

PRESENCE OF JAK2 V617F MUTATION AND EEC FORMATION IN PATIENTS WITH POLYCYTHAEMIA VERA – SINGLE CENTER EXPERIENCE

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Introduction: Polycitemia vera (PV) is a chronic myeloproliferative neoplasm characterized by increased red blood cellc. The most frequent genetic abnormality is the somatic mutation of Janus kinase 2 gene (JAK2 V617F) and it occurs in more than 90% of patients with PV. The aim of this study was to investigate the frequency of JAK2 V617F gene mutation in patients with PV and to compare results with presence of endogenous erythroid colony (EEC) formation.
Materials and Methods: Peripheral blood and bone marrow samples of 65 patients with PV were analyzed. The diagnosis of PV was established according to the bone marrow criteria of the World Health Organization. Mutation of JAK2 V617F was determined by allele specific pcr analysis. For detection of EEC formation we used assays of human clonogenic heamatopoietic progenitor cells with agar-leukocyte conditioned medium without recombinant human erythropoietin.
Results: Mutation of JAK2 V617F was found in the samples of the peripheral blood in 61/65 (93.8%) PV patients. EEC formation was obtained in the sample of bone marrow in 59/65 (90,8%) PV patients. In 57/65 (87,7%) patients we detected presence of EEC formation and mutation of JAK2 V617F at the same time.
Conclusions: Presence of JAK2 V617F mutation and EEC are essential characteristics of PV. Considering these results, we concluded that the EEC formation observed in PV could be partially due to the JAK2-dependent activation signaling pathway.

JAK2V617F, EEC

02 – 40 Poster

ONE INSTITUTION'S EXPERIENCE WITH GENETIC TESTING FOR FACTOR V LEIDEN (1691G>A) AND FACTOR FII (20210G>A) VARIANTS AMONG WOMEN WITH THROMBOPHILIA AND REPRODUCTIVE FAILURE

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Genetic testing for thrombophilia (predisposition to form blood clots in blood vessels) is one of the most common tests in clinical genetic laboratories. Factor V Leiden (1691G>A) and factor II (20210G>A) variants, for which it is considered to be the major genetic risk factors for the onset of inherited hypercoagulability, are among the most commonly requested. Most of our patients are women with a diagnosis of venous thromboembolism (VTE) that experienced IVF implantation failure or recurrent foetal loss. Here, we presented the results of genetic testing from 122 females that compared to data obtained from 133 age-matched fertile controls from our population. Genomic DNA was isolated from peripheral blood and genotyping was performed using PCR-RFLP and MAS-PCR methods. No homozygotes and double heterozygotes for genotyped variants were found. Although no significant differences in factor V and factor II alleles and corresponding heterozygous genotypes between affected and controls were found ($p>0.05$), we observed a nonsignificant 3.3-fold increase in thrombophilia risk for the factor II 20210A risk allele (OR=3.3, 95%CI=0.34-31.98). Analyzed genetic variants are linked with the onset of the thrombophilia in the literature and our data could not support an association between factor V Leiden (1691G>A) and factor II (20210G>A) and VTE in females with reproductive failure. According to our results, a larger study group is needed to establish a true relationship between these genetic risk factors, especially for factor II 20210G>A, and thrombophilia in women with reproductive failure.

THROMBOPHILIA, REPRODUCTIVE FAILURE, FACTOR V LEIDEN, FACTOR II, GENETIC TESTING

02 – 41 Poster

CYTOGENETIC FINDINGS IN SERBIAN PATIENTS WITH KLINEFELTER SYNDROME

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Introduction: Klinefelter syndrome (KS) describes the phenotype of the most common sex chromosome abnormality in humans (1/600 newborn males). The most widespread karyotype in affected patients is 47,XXY, but various other karyotypes have been described. The aim of this study was to examine the karyotypes of a group of our patients suspected of having Klinefelter's syndrome.
Materials and Methods: Since January 1993. To April 2018., 104 adult KS patients have been evaluated in the Laboratory of Cytogenetics and Molecular Genetics Clinic of Hematology, Clinical Center of Serbia. Cytogenetic analysis was carried out on metaphases obtained from phytohemagglutinin-stimulated peripheral lymphocytes using a standard procedure. Fluorescence in situ hybridization (FISH) analysis was performed on peripheral blood samples in patients with mosaic karyotype, as well as in patients with sex chromosomes disomy. Vysis CEP X/Y- alpha satellite DNA probes were used to detect X and Y chromosomes.
Results: We identified KS with the 'standard' 47,XXY karyotype in 83 (80%) patients, KS with a mosaic karyotype: 47,XXY/46,XX in 3 (3%) and 47,XXY/46,XY in 5 (5%) (confirmed by FISH), KS with the 'standard' karyotype together with other autosomal chromosomal abnormalities in 6 (6%), and KS with other numerical sex chromosome abnormalities: 48,XXYY in 2 (2%) (confirmed by FISH) and 47,XYY in 5 (5%) patients.
Conclusion: In our group of KS patients most had the 'standard' 47,XXY karyotype, but some men had diverse other karyotypes. It is important to determine the nature of the karyotype of every male with clinical features of KS in very early childhood in order to initiate an adequate, personalized, medical approach.

KLINEFELTER SYNDROME, SEX CHROMOSOME ABNORMALITIES

02 – 42 Poster

RING CHROMOSOME 22 - CASE REPORT

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Introduction: Ring chromosome 22 is rare chromosomopathy caused by deletion of segment on the short (p) arm and a segment on the long (q) arm of chromosome 22 forming a ring. The remaining ends of chromosome 22 have joined together to make a ring shape.
Clinical report: We report 15-year-old boy, born by healthy, non-consanguineous parents. His birth weight was 3050 g, length was 53 cm, he was cyanotic on birth, without breading. During the first year he had 3 times pneumonia and 6 times bronchitis. He had deprivations in the development of static and psychomotoric functions. There were present intracranial anomalies (NMR) and encephalographic abnormalities. He has shown aggressive behavior, and communication problems.
Materials/Methods: Standard G banding chromosomal analyses were performed on metaphase preparations obtained from peripheral blood lymphocytes of the child and his parents.
Results: 50 metaphases from the child were examined. All cells showed 46 chromosomes with a ring chromosome 22 (46,XY,r(22)). Parental karyotypes were normal.
Conclusions: Clinical manifestation of our patient is more serious than other patients published in the present literature. Futher investigations are necessary in order to understand the heterogeneity of clinical expressions. Chromosome analysis is an effective method for detecting uncommon chromosomal anomalies, but additional molecular biology techniques are important for better understanding of the problem.

HROMOSOME 22, RING, CHROMOSOMOPATHY

02 – 43 Poster

ANALISYS OF THE ASSOCIATION OF TNFA, IL1 B AND IL6 PROMOTOR GENE POLYMORPHISMS WITH THE DEVELOPMENT OF SEVERE FORM OF RETINOPATHY OF PREMATURITY

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Retinopathy of prematurity (ROP) is the main cause of visual impairment and blindness in premature infants. Beside well known risk factors, such as: short gestational age, low birth weight and early exposure to oxygen, genetic susceptibility is also a risk factor for development of ROP. Inflammation, which is partly, mediated by proinflammatory cytokines, such as Tumor necrosis factor alpha (TNFα), Interleukin 6 (IL-6) and Interleukin 1 (IL-1β) plays important role in development of normal and pathological angiogenesis in the retina. The aim of this study was to explore the association between TNFα -308 G/A, IL1β -511 G/A and IL6 -174 G/C gene polymorphisms with development of severe ROP forms, which demands therapeutic approach. There were 284 preterm infants included in this study, divided into two groups. First group included 101 patients with severe form of ROP which demands therapy for its reduction, and control group which included 166 patients without ROP or with mild forms of ROP which did not request therapy. TNFα -308 G/A, IL1β -511 G/A and IL6 -174 G/C polymorphisms were genotyped using Real-time RCR method. No differences have been observed in genotypes distribution of TNFα -308 G/A and IL-6 -174 G/C polymorphisms between two analyzed groups. There was statistically significant difference by recessive model (GG+GA/AA) for IL1β -511 G/A polymorphism, between two groups of premature infants. We observed that AA carriers are more prone for development of severe form of ROP than carriers of GG+GA genotype (p=0.034, OR 2.465, 95%CI 1.044-5.824). Logistic regression analysis confirmed statistically significant association between IL1β -511 AA genotype and development of severe form of ROP with gestation age, birth weight and intracranial hemorrhage as covariates (B-1.293, p=0.017).
Our findings suggest that IL1β -511 AA genotype is risk factor for development of severe form of ROP.

RETINOPATHY OF PREMATURITY, GENE POLYMORPHISM,TNFA, IL1 B, IL6

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02 – 44 Poster

ANALYSIS OF ENDOTHELIAL NITRIC OXIDE SYNTHASE T786C GENE POLYMORPHISM IN SURGICAL PATIENTS WITH DIFFUSE SECONDARY PERITONITIS

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Introduction: Secondary peritonitis is a peritoneal inflammation caused by perforation, necrosis or penetrant injuries of gastrointestinal tract. Although significant progress in treatment has been made it is still a life threatening condition. Nitric oxide (NO) synthesized by endothelial NO synthase (eNOS), plays significant role in pathogenesis of sepsis. NO production is important for maintaining convenient blood flow in vital organs and causes the upregulation of proinflammatory cytokines. This study aimed to assess the association of eNOS T786C gene polymorphism with severe complications and mortality in surgical patients with secondary peritonitis.
Material and methods: Our study included 92 surgical patients with diffuse secondary peritonitis. All patients underwent emergency laparotomy, surgical source control, antibiotic therapy and intensive care support. We evaluated the following surgical outcomes: septic shock, multiple organ dysfunction syndrome (MODS), respiratory complications, mechanical ventilation and mortality. The analysis of T786C polymorphism was performed by PCR-RFLPs method.
Results: Analysis showed that frequencies of CT and CC genotypes were higher in a group of patients that required mechanical ventilation (p=0.026) independently from etiology of peritonitis and perioperative patients characteristics. Association of analyzed polymorphism with septic shock and MODS was not statistically significant.
Conclusion: eNOS T786C polymorphism could be important for the assessment of the risk of respiratory failure which requires mechanical ventilation in patients with secondary peritonitis.

SECONDARY PERITONITIS, ENOS, GENE POLYMORPHISM

02 – 45 Poster

A CASE OF A NEWBORN WITH COMPLETE TRISOMY 8 IN PERIPHERAL BLOOD LYMPHOCYTES

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Only a few cases of complete trisomy 8 have been described, with a wide variety of congenital malformations. We present an additional case of complete trisomy 8 in peripheral blood lymphocytes.
Case report: A female newborn with prenatally detected agenesis of the corpus callosum, was delivered in 36th gestational week. Physical examination revealed prominent forehead, expressionless facies with a deep-set eyes, a broad upturned nose with prominent nares, prominent, low set ears, cleft palate, full lips, camptodactyly, deep creases on palms and soles and widely spaced nipples. She had poor visual tracking of the moving objects because of congenital corneal opacities revealed by ophthalmologic examination. She was hypotonic with reduced motor activity and had brisk deep tendon reflexes. Ultrasound and computed tomography of the brain confirmed agenesis of the corpus callosum and revealed mildly dilated lateral ventricles. Spinal clinical examination showed a subtle protrusion cowered by a skin in the midline of the back in the thoracal region of the spine. Computed tomography detected spina bifida occulta. Chromosome analysis of peripheral blood lymphocytes showed complete chromosome 8 trisomy.
We would like to emphasize the significance of fetal karyotyping in case of detection of the corpus callosum agenesis as the most important echographic criterion for the diagnosis of trisomy 8 syndrome.

TRISOMY 8, NEWBORN, CORPUS CALLOSUM AGENESIS

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ANALYSIS OF PGC1A GENE POLYMORPHISM IN COLORECTAL CANCER PATIENTS

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Background: Colorectal cancer is the second most commonly diagnosed cancer in men and third in women in Serbia. The prognosis depends significantly on tumor invasion, lymph node metastases and distant metastasis. Recent studies have shown that peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC1α) could represent a biomarker for prognosis in colorectal cancer and a possible target for cancer therapy. PGC1α has important role in mitochondrial biogenesis regulation, de novo lipid synthesis and glycolysis. Common polymorphism rs8192678 (1444 G>A, Gly482Ser) has been described in a highly conserved region of the gene. The aim of our study was to investigate the correlation between PGC1α polymorphism Gly482Ser and clinicopathological factors of tumor progression in colorectal cancer patients.
Material and methods: Our study included 146 patients with colorectal cancer who underwent surgical treatment at Clinic for digestive surgery, Clinical center of Serbia. Genotypes for the selected polymorphism were detected by Real-time PCR. Information about tumor location, histologic differentiation, preoperative serum carcinoembryonic antigen (CEA) level, lymph node metastases, the presence of distant metastases and stage of the disease was recorded.
Results: Our analysis revealed that the presence of distant metastases is significantly more frequent in A-allele carriers compared to patients with GG genotype (p=0.005). Also, A-allele carriers had significantly higher frequency of moderately and poorly differentiated tumors compared to patients with GG genotype (p=0.034). Correlation with other clinicopathological parameters such as tumor location, CEA level, lymph node metastases and disease stage was not statistically significant.
Conclusion: We found PGC1α polymorphism Gly482Ser associated with colorectal tumor differentiation and presence of distant metastases. These results are the basis for further studies on larger patient groups.

COLORECTAL CANCER, PGC1A, GENE POLYMORPHISM, TUMOR PROGRESSION

02 – 47 Poster

THE ROLE AND CHALLENGES OF A PROPER GENETIC COUNSELING: CASE PRESENTATION

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We present a couple with unsuccessful reproductive history including: five spontaneous abortions, a male child with Dandy-Walker syndrome who died after 17 months from acute liver failure, one medically indicated termination of pregnancy due to corpus callosum agenesis, ventriculomegaly and colpocephaly in fetus, and a premature born child (29. gestational week), today 13 years old, with cerebral paralysis and severe psychomotor retardation.
Genetic testing of a boy with Dandy-Walker syndrome was not performed.
In the female fetus with CNS anomalies karyotype from amniotic fluid was normal (46,XX), as well as the results of the next generation sequencing of fetal DNA for 374 genes (genes included in spontaneous abortions, intracranial haemorrhagia, ventriculomegaly, agenesis of corpus callosum, Dandy-Walker syndrome and death in early childhood).
For both parents, peripheral blood karyotype and next generation sequencing for the same genes as for the fetus with CNS anomalies were also done.
In the only live child, a 13 years old boy, karyotype from peripheral blood lymphocytes, high resolution microarray (CGH), testing for Fragile X syndrome and next generation sequencing for the same genes as in fetus with CNS anomalies, were performed.
The results of all genetic tests were normal, without any significant genetic markers for this reproductively challenging couple.
Regarding the fact that cerebral paralysis and psychomotor retardation may be an aftermath of a premature birth, we would like to emphasize the role of a proper genetic counseling in this case. In our opinion, it is necessary to perform high resolution microarray (CGH) in both parents and in every future pregnancy, also having on mind possible mutations of a mitochondrial DNA.

GENETIC COUNSELING, KARYOTYPE, MICROARRAY, TESTING

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ANALYSIS OF LMNB1 GENE IN SERBIAN PATIENTS WITH AUTOSOMAL DOMINANT LEUKODYSTROPHY

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Autosomal Dominant Leukodystrophy (ADLD) is a slow progressive, fatal, adult onset disorder affecting myelin in the central nervous system. It is caused by duplications involving LMNB1gene or, in a single family reported, deletion upstream of the gene. Although, the exact prevalence of the disease is not known, it is assumed that ADLD is a very rare and not specific to any region or ethnic group. This is the only disorder associated with increased LMNB1 gene expression, described to date.
The aim of our study was to reveal multiplications or deletions of the LMNB1 gene in 3 familial cases with clinical diagnosis of ADLD, and in additional 18 sporadic cases with leukodistrophy or leukoencephalopathy.
Using multiple ligation probe amplification (MLPA) method, duplication of whole LMNB1 gene was detected in three familial cases. Two siblings and one unrelated patient reported affected family members in multiple generations. Age at onset in all cases with LMNB1 duplication was in fifth decade, with progressive gait difficulties and autonomic dysfunction (orthostatic hypotension and bladder dysfunction), hallmark symptoms of ADLD. None of analysed sporadic cases carried the duplication or deletion of LMNB1 gene.
Results of our study are suggesting that, beside clinical presentation, positive family history of leukodystrophies with autosomal-dominant inheritance should be reported, when analysis of LMNB1 gene is considered in diagnostic purposes. Very recent study showed that allele-specific silencing by RNA interference is a promising therapeutic option for ADLD, and genetic confirmation of families with this condition can contribute to developing potential drugs for this incurable disease.

LMNB1, LEUKODYSTROPHY, ADLD

02 – 49 Poster

RESPONSIVENESS OF HUMAN U251 GLIOBLASTOMA CELLS TO ALL-TRANS RETINOIC ACID

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Gliomas are the most common primary brain tumors in humans. Glioblastoma, grade IV of glioma tumors, is the most common and lethal brain tumor in adults with the median survival time of 15 months despite aggressive treatment which include surgical resection, high-dose radiation and chemotherapy with temozolomide. Therefore, development of more effective therapeutic strategies for patients with GBM is warranted. Differentiation therapy, which main goal is to induce differentiation of cancer cells in order to eliminate tumor phenotypes, holds great promise for cancer treatment. Literature data about the effects of all-trans retinoic acid (ATRA), the most widely used differentiating therapeutic agent, on the malignant characteristics of glioblastoma cells are contradictory. Thus, we analysed whether ATRA treatment affects features of human glioblastoma U251 cells. To that end, the U251 cells, one of the most widely used in vitro model system for studying glioblastoma pathobiology, were treated with different concentrations of ATRA and effects on cell morphology, viability, expression of markers of neural differentiation, migratory and cell-matrix adhesion capabilities were analysed. Obtained results demonstrate that ATRA affected the viability of U251 glioblastoma cells in a dose- and time-dependent manner, induced changes in cell morphology and altered the mode of cell migration from collective to single cell motility. Also, pharmacologically relevant concentration of ATRA lowered the cell-matrix adhesion capability of U251 cells. At the same time this agent did not influence expression of markers of neural differentiation implying that ATRA treatment did not induce neural differentiation of U251 glioblastoma cells. Altogether, obtained results indicated that further studies are necessary before therapy with ATRA could be considered for treatment of glioblastoma.

GLIOBLASTOMA, ATRA, DIFFERENTIATION THERAPY

02 – 50 Poster

THE EXPRESSION OF HEDGEHOG PATHWAY GENES IN CANCER STEM CELLS OF BASAL CELL CARCINOMA

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Cutaneous basal cell carcinoma (BCC) is the most common human cancer and its incidence is rising worldwide. A major role in the pathogenesis of many tumors, including BCC is exerted by Sonic Hedgehog (SHH) signaling pathway which also plays an important role in embryonic development, adult tissue homeostasis and stem cell regulation. The PTCH1gene, a member of SHH pathway normally inhibits downstream signaling. The aim of this study was to investigate the role of SHH pathway in cancer stem cells of BCC and its margin. Primary cultures were generated from (a) tumor tissue, (b) its close surgical margin and (c) distant healthy margin (more than 5mm as control), obtained from patients with BCC. First and fifth passage cells were phenotypically characterized by flow-cytometry, spheroid formation test and differentiation assay. RNA isolation was performed by standard phenol-chloroform procedure followed by cDNA synthesis with reverse transcriptase. The relative expression of hedgehog signaling pathway genes PTCH1, GLI1, GLI2 was determined by real-time PCR. The level of PTCH1 was lower in the tumor compared to margin cells and controls pointing to its defective inhibitory activity. This reduced activity was further confirmed by higher levels of GLI1 both in tumors and margin cells, from the first and fifth passage. GLI2 expression seemed to be less affected by PTCH1 in our experimental settings. There was a statistically significant difference of GLI1 expression between the three types of cells ($p < 0.05$). Sonic Hedgehog signaling pathway appears to play a role in BCC cancer stem cells, and cells from close tumor margin as well.

CANCER STEM CELL, SHH SIGNALING PATHWAY, MARGIN, BASAL CELL CARCINOMA

02 – 51 Poster

THE ASSOCIATION OF THE GLUTATHIONE S-TRANSFERASE T1 AND M1 DELETION WITH MYOCARDIAL INFARCTION IN SERBIAN POPULATION

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Substantial findings implicate that oxidative stress (OS) plays an important role in the development of atherosclerosis. Glutathione S-transferases (GSTs) are the family of enzymes regarded as the second line of defence against OS and protect cellular macromolecules by catalyzing the conjugation of reduced glutathione (GSH) to endogenous harmful products of OS, as well as exogenous cytoxic compounds with potentially atherogenic effects. The aim of our study was to elucidate whether deletions in GSTM1 and GSTT1 genes are associated with myocardial infarction (MI) in Serbian population and subsequently with left ventricular maladaptive remodeling (LVMR) six months after the first acute MI. The deletion polymorphisms in these genes are responsible for loss of enzyme function and are denoted as GSTM1 null and GSTT1 null genotypes. A multiplex PCR method was used to detect the deletions in the genomic DNA of 185 patients and 329 controls. Patients with MI had significantly higher frequency of both, GSTT1 and GSTM1 null genotypes compared to controls (34.59 % vs. 21.88 %, 58.92 % vs. 47.42 %, p = 0.002, p = 0.008, respectively). After adjustment for risk factors (age, BMI, smoking status, TG, HDLC), association remained significant (for GSTT1 null genotype: adjusted OR 1.89, 95% CI=1.11-3.25, p=0.02; for GSTM1 null genotype: adjusted OR 1.86, 95 % CI = 1.14 - 3.03, p = 0.01). Also, these variants were associated with the presence of maladaptive left ventricular remodeling in patients who suffered from the first acute MI. Patients with LVMR had significantly higher frequency of double deletions compared to those without occurrence of LV (48.00% % vs. 27.42 %, respectively, OR = 2.44, 95% CI: 1.01–5.92, p = 0.046). Based on our results, we suggest that the deletions in GSTM1 and GSTT1 genes could be predictive markers for assessing the risk for MI and left ventricular maladaptive remodeling six months after the first MI.

GSTT1, GSTM1, OXIDATIVE STRESS, MYOCARDIAL INFARCTION, LEFT VENTRICULAR REMODELING

02 – 52 Poster

THE METHYLATION STATUS OF MGMT IN SERBIAN PATIENTS WITH DIFFUSE GLIOMA

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Promoter methylation of DNA-repair gene O6-methylguanine-DNA methyltransferase (MGMT) represents a widely accepted prognostic factor and a potent predictor of responsiveness to alkylating chemotherapy in diffuse glioma patients. The goal of our study was to investigate the relevance of methylation status MGMT for Serbian patients with diffuse glioma. Our preliminary results with 33 samples of diffuse glioma detected a positive methylation status in 17 patients (51.5%) by methylation-specific polymerase chain reaction. Hypermethylation of MGMT promoter did not correlate with overall survival of patients. Older patients (>50 years) had significantly lower overall survival in comparison to younger patients (7 and 19 months, respectively). Also, our study confirmed correlation between the extent of the tumour resection and overall survival. Patients with total resection had better overall survival in comparison to those with partial resection and biopsy (11, 7 and 1.5 months, respectively). Further evaluation of MGMT promoter methylation status and its association with other markers in larger-scale study is planned.

DIFFUSE GLIOMA, MGMT, METHYLATION, MSP, OVERALL SURVIVAL

02 – 53 Poster

MOLECULAR GENETIC TESTING OF HUNTINGTON'S DISEASE AND GENETIC COUNSELLING

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Huntington disease (HD) is a monogenic neurodegenerative disease with an autosomal – dominant pattern of inheritance. The prevalence of HD is around 3-7/100.000. It is characterized typically by onset in middle age and motor, cognitive and psychiatric features with irreversible progression of symptoms over 12 – 17 years as a result of neuronal death in subcortical parts of brain and basal ganglia. HD is caused by an expanded CAG triplet repeat in exon 1 of the HTT gene, with 35 uninterrupted repeats as normal cut off and more than 39 repeats as full mutation. Longer CAG repeat lengths correlate with an earlier age of disease onset and anticipation phenomenon is well known.
In a cohort of 76 patients from Neurology Clinic, Clinical Center of Serbia in Belgrade we performed qfPCR and fragment analysis to determine the exact CAG repeat number in the HD gene to confirm the clinical diagnosis.
We identified 19 patients with CAG repeats between 40 and 49 and they manifested classic middle age onset between the ages of 30 and 50. Repeat lengths larger than 50 with onset between 20 and 30 years of age, was detected in 4 cases. Juvenile HD, when disease onset occurs before the age of 20, was detected in one patient only as a result of anticipation with paternal transmission. In our cohort one older asymptomatic patient from HD family had expansion in the range between 36 and 39, generally characterised with incomplete penetrance.
In our center, molecular genetic testing is carried out on those people who are clinically consistent with HD and it is requested by neurologists after informed consent. The implication of both positive and negative test results for a patient should be explained. Predictive genetic testing as well as prenatal diagnosis in cases with a positive family history should be performed carefully after detail genetic information and counselling. However genetic testing of HD still carries great ethical dilemmas in this age of genetic medicine.

HUNTINGTON'S DISEASE, GENETIC TESTING, COUNSELLING

02 – 54 Poster

GENE EXPRESSION ANALYSIS OF EPITHELIAL TO MESENCHYMAL TRANSITION MARKERS IN ORAL CANCER AND ITS MARGINS

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Objective: Epithelial to mesenchymal transition (EMT) represents a feature of many human cancers, including oral squamous cell carcinoma (OSCC). The EMT phenomenon is closely related to cancer stem cells and represents a sign of tumor aggressiveness. In the present study tumor and margin cell cultures obtained from patients w ith O SCC were u sed to determine the expression patterns in the course of time, of EMT-related markers Vimentin, αSMA, SLUG and SNAIL, and EMT-related features, i.e. the clonal, proliferative and migratory potential of the two types of cells.
Design: Cell cultures were generated from tumor and margin tissue samples obtained from 6 patients and grown up to the 5th passage. EMT markers expression was assessed by real time qPCR. Cell proliferation, colony forming and scratch wound healing assays were conducted to characterize the two cell types in terms of proliferation rates, clonality, and motility.
Results: All the studied markers were expressed in both tumor and margin cells, without statistically significant difference (p>0 .05). With few exceptions, EMT markers expression was higher in the 5th passage compared to the 1st. Proliferation rates a nd c ell migration velocity decreased during passages, while the number of colonies increased in both types of cell cultures.
Conclusions: Tumor and margin cells showed remarkable similarities i n terms o f EMT characteristics, pointing to the necessaty of reconsidering the width of "safe surgical margins.

ORAL CANCER, EPITHELIAL TO MESENCHYMAL TRANSITION, SURGICAL MARGINS, GENE EXPRESSION, CELL PROLIFERATION AND MIGRATION

02 – 55 Poster

FREQUENCY OF GENETIC RISK FACTORS FOR THROMBOPHILIA AND GILBERT SYNDROME IN THE POPULATION OF REPUBLIC OF SRPSKA, BOSNIA AND HERCEGOVINA

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Variants in several genes (factor V-FV, prothrombin-PT and methylenetetrahydrofolate reductase-MTHFR), such as FVL c.1691 G>A (Leiden), PT c.20210 G>A and MTHFR c.677 C>T, have significant role in thrombophilia. UGT1A1 enzyme has a vital role in the metabolism of bilirubin and drugs. UGT1A1*28 promotor variant, with 7 TA repeats in TATA promoter box, is responsible for decreased gene expression and enzyme activity. UGT1A1*28 is a diagnostic marker for Gilbert syndrome and an important pharmacogenetic marker for numerous drugs, especially those used in the treatment of oncological patients. The aim of this study was to determine frequency of four genetic markers of high clinical significance in healthy population of Republic of Srpska, Bosnia and Hercegovina. In this study 100 healthy, unrelated individuals (86 women and 14 men) from the population from all regions of Republic of Srpska were included. We analyzed DNA samples using PCR-based methodology. Frequency of homozygous genotype of FVL wild type allele was 95% and 5% of heterozygotes for Leiden mutation were found. Frequency of homozygous genotype of prothrombin wild type allele was 98% and 2% heterozygotes for prothrombin PT c.20210 G>A variant allele were found. Frequency of homozygous genotype of MTHFR wild type allele was 50%, while 43% heterozygotes and 7% homozygotes for MTHFR c.677 T allele were found. Allele frequencies in healthy population were 2,5% for factor V Leiden mutation, 1% for prothrombin PT c.20210 G>A variant and 28,5% for MTHFR c.677 C>T variant. Genotypes UGT1A1 6/6 TA, 6/7 TA and 7/7 TA occur with a frequency of 37,19%, 37,19% and 25,6% respectively, and the frequency of UGT1A1*28 allele in the general population is 44%. Our data of frequency of genetic risk factors for thrombophilia and Gilbert syndrome are the first-of-its-kind for the population of Republic of Srpska and they can be used as reference values in future research association and pharmacogenetic studies.

GENETICS, THROMBOPHILIA, GILBERT SYNDROME, REPUBLIC OF SRPSKA

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GENE EXPRESSION ANALYSIS OF ODONTOGENIC AND OSTEOGENIC DIFFERENTIATION MARKERS IN HUMAN STEM CELLS FROM APICAL PAPILLA (SCAPS) SEEDED ON BONE SUBSTITUTE BASED ON HYDROXYAPATITE AND PLGA

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Novel bone substitute named ALBO-OS, based on hydroxyapatite porous scaffold covered with thin polymeric film of poly(lactide-co-glycolide) (PLGA), has been synthesized recently. The aim of this study was to investigate osteoinductive potential of ALBO-OS, *in vitro*, on stem cells from apical papilla (SCAPs).
SCAPs were characterized by flow cytometry and multilinear differentiation. ALBO-OS discs (10x10x5 mm) were placed in 12 well plates and SCAPs were seeded on to scaffolds and in control wells. Cells were cultured in growth and osteogenic medium, and cells cultured in complete medium served as control. Twenty-one days after, the total RNA was isolated from cells by the standard phenol-chloroform procedure. The relative target gene expression of odontogenic marker matrilin-4 (MTNR-4), and osteogenic markers osteocalcin (OCN), and bone morphogenetic protein 2 (BMP-2) were determined by real-time reverse-transcription polymerase chain reaction.
The 98 – 99% of cells were positive for mesenchymal stem cells markers and successfully differenced into osteogenic, adipogenic and chondrogenic lineage. The relative expression of selected markers of osteogenic differentiation (OCN, BMP-2) showed to be upregulated in the presence of ALBO-OS, both in cells cultivated in growth ($p<0.01$) and in osteogenic medium ($p<0.01$, $p<0.0001$). Upregulation of MTNR-4 was noticed only in group SCAPs cultured in osteogenic medium alone ($p<0.0001$), while presence of ALBO-OS influenced downregulation of odontogenic marker, in both mediums.
Results of this study revealed excellent osteoinductive properties of ALBO-OS alone, while by adding induction medium osteogenic properties were further induced. Upregulation of MTNR-4 only of SCAPs cultured in osteogenic medium alone could suggest that, since their dental tissue origin, SCAPs spontaneously different to odonto/osteogenic lineage, but in the presence of scaffold pure osteogenic differentiation occurs.

BONE SUBSTITUTE, ALBO-OS, OSTEOINDUCTIVE PROPERTIES, DENTAL STEM CELLS, OSTEOGENIC DIFFERENTIATION

02 – 57 Poster

eNOS POLYMORPHISMS AND RISK OF OSCC DEVELOPMENT IN SERBIAN POPULATION

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Studies have shown that nitric oxide (NO) has an important but contrasting role in cancerogenesis, both tumor-promoting and antitumor effects. Endothelial NO synthase (eNOS) is one of the three classes of NOS enzymes and is expressed by certain cancers, including oral squamous cell carcinomas (OSCCs). It has been reported that genetic polymorphisms in the eNOS gene regulate its transcription and mediate NO production, but the results were inconsistent. The aim of this study was to assess the possible association of eNOS polymorphisms (-786 T/C, 894G/T and intron 4b/a VNTR) and their haplotypes with OSCC risk in Serbian population.
In a case-control study of 50 OSCC cases and 110 cancer-free controls, eNOS polymorphisms were genotyped by polymerase chain reaction/restriction fragment length polymorphism analysis (PCR-RFLP). Logistic regression has been used for cancer risk assessment.
Patients with -786 T/C CC genotype and C allele were under significantly higher risk for OSCC: OR=3.63, 95% CI 1.08-12.21, P=0.045, and OR=1.70, 95% CI 1.03-2.81, P=0.035 respectively. Carriers of intron 4b/a VNTR 4b4a genotype, 4a4a genotype and 4a allele showed the following risk: OR=3.03, 95% CI 1.45-6.32, P=0.003, OR=11.29, 95%CI 2.71-47.11, P<0.001, and OR=3.03 95% CI 1.80-5.12, P<0.001, respectively. None of the investigated polymorphisms were in Linkage Disequilibrium (LD). However, T–G–4a, T–T-4b and C–G–4b haplotypes were associated with significantly higher risk for OSCC: OR=3.84, 95% 1.61-9.14, P=0.002, OR=3.17 95% CI 1.13-8.91, P=0.025, and OR=11.52, 95% CI 3.54-37.49, P<0.001, respectively.
These results implicate eNOS polymorphisms −786 T>C and intron 4b/a VNTR as risk modulators of OSCC development, but further studies are needed to validate our findings.

ORAL SQUAMOUS CELL CARCINOMA, ENDOTHELIAL NITRIC OXIDE SYNTHASE, GENE POLYMORPHISM

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ASSOCIATION BETWEEN METHYLENETETRAHYDROFOLATE REDUCTASE GENE POLYMORPHISMS C677T (rs1801133) AND A1298C (rs1801131) AND RELATIVE TELOMERE LENGTH IN HIV-INFECTED PATIENTS ON cART

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Mutations in methylenetetrahydrofolate reductase gene (MTHFR) can lead to high levels of homocysteine in the blood which produces homocysteine accumulation and causes DNA hypomethylation. When TERT gene promoter is hypomethylated, telomerase catalytic subunit's activity is hampered leading to shorter telomeres. Individuals with single nucleotide polymorphisms (SNPs) in MTHFR genes, C677T and A1298C, can face up to 80-90% reduction in MTHFR activity. Telomere length can also be altered in response to HIV exposure and combined antiretroviral therapy (cART). The aim of our study was to compare relative telomere length (RTL) in HIV-infected patients with different MTHFR genotypes. The study was conducted on blood samples collected from 126 HIV-infected patients older than 18 years who had been receiving cART for at least 12 months. Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method was used for MTHFR genotyping, while Relative Telomere Length (RTL) was measured by quantitative PCR. Collected data were analyzed by EZR software. Genotype distribution of rs 1801133 was as follows: 52 (41.27%) patients were wild type homozygotes (CC genotype), 68 (53.97%) were heterozygotes (CT) and 6 (4.76%) were homozygotes for the variant allele (TT). The genotypes for the rs 1801131 polymorphism were the following: 23 (18.25%) of patients were wild type homozygotes (AA genotype), while there were 66 (52.38%) of heterozygotes and 37 (29.37%) with CC genotype. There was no difference (p = 0,276) in RTL between patients with the rs 1801133 CC genotype (median RTL = 2.055, iqr = 1.377-3.165), patients with CT (median RTL = 2.105, iqr = 1.175-3.295) and TT genotypes (median RTL = 1.285, iqr = 1.132-1.407). Also, significance was not found (p=0,458) for the rs 1801131 polymorphism between carriers of the AA genotype (median RTL = 3.265, iqr = 1.5650-3.265), heterozygotes (median RTL = 1.945, iqr = 1.322-3.045) and variant homozygotes (median RTL = 1.870, iqr = 1.120-3.460). In conclusion, from the present study, it appears that MTHFR genotypes do not have considerable impact on telomere length in HIV-infected patients, but HIV infection and cART might have.

MTHFR, RTL, HIV, SNP

02 – 59 Poster

GENE POLYMORPHISMS OF TNF-A, IL-1B, GSTT AND GSTM AS RISK FACTORS FOR EPSTEIN - BARR VIRUS INFECTION IN APICAL PERIODONTITIS

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Genetic predisposition for EBV infection has been reported in several inflammatory and malignant diseases. Therefore, this study aimed to investigate whether single nucleotide polymorphisms (SNPs) in tumor necrosis factor-alpha (TNF-α) (-308G>A) and interleukin-1 beta (IL-1β) (-511C>T) genes, and deletion polymorphisms in glutathione S-transferase T and M (GSTT and GSTM) genes are associated with the occurrence of EBV infection in AP. One hundred and twenty AP lesions were collected following standard apicoectomy. A nested polymerase chain reaction (PCR) was used to determine the presence of EBV. Based on those findings, all AP lesions were further divided into two groups, i.e. 80 EBV positive (EBV +) and 40 EBV negative (EBV-) lesions. PCR and restriction fragment length polymorphism analysis were used for genotyping. Allele and genotypic frequencies, Hardy–Weinberg equilibrium (HWE) and study power (SP) were calculated using the SPSS and R-statistical software. Logistic regression analysis (LRA) was used to calculate odds ratio (OR) and 95% confidence interval (CI). There was no evidence of deviation from HWE for any of the investigated polymorphisms within the groups (p>0.05). A significant difference in the GSTT deletion genotype was observed between EBV+ and EBV- lesions (66.25% vs. 47.5%), and LRA showed an increased risk for EBV infection in GSTT null genotype carriers (OR=2.17, 95% CI=1-4.7, p=0.048). LRA also showed a lower risk for EBV infection in both GA heterozygote and AA homozygote genotypes for TNF-α (OR=0.20, 95% CI=0.08-0.48, p<0.001; OR=0.07, 95% CI=0.01-0.37, p=0.001, respectively). The sample size was sufficient to achieve a SP above 80% for the analyzed parameters. Beside microorganisms, heredity may contribute to variation in AP pathogenesis. The present study has demonstrated that a deletion polymorphism in GSTT and a SNP in TNF-α genes modulate the risk for EBV infection in AP.

APICAL PERIODONTITIS, EPSTEIN-BARR VIRUS, GENE POLYMORPHISM, INTERLEUKIN, GLUTATHIONE S-TRANSFERASES

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02 – 60 Poster

ASSOCIATION BETWEEN CLASS 1 MYOSIN (MYO1H) SINGLE NUCLEOTIDE POLYMORPHISM (rs3825393) AND MANDIBULAR PROGNATHISM

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Mandibular prognathism is a deformity of the dento/cranio/facial complex characterized by a protrusion of the mandible. It is also called skeletal class III. In cases of severe prognathism the quality of life may considerably be affected due to social misfunctioning, in addition to difficulties in mastication and speech. Cephalometric analysis is the most accurate technique for determining the specific subtypes of prognathism. It includes assessments of skeletal base, occlusal plane angulation, anterior dental angulation, facial height and soft tissue assessment. Mandibular prognathism is a multifactorial disorder, with a still unclear genetic etiology. The aim of the present study was to evaluate the potential role of single nucleotide polymorphisms in two genes (MATN1 and MYO1H) whose products, the cartilage matrix protein matrilin and class 1 myosin, are involved in the development of the craniofacial complex. Genotyping of -1878 G/A; rs1149048 single nucleotide polymorphisms (SNP) in MATN1 and 1001 G/A; rs3825393 SNP in MYO1H was done on 80 patients (50 class III patients and 30 class I controls), using the PCR/RFLP technique.
There were no statistically significant differences in genotype/allele frequencies between patients and controls for the MATN1 polymorphism. However, the SNP in MYO1H gene, despite the small sample size, appeared to be significantly associated with the risk of mandibular prognathism. Namely, carriers of the variant allele A, either as heterozygous or homozygous, had an almost 3-fold risk increase to develop prognathism compared to wild type homozygotes (OR 2.73, 95% CI 0.93-7.98, p=0.05). A larger cohort of patients and controls is needed, along with other DNA polymorphisms studies in order to better understand this complex disorder.

MANDIBULAR PROGNATHISM, SINGLE NUCLEOTIDE POLYMORPHISM, MATRILIN 1, CLASS 1 MYOSIN

02 – 61 Poster

RS9349379 AFFECTS PHACTR1 mRNA EXPRESSION IN CAROTID ATHEROSCLEROTIC PLAQUE

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Background: Carotid plaque is a hallmark of advanced carotid atherosclerosis. Phosphatase and actin regulator 1 (PHACTR1) is involved in the processes that lead to atherosclerosis. PHACTR1 intronic variants have been associated with coronary artery disease, ischemic stroke and carotid dissection. It has been shown that rs9349379 is an eQTL for PHACTR1 in coronary arteries. The aims of this study were to investigate possible association of PHACTR1 rs9349379 with carotid plaque presence (CPP) in patients undergoing carotid endarterectomy and to analyze its possible effect on PHACTR1 mRNA expression in carotid plaque tissue specimens.
Methods: The study group consisted of 380 patients with evidence of carotid plaque presence admitted for carotid endarterectomy and 250 healthy controls. Out of 40 carotid plaque specimens obtained, 33 provided the total RNA with satisfactory quality. PHACTR1 rs9349379 and relative mRNA expression were detected by TaqMan® technology. The relative PHACTR1 mRNA levels were analyzed using REST software.
Results: PHACTR1 rs9349379 was not associated with carotid plaque presence (p=0.15). We have shown that PHACTR1 mRNA was significantly down-regulated in G allele carriers (AG+GG vs AA) by a mean factor of 0,546 (S.E. range 0,214 - 1,453), p=0,019.
Conclusion: PHACTR1 rs9349379 could be relevant for the atherosclerosis development and/or progression through its effect on PHACTR1 mRNA level. Further studies in a larger sample size are required, as well as, the analysis of this variant in haplotype with other PHACTR1 variants.

PHACTR1, HAPLOTYPE, CAROTID PLAQUE, MRNA EXPRESSION, ATHEROSCLEROSIS

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02 – 62 Poster

ASSOCIATION OF IFI30 rs11554159 WITH SEVERITY OF RELAPSE REMITTING MULTIPLE SCLEROSIS (RRMS) IN PATIENTS FROM SERBIA

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The genetics of antigen presentation was thoroughly investigated for the purpose of understanding the genetic basis of multiple sclerosis (MS) onset and progression. Yet, the effects of genetic variants beyond the function of MHC genes, which present the antigen epitopes are still fairly obscure. Gene IFI30 codes for the gamma inducible thiol reductase, which is the only known enzyme to reduce disulfate bound in lysosomes significantly influencing the peptide epitope repertoire. The non synonymous variant rs11554159 results in an amino acid change from to Arg to Gln at codon 76, near the active site of the enzyme potentially affecting protein structure and function. We aim to analyze the association of IFI30 rs11554159 with clinical parameters of MS. Therefore 474 patients with MS were recruited from the Clinic for Neurology at the Military Medical Academy in Belgrade and genotyped for IFI30 rs11554159 using TaqMan® based allelic discrimination methodology. The rs11554159 genotypes (GG 59.5%, AG 35% and AA 5.5%) were analyzed in context of multiple sclerosis severity score (MSSS) (GG MSSS=4.92±2.47, MSSS=AG 4.66±2.39, AA MSSS=5.38±2.27, p=0.25) and age of disease onset (GG 30.7±9.6 years, AG 31.3±8.9 years, AA 31.5±8.17 years, p=0.76). Analyzed by the recessive model of inheritance, rs11554159 AA genotype was associated with significantly higher MSSS compared to common G allele carriers, but only in the group of patients with RRMS and disease duration greater than 10 years (GG+AG MSSS=2.7±1.39, AA MSSS=4.55±1.56, p=0.01, n= 65). No significant difference was found in genotype distribution between RRMS patients with disease duration greater and lesser than 10 years (p=0.16). In conclusion, our results indicate that rs11554159 AA genotype might be unfavorable in the context of MS severity in RRMS patients with disease duration of more than ten years, but further investigation on larger group of patients is needed.

MULTIPLE SCLEROSIS, IFI30, rs11554159, DISEASE SEVERITY

02 – 63 Poster

"MINING" FOR THE RARE SERPINA1 VARIANTS IN POPULATION OF SERBIA – ANALYSIS OF THE PUBLISHED DATA

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Background: SERPINA1 is encoding for alpha-1-antitrypsin. Although some of rare SERPINA1 variants can be associated with alpha-1-antitrypsin deficiency (AATD), little is known about their epidemiology and clinical phenotype, due to their low occurrence. Our aim was to assess the availability of published data about rare SERPINA1 variants in population of Serbia.
Methodology: We used "alpha-1-antitrypsin" and "Serbia" as key-words to search PubMed.
Results: The search retrieved 24 studies and rare variants were reported in 10. Study about AAT polymorphism in healthy adult population identified 17 heterozygous carriers (9 of P and 8 F allele). Among healthy newborns the frequencies were 0.031 for P and 0.0073 for other variants. Three studies addressed the identification and functional characterization of two variants, G320R and V321F, detected in patients with emphysema and lung cancer. In the study enrolling 50 patients with premature development of chronic obstructive pulmonary disease, 2 cases were detected (Mmalton and Q0amersfoort). Two studies on patients with lung cancer reported frequencies for P (0.0076) i.e. other rare variants (0.00027). When patients with monoclonal gammopathies were studied 5 carriers of rare variants were found. Finally, in a group of children with liver disease, the frequency of P variant was 0.0112, while for other rare variants it exceeded 0.0056.
Conclusion: Currently available literature provides certain data about the presence of rare SERPINA1 variants in different age and health-status groups in the population of Serbia. Nevertheless, further studies are warranted to assure a more comprehensive evaluation of their epidemiology and clinical features.

SERPINA1, RARE VARIANTS, SERBIA

02 – 64 Poster

GENETIC DIAGNOSIS BY aCGH IN PATIENT WITH DEVELOPMENTAL DELAY AND CONGENITAL ANOMALIES - CASE REPORT

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Array Comparative Genomic Hybridization (aCGH) is becoming the first choice of clinical genetic test for patients with developmental delay/intellectual disability and multiple congenital anomalies. Array CGH offers a much higher diagnostic yield for this group of patients in respect to conventional karyotyping with a G-banded karyotype. Better resolution of microarray technology allows identification of chromosomal imbalances with greater precision, accuracy, and technical sensitivity, primarily because of its higher sensitivity for sub-microscopic deletions and duplications. In this case report, patient is a 4-year-old girl with diagnosed developmental delay. Patient does not speak, has persistent ductus arteriosus, ventricular septal defect, microcephaly, retrognathia and small jaw. Patient had a karyotype interpreted as 46,XX, t (2;14) (q23;q24) using GTG band analysis. Array CGH was performed using Agilent SurePrint G3 custom CGH+SNP Microarray 8x60K (UCSC, hg19, NCBI Build 37, February,2009). Results were analyzed by CytoGenomics 3.0 Agilent software. Results of aCGH revealed clinically significant deletion of 2q23.3-q24.1 region with the size of ~7.25Mb. Within the deleted region 42 genes are present of which 4 are described as OMIM morbid. The OMIM morbid genes include ACVR1, CACNB4, GPD2, and NEB. NR4X2 gene within this region is not described as OMIM morbid, but it is linked to the autistic spectrum disorder. Deletion of similar size is described in ISCA database in patients with psychomotor and speech delay as well as with autism. Also, a ~221 kb deletion of unknown clinical significance was detected in the 14q24.1 region. This deletion includes 4 genes of which 3 are protein coding genes; DCAF5, EXD2 and GALNT16. None of them is described as OMIM morbid. Analysis also revealed few copy number variations which are described as normal variations in Database of Genomic Variations.

ARRAY COMPARATIVE GENOMIC HYBRIDIZATION, PSYCHOMOTOR DELAY, DELETION, DUPLICATION

02 – 65 Poster

EXPRESSION OF PROINFLAMMATORY CYTOKINES IN PREGNANT WOMEN WITH GESTATIONAL DIABETES MELLITUS

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Gestational diabetes mellitus (GDM) is one of the most common pathological conditions that can develop during pregnancy and is associated with high risk of health complications and adverse outcomes for both the mother and the fetus. It is also well known that gestational diabetes is characterized by increased inflammation. However, the implication of inflammatory cytokines in GDM pathophysiology remains unclear. The aim of the present study was to determine whether there are differences in the expression levels of proinflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-17) between GDM and normal pregnancies.
This pilot study included 15 GDM patients and 15 healthy pregnant women (control group, CG). RNA was extracted from leukocytes, and real-time PCR was used to analyze relative gene expression of examined cytokines.
The results showed a statistically significant increase of IL-6 and IL-17 expression in GDM compared to controls (p=0.016 and p=0.036, respectively). There were no statistically significant differences between expression levels of TNF-α and IL-1β in GDM and CG groups (p>0.05).
Our study points to a possible role of IL-6 and IL-17 in the pathophysiology of GDM. However, more research is needed for better understanding of inflammatory mechanisms underlying GDM. This is of vital importance for the development of preventive strategies and optimal management of GDM.

This research was funded by Ministry of Education, Science and Technological Development of the Republic of Serbia (Project OI175075).

GESTATIONAL DIABETES MELLITUS, PROINFLAMMATORY CYTOKINES, IL-6, IL-17

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02 – 66 Poster

FIRST TRIMESTER SCREENING MARKERS FOR DOWN SYNDROME FOR PREDICTION INTRAUTERINE FETAL GROW RESTRICTION

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Background/Aim: Intrauterine fetal growth restriction (IUGR) is a pathological condition of pregnancy characterized by fetal weight below the 10th percentile for gestational age. This condition is one of the leading causes of perinatal mortality and morbidity. Early identification of fetuses developing IUGR is one of the priority tasks of perinatal care. The aim of this study was to examine the association between first trimester maternal serum analytes (PAPP-A and free βhCG) levels with intrauterine growth restriction in chromosomally normal pregnancies.
Materials and Methods: In this study we retrospectively analyzed data from 36 expectant mothers with a diagnosis of IUGR and 30 control subjects with singleton pregnancies who attended the prenatal biochemical screening program at Clinical Center of Montenegro, during 2-years period. Serum PAPP-A and free βhCG levels were measured in pregnant women at 11 to 13 gestational weeks by ELISA autoDelfia tests. Statistical analysis was performed using SPSS version 20 for Windows. For determining the influence of biochemical parameters on the occurrence of IUGR, a binary logistic regression, analysis, Hosmer-Lemeshow Goodness of fit Test and ROC curve analysis have been used.
Results: The level of maternal serum PAPP-A in the experimental group was lower (2.4±1.8) than that in the control group (3.1±2.2), but the difference was not statistically significant. Also, there was no established statistically significant difference between the serum levels of free βhCG in the experimental (87.0±108.7) and control (66.5 ± 40.4) groups. The sensitivity of the model was 75%, Specificity 40%, positive predictive value 57% and negative predictive value of 60%. There were no significant differences in demographic characteristics of expectant mothers with and without IUGR.
Conclusion: Predictive model (PAPP-A and free βhCG) is not capable to differentiate pregnancy with and without intrauterine growth restriction.

PAPP-A, FREE βhCG, FIRST TRIMESTER BIOCHEMICAL SCREENING, INTRAUTERINE GROWTH RESTRICTION

SESSION 3

Genetic toxicology: from cell to ecosystem

VI CONGRESS OF THE SERBIAN GENETIC SOCIETY

03 – 01 Invited lecture

GENOTOXICITY AND BIOCOMPATIBILITY OF NEW NANOSTRUCTURAL DENTAL BIOMATERIALS BASED ON ACTIVE CALCIUM SILICATE SYSTEM AND HYDROXYAPATITE

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Noticeable progress in the development and application of new dental biomaterials was achieved by applying nanotechnology in the field of regenerative endodontics. The main advantage of nanomaterials compared to conventional lies in different particle activity, as a result of a large reaction surface of particle in relation to its total weight and volume. Smaller sizes of particles enhance the hydration of the material and exhibit a positive effect on hardening and setting time. However, biomaterials should not only have adequate physical properties, but also have to be safe for human use. Therefore, the characterization and investigation of *in vitro* cytotoxic and genotoxic effects of the new nanostructural, noncommercial endodontic cement, based on dicalcium/tricalcium-silicate (CS), hydroxyapatite (HA), as well as the new material consisting of both components (HA-CS), were investigated. For this purpose human fetal fibroblasts (MRC-5) and human peripheral blood lymphocytes were used. In addition, *in vivo* biocompatibility test on Wistar albino male rats was performed. As a control, MTA material, being the gold standard in endodontics, was used. Results obtained showed that CS and HA-CS had lower cytotoxicity than MTA in MRC-5 cells. Moreover, no cytotoxicity was obtained in the study performed on the human lymphocytes. Genotoxicity testing of materials clearly indicated that HA, CS and HA-CS induced lower effect than MTA, with HA-CS exhibiting the lowest genotoxicity. Histopathological examination indicated that mild inflammatory reaction, without vascular congestion and well preserved connective tissue were associated with rat's treatment with CS and HA-CS. Taking together, the new materials, especially HA-CS, could be considered as good candidates for possible endodontic use.

NANOMATERIALS, BIOCOMPATIBILITY, COMET TEST, *IN VIVO* BIOCOMPATIBILITY TEST

03 – 02 Invited lecture

CURRENT APPROACHES AND BIOASSAYS IN THE ASSESSMENT OF ENVIRONMENTAL POLLUTION

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The integrity of cellular DNA is continuously attacked by various genotoxic agents that block DNA replication, cause mutations and provoke cell death. These processes can be associated with reduced growth, abnormal development, and reduced survival of embryos and adults with a biological impact at the different levels: cellular, organ, whole organism, and finally community and population.

The Center of Genotoxicology and Ecogenotoxicology was founded at the Faculty of Biology, University of Belgrade, at the end of 2012. Scientific approach implemented in the Center is based on the principles and methodologies of genetic toxicology, and includes prokaryotic (*E. coli*, *S. typhimurium*) and eukaryotic models (cultures of mammalian cell lines, tissues of mammals, fish, mussels, worms, arthropods) and well-validated *in vivo* and *in vitro* bioassays with different end-points (DNA damage, SOS induction, reverse mutations, micronuclei). The biological activities of tested substances (natural products or various pollutants) detected in these assays can be attributed to their cytotoxic, cytostatic, genotoxic or antigenotoxic potential, as a consequence of their interactions with the DNA molecule or other cellular structures. In this presentation we will review our results obtained during national, bilateral and international projects, dealing with cytostatics as emerging water pollutants, fish as water quality indicators in open waters of Serbia and bioactive natural products as potential sources of new pharmaceuticals and food supplements.

GENOTOXICOLOGY AND ECOGENOTOXICOLOGY, PROKARYOTIC AND EUKARYOTIC TESTS, ENVIRONMENTAL POLLUTANTS, NATURAL PRODUCTS

03 – 03 Invited lecture

GENOTOXIC EFFECTS OF NONSTEROIDAL HORMONES

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Despite numerous overall data about genotoxic effects of xenobiotics, endogenous substances, including hormones, are not thoroughly investigated. Hormones are involved in regulation of various biochemical, physiological and developmental processes. Since hormones are natural compounds normally present in the organism, it seems unlikely that they can have deleterious effects on genetic material. However, it is well established that elevated tissue concentrations of steroidal oestrogens can overshadow their hormonal effects leading to genotoxic and mutagenic effects. On the other hand, although possible genotoxic effects of nonsteroidal hormones are not thoroughly studied, in last couple of decades it has been realized that they may also have genotoxic effects, although it seems it cannot be generalized for all nonsteroidal hormones. Among nonsteroidal hormones, the best studied groups are thyroid hormone and catecholamines. So far, we know that thyroid hormones can exhibit genotoxic effects due to oxidation of their phenolic groups and also their capability to accelerate oxidative metabolism in mitochondria leading to increased production of reactive oxygen species (ROS). Catecholamines (adrenaline, noradrenaline, dopamine etc.) have catechol moieties which are critical for their genotoxic action. Namely, redox cycling of catechol group is accompanied by generation of ROS and oxidative stress. Among protein hormones it has been shown that insulin leads to increased ROS production via NADPH-dependent H_2O_2 generation. Apart from insulin, other protein hormones are not studied enough. There are experimental findings that neuroendocrine oligopeptide oxytocin was not genotoxic at cytogenetic level (chromosome aberration and sister-chromatid exchange tests). In conclusion, further investigations of nonsteroidal hormones, especially proteinaceous ones, are needed to elucidate their possible roles in the processes of mutagenesis and carcinogenesis.

HORMONES, GENOTOXICITY, MUTAGENESIS, OXIDATIVE STRESS

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03 – 04 Oral

BIOCOMPATIBILITY OF RESIN-BASED COMPOSITES: COMPARATIVE ANALYSIS OF CYTOTOXICITY AND GENOTOXICITY OF CONSTITUENTS AND THEIR COMBINATIONS

Biljana Nikolić [1], Dragica Manojlovic [2], Miroslav D. Dramićanin [3], Vesna Miletic [2], Jelena Knežević-Vukčević [1], Bojana Vasilijević [1], Dragana Mitić-Ćulafić [1]

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Resin-based composites are used in dentistry as restorative materials or adhesives. Their unbound components may diffuse through dentin into the pulp or be swallowed with saliva; in both cases they could be systemically distributed by blood circulation. To prevent possible adverse effects of such uptake, the study of biocompatibility is extremely important. In this work we studied cytotoxicity and genotoxicity of dental materials on human fetal lung fibroblasts MRC-5, by MTT and comet assay. Tested materials were novel urethane -based monomer FIT and the conventional one BisGMA, co-monomer TEGDMA, and photoinitiator systems - the new Lucirin TPO (TPO), and conventional photoiniator camphor quinone (CQ) and its co-iniator DMAEMA. They were tested individually and in mixtures of composites that are actually being used in dental practice: FIT+TEGDMA and CQ+DMAEMA (C-1), FIT+TEGDMA and TPO (C-2), BisGMA+TEGDMA and CQ+DMAEMA (C-3), and BisGMA+TEGDMA and TPO (C-4).
Results obtained revealed that cytotoxicity and genotoxicity increased in the following orders: TEGDMA<DMAEMA<CQ<FIT<TPO<BisGMA, and TEGDMA< CQ<FIT<BisGMA<TPO, respectively. Moreover, DMAEMA was not genotoxic (up to 75 μg mL-1). Concerning the effects of the composite mixtures, the manner in which cytotoxicity increased was C-2<C-1<C-3<C-4, while the pattern in which genotoxicity increased was C-2<C-3<C-1<C-4. The results obtained favored the novel monomer FIT, due to lower cytotoxicity and genotoxicity than the conventional monomer BisGMA. On the contrary, new photoiniator TPO could not be recommended, due to the multifold higher genotoxicity than both CQ and DMAEMA. Results support the importance of genotoxicity testing, due to different ranges of genotoxic and cytotoxic activity. Furthermore, the study of complex composites seems to be of special interest, due to the fact that both antagonism and synergism were observed for certain mixtures.

BIOCOMPATIBILITY, RESIN-BASED COMPOSITES, MTT ASSAY, COMET ASSAY

03 – 05 Oral

PROTECTIVE EFFECT OF *GENTIANA LUTEA* EXTRACTS AGAINST UV-INDUCED GENOTOXICITY

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Exposure of the skin to ultraviolet (UV) radiation is a major factor in the development of skin cancer. UV radiation, when absorbed by the skin, can result in direct damage, by formation of pyrimidine dimers and in indirect damage, mainly mediated by reactive oxygen species (ROS). One of the approaches to protect human health is to use natural compounds that can be applied in diet or added in sunscreens. Compounds that are potent antioxidants could be used to protect against indirect ROS-mediated damage. Taking all the above mentioned into account, the aim of this study was to examine the antioxidant and antigenotoxic effect of chemically characterized *Gentiana lutea* methanolic root and leaf extracts against UV-C radiation, on human melanoma cells (Hs294T) *in vitro*. Preliminary research determined the non-cytotoxic concentrations of extracts in MTT assay, as well as the genotoxicity of UV and extracts using the alkaline comet assay. Antioxidant potential, tested in DPPH assay, revealed that extracts manifested moderate antioxidant potential with IC50 values at 215 µg mL-1 and 57 µg mL-1 for root and leaf extract, respectively. Genotoxicity testing established the dose of UV of 63 J/m2 as the one that induced sufficient DNA damage in used experimental model (tail intensity 29.3%), but did not have a remarkable lethal effect (survived cell fraction 60%). In the applied concentration range (up to 2 mg mL-1) root extract showed no genotoxicity, while the highest non-genotoxic concentration of leaf extract was 0.5 mg mL-1. Results of antigenotoxicity testing showed significant protective potential of extracts with the inhibition of UV-induced genotoxicity recorded at 71% and 66% for root and leaf extracts, respectively. Data obtained is encouraging and could stimulate further research of the protective role of *G. lutea* and its potential application in reducing the UV-induced DNA damage.

GENTIANA LUTEA, UV RADIATION, ANTIGENOTOXICITY, COMET ASSAY

03 – 06 Oral

THE MODULATION OF Keap1-Nrf2 SIGNALING PATHWAY: *IN VITRO* STUDIES OF COMMERCIAL CYTOSTATIC AND POLYGONOIDEAE WEEDS WITH CHEMOTHERAPEUTIC POTENTIALS

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Frequent occurrence of chemoresistance as a result of disrupted Keap1 activity that leads to Nrf2 overexpression was detected in various types of cancers including hepatocellular carcinoma and lung adenocarcinoma. Some phytochemicals have the potential to sensitize chemoresistant cells and enhance efficacy of cytostatics by reducing the Nrf2 expression. *Polygonum aviculare*, *Persicaria amphibia* and *Persicaria maculosa* are described as edible and healing weeds. Characterized by rich phenolics content, extracts obtained from these plants may influence the oxidant and xenobiotic stress response in malignant cells. The purpose of this study was to investigate the effects of *P. aviculare* (POA), *P. amphibia* (PEA) and *P. maculosa* (PEM) ethanol extracts combined with cytostatic doxorubicin (Dox) on Nrf2 and Keap1 gene expression and modulation of antioxidant protein level, as well as to establish were there any correlation between these effects. In this research human hepatocarcinoma (HepG2) and adenocarcinoma (A549) cells were used. Cells growth was measured by MTT assay and concentration that gave 75% viability were used in RT-PCR for estimation of gene expression, and in immunoblot for protein level analysis. Results of the study revealed that all tested cotreatments decrease the expression of Nrf2, while simultaneously increasing Keap1 expression in HepG2 cells. In A549 cells PEA+Dox also significantly reduces Nrf2 activity that likely causes the increase of Keap1 expression. Additionally, combination of extracts/Dox modulates antioxidative enzyme activity. Concerning POA+Dox and PEA+Dox it appears that different signaling pathway(s) have been employed in regulation of SOD1 expression, while the expression of SOD2 after PEM+Dox cotreatment is regulated entirely by the Nrf2-Keap1 regulatory network. Further research considering chemotherapeutic properties of selected plant extracts combined with Dox could be recommended due to their potential usages as Nrf2 inhibitors.

DOXORUBICIN, POLYGONOIDEAE, ANTIOXIDATIVE ENZYMES, KEAP1-NRF2 SIGNALING PATHWAY

03 – 07 Poster

IN VITRO DNA PROTECTIVE ACTIVITY OF SELECTED PYRAZOLINES

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Pyrazolines are well known nitrogen-containing five-membered heterocyclic compounds with various pharmaceutical activities such as analgesic, antibacterial, antifungal, anticancer, antiviral, anti-inflammatory, antitubercular, antidepressant, anticonvulsant, antihyperglycemic, antipyretic, etc. This study aims to investigate the in vitro DNA protective effect of twenty-four pyrazoline derivatives with vanillic and ferrocenyl fragments. Our previous results showed that both fragments are responsible for various biological activities, and slight changes in their structure induce sometimes dramatic difference in activities. Those compounds designated as 3a-3f, 4a-4f, 5a-5f, and 6a-6f, have been tested on hydroxyl radical-induced DNA damage in various concentrations (25, 50, 100, 200, and 400 μg/mL). The decreasing order in the reduction of DNA damage among the compounds was found to be: 3d > 3e > 3a > 3b > 3f > 3c; 4d > 4e > 4a > 4c > 4f > 4b; 5e > 5a > 5c > 5f > 5d > 5b; and 6d > 6c > 6f > 6e > 6a. Results showed that the best *in vitro* DNA protective potential against hydroxyl radicals have propyl or butyl derivatives (3d and 6d followed by 4d and 5e) and can be identified as the most promising substrates. From this point of view length of carbon chain in this case could be a key factor for in vitro DNA protective activity of selected pyrazolines.

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DNA, PYRAZOLINES, HYDROXYL RADICAL

03 – 08 Poster

DNA DAMAGE INDUCED BY SELECTED PYRAZOLINES IN RAT LIVER USING COMET ASSAY

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Pyrazolines are well known biological agents with various pharmacologically activities. As far as we know there is no previous study aimed at determining the genotoxicity of pyrazolines. The *in vivo* comet assay was performed to evaluate, whether or not eight selected pyrazoline derivatives with vanillic and ferrocenyl fragments, namely 3c, 3f, 4d, 4e, 5e, 5f, 6c, and 6d can damage the DNA in rat livers. No statistically significant difference was observed in the 4d, 4e, 5e, and 6d-treated groups upon comparison with the negative control. Significantly increase of the total comet score was detected in the 3c, 3f, 5f, and 6c treatment groups in comparison with the rats in the negative control group and significant less total score than those of the positive control group. According to the *in vivo* genotoxicity assays, propyl or butyl derivatives (4d, 4e, 5e, and 6d) seem to be the most promising substrates due to their non-genotoxic effect.

This work was financed by the Ministry of Education, Science and Technological Development, Republic of Serbia, Grants No. III 43004 and III 41010.

DNA, PYRAZOLINES, COMET ASSAY

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03 – 09 Poster

NAJAS MARINA EXTRACT PROTECTS AGAINST MITOMYCIN C INCREASED MUTAGENICITY IN HUMAN LYMPHOCYTES *IN VITRO*

Jovana Tubić [1], Olivera Milošević-Djordjević [1, 2], Aleksandra Marković [1], Darko Grujičić [1], Marina Topuzović [1], Ljiljana Čomić [1], Ivana Radojević [1]

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Biological systems are frequently exposed to reactive oxygen species, which cause disorders in the cells' natural antioxidant defense systems and finally resulting in DNA damages. Plant extracts represent important source of compounds with protective and antioxidative activities, such as phenolics and flavonoids. Thus, the aim of the study was to investigate the antioxidant and antimutagenic activities of *Najas marina* acetone extract. The total phenolic and flavonoid contents were measured by spectrophotometry, while antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. The antimutagenic activity was evaluated using comet assay on cultured human lymphocytes in treatment with four different concentrations of the extract (125, 250, 500 and 1000 μg/ml) against mitomycin C (MMC). The total phenolic content was 23.57 ± 0.05 mg GA/g, while total flavonoid content was 27.55 ± 0.03 mg RU/g, of the acetone extract. The extract showed moderate DPPH scavenging activity (IC_{50} 2164.46 ± 18.30 μg/ml), and in all tested concentrations decreased MMC-induced genetic damage index (GDI), but significantly in higher tested concentrations (1.71 ± 0.04 for 500 μg/ml; 1.54 ± 0.11 for 1000 μg/ml) in comparison to the positive control (only MMC, 3.14 ± 0.07). With the increase of extract concentrations, the percentage of undamaged cells (without tail) increased almost five times (from 3.50 to 17%) in comparison to positive control (1.50%). In conclusion, our results suggest that the acetone extract of *N. marina* possesses both antioxidant and antimutagenic activities, which are why it can be safely used against increased mutagenicity induced by chemotherapeutic agents.

NAJAS MARINA, TOTAL PHENOLICS AND FLAVONOIDS, ANTIOXIDANT ACTIVITY, ANTIMUTAGENIC ACTIVITY

03 – 10 Poster

CYTOTOXIC AND GENOTOXIC PROPERTIES OF PLANT *ARTEMISIA VULGARIS*

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Since plant *Artemisia vulgaris* L. is often applied in folk medicine, and that there is insufficient data in the literature about its genotoxic and cytotoxic properties, the aim of the study was to evaluate these biological effects of methanolic, acetone and aqueous extracts obtained from this plant. Phytochemical composition was determined by high-performance liquid chromatography (HPLC). For the analysis of cytotoxic potential, MTT assay was performed on SW-480 human colon cancer cells and stem cells (as controls) derived from human periodontal ligament (PDLSCs). Genotoxic effect was determined by using the cytokinesis-block micronucleus (MN) assay on cultured human peripheral blood lymphocytes (PBLs). HPLC showed that chlorogenic acid was the dominant component in methanolic extract, and that in acetone and aqueous extracts were the most trans-cinnamic and syringic acids. Among three extracts, only the acetone extract decreased SW-480 cell viability, with IC50 values of 283.62 ± 51.76 μg/mL for 24 h and 240.12 ± 25.49 μg/mL for 72 h. The tested extracts did not significantly affect the viability of the PDLSCs line. The separate treatments of cultured PBLs with 10, 50, 100 and 250 μg/ml concentrations of extracts showed statistically significant increase of the MN frequency ($p < 0.05$) in all tested concentrations, except the lowest concentration of methanolic extract. All three extracts reduced the nuclear division index (NDI), but significantly only at the highest tested acetone and aqueous extracts concentrations. In conclusion, acetonic extract of *Artemisia vulgaris* L. showed both cytotoxic effect against SW-480 colon cancer cells and genotoxic effect in cultured human PBLs, while the methanolic and aqueous extracts were only genotoxic.

ARTEMISIA VULGARIS, CYTOTOXICITY, GENOTOXICITY, PHYTOCHEMICAL COMPOSITION

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03 – 11 Poster

EVALUATION OF THE GENOTOXIC EFFECT OF METHANOLIC EXTRACTS OF DIFFERENT PARTS OF *ACHILLEA AGERATIFOLIA* VAR. *SERBICA*

Aleksandra Marković [1], Jovana Tubić [1], Darko Grujičić 1, Ivana Radojević [1], Ivana Raković [2], Gorica Đelić [3], Nevena Mihailović [3], Olivera Milošević-Djordjević [1, 2]

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Plants from *Achillea* genus are often used in folk medicine in the treatment of stomach problems, inflammatory processes, thrombosis and the treatment of wounds and purulent processes. The aim of this study was to evaluate genotoxic potential and possible correlation with polyphenolic contents of methanolic extracts obtained from different parts (aerial part and root) of an endemic species from Serbia, *Achillea ageratifolia* var. *serbica*. The total phenol and flavonoid contents determined using spectrophotometric method, and identification and quantification of polyphenols were performed on a high performance liquid chromatography (HPLC). The genotoxic effect of extracts in four different concentrations (125, 250, 500 and 1000 µg/ml) was evaluated by comet assay in human peripheral blood lymphocytes. There were 39.83 mg GA/g of total phenols and 20.33 mg RU/g of total flavonoids in aerial part extract, and 70.78 mg GA/g of total phenols and 7.79 mg RU/g of total flavonoids in the root extract. The HPLC analysis showed the partly different composition of extracts, aerial part extract contained chlorogenic acid, rutin, quercetin, apigenin and luteolin, while root extract contained chlorogenic acid, epicatechin, apigenin and luteolin. Results of genotoxic analysis showed that both extracts separately tested in different concentrations increased genetic damage index (GDI), but significantly only in the highest concentration of aerial part extract ($p = 0.03$). The comet distribution analyses showed that increase in the percentage of cells with tail was significantly correlated with an increase in the concentrations of extracts (Pearson, $r = 0.83$, $p = 0.01$ for aerial part extract; $r = 0.81$, $p = 0.02$ for root extract). In conclusion, both methanolic extracts obtained from different part of the *A. ageratifolia* var. *serbica* were not genotoxic in tested concentrations, except the highest concentration of aerial part extract, so caution is advised when using high concentrations.

ACHILLEA AGERATIFOLIA, PHENOLS, FLAVONOIDS, COMET ASSAY, GENOTOXIC EFFECT

03 – 12 Poster

INVESTIGATING THE APOPTOTIC EFFECTS OF STENOENDEMIC *ACINOS ORONTIUS* PLANT EXTRACTS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES *IN VITRO*

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Acinos orontius (K. Maly) Silić is a Bosnian-Herzegovinian stenoendemic species limited to the dolomite complex around the area of Konjic. The composition of the essential oils in different species of the genus *Acinos* is a promising source of natural antioxidants due to the high content of flavonoids and linolenic acid. According to available literature data, there are only three papers that indicate antioxidant and antimicrobial activity of some species of *Acinos*, but there is lack of data that shows the effect of *Acinos* herbal extracts on cytotoxic, apoptosis and antiproliferative activity. The aim of this study was to evaluate relative gene expression and influence on apoptosis signal pathways in cultivated human lymphocytes, after the treatment with *Acinos orontius* (K. Maly) Silić aqueous and DMSO extracts.

Fresh plant material was collected from five localities in Herzegovina and used for aqueous and DMSO extracts preparation. Cultured human peripheral blood lymphocytes were treated with the plant extracts in the final concentrations of 0.01, 0.05, 0.1 and 0.2 mg/mL. RNA was extracted by using NucleoSpin® RNA extraction kit. Relative expression of the apoptotic genes was measured using SALSA RT-MLPA R011-C1 Apoptosis assay (MRC Holland). Electrophoresis was performed on Genetic Analyzer 3500 (Applied Biosystems).

Preliminary results showed that extracts diluted in water as well as in DMSO had the effect in regulating different pro- and anti-apoptotic genes in all tested concentrations. Genes belonging to the BCL-2 family: BCL2L1 and BID were upregulated and BCL2L13 gene was downregulated. BIRC3 and XIAP are anti-apoptotic genes of the IAP family, and they were upregulated, while proapoptotic APAF1 and AIFM1 genes were downregulated.

Human lymphocytes treated with *A. orontius* extracts, regardless of solvent or concentration applied, showed certain antiapoptotic potential suggesting that it should be further investigated in different cell cultures.

STENOENDEMIC SPECIES, PLANT EXTRACTS, APOPTOSIS, GENE EXPRESSION

03 – 13 Poster

K2(B3O3F4OH) REDUCED CELL VIABILITY AND DOWNREGULATED TUMOR NECROSIS FACTOR SUPERFAMILY MEMBERS *IN VITRO*

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The number of new natural or synthetic compounds tested as a potential therapeutics increases every day. Halogenated boroxine, K2(B3O3F4OH) (HB) is a synthetic compound developed for the treatment of skin changes with various proven biological activity in many test models *in vitro* and *in vivo*. The effects of HB on cellular and genes' expression level in different tumor cell lines were previously evaluated but there is still no clear explanation of its mechanism of action. We aimed to analyze the impact of HB on the cell viability and expression level of selected tumor necrosis factor (TNF) superfamily genes in cultured human peripheral blood mononuclear cells (PBMCs). These genes regulate cell death and proliferation thus their expression level may be associated with the induction of apoptosis.
PBMCs cultures were treated with HB in series of concentrations (0.1, 0.2, 0.4 mg/mL) followed by incubation period of 72 h. Trypan Blue exclusion assay was used for the evaluation of cell viability. For the analysis of transcriptional activity of five genes from TNF superfamily (TNFRSF10A, TNFRSF10B, TNFRSF1B, TNFRSF25, TNFSF10) in PBMCs after treatments, isolation of total RNA, RT-PCR and Real-Time PCR were done. The average Ct values and 2-ΔΔCt were used as an input for the correlation assessment.
As HB concentration increased, viability of PBMCs decreased from 73.6% to 54.4% in comparison to negative control. Comparative analysis of gene expression level between positive control (5-Fluorouracil) and HB treatments showed downregulation of TNF genes after treatment at two higher HB concentrations. Our results suggest that antiproliferative effects of HB on cellular level probably are not mediated via TNF receptors and ligands.

HALOGENATED BOROXINE, CELL VIABILITY, TNF SUPERFAMILY GENES, PBMCS

03 – 14 Poster

ECOGENOTOXICOLOGICAL ASSESSMENT OF THE WATER QUALITY OF THE DANUBE RIVER (SITE VIŠNJICA) BASED ON DNA DAMAGE IN VARIOUS FISH SPECIES

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This work represents the study of quality of the Danube River, in Belgrade, at the Višnjica site, by analyzing the genotoxicity of water by using fish as bioindicators. With a population of about 2 million inhabitants (Belgrade) and poor legislation regarding the discharge of wastewaters, monitoring is the first step in approaching this serious problem. To obtain information on the genotoxicity of water as a measure of DNA damage, the comet assay, which detects the effects of exposure, and micronucleus test, which detects permanent effect, were used. For the comet assay we have used three types of cells: blood, liver, and gills, while the micronucleus test was done on blood cells. The fish used as bioindicators are the common perch (*Perca fluviatilis*), vimba bream (*Vimba vimba*), common barbell (*Barbus barbus*), and white bream (*Blicca bjoerkna*).
Regarding the comet assay, significant difference in DNA damage was observed between species in all tested tissues. Vimba bream and common barbell had the highest level of DNA damage in gills, white bream in liver and common perch in blood. In the case of the micronucleus test, the highest number of micronuclei was detected in white bream blood cells. The obtained results showed that there is no correlation between comet assay and the micronucleus test suggesting the importance of performing different bioassays on multiple types of fish tissues in order to find the best biomarker for assessing the genotoxic potential of water.

ECOGENOTOXICOLOGY, DNA DAMAGE, COMET ASSAY, MICRONUCLEUS TEST

03 – 15 Poster

BIOLOGICAL PROPERTIES OF ETHANOLIC EXTRACTS OF *TARAXACUM OFFICINALE*, *HYSSOPUS OFFICINALIS* AND *CHELIDONIUM MAJUS* ON SELECTED CELL LINES

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Despite numerous cytostatic and treatments, cancer is still leading cause of deaths in the world. Moreover, cancer cells are developing resistance to different cytostatic which leads to constant need to develop new and effective anticancer drugs. Since ancient time, plants have been used to treat various diseases. Therefore, new therapeutic drugs can be found among plants, thus the aim of this study was to test cytotoxic and genotoxic potential of ethanolic extracts of *Taraxacum officinale* (dandelion), *Hyssopus officinalis* (hyssop) and *Chelidonium majus* (greater celandine).

To determine cytotoxic potential of extracts, MTT assay was done on human fetal fibroblasts (MRC-5) and lung adenocarcinoma cells (A549) in concentration range of 0.125 mg/mL – 4 mg/mL. Furthermore, to determine the prospective mechanism of action of plant extracts, the measurement of mitochondrial membrane potential was performed using flow cytometry. To quantify DNA damage induced by extracts, comet assay was used.

Extract of dandelion did not show cytotoxic potential towards used cell lines, while extracts of greater celandine and hyssop exhibited a cytotoxic effect on A549 cell line. In order to determine the effect on membrane mitochondrial potential and genotoxicity, the highest non-cytoxic concentrations were selected (4 and 2mg/mL). None of the extracts had an effect on the membrane potential on MRC-5 cells. Dandelion extract did not have effect on membrane potential of A549 cells, while two other extracts changed the membrane potential. All tested extracts induced DNA damage in MRC-5 cells, while in A549 cells greater celandine and hyssop were genotoxic.

According to obtained results it can be notice that tested extract possess different mechanism of action. Future research is necessary to determine underlie mechanisms as well as to detect the active substances that may be responsible for the extracts activity.

PLANT EXTRACTS, HUMAN CELLS, CYTOTOXICITY, GENOTOXICITY

03 – 16 Poster

INFLUENCE OF TOWN POLLUTIONS ON LEVELS OF WATER GENOTOXICITY DURING DIFFERENT WATER LEVEL REGIMES

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Water, being of critical importance for the survival of life on the planet, requires our permanent care and attention. There is a growing concern about the genotoxicity of complex environmental mixtures present in surface waters, due to the risk of genetic damage and cancer, both in aquatic organisms and humans. *Allium* anaphase-telophase test has been accepted as a promising tool to detect, among other things, toxicity and genotoxicity of river water. The present study has focused on exploring the status of water pollution in areas with combined industrial and agricultural activities in order to estimate the magnitude of toxicity and genotoxicity using the *Allium* test. We collected samples of surface water from the Sava River, upstream and downstream from the town of Šabac and at the Danube River upstream and downstream from the town Smederevo. We analyzed using the *Allium* anaphase-telophase test. In both rivers sampling was done in periods of low and high water level. While at low water level genotoxicity was higher at points downstream of Šabac and Smederevo, samples collected in periods of highwater level were more genotoxic upstream from the towns. This can be explained as a consequence of agricultural activities.

ALLIUM TEST, GENOTOXICITY, RIVER WATER, TOWN POLLUTIONS, AGRICULTURE

SESSION 4

Adaptation and ecological genetics

VI CONGRESS OF THE SERBIAN GENETIC SOCIETY

04 – 01 Oral

DO DENDROPHENOTYPIC TRAITS INFLUENCE INDIVIDUAL REPRODUCTIVE SUCCESS? AN EMPIRICAL ASSESSMENT IN NORWAY SPRUCE NATURAL POPULATIONS

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Despite the critical importance of reproductive dynamics on the adaptive potential of forest tree populations, the determinants of individual reproductive success are still largely under-documented. Here, we combine dendrochronological and genetic data to assess how dendrophenotypes influence Norway spruce reproductive success. A total of 518 adult trees and 604 seedlings were genotyped and phenotyped within five plots from two populations in southern and central Europe. Parentage analysis was used to estimate individual female and male reproductive success, expressed as the number of gametes produced by each adult tree. The effects of a large set of dendrophenotypic traits on reproductive success were tested i) through selection gradients estimated within the neighbourhood model framework and ii) by fitting a generalized linear model on the most likely genealogies from the neighbourhood model run without selection gradients. We consistently found that female and male reproductive success were positively associated with age and average basal area increment and that female reproductive success was also positively influenced by growth-climate correlations with mean temperature of the previous vegetative season. Our results suggest that individuals which grew more throughout their entire lifetime might have a higher fitness because they may better compensate costs of reproduction by increasing their resource intake and/or by developing other compensatory mechanisms. We deem that combining dendrochronological and genetic data is a fruitful strategy to link forest trees' growth performances and their evolutionary gains, thus shedding some light on the phenotypic mechanisms that shape the transmission of genetic information across generations in Norway spruce.

GENE FLOW, PARENTAGE ANALYSIS, FOREST TREE SPECIES, DENDROCHRONOLOGY, REPRODUCTIVE COSTS

04 – 02 Oral

CHANGE IN MICROBIOTA DIVERSITY IN TWO *DROSOPHILA* SPECIES UNDER EXPERIMENTAL CONDITIONS ON LEAD

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Gut microbiota is found to have a large influence on all aspects of host's life. The composition of the microbiota affects different traits throughout the life cycle of *Drosophila*, such as: larvae growth, development, stress resistance, immune response, metabolism, lifespan, etc. Changes in composition of microbiota can have large negative impact on life history traits. Having in mind such a strong impact of microbiota, it is important to identify factors that are shaping microbial community. Regarding the fact that our environment is exposed to high levels of pollution which causes the accumulation of heavy metals in living habitat, we investigated the influence of lead, added to feeding substrate, on microbiota diversity in two species, *Drosophila melanogaster* and *Drosophila subobscura*. Flies were reared for 10 generations at experimental and standard (control) substrate under the same conditions and controlled population density. Identification of the main bacterial genera represented in their microbiota was performed by metagenomic sequencing of V3-V4 variable region of 16S rRNA gene. The results indicated differences in bacterial community between the substrates, but also between the species. The increased concentration of heavy metals in the habitat leads to the extinction of many species and populations, and influences numerous obligatory interactions among species. One of the most important interactions is the one between the host and its microbial community in maintaining developmental homeostasis. The presence of heavy metals in the environment affects the populations' ability to adapt using different mechanisms at genetic and epigenetic levels. In this respect, the composition of microbiota affects the interactions of organisms and the environment, and changes in this interaction are significant from the conservation aspect.

DROSOPHILA, MICROBIOTA, DIVERSITY, LEAD

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04 – 03 Oral

DOES SEX-SPECIFIC SELECTION ON SYMPATRIC MITO-NUCLEAR VARIATION ACT ON ADULT *DROSOPHILA SUBOBSCURA* IN REGARD TO DESICCATION RESISTANCE?

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It is currently thought that different types of balancing selection can maintain sympatric mtDNA variation in populations. In particular, sex-specific selection has been proposed as a mechanism maintaining this variation, but supporting experimental data are scarce. *Drosophila subobscura*, having two, almost equally frequent haplotypes across its whole range is a perfect model for testing this hypothesis. The frequency of two aforementioned haplotypes varies to a small extent annually, possibly due to temperature variation throughout the year. Using 11 pairs of isofemale lines and a backcrossing design, we constructed a set of 44 mito-nuclear introgression lines representing four distinguishable cross types each being a combination of either of the two main mtDNA haplotypes (I or II) and nuclear genetic background. We measured desiccation resistance for both males and females of this set of mito-nuclear lines on two different temperatures (19°C and 24°C). The results shed light on the role of sexually antagonistic selection in preserving mito-nuclear variation. They reveal whether mito-nuclear interactions are sensitive to temperature variation.

SEX-SPECIFIC SELECTION, mtDNA HAPLOTYPES, NUCLEAR GENETIC BACKGROUND, DESICCATION

04 – 04 Poster

PRECIPITATION EFFECTS ON THE NADH DEHYDROGENASE SUBUNIT 6 GENE IN BROWN HARES (*LEPUS EUROPAEUS*) FROM EUROPE AND ASIA MINOR

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Previous studies in hares indicated the role of positive selection in shaping the diversity of protein-coding mitochondrial (mt) genes involved in oxidative phosphorylation (OXPHOS). Those genes affect cellular energy production and may be involved in adaptations to different environmental (climatic) pressures. Here, we sequenced the NADH dehydrogenase subunit 6 (MT-ND6) gene of 267 hares from Europe and Asia Minor. Molecular diversity indices were calculated in DnaSP, while the presence of selection signals was tested by codeml in PAML, and the "Datamonkey Adaptive Evolution" web server. Moreover, SPSS was used to run multinomial regression models to test for possible effects of climate parameters on the currently obtained protein variants. Fifty-eight haplotypes were revealed with a nucleotide diversity of 0.817, coding for 17 different amino-acid sequences (protein variants). Based on several selection tests, only codon position 102 was consistently proved to be under positive selection. Our multinomial regression models indicated that certain protein variant occurring in south-eastern Europe (mainly Bulgaria, North Macedonia and central Serbia) was favored at locations where precipitation values during the wettest and coldest period of the year were lower, or where the precipitation values during the driest and warmest period of the year were higher, whereas another protein variant occurring exclusively in Bulgaria was favored at locations where precipitation values during the warmest and driest period of the year were high. Altitudinal variation leading to more pronounced changes in precipitation across the Balkans may have generated novel selective pressure on protein variants and regional adaptation, in the course of colonization of southeastern Europe by the hares from the Anatolian Peninsula, where more ancestral lineages show less protein diversity and no signal of positive selection.

BROWN HARES, MITOCHONDRIAL DNA, OXPHOS, POSITIVE SELECTION

04 – 05 Poster

ALTITUDINAL VARIATION IN CHROMOSOME INVERSION FREQUENCY OF *D. SUBOBSCURA* POPULATIONAS FROM STARA PLANINA IN SERBIA

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Drosophila subobscura is a species with rich chromosomal inversion polymorphism with well-established and documented latitudinal clines. Most of latitudinal clines follow a southwest - northereast axis, starting from the Iberian Penninsula. This type of polimorphysm has rapid response to environmental conditions and can be used as a good candidate to measure the effects of global warming on the genetic structure of populations. The long term variation of chromosomal inversion polymorphism shows a general increase in the frequency of "warm - related" low latitudes inversions, and decrease of those associated with higher latitudes. However, altitudinal patterns in inversion polymorphism have rarely been investigated, and it has been expected to follow similar pattern as latitudinal, where "cold - related" inversions (A_{ST}, J_{ST}, U_{ST}, E_{ST}, O_{ST}) are most abundant in the higher latitude/altitude and "warm - related" (as J_1, U_{1+2}, U_{1+2+6}, E_{1+2+9} and O_{3+4}) in the lower latititude/altitude. In order to explore the effects of altitudinal variation and to evaluate clinal trends of chromosome arrangement frequencies in *D. subobscura* we analyzed and compared inversion polymorphism data from three different altitudes (500 m a.s.l, 1000 m a.s.l and 1600 m a.s.l) in Stara Planina in Serbia. Preliminary results suggest that the observed altitudinal inversion frequency in studied populations follows general latitudinal trend.

D. SUBOBSCURA, CHROMOSOMAL INVERSION POLYMORPHISM, ALTITUDE

04 – 06 Poster

ADAPTATION TO LEAD POLLUTION IN *DROSOPHILA SUBOBSCURA* IS NOT WITHOUT A COST

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Selection can alternate paths of reproduction and development of the organisms and shape the genetic basic of adaptation and resistance to different types of pollution, including lead. On the other hand, adaptation and resulting resistance occasionally comes with the cost for resistant individuals. Previous analysis showed that lead alters patterns of oviposition rate and fecundity in *Drosophila subobscura* females and that adapted individuals fare better in polluted environment. In order to determinate cost and/or benefits of the adaptation to lead pollution, series of inbred lines were made from flies selected on high lead concentration and ones maintained under optimal conditions. Females from each inbred line were left to individually lay eggs on standard and media with 1000µg/mL of lead acetate. Fitness assay was performed and fecundity and oviposition rate were scored. Results suggest that previous adaptation significantly influences measured components of fitness but there is strong individual footprint of selection observed as altered response of some inbred lines.

LEAD POLLUTION, ADAPTATION, SELECTION, OVIPOSITION RATE, FECUNDITY

04 – 07 Poster

LOCAL ADAPTATION OF *MEDICAGO LUPULINA* TO HEAVY METAL CONTAMINATED SOILS

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Metalliferous soils are restrictive habitats for the majority of plant species due to metal phytotoxicity, however, some species evolved tolerance mechanisms, which enable surviving in these hostile environments without developing toxicity symptoms. Pseudometallophytes can thrive in both metalliferous and non-metalifferous soils thus representing excellent model plants to study local plant adaptation. The objective of our study was to determine metal accumulation efficacy and biochemical and physiological status of selected populations of *Medicago lupulina* L. from Bosnia and Herzegovina. Plant material and surrounding rhizosphere were collected during flowering season from serpentine outcrops located in Central Bosnia and from urban area of Sarajevo. Content of heavy metals in plant material and soil samples were measured using flame atomic absorption spectroscopy. Concentrations of total phenolics, phenolic acids, flavonoids, proline and chlorophyll were determined in plant extracts using spectrophotometric methods. Analysed soil samples showed concentrations of Cr, Ni and Co considerably above the threshold provided by the legal framework for agricultural soil. Content of heavy metals in herb did not exceed the values considered toxic except in Bljuva site where nickel content exceeded 30 ppm. Regardless high concentrations of heavy metals in soil analysed samples did not show high levels of primary and secondary metabolites indicating strong tolerance to heavy metals and potential application of *M. lupulina* for phytoremediation. Extreme edaphic conditions of heavy metal contaminated areas exert strong selection pressure therefore AFLP (Amplified Fragment Length Polymorphism) analysis of interpopulation and intrapopulation genetic diversity could provide information on potential development of different ecotypes.

HEAVY METALS, SOIL, TOLERANCE, ADAPTATION

04 – 08 Poster

DEVELOPMENTAL STABILITY, B CHROMOSOMES AND SUSCEPTIBILITY TO PARASITISM IN THE YELLOW-NECKED MOUSE *APODEMUS FLAVICOLLIS*

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Developmental stability (DS), along with canalization, represents an element of developmental homeostasis (DH). DH is defined as the mechanism responsible for ensuring phenotypic constancy in organisms despite the great variability of genetic and environmental features. DS is usually measured by fluctuating asymmetry (FA) which refers to the minor, random differences between the two sides in bilaterally symmetric traits. The factors that cause FA can be either genetic or environmental in origin. In this study we used 276 mandibles and 323 crania of adult yellow-necked mice (*Apodemus flavicollis*) featured by the frequent presence of supernumerary B chromosomes (Bs). We investigated the associations between developmental stability and susceptibility to nematode parasitism in this species in the context of Bs presence or absence. In agreement with prevailing view that Bs are genomic parasites, B carriers would possess lower level of DS, i.e. higher level of FA, compared to non-carriers. We hypothesized that parasitized individuals should be more asymmetric as well. By applying landmark-based geometric morphometrics, we estimated the levels of FA (FA10a indices) for mandibular size and shape and cranial shape in non-parasitized B non-carriers (NPB0), parasitized B non-carriers (PB0), non-parasitized B carriers (NPB+) and parasitized B carriers (PB+). According to the hypotheses mentioned above, NPB0 mice would possess the lowest FA10a indices. Although our results revealed no significant differences in the levels of FA, NPB0 individuals are characterized by the lowest FA10a index for mandibular size, but the highest FA10a index for cranial shape. Additionally, the similar levels of FA estimated for PB0 and PB+ mice indicated that B chromosomes are not linked to developmental stability and susceptibility to nematode parasitism in this species.

CRANIUM, DEVELOPMENTAL HOMEOSTASIS, FLUCTUATING ASYMMETRY, GEOMETRIC MORPHOMETRICS, MANDIBLE

04 – 09 Poster

EXPRESSION OF METALLOTHIONEIN GENES IN *DROSOPHILA SUBOBSCURA* LARVAE EXPOSED TO HEAVY METALS

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Anthropogenic contaminants may have detrimental effects on species and their populations. However, individuals and populations can have different responses to pollutants exposure. We investigated the influence of heavy metals on expression of metallothionein genes (Mtn) in *Drosophila subobscura* larvae. The goal was to show to which extent concentration of heavy metals and duration of exposure influence Mtn genes expression, as well as potential correlation of certain metallothioneins to a specific metal or combination of them. In order to analyze biological responses to heavy metal pollution, larvae of *D. subobscura* were grown in substrate enriched with heavy metals in different concentrations, for variable period of time. RNA isolation was performed with TRIzol, after which it was converted to cDNA and quantified with RT-PCR. Level of Mtn genes expression is showing to be promising indicator of both heavy metal pollution in environment as well as the ability of the organisms and species to cope with this type of anthropogenic influence.

HEAVY METALS, METALLOTHIONEINS, *DROSOPHILA SUBOBSCURA*

SESSION 5

Genetic diversity, phylogeny and conservation

VI CONGRESS OF THE SERBIAN GENETIC SOCIETY

05 – 01 Invited lecture

THE UTILITY OF MOLECULAR MARKERS IN POPULATION AND CONSERVATION GENETICS IN DIPTERA

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In molecular genetic studies of insects at both intra- and interspecific levels, two widely used markers are mitochondrial DNA cytochrome c-oxidase subunit I gene (COI mtDNA) and nuclear ribosomal DNA internal transcribed spacer 2 (ITS2 rDNA). Given different features of these markers (e.g. structure, inheritance, mutation rate), discordant patterns and levels of variation could be observed in some taxa. With the aim to illustrate (in)congruent patterns of variability of different molecular markers (COI mtDNA and ITS2 rDNA) in selected dipteran taxa, the data from several studies dealing with the species of family *Culicidae* (mosquitoes) and family *Syrphidae* (hoverflies) were selected and presented herein.
Assessing population structure and spatial distribution of genetic diversity is of great importance in understanding microevolutionary processes involved in shaping intra- and interpopulation variability of species. Thus, phylogeographic structure of the mosquito taxa, *Ochlerotatus caspius* and *O. dorsalis*, and taxonomic value of both COI mtDNA and ITS2 rDNA markers in delimitation of these two cryptic species were estimated. In addition, identification of genetic uniqueness, cryptic species and evolutionary diversification of taxonomically diverse and challenging taxa revealed by molecular markers has also conservation implications. In this regard, molecular diversity of the hoverfly species belonging to the *Cheilosia longula* group: *C. longula, C. aff. longula, C. flavissima, C. scutellata, C. soror* and *C. thessala* was studied and independent gene pools were recognized. Finally, the usefulness of COI mtDNA in resolving phylogenetic relationships of taxa within hoverfly genera *Helophilus* and *Sphaerophoria* as well as patterns of intraspecific molecular variability of COI mtDNA and ITS2 rDNA of the *Helophilus trivittatus* and *Sphaerophoria scripta* species were compared.

COI mtDNA, ITS2 rDNA, MOLECULAR DIVERSITY, CULICIDAE, SYRPHIDAE

05 – 02 Invited lecture

EVOLUTIONARY HISTORY AND PHYLOGEOGRAPHY OF GENUS *APODEMUS* (MURIDAE, RODENTIA)

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The genus of wood and field mice, *Apodemus* Kaup, 1829, is composed of 21 extant species distributed in Palearctic. Many of them are found in Europe, and five are present in the Balkan Peninsula. The complex evolutionary history of this genus started in the late Miocene and included several instances of vicariance and morphological parallelism. Therefore, the studies of *Apodemus* often deal with topics of species distinction and the description of new species and forms.
Here we present several approaches to decipher the historical biogeography and contemporary diversity of *Apodemus*: paleontological, ecological and genetic perspective. Starting from several types of molecular sequences, we built and dated a "guiding" Bayesian phylogenetic tree. We complemented these molecular findings with geological events and known fossil records, elucidating the comprehensive evolutionary history of the genus and interrelationship among species. For chosen extant species, we modelled the habitats and predicted potential species distribution in the present and during the last Pleistocene glaciation based on the known contemporary occurrences. We then contrasted the obtained habitat preferences among species and further incorporated the information into the speciation assumptions. The ecological preferences were particularly interesting for sister species (e.g. *A. flavicollis* and *A. ponticus*) and for the explanation of their divergence.
The phylogeography of mammal species is often based on mitochondrial (maternal) haplotype distribution. Hence, we chose to calculate the haplotype diversity of cytochrome b gene (mt-Cytb), and to construct the haplotype networks for all species with more than ten available mt-Cytb sequences. The investigated species differed in the genetic diversity and phylogeographic patterns. The within-species phylogeographic patterns are explained in relation to dramatical Pleistocene climate shifts.

APODEMUS, PHYLOGEOGRAPHY, PLEISTOCENE, SPECIATION

05 – 03 Invited lecture

THE FIRST INTEGRATIVE ASSESSMENT OF THE INVASIVE POPULATION OF *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE) FROM THE CENTRAL BALKANS

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The Asian tiger mosquito, *Aedes (Stegomya) albopictus* (Skuse 1984), represents one of the medically most important mosquito vectors. Although native to South East Asia, the species has recently spread globally, and was registered in the city of Novi Sad (Serbia, the Central Balkans) in August 2018. The knowledge about the microevolutionary patterns characterizing the introduced populations can help evaluate the invasion model and the population establishment likelihood, as well as the introduction route. We therefore characterized the invasive population using phenotypic (wing size and shape) and molecular (nuclear, internal transcribed spacer 2- ITS2, and mitochondrial, cytochrome c oxidase subunit I- COI) markers, also including CLIC and GenBank repository data, respectively. The results of phenotypic analyses indicated that the Serbian population was differentiated from the native (Thailand) and invasive (Hawaii and Florida) populations, which might be due to restricted gene flow, founder effect, and supposed different strain origin. Concerning the molecular analyses, the Serbian population showed genetic homogeneity, indicative of a small founder number (bottleneck invasion model). Despite the incorporation of ITS2 GenBank sequences into the data set, neither spatial (Geneland) nor nonspatial (BAPS) genetic structuring analyses helped infer the Serbian population origin. However, the comparison of the retrieved COI haplotype with previously characterized mitogenomes indicated a temperate strain origin, capable of overwintering. Such findings suggest that the newly registered *Ae. albopictus* population could be able to establish itself since previous studies outlined Novi Sad as a suitable area.

ASIAN TIGER MOSQUITO, COI, ITS2, WING GEOMETRIC MORPHOMETRICS

05 – 04 Oral

GENETIC VARIABILITY OF *CHEILOSIA URBANA* (DIPTERA, SYRPHIDAE)

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Cheilosia urbana (Meigen, 1822) (Diptera, Syrphidae) belongs to the genus *Cheilosia* which comprises 445 currently described species worldwide. Despite the lack of morphological differences, previous research has revealed high intraspecific genetic variability of *C. urbana* suggesting the existence of potential hidden or cryptic taxa. In order to provide additional information on this subject, the 5' and 3' ends of the mitochondrial cytochrome c oxidase subunit I gene (COI) were analyzed. In total, 41 *C. urbana* specimens were collected from 14 different localities in 7 European countries (Greece, Spain, Serbia, Montenegro, Hungary, Switzerland, Slovenia) and Turkey. The results have shown high haplotype diversity for both analyzed regions of the COI gene (Hd = 0,96 for both 3' and 5' COI regions). Additionally, the construction of phylogenetic trees revealed that certain specimens from different geographically distant localities are clustered together into monophyletic clades with often moderate to high bootstrap support. Furthermore, in some cases, specimens from the same localities are placed in multiple independent clades (e.g. specimens from Simplon Dorf, Switzerland are placed with those from Greece and Slovenia in one monophyletic clade, with specimens from Hungary and Serbia into a second monophyletic clade, while one of the specimens from Switzerland forms a separate independent branch). Similar cluster formation is observed for specimens from other localities as well. These findings indicate possible presence of cryptic species with overlapping distribution and stress the importance of the application of molecular markers in the assessment of hoverfly species diversity.

CHEILOSIA URBANA, COI GENE SEQUENCES, CRYPTIC SPECIES

05 – 05 Oral

THE EXTENT OF RECENT MATERIAL TRANSFERS AND PAST DEMOGRAPHIC HISTORY IN NORWAY SPRUCE INFERRED FROM GENOMIC DATA

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The transfer of forest reproductive material for afforestation and improvement, which has been very common in Europe especially over the last two centuries, may bias inferences on past population movements in response to the last glacial maximum (LGM), some 18,000 years ago. In the present study, we genotyped 1,672 individuals belonging to three *Picea* species (*P. abies, P. obovata*, and *P. omorika*) at 400,000 SNPs using exome capture to infer the past demographic history of Norway spruce (*P. abies*) and estimate the amount of recent introduction used to establish the Norway spruce breeding program in southern Sweden. Most of these trees belong to *P. abies* and originate from the base populations of the Swedish breeding program. Others originate from populations across the natural ranges of the three species. Of the 1,499 individuals stemming from the breeding program, a large proportion corresponds to recent introductions from mainland Europe. The split of *P. omorika* occurred 23 million years ago (mya), while the divergence between *P. obovata* and *P. abies* began 17.6 mya. Demographic inferences retrieved the same main clusters within *P. abies* as previous studies, that is, a vast northern domain ranging from Norway to central Russia, where the species is progressively replaced by Siberian spruce (*P. obovata*) and two smaller domains, an Alpine domain and a Carpathian one, but also revealed further subdivision and gene flow among clusters. The three main domains divergence was ancient (15 mya), and all three went through a bottleneck corresponding to the LGM. Approximately 17% of *P. abies* Nordic domain migrated from *P. obovata* ~0.103 mya, when both species had much larger effective population sizes. Our analysis of genome-wide polymorphism data thus revealed the complex demographic history of *Picea* genus in Western Europe and highlighted the importance of material transfer in Swedish breeding program.

DEMOGRAPHIC INFERENCES, EXOME CAPTURE SEQUENCING, FOREST MANAGEMENT, *PICEA ABIES*, POPULATION TRANSFER, SNPS

05 – 06 Oral

MULTILOCUS SEQUENCE CHARACTERIZATION OF *GNOMONIOPSIS IDAEICOLA*

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Serbia is the fourth largest producer of blackberry in the world, participating in annual production with approximately 18%. Blackberry cane disease is largely understudied despite causing annual yield loses. Recently a new pathogen *Gnomoniopsis idaeicola* (*Gnomoniaceae*) occurred in Serbia causing blackberry canker and wilting. *G. idaeicola* was so far detected only in several producing areas, including Finland, France, USA – California, Oregon and Washington, and Australia, and the total number of isolates characterized so far - is limited. During 2013-2016, the presence of *G. idaeicola* in Serbia was confirmed on 11 out of 24 localities, on three out of five blackberry cultivars, with disease incidence estimated at 10-80%, plant death rate over 40% and yield loses up to 50%. Total of 18 single-spore isolates originating from different localities were identified on the bases of morphology, pathogenicity and sequencing of the ITS region of rDNA. Further sequence analyses of Serbian and all available isolates of *G. idaeicola* were conducted using BLAST and phylogenetic analyses of four genetic markers including β-tubulin, tef-1α, FG1093 gene and the ITS rDNA region. In this study molecular identification and phylogeny supported the conventional identification of Serbian *G. idaeicola* isolates. One well-defined and separated species-specific branch included all isolates available from different hosts and parts of the world. On the other hand, phylogeny based on combined sequences of four markers provided additional information and represents the first ever demonstrated insight into possible intra-species variability of *G.idaeicola*. One Serbian isolate (KSV1-16) positioned separately, indicating its different origin and possible pathway of introduction into Serbia probably via infected planting material.

Financed by grants TR 31018 and III-43001 of the Ministry of Education, Science and Technological Development of the Republic of Serbia.

BLACKBERRY, STEAM DISEASES, IDENTIFICATION, CHARACTERIZATION, PHYLOGENY

05 – 07 Oral

DYNAMIC CONSERVATION OF *PICEA OMORIKA* POPULATIONS IN THE REPUBLIC OF SRPSKA, BOSNIA AND HERZEGOVINA

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Serbian spruce *Picea omorika* (Panč.) Purk. is a rare, IUCN red-listed European conifer endemic to the refugial Balkan region. Current rigid conservation (without any intervention allowed) and designation of seven Genetic Conservation Units (GCUs) included into pan-European network for dynamic conservation of forest genetic resources (four natural populations and three planted stands) are not based on genetic data. We carried out a comprehensive field survey in the Republic of Srpska, Bosnia and Herzegovina (RS-BH), and genotyped 689 individuals from 14 populations with ten highly informative nuclear EST-SSRs. Seed sources for the re-establishment of populations at sites fully burned over the past 100 years, were identified and we applied analytical methods for prioritizing populations for conservation based on their contribution to the geographical structuring of genetic diversity. The levels of genetic diversity of studied Serbian spruce populations (Ae= 2.524, H_O=0.465, H_E=0.451) are slightly lower than those found in eastern populations analysed previously with the same molecular markers, and effective population size is generally ≥15. Populations are highly genetically differentiated [Hedrick's G'ST=0.186 (± 0.044); Jost's D=0.097 (±0.040)] and comprise ten distinct gene pools. Although wildfires contribute to admixture of gene pools within populations, re-establishment from seeds from extirpated populations has likely prevailed. As much as 14% of alleles are not preserved in the extant network of four GCUs (natural populations only) which does not include eastern, genetically distinct and more diverse populations. Seven populations positively contribute to within-population genetic diversity, four to genetic differentiation, and two are globally important in terms of diversity and differentiation. The high conservation value of two populations was not evident from nuclear but from available knowledge on mitochondrial diversity.

FOREST TREE CONSERVATION GENETICS, GENETIC STRUCTURE, GENETIC CONSERVATION UNITS, NUCLEAR MICROSATELLITES, RARE AND ENDANGERED SPECIES

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05 – 08 Oral

WAS THE BALKAN PENINSULA A GLACIAL REFUGIUM FOR THE MEDITERRANEAN HORSESHOE BAT, *RHINOLOPHUS EURYALE* (BLASIUS, 1853)?

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Large Mediterranean peninsulas of Europe like the Balkans, Italian and the Iberian Peninsula have been recognized as Pleistocene glacial refugia for many temperate species. The aim of this study was to investigate demographic history and genetic structure of Mediterranean horseshoe bat populations on the Balkan Peninsula. *R. euryale* is a cave-dwelling species distributed throughout the Mediterranean region. We collected 82 samples from 20 localities in the Balkans and Italian Peninsula and analysed genetic diversity of mitochondrial D-loop sequences. Our results revealed low nucleotide but high haplotype diversity, and 20 haplotypes were reported for the first time. Phylogenetic reconstructions showed that all haplotypes obtained from both Peninsulas belong to the same lineage together with the previously published samples from Turkey, southern France and North Africa. All haplotypes from the current study represent single haplogroup and haplotype network had a star-like topology that is indicative of recent population expansion. Scenario of sudden demographic expansion was also supported by shallow genetic differentiation and mismatch distribution analysis, and we estimate that expansion within this lineage started after the last glacial maximum. We present the new data on genetic variation in this species, and highlight the importance of the Balkans in the demographic history of Mediterranean horseshoe bat. The obtained results support the hypothesis that the Balkan Peninsula was a glacial refugium for *R. euryale* during the Pleistocene.

D-LOOP, EXPANSION, mtDNA, REFUGIUM

05 – 09 Poster

GLIADIN ALLELE COMPOSITION AND TECHNOLOGICAL QUALITY TRAITS IN BREAD WHEAT GENOTYPES

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Gliadins are endosperm storage proteins of wheat seed, encoded by six genes which are located at the short arm of chromosomes 1A, 1B, 1D, 6A, 6B and 6D. Gliadins are important in determining baking quality traits of the flour. The aim of this work was identification of allele at Gli-1 and Gli-2 loci and their association with grain protein content, sedimentation volume and bread volume in wheat. The 10 wheat genotypes (G-3644-4, G-3619-3, G-3601-4, G-3626-2, G-3622-1, G-3617-1, G-3611-2, G-3634-2, G-3639-1, G-3615-1) were included in this study. Gliadins extracted with 70% ethanol from endosperm of 20 seeds. The separation of gliadins conducted by using electrophoresis method on polyacrylamide gel in buffer at pH=3.1. Electrophoregrams used for determining Gli-1 and Gli-2 alleles. Technological quality parameters analyzed by standard laboratory methods. In analyzed wheat genotypes were identified 28 alleles at the six Gli- loci. The 4 alleles at the Gli-A1, 5 alleles at the Gli-B1, 3 alleles at the Gli-D1, 5 alleles at the Gli-A2, 6 alleles at the Gli-B2 and 5 alleles at the Gli-D2 locus were identified. The grain protein content was the highest in G-3634-2 (15.20%) while the lowest GPC had G-3601-4 (12.20%). The highest protein sedimentation volume had wheat genotype G-3619-3 in (48.0 ml) while the lowest sedimentation volume had G-3644-4 (35.0 ml). Loaf volume was the highest in G-3634-2 (550 ml) while the lowest was in G-3601-4 (440 ml). The analysed wheat genotypes are genetically divergent according to Gli-allele composition and quality traits which characteristic of genotypes.

GLIADIN, ALLELES, POLYMORPHISM, QUALITY, WHEAT

05 – 10 Poster

16S rRNA GENE POLYMORPHISM SUPPORTS CRYPTIC SPECIATION WITHIN THE LESSER BLIND MOLE RAT *NANNOSPALAX LEUCODON* SUPERSPECIES (RODENTIA: *SPALACIDAE*)

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Among 26 genera of Palaearctic mammals, the genus *Nannospalax* has the highest karyotype variability with 74 chromosomal forms (CFs). Taxonomic effects i.e. implications to phylogeny and speciation process of such chromosomal variety are still lacking, especially among 25 reported CFs of South-European *N. leucodon* superspecies. Many cryptic species are under serious threat of complete disappearance, with population declines in Europe. As genetic discrepancies for the majority of them are missing, we analyzed nucleotide sequence polymorphism of the mitochondrial 16S rRNA gene between eight *N. leucodon* CFs and also add for the first time nucleotide sequence data for three CFs: monticola, montanoserbicus and syrmiensis. Further, including 40-57 years old teeth we evaluate the usefulness of the archived samples, e.g. from museums and other old collections as starting material for phylogenetic analysis. The topology of the Bayesian Inference tree is in agreement with the traditional taxonomic separation of recent blind mole rats. Among the three superspecies, the genetic diversity was lowest in *N. ehrenbergi* (0.004-0.031), highest in *N. xanthodon* (0.009-0.063) and intermediate in *N. leucodon* (0.008-0.055). The comparable scale of evolutionary divergence among *N. leucodon* CFs and among species from the genus *Spalax* supports our previous proposal that seven reproductively isolated CFs should be considered to be cryptic species and thus protected from extinction in their natural habitat.

16S rRNA GENE, CRYPTIC SPECIATION, KARYOTYPE EVOLUTION, CONSERVATION, MUSEUM ARCHIVED SAMPLES

05 – 11 Poster

VARIABILITY OF NEEDLE MORPHO-ANATOMY OF NATURAL *PINUS HELDREICHII* POPULATIONS FROM SCARDO-PINDIC MOUNTAINS

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On the cross-section Bosnian pine needles are lunate, rarely triangular (when three needles are in the same sheet). Resin ducts are immersed in mesophyll (internal type). Eight morpho-anatomical properties of two-year old needles of the *Pinus heldreichii* (Bosnian pine) from Scardo-Pindic mountain massif in Serbia (Mt. Šara) and Republic of North Macedonia (Mt. Galičica) were investigated. The mean values of analyzed characters were as follows: 5.91 cm (needle length), 1.49 mm (needle width), 0.85 mm (needle thickness), 25.05 µm (cuticle + epidermis thickness), 69.90 µm (height of hypodermal cells), and 46.91 µm (resin duct diameter). *Pinus heldreichii* needles also had 2-5 hypodermis layers and 0-12 resin ducts. Variance analysis (P≤0.05, LSD test) established the significant differences among populations in all needle characteristics analysed, especially in needle length and width.
In comparison to previously investigated needles of Dinaric mountains, *P. heldreichii* needles from Scardo-Pindic massif are shorter, and with thicker hypodermis layer. PCA and CA visualize segregation of *P. heldreichii* populations between two mountain massifs. Population from Mt. Ošljak has significantly longer and narrower needles, as well as lower needle thickness, height of hypodermal cells and resin duct diameter. Population from Mt. Galičica has slightly longer cuticle+epidermis thickness. Needle width and thickness, height of hypodermal cells, height of hypodermis cells and resin duct diameter tend to increase along the north-east distribution of populations.

BOSNIAN PINE, NEEDLE MORPHOLOGY, NEEDLE ANATOMY, PCA, CLUSTER ANALYSIS

05 – 12 Poster

PHENOTYPIC AND MOLECULAR CHARACTERIZATION TOWARDS GENETIC PURITY DETERMINATION OF MAIZE INBREDS

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Maintenance of genetic purity and uniformity of lines and hybrids is a prerequisite for successful production and placement of commercial hybrid seed on the market. The aim of this study was to compare the efficiency of different marker type regarding genetic purity determination. Morphological characterization of three maize inbred lines was performed according to the UPOV (Union Internationale pour la Protection des Obtentions Végétales) markers in three-year field experiment. For uniformity and stability testing, 16 measured (MS) agro-morphological traits were evaluated. For all observed MS agro-morphological traits based upon three-way Analyses of Variance (ANOVA), the best performing was inbred L3. However, less uniformity level was found for L1 and L2 inbred lines. In parallel, 12 informative SSR markers were used for genetic purity analysis. Initial molecular marker analysis of the genotypes was done on bulked seed samples. Two out of 12 SSR markers revealed possible problems with genetic purity of L1 and L2 genotypes. Since for these genotypes double band was scored upon visualization of PAGE electrophoresis, analyses were repeated with the two mentioned SSR markers on individual seeds of each genotype. The electrophoretic pattern of L1 individual seeds revealed that genotype is not uniform, with upper or lower band present. Electrophoregram of L2 individuals showed the presence of double band in each seed, the same as in the bulk, which confirms its genetic homogeneity. Based on results obtained, it could be concluded that both marker types used, confirmed quite the same level of genetic purity (i.e. L3 >L2 > L1) and could be applied for genetic purity estimation.

STABILITY, UNIFORMITY, MORPHOLOGICAL MARKERS, SSRS, *ZEA MAYS* L.

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05 – 13 Poster

GREY WOLVES IN SERBIA – GENETIC DIVERSITY AS INFERRED FROM MICROSATELLITES

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In the past, grey wolf (*Canis lupus*) was widespread throughout Europe but at the end of the 19th century, following the habitat destruction and decline in prey abundance due to human influence, their numbers have dwindled and large continuous population became fragmented. Drastic reduction in their population size has continued in the 20th century after which the need for protection has become apparent. Grey wolf represents a highly important species in Serbia, whose conservation efforts largely depend on genetic monitoring. The main objective of this study is to evaluate the present genetic diversity in grey wolf population from Serbia. For this purpose the panel of microsatellite markers was used. Genomic DNA was extracted from 59 grey wolves muscle tissue samples collected during 2019. Individuals were genotyped by panel of 19 markers, 18 autosomal microsatellite markers and amelogenin sex determination locus (The Canine Genotypes™ Panel 1.1 kit). Genetic variability parameters were calculated in GENEPOP and ARLEQUIN. The number of alleles per locus varied between 16 in the case of AHT121 and 7 alleles for loci REN54P11, REN162C04 and INRA21, with the average number of alleles per locus at 10.278. Overall expected heterozygosity (0.772) was higher than the observed heterozygosity (0.693). The highest observed heterozygosity was found at AHTh171 (0.84746), while the lowest value was detected at INRA21 locus (0.424). Estimated effective population size calculated in LDNe was 120.9 (with exclusion of rare alleles 107.7). Comparison to previous results from the genetic monitoring of the same population, it can be concluded that this population shows high level of genetic diversity and constant effective population size.

GREY WOLF, GENETIC DIVERSITY, MICROSATELLITES

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05 – 14 Poster

WHOLE MITOCHONDRIAL GENOME DIVERSITY IN SERBIAN POPULATION: PHYLOGENETIC AND FORENSIC ASPECTS

Natasa Kovacevic-Grujicic [1], Slobodan Davidovic [1, 2], Boris Malyarchuk [3], Tomasz Grzybowski [4], Jelena M Aleksic [1], Miroslava Derenko [3], Andrey Litvinov [3], Urszula Rogalla-Ładniak [4], Milena Stevanovic [1, 5, 6]

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Mitochondrial DNA (mtDNA) is used in forensics for over three decades and is particularly suitable when STR profiling cannot be performed due to the degraded and/or scarce nuclear DNA. Traditionally, mtDNA typing is based on ~600 bp of the hypervariable segments I and II (HVS-I and HVS-II) of the control region (CR, ~1100 bp). Nowadays, it is possible to use variability of complete mtDNAs which enables maximum resolution of distinct maternal lineages. However, the number of complete mitogenomes in reference databases such as EMPOP is still insufficient, and that hampers their wider usage in forensic casework. In order to fill in the gap in the reference database, which, considering Slavic-speaking populations, currently comprises only mitogenomes of East and West Slavs, we present population data for 226 Serbian mitogenomes, representatives of South Slavs from the Balkan Peninsula. We support previous findings on both high levels of genetic diversity in the Serbian population and patterns of genetic differentiation among Serbian and ten studied European populations. However, increased genetic differentiation was observed among Serbian and two European populations (Russians and Poles) with our high resolution data. We demonstrate that the inclusion of indel polymorphisms into analysis contributed towards nearly complete resolution of mtDNA haplotypes (97.1% vs. 86.3% without indels), and that the random match probability was as low as 0.53%. Bayesian skyline analysis of Serbian mitogenomes revealed population expansion after the Last Glacial Maximum and during the Migration period (IV-IX century A.D.). Phylogenetic analysis of the Serbian and relevant West Eurasian haplotypes contributed towards the improvement of the worldwide mtDNA phylogeny to the certain extent, which is essential for the interpretation of the mtDNA casework. Lineages of a putative Balkan origin as well as those shared among Serbian and other European populations were observed.

COMPLETE MITOGENOMES; DEMOGRAPHIC CHANGES; MOLECULAR PHYLOGEOGRAPHY; SERBIAN POPULATION

05 – 15 Poster

DIVERSITY OF MONTENEGRIN MAIZE GENE POOL FROM MRIZP GENE BANK

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Numerous studies emphasize the importance of gene banks maintained accessions regarding conservation of traits of importance, both to overcome various types of biotic and abiotic stress, and sources of desirable technological and nutritional properties. The gene bank of the Maize Research Institute „Zemun Polje" (MRIZP) currently preserves and maintains 320 samples of maize landraces, collected in Montenegro (64 samples collected in the 1960's, 225 in 1970's and 31 samples in 1980's). A large number of collected samples from the small area open the question of possible existence of duplicate samples. The aim of the study was to compare the stored samples, at morphological and molecular level, in order to identify possible duplicates. Based on 27 phenotypic traits, using hierarchical cluster analysis, landraces are classified into seven morphologically similar groups. The maximum morphological distance was 353.3, minimum 3.4, and average 56.0. By observing the groups of landraces that are most closely related to each other, it can be noticed that these are mainly landraces of similar kernel color and hardness, collected on different sites and time and with usually different local names. Since morphological markers are insufficiently reliable indications of genetic similarity, it is expected that the molecular characterization would give more clear insight of the diversity of the Montenegrin maize landraces gene pool stored in the MRIZP gene bank.

DUPLICATE ACCESSIONS, LANDRACE, MORPHOLOGICAL MARKERS, *ZEA MAYS* L.

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05 – 16 Poster

INTRA-POPULATION GENETIC DIVERSITY OF BEECH FROM GOČ MOUNTAIN ASSESSED BY MOLECULAR MARKERS

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Genetic studies of beech species were subjects of numerous researches performed in different populations over a wide range of the species distribution. Gene pool of beech (*Fagus sylvatica* L.) in Serbia is characterized by the variability of different morphological, physiological and genetic traits. The aim of this research was to determine the intra-population genetic diversity of beech population „Goč-Gvozdac" at the molecular-genetic level. The population is located at Goč Mountain in Serbia. Five primer pairs of microsatellite loci (mfc5, sfc_0036, csolfagus_19, csolfagus_31, and DE576_A_0) were used for the analysis. The samples of 45 genotypes (juvenile beech plants aged between 3 and 5 years) from the population were selected and dormant buds were collected from each individual. After DNA isolation, PCR amplification, fragment length size and allele determination of the obtained PCR products were performed using an automatic sequencer CEQ 8000 Genetic Analyzer. Data analysis was carried out using the GenAlEx 6.5 software. The number of alleles per locus was in the range from 6 (DE576_A_0) to 11 (csolfagus19 and mfc5), while the average effective number of alleles per locus was 4.271. Both observed and expected heterozygosity in the studied population were high (mean Ho = 0.711; mean He = 0.734), which indicates a considerable amount of genetic diversity within the population.

GENETIC DIVERSITY, *FAGUS SYLVATICA* L., MICROSATELLITE MARKERS

05 – 17 Poster

BIOMONITORING CYTOGENOME DIVERSITY USING WETLAND PLANTS: NATURAL HERITAGE RISK ASSESSMENT CASE STUDY

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New advances in efficient use of biomonitoring of vulnerable plants through implementation of pollen grain deformation bioassays are helping to preserve last refuge of natural heritage in wetland habitats of Ljubljansko barje, Slovenia. Ljubljansko barje has changed from a useless moor into a cultural landscape. Located to the South of Ljubljana, the capital of Slovenia, the wetland complex of meadows and fields spans about 150 km2 and is in its entirety managed by Ljubljana Marsh Nature Park. Regarding the areas of threatened species from the national Red List and the locations of the exceptional archaeological discoveries, they all lie in the core protection zone of the Park. Despite having successfully protected such findings of great cultural significance until the present day, the still existing wetland habitats are very vulnerable to pollution and climate change. Tracing out their capacity to sustain plant diversity is a key to ensure the continued survival of both natural and cultural heritage. One example where biomonitoring of plants is used with great success is the UNESCO World Heritage Site of the pile-dwelling remains at Ljubljansko barje. Despite being overgrown with invasive aliens (*Solidago canadensis*, *S. gigantea*, *Reynoutria japonica*, *Impatiens glandulifera*), these wetlands show good plant conditions, i.e. the pollen grain deformation (PGD) bioassay is indicating normal reproduction capacity. More than 20 years worth of PGD data, many of them directly resulting from biomonitoring of *Fritillaria meleagris* and *Allium angulosum* using new cytogenome balance risk schemes, suggest that pollen grains are mainly viable and fully nutritious. Such results indicate that the pollen from wetland areas but only when traditionally cultivated is of good enough quality even for apiculture. All of the above indicates that despite many environmental changes Ljubljansko barje as a whole still functions as a working ecosystem that will continue to help preserve a World Class Heritage.

WETLAND PLANTS, POLLEN GRAIN DEFORMATION BIOASSAY, BIODIVERSITY, RISK ASSESSMENT, CONSERVATION

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05 – 18 Poster

THE GENETIC MONITORING OF BROWN HARES (*LEPUS EUROPAEUS*) FROM VOJVODINA

Iva Gorše [1], Milomir Stefanović [1], Ivana Matić [1], Dejan Beuković [2], Zoran Popović [3], Nevena Veličković [1], Mihajla Đan [1]

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The European brown hare (*Lepus europaeus*) is one of the most important game species, recognized as the keystone species in agro-ecosystems. Its strong population decline during the last few decades may influence population's genetic variability, thereby disturbing the ability for a long term survival. Therefore, genetic monitoring of brown hare population was established, aiming to provide insight in genetic signals of possible reduction of genetic diversity. The long-term genetic monitoring represents a crucial step in improving management strategy.
Here we used panel of six microsatellite loci to assess the genetic variability. During hunting season 2018/19 tissue of 89 brown hares from three different geographical regions (Backa, Banat and Srem) in northern part of Serbia were sampled. Molecular diversity indices were calculated in Genetix, Arlequin and GenAlEx. Genetic diversity parameters were compared with the previously detected parameters for the same population ten years ago. An average of 10.83 alleles per locus was detected, with a range from 2 to 19, while the number of private alleles ranged from 4 in Backa and Srem, to 10 in Banat. The observed heterozygosity (0.499) was lower than expected (0.707), with the overall FIS value of 0.3 ranging from 0.252 to 0.368 in different geographical regions. Number of loci deviating from Hardy-Weinberg equilibrium ranged from 2 to 5, in different geographical regions. Genetic differentiation was low with a mean FST of 0.023, while FST values ranged from 0.016 to 0.029. Percentage of variation among geographical regions was 2.34%, and 97.66% within. The results show maintenance of moderate genetic diversity over a ten-year period, supporting prior measurements, such as reduction of hunting season period, as justified for the long-term sustainability of the population.

BROWN HARES, GENETIC MONITORING, GENETIC DIVERSITY, MICROSATELLITES, GAME MANAGEMENT

05 – 19 Poster

CHARACTERIZATION AND EVALUATION OF POTATO GENETIC RESOURCES IN MONTENEGRO

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Potato was introduced in Montenegro in the late 18th century. Over the course of more than 230 years of cultivation, potatoes in these areas have been differentiated into a large number of forms of different lengths of vegetation. The cultivation of local potato varieties lasted until the 1970s when massive introduction of new high-productive selections began. Right at that time, work on the conservation of these resources begins. The most important measures for their conservation were made in the period from 2008 to 2010, when 52 local populations of potatoes were collected from more than 150 sites.
In order to get a clear estimate of the value of this collection, but also to identify duplicates, a program of characterization and evaluation began in 2016. The morphological characterization of the sprout was made on the basis of the UPOV descriptor for the 11 characteristics of the sprout. Sixteen different phenotypes were identified. Morphological examination selected 23 samples for DNA analysis.
Molecular evaluation, using 12 microsatellite markers (SSR), confirmed the existence of 13 groups. Comparison of the DNA materials of Montenegrin populations with data base of over 8000 varieties at the SASA Institute in Scotland, found the existence of 5 unique genotypes, two of which were duplicates (genetic profile 1 - Ljubičasti šareni and Kromir rozi, genetic profile 2 - Koprivuša, genetic profile 3 - Maus and genetic profile 4 - Cvjetaš. All other Montenegrin populations were known varieties.
The characterization performed will significantly contribute to the reduction of the maintenance cost of the collection, but also raise the quality of its conservation to a significantly higher level.

POTATO, LOCAL POPULATIONS, CHARACTERIZATION, EVALUATION

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05 – 20 Poster

GENETIC DIVERSITY OF SERBIAN ISOLATES OF *XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS* ORIGINATED FROM WINTER OILSEED RAPE

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Oilseed rape (*Brassica napus* L.) is a hybrid species within Brassicaceae family, significant for its oil-rich seed, mainly used for vegetable oil and biodiesel production, but also for consumption as livestock feed. Yield of oilseed rape and other cruciferous crops can be reduced due to different plant pathogenic bacteria, fungi and pests. One of the most significant bacteria affecting crucifers is *Xanthomonas campestris pv. campestris* (Xcc) causing black rot disease. Xcc isolates from diseased winter oilseed rape plants showing bacterial blight symptom on leaves were collected in nine-year period (2010-2018), from different localities in Serbia, with the aim to examine their genetic features, through sequencing of housekeeping genes. DNA of the obtained isolates was therefore amplified with six primers (dnaK, fyuA, gapA, gyrB, lepA, rpoD) and sent for sequencing. The obtained sequences were checked for homology with strains available in NCBI database. Multilocus sequence analysis (MLSA) was then performed to determine relatedness among the tested isolates. Based on six genes, tested isolates were identified as Xcc using NCBI BLAST, showing 99-100% homology with the available data. Winter oilseed rape Xcc isolates were divided into five groups on Neighbour-joining tree, revealing intrapathovar diversity within isolates from this host. These results could be connected with pathogen adaptation to winter oilseed rape as a new host, providing completely distinct ecological niche from *B. oleracea* vegetable crops (broccoli, cabbage, cauliflower, etc.) which are common hosts for this bacteria. This observation on Serbian Xcc isolates gives a significant input on genetic variation and constitutes a highly informative sample of *X. campestris* diversity.

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. III43010).

OILSEED RAPE, *XANTHOMONAS*, BACTERIAL BLIGHT, MULTILOCUS SEQUENCE ANALYSIS (MLSA)

05 – 21 Poster

MULTILOCUS SEQUENCE ANALYSIS OF *RALSTONIA SOLANACEARUM* ISOLATES ORIGINATED FROM POTATO IN SERBIA

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Ralstonia solanacearum is a soil borne bacterium which affects more than 450 plant species including a wide range of crop plants, ornamentals and weeds. This bacterium is causing bacterial wilt disease which leads to severe economic losses. On potato, disease is known as brown rot. Even though this phytopathogen originates from tropic, subtropics and warm temperate regions, in recent years cold-adapted strains dramatically enhanced the threat of European potato crops, including Serbia. *R. solanacearum* is at A2 list of quarantine plant pathogens in Europe. During six year period (2013-2018), isolates from diseased potato tubers were collected, identified using Multilocus sequence analysis (MLSA) and checked for certain phylotype affiliation. DNA from the obtained isolates was amplified using seven housekeeping genes (*adk, fliC, gapA, gdhA, gyrB, hrpB, ppsA*) and sequenced. Phylogenetic analysis was performed with concatenated sequences of all tested isolates, and compared with the most similar *R. solanacearum* strains from PAMDB database. All Serbian isolates were identified as *R. solanacearum* using PAMDB BLAST. Neighbor-joining phylogenetic analysis placed tested isolates in the same cluster with *R. solanacearum* strains belonging to race 3, biovar 2, and phylotype II obtained from PAMDB. Although, bacterial wilt caused by this plant pathogenic bacteria is appearing in different locations and on different potato cultivars in Serbia it remained genetically homogenous. Currently, *R. solanacearum* is a quarantine bacteria occurring only on potato in our country, but considering its wide host range, there is a possibility of it causing disease on other significant crops with devastating consequences. Therefore, its fast and accurate identification and mapping is of main interest for stopping the disease spread.

This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Project No. III43010).

RALSTONIA SOLANACEARUM, BACTERIAL WILT, BROWN ROT, MULTILOCUS SEQUENCE ANALYSIS (MLSA)

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GENETIC INSIGHT INTO THE ISOLATES CAUSING BLACKLEG DISEASE ON POTATO

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Blackleg disease, caused by pectolytic bacteria from genus *Dickeya* and *Pectobacterium* is currently being one of the main concerns in potato production. Yield loss and reduced quality, visible through potato black rotting at the stem base, as well as whole plant decaying, makes potato cultivation less profitable. Blackleg disease outbreak was observed in potato field (cv. Lady Claire) in Bačka (northern Serbia) in June 2018. The percent of the infected plants, estimated during the vegetation season was 45%, with yield reduction of about 20%. In this study the causal pathogens were genetically characterized using multilocus sequence analysis (MLSA). DNA of the obtained isolates was amplified with primers made based on the sequences of five housekeeping genes - *gapA*, *icdA*, *mdh*, *pgi* and *proA* and sequenced. Phylogenetic analysis was performed to compare the obtained isolates with the ones already deposited in NCBI database. NCBI BLAST identified isolates as *Dickeya dianthicola* and *Pectobacterium carotovorum* subsp. *brasiliensis*, indicating on the persistence of mix infection on observed potato crop. According to all genes, isolates appeared to be the most similar (99-100%) to the reference strains - *D. dianthicola* (CFBP 1200) and *P. carotovorum* subsp. *brasiliensis* (BC1). Blast results were confirmed after phylogenetic analysis, where isolates of both identified species were clustering with the rest of the strains of the same species, obtained from NCBI. Our findings confirm the presence of new blackleg causal agents, *D. dianthicola* and *P. carotovorum* subsp. *brasiliensis* on potato in Serbia. Their isolation and localization is of great importance, especially *D. dianthicola* which has quarantine status, and therefore it gives a signal for pathogen eradication and prevention of its future spreading.

This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Project No. III43010).

POTATO, BLACKLEG, *DICKEYA*, *PECTOBACTERIUM*, MULTILOCUS SEQUENCE ANALYSIS (MLSA)

05 – 23 Poster

SOME ARE GOOD, SOME ARE BAD: AN OVERVIEW OF AVAILABLE NUCLEAR MICROSATELLITES IN *FAGUS* SPECIES, AND THEIR UTILITY IN *F. SYLVATICA* FROM THE CENTRAL BALKANS (SERBIA)

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Selectively neutral nuclear microsatellites (SSRs) are among the most commonly used molecular markers for genotyping in plant and animal species. They are also used in studies focusing on adaptation along with adaptive markers because available methods for identification of loci related to adaptation are sensitive to evolutionary forces that can mimic selection, such as population structure. In studies of this kind, the usage of selectively neutral molecular markers may provide insights into among- and within-population genetic structure, and thus, may be used for the distinction of effects of these phenomena from the effects of selection. Nuclear microsatellites have been developed for *Fagus sp.*, and have been used in *F. sylvatica*, an ecologically and economically most important European *Fagus* species. We provide a comprehensive overview of available *Fagus* sp. SSRs (185 loci), carry out meta-analysis of loci used in *F. sylvatica* (62 loci employed in 62 surveys), and validate a set of 16 loci in 45 individuals of this species from the central Balkans (Serbia). Erroneous usage of marker's names/authors is rather frequent, and loci successfully used in a number of studies are characterized by other authors by high prevalence of null alleles and even multilocus amplification products. Frequent occurrence of null alleles at FS4-46, used in 26 surveys to date, most likely indicates a failure to record multiple alleles at this locus. Twelve loci are reliable/informative in *F. sylvatica* from the Balkans (5-18 alleles/locus, HE ranging from 0.523 to 0.850), while four loci are characterized by high prevalence of null alleles (sfc0161 and sfc1063) and multilocus amplification products (FS4-46 and Fagsyl_007038). Our findings are important for future population genetic analyses and studies on adaptation of *F. sylvatica* to its environment, because the latter rely on both selectively neutral and markers under selection.

BALKAN PENINSULA; COMMON BEECH; GENOTYPING; NULL ALLELES; MULTILOCUS AMPLIFICATION PRODUCTS

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05 – 24 Poster

VARIABILITY OF BEECH (*FAGUS SYLAVTICA* L.) POPULATION IN SERBIA BASED ON MORPHOLOGICAL CHARACTERISTICS OF CUPULES

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Beech (*Fagus sylavtica* L.) as a species of wide utilization is one of the most important trees in Europe. Taxonomic status of this species is a decades-old problem in the southeastern part of its European distribution. Based on the numerous studies, the analysis of the morphological characteristics of the cupules proved to be informative in terms of selecting certain ecotypes in this area. In order to determine variability between populations we analyzed cupules from 12 natural beech populations in Serbia, from different altitude (300 to 1250 m.a.s.l). Five quantitative morphological parameters were analyzed: the length of the cupule (without the petiole), the length of the longest valve, the width of the longest valve, the length of the petiole and the distance between the base of the longest valve and the petiole ("connecting piece"). Data obtained by measuring were processed by the software program STATISTICA 7. The length of the petiole and "connecting piece" were shown to be the most variable characteristics. All pairwise correlations were positive, except for the "connecting piece" and the length and width of the valve. The variance analysis showed that the differences in the average values among the populations in all measured characteristics are statistically significant ($p<0.05$). The LSD test did not show a clear separation of homogeneous groups, which was also demonstrated by the dendrogram cluster analysis. The results of this research can serve as an additional knowledge of the variability of beech in Serbia. If they are combined with other analyzes (morphological, anatomical and molecular) they can contribute to solving specific problems in forestry related to this species.

COMMON BEECH; MORPHOLOGICAL CHARACTERISTICS; CUPULES; NATURAL POPULATIONS; VARIABILITY

05 – 25 Poster

VARIABILITY OF STOMATAL CHARACTERISTICS OF THREE LEAF-ORNAMENTAL BEECH CULTIVARS

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In Serbia, the leaf stomatal traits of beech cultivars have been studied by a small number of researchers. The aim of this research was to study the variability of the stomatal characteristics of different leaf-ornamental beech cultivars and compare it with one *Fagus sylvatica* L. genotype. Three European beech cultivars, representing mutants in leaf shape and color (*Fagus sylvatica* 'Purpurea', *Fagus sylvatica* 'Tricolor' and *Fagus sylvatica* 'Purpurea Tricolor') together with the wild type (*Fagus sylvatica* L.) were selected in Belgrade. Nine fully expanded leaves were collected from each of 10 genotypes and 90 stomatal imprints were made using the „Collodion" method. In order to determine the variability of the stomata, three basic stomatal parameters: stomatal length, stomatal width, and stomatal density and one derived parameter - stomatal shape coefficient, were analyzed. The analysis was done using a microscope with a digital camera (MAGNUM-T-Trinocular Microscope) and specialized software. The collected data were statistically processed using the Statistica 6.0 and Statgraphics Plus 5.0 software. The obtained values showed statistically significant differences in the size and density of stomata. Based on the results of the study of stomatal size and density, the trees presenting the same cultivar were not always in the same group, which may indicate that these stomatal characteristics are under genetic control.

VARIABILITY, STOMATAL SIZE AND DENSITY, BEECH CULTIVARS

05 – 26 Poster

GENETIC DIVERSITY OF MAIZE INBRED LINES WITH DIFFERENT KERNEL TYPES ACCORDING TO MICROSATELLITE ANALYSIS

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Molecular characterization is important for efficient utilization of maize germplasm and development of improved varieties. The objective of the study was to assess genetic diversity among maize inbred lines with different kernel type (dent, flint, popcorn, sweet maize) and kernel color (white, yellow, orange) with microsatellite markers. Total number of alleles detected in 24 inbred lines using 21 SSR markers was 139 with 23% unique alleles. Eight, five, four and ten of them were found among genotypes with orange kernel color, white maize and popcorn, sweet, and yellow maize genotypes, respectively. These alleles are important because they could be used in identification of genome regions specific for a certain type of maize. Number of alleles per marker ranged from 4 (umc1887, umc1172, phi080) to 14 (umc2002) with an average of 6.62. Expected heterozygosity varied from 0.62 to 0.89, with an average of 0.77. PIC values of tested microsatellites were high (from 0.57 to 0.89), with average value of 0.73. The lowest genetic distance calculated according to Rogers' coefficient was 0.38 while the highest was 0.99. UPGMA cluster and principal coordinate analyses revealed the presence of two to three main groups. Sweet maize and popcorn genotypes grouped together with standard maize genotypes. Classification of mentioned groups is not surprising since inclusion of standard maize germplasm in breeding of speciality maize is not uncommon practice. It could be concluded that grouping of all tested genotypes was not completely in agreement with kernel type/color. Also, absence of complete data for origin and pedigree affected potentially a better explanation of the presented classification.

MAIZE, INBRED LINES, SSR, GENETIC DIVERSITY

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05 – 27 Poster

APPLICATION OF SSR MARKERS FOR ASSESSMENT OF GENETIC DIVERSITY OF THE HUNGARIAN OAK (*QUERCUS FRAINETTO* TEN) AT THE LEVEL OF THE SEED STAND RS-2-2-qfr-00-806

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Knowing the level of genetic diversity is of particular importance for the process of plant breeding and conservation of available gene pool. Knowing the level of genetic diversity of an individual seed stand is of great importance when choosing the source of reproductive material. The objective of this work was to analyze population structure and genetic diversity of *Quercus frainetto* RS-2-2-qfr-00-806 seed stands using SSR (Simple Sequence Repeat) markers. DNA was extracted from the buds of 20 test trees of the Hungarian oak which were sampled during the hibernation. The test trees were evenly distributed throughout seed stand and the distance between them was more than 50 m.
Seven SSR markers were analyzed and all were polymorphic. Total number of alleles revealed for all analyzed individuals were 23, ranging from two (QpZAG112, QpZAG36 and QpZAG15) to five (QpZAG9). Genetic similarities were calculated on binary data using Dice's coefficient by NTSYSpc2.1 program package. High genetic variation was observed among analyzed genotypes, as genetic similarity covered a larger range of values (0.23-0.93).
The obtained level of genetic variability of the seed stand RS-2-2-qfr-00-806 represent a good starting point for future experiments on breeding of the species. This is basic research for the improvement of mass production of quality reproductive material of Hungarian oak in Serbia.

HUNGARIAN OAK, SSR (SIMPLE SEQUENCE REPEAT) MARKERS, POPULATION, SEED STAND

05 – 28 Poster

GENETIC RESOURCES EXPLOITATION IN WHEAT BREEDING AND IMPROVEMENT AT THE IFVCNS

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Modern agriculture depends on improved forms of crop plants, created by breeders from germplasm which provides the genetic information needed to increase yield and quality and add resistance to pests, diseases and adverse environmental factors. Genetic resources are maintained and evaluated in collections located worldwide and supported by national and international public funds. Institute of Field and Vegetable Crops, Novi Sad (IFVCNS) has its own collection of wheat genotypes that comprises around 2800 accessions originating from more than 30 countries, as well as 65 wheat ancestors and related species. For the maintenance purpose each year one third of the collection is sown and evaluated for some important characters, enabling us to refresh the seeds of the entire collection in three years. According to the evaluation results the genetic (core) collection with more than 850 entries is extracted from the world collection. From this collection the material for different projects and experiments was chosen and according to their objectives it was extensively evaluated for different agronomical, morphological, physiological and other traits in field and controlled conditions. It was also used for molecular diversity studies, association panels, and genome-wide association analyses. One of our international projects is dedicated to redesigning the exploitation of small grains genetic resources towards increased sustainability of grain-value chain (FAO project PR-166 - GRAINEFIT). The project addresses some of FAO sustainability goals, such as to end poverty, achieve food security and improved nutrition, promote sustainable agriculture, combat climate change and its impacts, halt biodiversity loss, promote inclusive societies and revitalize the global partnership for sustainable development.

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GENETIC RESOURCES, WHEAT GERMPLASM, MAINTENANCE, EVALUATION

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05 – 29 Poster

PHYLOGEOGRAPHY, PHENOLOGY AND MORPHOLOGY OF BALKAN SNOWDROPS (*GALANTHUS SPP.*)

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The genus *Galanthus* L. (Amaryllidaceae) comprises 22 species, which are perennial bulbous petaloid monocots, commonly known as snowdrops. It is distributed throughout Europe, Asia Minor and the Near East. *Galathus spp*. are cherished garden plants and the world's most traded wild-sourced ornamental bulb genus, threatened by illegal collecting and habitat destruction and thus listed in Appendix II of the CITES. However, species delimitation is problematic and the infrageneric classification uncertain, mainly due to the overall morphological similarity within the genus, and lack of easily discernible distinguishing characters. In order to shed light on taxonomy and evolutionary history of *Galathus* species from the Central Balkans, we analyzed variability of three plastid regions (*rps16-trnK, trnL-trnF* and *trnE-trnT*) in 119 individuals from 40 populations representing six species (*G. nivalis*, *G. elwesii* and *G. reginae-olgae* occurring naturally in this region and three allochthonous species, phenology in 21 populations representing two species (*G. nivalis* and *G. elwesii*), and variability of 13 quantitative characters in 510 individuals from 17 populations belonging to one species (*G. nivalis*). Although evolutionary relationships among species were not fully resolved, they were concordant with those based on morphological data (sensu Davis), with the exception of *G. gracilis* which was not grouped with *G. elwesii* and *G. woronowii*. Interspecific hybridization between *G. nivalis* and *G. reginae-olge* was detected. We observed a phylogeographic structure in the Balkans, with eight lineages distributed throughout this region. The most recent common ancestor of *G. nivalis* lineages was dated to the 1.683 Mya (1.089 Mya - 2.376 Mya), and diversification of more or less all *G. nivalis* lineages took place during the past 0.500 Mya. Phenological differences were observed among six *G. nivalis* lineages, of which one was characterized also by specific morphological traits.

CITES, *GALANTHUS SP.*, MOLECULAR DATING, MORPHOLOGY, PHYLOGEOGRAPHY, PLASTID GENOME, PHENOLOGY

05 – 30 Poster

POSSIBLE EVOLUTIONARY PATHWAY OF B CHROMOSOMES IN YELLOW-NECKED MOUSE, *APODEMUS FLAVICOLLIS*

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B chromosomes (Bs) are supernumerary chromosomes in standard karyotype, unnecessary for normal growth and development. Bs are present in 3% of all analyzed eukaryote species. The most specific characteristic of those elements is great heterogeneity considering: origin, morphology, size, number and molecular content. This heterogeneity goes from individual to population level. Our recent studies, revealed the molecular structure and potential origin of these supernumeraries from sex chromosomes in yellow-necked mouse, *Apodemus flavicollis*. Here, we analyse structure of Bs in specimens from geographically distant populations. Our findings indicate that regions of almost all chromosomes from standard karyotype can be found on Bs. Detected differences among Bs from distant populations are unexpectedly small. In this content we consider possible evolutionary scenario in order to explain this lack of Bs' heterogeneity in *A. flavicollis*.

B CHROMOSOMES, *APODEMUS FLAVICOLLIS*, EVOLUTION

05 – 31 Poster

MORPHOLOGICAL AND GENETIC ANALYSIS OF FRESHWATER SPONGES IN SERBIA

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Sponges in Serbian rivers and lakes have not been extensively studied. Hence, the aim of this work was to undertake an investigation of the distribution and phylogenetics of sponge species in Serbian waterbodies. A total of 83 localities on 17 rivers and 10 lakes have been investigated. Sponges (62 specimens) were found at 22 localities only. Sponge determination was done using a combination of morphological and genetic studies. Light microscopy and scanning electron microscopy were applied for spicule analysis while the D3 domain of 28S DNA was amplified and sequenced for genetic determination. The following five sponge species were identified*: E. fluviatilis*, *S. lacustris*, *E. muelleri*, *T. horrida* and *E. fragilis*. The sequence of the Serbian *E. fragilis* differed in two base pairs compared to Estonian *E. fragilis* (sequence obtained from the database of the National Center for Biotechnology Information, U.S. National Library of Medicine – NCBI), the previously deposited sequence on NCBI from Estonian *E. fragilis*. The sequence of *T. horrida* from our study is the first partial 28S sequence deposited for this species in the NCBI. A phylogenetic tree based on the 340bp sequences was also generated. It showed distinctive clades and was concordant with the results of the morphological analysis.

FRESHWATER SPONGES, 28S rDNA, PHYLOGENETICS

05 – 32 Poster

GENETIC DIVERSITY OF BAT-FLIES AND MITES IN TWO CAVE-DWELLING BAT SPECIES IN SERBIA

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Bats (Chiroptera) can differ widely in their social organisation, from living in small groups to large colonies counting several thousands of individuals. Such ecological differences can lead to drastic discrepancies in the population dynamics of parasites they carry. European bat species are infected by a wide range of specialized ectoparasites. Bat flies (Diptera: *Hippoboscoidea* – fam. *Nycteribiidae*) and wing-mites (Acari: *Mesostigmata* - fam. *Spinturnicidae*) differ strongly in their phenology and life-histories. The objective of this study was to characterize the species assemblage, genetic diversity and host specificity of bat flies and mites collected from two cavernicolous bat species commonly found in Serbia, which have different migration potentials, and can be found both separate and in shared roosts. A total of 219 flies and 205 mite specimens were collected from the common bentwing bat (*Miniopterus schreibersii*) and greater horseshoe bat (*Rhinolophus ferrumequinum*) at eight localities in Serbia and one in Bosnia and Herzegovina. Flies were morphologically identified and all samples were sequenced for a single mitochondrial gene (16S in mites and COI in flies), allowing for a comparison of within and between-colony genetic diversity among the dominant ectoparasite species in the sample (*Nycteribia schmidlii*, *Phthiridium biarticulatum*, *Spinturnix psi* and *Eyndhovenia euryalis*). Notably, all ectoparasite species showed marked specificity towards their primary hosts, even in roosts that were shared by both bat host-species. Additionally, this data constitutes some of the first reference sequences for commonly used barcoding sequence fragments for several of the investigated species.

NYCTERIBIIDAE, SPINTURNICIDAE, mtDNA, CHIROPTERA

05 – 33 Poster

GENETIC LAYOUT AND GENETIC STRUCTURE OF THE GREENHOUSE WHITEFLY *TRIALEURODES VAPORARIORUM* FROM SERBIA

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Trialeurodes vaporariorum Westwood (Hemiptera: Aleyrodidae), a haplo-diploid sap feeding insect species, is a vector of many plant viruses and a serious polyphagous pest of greenhouse-grown vegetables and ornamental plants worldwide. To explore the populations of this pest and recognize potential invasion routes, the genetic diversity and genetic structure of *T. vaporariorum* from Serbia were analysed using six microsatellite loci. PCR multiplex conditions were optimized and tested using 88 *T. vaporariorum* females collected from natural populations in 10 sampling locations in Serbia: Apatin (AP), Beograd (BG), Kovin (KO), Negotin (NE), Pirot (PI), Smederevska Palanka (SP), Subotica (SU), Svilajnac (SV), Trbušan (TR) and Vranjska Banja (VB). Observed heterozygosity ranged from 0.105 to 0.796, deviations of the frequency of genotypes from the expected Hardy–Weinberg equilibrium were detected in all populations. The values of the Fst parameters ranged from 0.011 between populations of AP and SU, up to 0.286 among the SV and BG populations. The obtained results for Fst values were used to explore genetic structure using several analyzes: PAST, Principal Coordinate Analysis – PCoA and SAMOVA. The analyzed *T. vaporariorum* populations were grouped in three distinct genetic clusters based on these population-genetic analyses: (1) BG, (2) AP, KO, SP, TR, VB and SU, and (3) SV, NE and PI. Also, BOTTLENECK identified reductions in effective size of SP population in the recent past and the significant heterozygosity deficiency was detected in the four populations VB, SU, BG and NE. These results demonstrate that populations of *T. vaporariorum* in Serbia show significant genetic differentiation, indicating the likelihood of multiple introductions of this important pest into Serbia, as well as the influence of crop protection and pest management on their genetic variability.

GREENHOUSE WHITEFLY; GENETIC DIFFERENTIATION; GENETIC STRUCTURE

05 – 34 Poster

VARIABILITY AND RELATIONSHIP BETWEEN PLANT AND EAR HEIGHT IN MAIZE TOP CROSS HYBRIDS

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In numerous plant species, including maize, phenotypic traits, such as plant height and plant height to upper most ear, have undergone major changes through breeding, resulting in the development of varieties and hybrids with good agronomic performances. The aim of this study was to cross 31 landraces to genetically divergent testers (L217, L73B013 and L255/75-5) in order to determine variability and general combining ability for quantitative traits: plant height and plant height to upper most ear. Developed top cross hybrids were tested in the two-year trial, at four locations, in two replications. The grand mean of top cross hybrids amounted to 255.8 and 101.8 cm for the plant and ear height, respectively. The analysis of variance showed that all sources of variation (environment, effect of female component - landraces, effect of male component - tester, as well as their interactions) were highly significant ($p \leq 0.01$) for both traits, except for the environment × female component × male component interaction. The coefficient of variation amounted to 4.76% and 8.87% for plant and ear height, respectively. The obtained results point out to small variability within each top cross hybrid. The correlation between observed traits was highly significant ($r=0.785$; $p<0.01$). Linear regression for observed traits indicates that plant height increase for 1cm is followed by plant height to upper most ear increase for 0.5 cm. Results of general combining ability (GCA) of the landraces for the traits evaluated indicated that GCA is under additive genetic effects, i.e. high populations *per se* crossed to all three testers resulted in high hybrids and *vice versa*.

GENERAL COMBINING ABILITY, LANDRACE, INBRED LINE, PHENOTYPIC CORRELATIONS, *ZEA MAYS* L.

05 – 35 Poster

VARIABILITY OF SEED AND SEEDLINGS OF COMMON OAK (*QUERCUS ROBUR* L.) FROM BOSNIA AND HERZEGOVINA AND CROATIA

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Common oak (*Q. robur*) is one of the most valuable forest tree species in the SE Europe. The most valuable forests are located in flatland areas around the Sava River. In Bosnia and Herzegovina, common oak forests are mostly devastated and located in small areas. For the purpose of species reintroduction we compared phenotypic variability of material originating from existing populations in Bosnia and Herzegovina (BH) and material introduced from Croatia (CRO), where common oak is preserved.
In 2015, seeds from 4 Croatian populations (Sisak, Našice, Nova Gradiška and Koprivnica) and 4 Bosnian-Herzegovinian populations (Prijedor, Karanovac, Podgradci and Srbac) were collected for the purpose of variability investigation and reintroduction. The variability of seed was investigated as well as the variability of one-year and two-year seedlings.
The results of morphometric seed analyses indicate statistically significant differences between all measured parameters. Summing up all dimensions in one - volume, it was found that the average volume of seeds from Srbac (BH) population attained the highest value.
Survival of seedlings in the first year after sowing ranged from 26 to 72%. In the first year of observation, the highest seedlings were recorded for Podgradci (BH) and Nova Gradiška (CRO) populations. In the second year, the dominant population was Prijedor (BH) population. The height and the root collar in the first and second year were different among populations, whereby the population order changes, which indicates that the conclusions cannot be made after just one-year seedling measurement. Activities need to be carried out continuously in successive years.

COMMON OAK, SEED, SEEDLINGS, VARIABILITY

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05 – 36 Poster

GENETIC DIVERSITY OF NATURAL BOSNIAN AND HERZEGOVINIAN POPULATIONS OF SWEET CHESTNUT (*CASTANEA SATIVA* MILL.) AS REVEALED BY SSR MARKERS

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Sweet chestnut (*Castanea sativa* Mill.) is a widely distributed European forest tree species of great economic importance due to its wide range of use. In Bosnia and Herzegovina sweet chestnut grows in very diverse ecological conditions, in various forest communities.
Total 287 samples from six populations (Bužim, Kostajnica, Banja Luka, Prijedor, Bratunac and Konjic) were tested with six nuclear microsatellite markers (ICMA004, ICMA007, ICMA018, ICMA020, ICMA023 and QPZAG7). DNA was extracted from buds using the CTAB protocol of Doyle and Doyle (1990).
A total of 49 different alleles were identified and the number of detected alleles varied between 5 and 13, with mean of 8,17 alleles per locus. The mean number of effective alleles (Ne) varied between 1,974 (Konjic) and 3,502 (Kostajnica). Private alleles were found in four populations. Null alleles were not detected in any of the investigated populations. The observed heterozygosity ranged from 0,503 (Konjic) to 0,677 (Prijedor). Values for expected heterozygosity varied between 0,464 (Konjic) and 0,689 (Kostajnica).
The UPGMA method was used for the classification of populations into groups. Two clusters were established. The cluster I comprises populations from Bužim, Prijedor, Kostajnica and Bratunac. The cluster II comprises two populations from Banja Luka and Konjic.
The Principal Coordinate Analysis (PCoA) confirmed the previous results obtained by UPGMA. The outcome of the research point to the fact that the Bosnian and Herzegovinian chestnut is a rich source of genetic diversity and is very suitable for further breeding purposes.

CASTANEA SATIVA MILL., SWEET CHESTNUT, GENETIC DIVERSITY, SSR (SIMPLE SEQUENCE REPEAT) MARKERS

SESSION 6

Breeding for changing environments

VI CONGRESS OF THE SERBIAN GENETIC SOCIETY

06 – 01 Invited lectures

USE OF BARLEY AND WHEAT REFERENCE SEQUENCES: DOWNSTREAM APPLICATIONS IN BREEDING, GENE ISOLATION, GWAS AND EVOLUTION

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The decoded blue prints of the barley and wheat genome open new avenues in exploring genome sequences in two important cereal crops for both applied and basic research. Barley and wheat geneticist and breeders are nowadays in a position in which users of the first sequenced model plants, rice, and Arabidopsis, had been more than fourteen years ago. Knowledge of whole barley and wheat genomes not only promotes applied breeding but also provides an extraordinary opportunity for basic biological research i.e. accelerated isolation of novel genes and rapid detection of natural variation. The availability of genome sequences will revolutionize barley and wheat genetics, accelerate identification and use of rare allelic variants in classical breeding schemes, such as marker-assisted backcrossing (MABC), marker-assisted selection (MAS) and pyramiding of genetic factors responsible for important traits. Positional isolation of genes will be among those activities having the full profit when a genome reference sequence is available. Accordingly, gene isolation will be cheaper and faster as the time from gene mapping to the identification and functional validation of candidate gene will be reduced. After sequencing of more accessions, as a result of PanGenome projects, it will be possible to do direct targeting of important gene variants and introduce them into cultivars in order to exploit the rich germplasms for breeding purposes.

In this research topic, we present an efficient use of genetics and breeding methods in harnessing genetic resources of barley and wheat that promote the rapid improvement of cultivars. This themed article collection covers all aspects of the use of two reference sequences i.e. the discovery and isolation of new genes, allele-mining, development of large-scale SNP genotyping platforms, integration of metabolomic, proteomic and phenomic data and platforms in trait discovery, GWAS, genomics breeding.

BARLEY, WHEAT, GENOME REFERENCE SEQUENCE, BREEDING, GENE ISOLATION

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06 – 02 Invited lectures

UTILIZATION AND TRANSFER OF FOREST GENETIC RESOURCES BY INTRODUCTION OF ALIEN SPECIES

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Emerging needs for wood, non-wood forest products, different ecosystem services and research programs in the last two centuries have affected the transfer of forest genetic resources within and outside of their natural distribution area. The research aim in this paper is to study the environmental conditions at the allochthonous species test sites, aiming at the more reliable selection of the forest-economic works on the establishment, silviculture, tending and utilization of the alien species. The transfer of species to the new habitats outside their range of distribution involves the well-known risks - reduced growth and (or) dieback as a result of the low adaptive potential of the introduced species to the new environmental conditions. The methods of close and distant - intraspecific and interspecific hybridization have been applied together with the establishment and analysis of the provenance tests throughout the 20th century in Serbia in order to provide a reliable assessment of the adaptive, productive and reproductive potential of the introduced species. The extensive establishment of the plantations for the production of timber for mechanical and chemical wood processing has been one of the main reasons for the introduction of alien species, especially conifers **of** *Pinus*, *Picea*, *Pseudotsuga* genera and the species of *Populus* and *Salix* genera. This paper deals with the attitude of the human population towards the introduction of alien species, their effects on native habitats and indirect influence on the progress of woody plant improvement.

ALIEN SPECIES, FOREST GENETIC RESOURCES

06 – 03 Invited lectures

BREEDING OF MAIZE HYBRIDS WITH SPECIFIC TRAITS FOR ENHANCED AGRONOMIC AND NUTRITIONAL PROPERTIES

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Maize stands out by its high genetic variability, thus it provides the possibility to develop different types for various purposes in the breeding process. Maize breeding provides significant modifications in the grain composition in the respect of quantity and quality of certain components. It is mainly grown as an energetic crop, but there is a broad use of types with specific traits, such are sweet corn and popcorn that are primarily used for human consumption. Although breeding methods are the same as in breeding standard quality grain, special attention is necessary in the process of breeding, seed production, but also in the commercial production and processing. Breeding strategies are based on the application of conventional breeding methods and also on the application of molecular markers, especially due to the fact that the genetic base of these two types of maize is very narrow. Beside the increase in yielding potential and other agronomic traits, great challenge is the improvement of quality parameters and nutritional composition of sweet corn and popcorn. Quality and sensory characteristics are of great importance for the commercial value, therefore several methods are applied in order to estimate them. On the other hand these traits are often in negative correlation with the yield, which presents a great challenge in breeding of specialty maize. Popcorn and sweet corn are very popular in diet because of their high nutritional values. Popcorn contains very powerful antioxidants such as tocopherols and carotenoids, and the compounds with the highest biological activity like tocopherols, are mainly retained in popcorn after heat treatment. Sweet corn also has very rich nutrient composition containing main nutrients, such as: starch, sugar, protein, oil and cellulose, and phenolic acids: galic, protocatechuic, vanilic, sinapic, p-coumaric, ferulic and cinnamic.

SPECIFIC TRAITS, SWEET CORN, POPCORN, NUTRITIONAL PROPERTIES

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06 – 04 Invited lectures

GENOMIC PREDICTION – NEW TOOL IN SOYBEAN BREEDING

Vuk Đorđević, Marina Ćeran, Jegor Miladinović, Svetlana Balešević-Tubić, Kristina Petrović, Predrag Ranđeliović, Jelena Marinković

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Yield and most of the agronomical important traits are quantitatively inherited, influenced by many loci with a small effect and affected by environmental conditions. When dealing with the improvement of quantitative traits, it is not particularly useful to perform the selection by using few major-effect loci as in traditional marker-assisted selection, but to simultaneously use genome-wide molecular markers able to capture all small effect loci influencing a trait.

The training population consisted of 227 diverse soybean linesthat were used for genomic prediction model development. Training population was evaluated for yield at three consecutive years. DNA of each genotype was sequencing on Illumina HiSeq 2500, using GBS Discovery Pipeline for SNP calling. Prediction ability was evaluated using six mathematical models, including parametric and non-parametric and were validated on three different levels: self-prediction, cross-validation (5-fold) and external validation (historical data).

Overall, genomic prediction ability for soybean yield was relatively high (0.60) and the results indicate a modest influence of mathematical model and marker number on the prediction ability using cross-validation and external validation. However, model had variable ability to predict phenotypic performance in separate environments, with especially high prediction ability in years not impacted by yield-limiting factors, when the genetic potential was fully achieved. Improvement of model performance in cross-validation and external validation was achieved by increasing the phenotyping intensity that must reflect the target environment variability.

Obtained results indicate that genomic prediction can be integrating part of breeding process as useful tool that can increase breeding efficiency and decrees breeding time. Particular implementations are diverse, from germplasm screening and parental choice to the forward breeding and direct section based on genomic prediction.

SOYBEAN, GENOMIC PREDICTION, YIELD, MODEL

06 – 05 Oral

MOLECULAR MARKERS FOR DETECTION OF Rf1 GENE DEVELOPED FROM BAC-END SEQUENCES IN SUNFLOWER

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Sunflower is the second most common crop among hybrids, worldwide. Development of cytoplasmic male sterile and fertility restoration lines enables creation of hybrids. Sunflower hybrid breeding most frequently relies on the combination of the CMS PET1 cytoplasm and the fertility restoration gene Rf1. Use of molecular markers can accelerate creation of restorer lines. Previous work on this subject included mapping of the Rf1 gene on chromosome 13 and development of bacterial artificial chromosome (BAC) libraries for the restorer line RHA325 and the maintainer line HA383, which enabled positioning of BAC clones surrounding the Rf1 gene in the cross RHA325 x HA342. In this study, BAC-end sequences were used to derive primers in order to amplify selected regions from RHA325 and the maintainer line, HA342. While the majority of primer combinations were monomorphic, some were polymorphic between RHA325 and HA342. Previously reported markers for detection of Rf1 gene and newly developed ones based on BAC-end sequences were further tested on hybrids and its components (A, B and R lines) created at the Institute of Field and Vegetable Crops, Novi Sad.

So far, the majority of markers designed for identification of Rf1 gene are dominant, with just a few being co-dominant. In the present research we increased the number of available markers that can be used for detection of the Rf1 gene in marker assisted selection (MAS). However, none of the newly designed markers could be universally used for all tested restorer lines, thus verification of the reported markers is required before application in MAS in a specific cross combination.

This study was supported by German-Serbian bilateral cooperation project - (No. 451-03-01732/2017-09/3 and project code 57393592), Deutsche Forschungsgemeinschaft (DFG) projects HO 1593/5-1 and HO 1593/5-2, Ministry of Education, Science and Technological Development of Republic of Serbia, project TR31025 and COST Action CA18111.

HELIANTHUS ANNUUS L., MARKER ASSISTED SELECTION, FERTILITY RESTORATION, HYBRID, BREEDING

06 – 06 Oral

EFFECT OF GENOTYPE AND ENVIRONMENT ON VARIABILITY OF PRODUCTIVE TILLERING IN BREAD WHEAT (*TRITICUM AESTIVUM* L.)

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Productive tillering of wheat plants has impact on determination of crop density, number of spikes and number of kernels per unit area (m-2), which is related with grain yield. The aim of the work was to investigate variability of productive tillering of genetically divergent wheat cultivars grown under different environmental conditions. Twenty genetically divergent winter wheat cultivars were investigated during a two-year period in a randomized block design experiment, in three replications. Seeds of the cultivars were sown in rows 1.0 m in length. The distance between seeds was 0.10 m in rows and the distance between rows was 0.2m. The 60 plants in full maturity stage (20 plants per replication) were used for analysis of the number of productive tillers plant-1. The analysis of variance of obtained data were processed using MSTAT C (5.0 version). The significant differences between values were estimated by F-test and LSD (0.01; 0.05). The results showed differences among genotypes for productive tillering and significant differences between years. In the first year of experiment, the number of productive tillers varied between 6.13 in Proteinka and 9.01 in Kompas, and in the second between 8.26 in Tanjugovka and 10.68 tillers in Kompas cultivar. The average productive tillering was 7.39 in the first year, while in the second it was 9.40. The differences between cultivars were affected by genetic and environmental factors with greater influence by the latter.

WHEAT, GENOTYPE, VARIABILITY, TILLERS, ENVIRONMENT

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06 – 07 Oral

DIALLEL ANALYSIS FOR MOST IMPORTANT TRAITSOF RED FESCUE (*FESTUCA RUBRA* L.)

Dejan Sokolović, Snežana Babić, Jasmina Radović, Zoran Lugić,
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Phenotypic recurrent selection is the most used and successful method in perennial grasses breeding. Direct or polycross hybridization of chosen genotypes is quite often step within this method of selection. In general, most important goal in grass breeding is to find genotypes which will be superior in accumulation as much additive genetic components as it is possible within their inercrossings. Therefore, characteristics of genotypes after hybridization are extremely important. It could be evaluated by progeny testing of obtained hybrids, but plot trials of lot of genotypes could be laborious and expensive job. To reduce number of possible crossings and to make progeny testing cost efficient it is important to know in advance what are combining abilities of chosen genotypes. The information about effects of genes and traits genetic control, as well as general and specific combining ability heritability and heterotic and maternal effects can be provided by diallel analysis

Complete diallel analysis on 8 promising red fescue genotypes from 2013 to 2016 was performed. To obtain the F1 generation each cross was made under bag isolation of tillers from plants which are planted in pentagonal space plant design.

A spaced plant trial of obtained diallel hybrids (full sibs) was established in 2014. including 28 crosses in F1 (56 diallel hybrids with reciprocals). During two years of full plant development and utilization we analyzed 8 traits (time of heading, plant height, number, width and length of leaves, number of tillers, green mass and dry matter yield per plant). Data were computed with Griffing's method 3 without inbred parents. The results of the diallel analysis showed that most of analysed traits are determined by interaction of both additive genes (GCA effects) and non additive genes (SCA effects). There were some hybrid combinations with high-parent heterosis. Hybrid combinations with best combining ability were selected for future breeding of red fescue.

DIALLEL ANALYSIS, RED FESCUE, HYBRIDS, COMBINING ABILITY, HETEROSIS

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06 – 08 Poster

GENETIC VARIABILITY AND ANTIOXIDANT RESPONSES OF WHEAT UNDER SALINITY STRESS I*N SITU*

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Unfavorable environment, for most cultivated plants is the soil, which is characterized by high concentrations of salt. Stress caused by high salt concentrations leads to disorders in the transport of electrons in certain cell organelles, creating reactive oxygen species that cause lipid peroxidation, oxidative protein degradation, and DNA mutations. Trail was set in Banat, locality Kumane, where the complex stress environment is caused by alkaline soil of solonetz type, soil of poorer productivity, represents a production environment that can be considered stressful for plants. The research included 12 genotypes, of which 10 varieties and one local population of hexaploid wheat and one variety of triticale. The enzymatic activity of the antioxidant system (SOD, GPx, PPx), PAL, non-enzymatic components of the antioxidative system (GSH, phenols, tannins, DPPH-test) as well as lipid peroxidation have been determined. From the standpoint of both groups of investigated components, genotypes of Banatka and Bankut 1205 were the most stress tolerant. The genotype Rapsodija has stood out according to a good reaction when the enzymatic component is observed and the genotype Renesansa when considering non-enzymatic activity. The most tolerant of stress. The genotype Rapsodija has separated with a good reaction when the enzymatic component is observed and the genotype Renesansa when considering non-enzymatic activity. The most intense lipid peroxidation was observed in the Bankut 1205 genotype and Odisej, and the slightest damage to cell membranes was the Nevesinjka genotype. Understanding the way in which oxygen radicals are formed in the plant tissue and disappearing from it contributes to the creation of new varieties of wheat, which will be more tolerant to stress. The selection of better-adapted genotypes to growing conditions at the solonetz may allow economically justified production of wheat and more intensive use of such soil in agricultural production.

WHEAT, SOLONETZ, STRESS, ANTIOXIDATIVE SYSTEM, VARIABILITY

06 – 09 Poster

COMPONENTS OF VARIANCE AND GENETIC PARAMETERS OF FERTILITY TRAITS IN BLACK AND WHITE CATLLE BREED

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Cattle fertility is quite complex and one of the most important traits with a strong influence on production's profitability. The intent of this paper is determining the variance components, genetic correlations and heritability values for two observed fertility traits, service period (SP1 and SP2) and calving interval (CI). Genetic parameters of the fertility traits were examined on a sample which counted 498 black and white cows, raised on 6 farms of Agricultural Corporation Belgrade. All cattle had concluded first two lactations, and the research included 996 lactations. They examined the farm effect, calving years, seasons and the order of lactations relative to fertility of analyzed cows. The variance components used for the estimation of heritability of each trait were obtained by the SAS, PROC VARCOMP (2012) by means of the Restricted Maximum Likelihood Method.

Estimated heritability values are 0.109 during the first service period; 0.052 during the second service period and 0.109 during calving interval. Analysis determined that fertility traits are mostly affected by the years/seasons which showed the highest statistical value ($P<0.01$).

BLACK AND WHITE BREED, FERTILITY TRAITS, HERITABILITY

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06 – 10 Poster

INVOLVEMENT OF CHLOROPHYLL AND FLAVONOIDS CONTENT IN RESPONSE TO CONTRASTING WATER SUPPLY CONDITIONS

Natalija Kravić, Vesna Dragičević, Danijela Ristić, Jelena Mesarović, Jelena Srdić, Vojka Babić, Violeta Anđelković

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In temperate regions worldwide, the ongoing climatic changes cause frequent and severe summer droughts which seriously impaire maize grain yield. In breeding of superior genotypes that are able to endure such conditions, evaluation of leaf chlorophyll content, as an indicator of photosynthetic capability of plant tissues, growth and productivity, could assist. Likewise, evaluation of status regarding flavonoids (including anthocyanins and flavonols), as a class of specialized secondary metabolites with strong radical scavenging activity, contributes to mitigation of drought stress. Accordingly, the aim of this study was to determine whether the tolerance/susceptibility to drought stress can be attributed to the level of phenolic compounds and chlorophyll in maize leaves. For this purpose, five maize inbreds differing in drought tolerance were evaluated in two sets of field experiment: under well-watered and rain-fed conditions. At flowering, chlorophyll, flavonoid and anthocyanin indexes, as well as the nitrogen balance index (NBI) that represents the ratio between the mesophyll chlorophyll and epidermal flavone leaf contents, were analyzed. In addition, anthesis-silking interval (ASI) as secondary trait relevant to drought tolerance, and grain yield were determined. In drought tolerant lines, effect of water deficit was reflected through NBI and chlorophyll increase, followed by flavonoid content decrease. However, the opposite trend was noticed in drought susceptible maize inbreds. Under rain-fed conditions, anthocyanins content increased or remained unchanged in the majority of inbreds evaluated regardless to their tolerance to drought. Compared to well-watered set of field experiment, quite opposite trend in correlations between grain yield and indexes measured was found under water deficit conditions. The results obtained indicated the activation of different metabolic pathways in defence against existing water stress.

ASI, PHENOLIC COMPOUNDS, GRAIN YIELD, WATER STRESS, *ZEA MAYS* L.

06 – 11 Poster

THE PHOTOSYNTHETIC EFFICIENCY IN SUNFLOWER PLANTS AT STRESSFUL GROWTH CONDITIONS

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Lately, we are witnessing climate crisis that are disrupting the cultivation and production of agricultural crops. Consequently, the conducted study's objective is to clarify the influence of climate changes on the photosynthetic efficiency of sunflower plants (*Helianthus annuus* L). The investigation focuses on the influence of heat and light stress during the flowering of two selected sunflowers genotypes. The analysis of variance shows statistically significant differences between genotypes for all tested parameters of photosynthesis, as well as between treatments except for parameter variable fluorescence at step J. Based on the results, genotype 1 was less sensitive to the changes in the photosynthetic apparatus caused by stressors than genotype 2. The heat and light stress caused the difference between treatments in genotype 2 for the parameter initial intensity of the fluorescence to be larger. The inhibition of the oxygen-evolving centre was stronger in genotype 2 as well, and this resulted in lower values of the maximum fluorescence intensity. The genotype 2 had a larger increase in the re-oxidation of the plastoquinone than genotype 1, i.e. the values of the variable fluorescence at step J were higher for genotype 2. The plants adapted to stressful conditions by lowering the values of the maximum quantum yield of photosystem II, but its values in the genotype 2 decreased below 0.75 relative units indicating damage to the photosynthetic apparatus' functioning. At the same time, performance index on absorption basis, which gives insight into the plant's vitality, as well as the parameters from which the index was calculated, confirmed a larger impact of stress on genotype 2.

HELIANTHUS ANNUUS, HEAT STRESS, LIGHT STRESS, PIABS, FV/FM

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ADAPTIVE VALUE OF CONVENTIONAL RAPESEED CULTIVARS IN ORGANIC AND CONVENTIONAL CULTIVATION ENVIRONMENTS

Sreten Terzić, Ana Marjanović-Jeromela, Miroslav Zorić, Vladimir Sikora, Željko Milovac, Petar Mitrović, Igor Balalić, Velimir Radić

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Rapeseed is one of the major oil crops, grown in various environments. Interest in organic rapeseed is rising, with increasing importance to breeders to determine the need for specific organic breeding programs. The objective of this study was to determine the adaptive value of conventionally developed rapeseed cultivars in organic cultivation environments.

Five winter rapeseed cultivars were grown in conventional and organic plots, each with three sowing dates and four replications. The trials were organized in a randomized block design and the effect of cultivar and farming system on emergence, survival rate, yield, oil and protein content was evaluated. Locally recommended agricultural practices were used to keep the fields free from weeds, insects and diseases. In organic field, weeds were removed mechanically while insects were treated with an insecticide used for organic production. The seed samples for analysis of oil and protein content were taken during harvest.

Considering agricultural practices, it was found that rapeseed can be successfully grown in organic agriculture, but further improvements are needed to increase stability of production. The cultivars had higher oil content in the conventional environment, while there was no significant effect on protein content. Cultivar Slavica reacted to organic cultivation with largest increase of yield, while cultivars Banaćanka and Nena had high yield in both environments. The results suggest that some of the conventional cultivars can be successfully used in organic cultivation and that the existing breeding material can be used as a good starting point for further specific trait improvements.

RAPESEED, ORGANIC, CONVENTIONAL, YIELD, OIL, PROTEIN

06 – 13 Poster

GRAIN YIELD VARIABILITY IN MAIZE HYBRIDS BELONGING TO FAO MATURITY GROUPS 300 AND 400

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Maize is the most widely grown grain crop in the Republic of Serbia, covering over million hectares, with an average yield of 5.9 t ha^{-1}. As there is no universal cultural practice for maize growing areas, production methods should be adapted to the specific climate conditions, soil and other environmental factors for maximum possible utilization of environmental and genotype potentials. To determine grain yield variability in maize hybrids in two locations in Southwestern Serbia (Ivanjica), trials were conducted during 2013–2014. Although these locations are outside the main growing regions, maize is traditionally cultivated in them on relatively small plots. Given the climatic conditions (mountain climate), maize hybrids (ZPSC341, ZPSC360, ZPSC42a, ZPSC434, NS 444 ultra, NS4015 and NS4030) having short growing seasons (110–130 days) were selected for the experiment. Recommended seeding rates (plants ha^{-1}) were used for all hybrids. Climate conditions for maize cultivation were more favorable in 2013, i.e. moderate temperatures during the growing season, evenly distributed precipitation, a great number of rainy days, whereas 2014 was unfavorable due to huge amounts of precipitation, particularly during, germination, emergence but also during subsequent growth stages. Hybrids of the FAO maturity group 400 had significantly higher yields compared to FAO 300 hybrids. The average yield of FAO 400 and FAO 300 hybrids was 5.3 t ha^{-1} and 4.3 t ha^{-1}, respectively, with the highest yield in NS4030 (5.8 t ha^{-1}). The total average yield was 4.8 t ha^{-1}. The average grain yield was lower by 1.1 t ha^{-1} than the average for yield Serbia (5.9 t ha^{-1}) and by 0.2 t ha^{-1} than for Central Serbia (5.0 t ha^{-1}). Despite quite good average yields of some hybrids, a realistic estimate of a maize hybrid in a given area can be made only based on long-term field trials.

MAIZE, HYBRIDS, GRAIN YIELD

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CHANGES OCCURRING IN FATTY ACID CONTENT AND TOCOPHEROL COMPOSITION IN OILSEED RAPE SEED DURING ACCELERATED AGEING

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The high content of polyunsaturated and low content of saturated fatty acids gives the oilseed rape seed oil of high nutritional value and a special place in human nutrition. Considering their chemical composition, oily seeds have limited longevity. In the course of aging (both natural and accelerated) there occur changes in the molecule (peroxidation of fatty acids, protein oxidation, activation of nuclease, and DNA damage) that affect both cell membrane and organelles. Seeds of five varieties of oilseed rape were exposed to accelerated ageing (100% relative humidity, 41°C-72h) and seed quality tests of the seed material were performed (mean germination percentage, shoot and root length, shoot and root fresh weight) before and after accelerated ageing treatment. Additionally, lipid composition was determined before and after accelerated ageing. Fatty acid (FA) composition was determined by gas chromatography (Konik HRGC 4000) coupled with a flame ionizing detector, after derivatization to their volatile methylesters (FAME). Quantification of tocopherols was carried out using high-performance liquid chromatography on a column Nucleosil 100-5 NH2 with fluorescence detection (λex=280 nm, λem=340 nm) in oil. The aim of this study is to determine the mechanisms of oilseed rape seed deterioration, by analyzing changes in the lipid composition during the conditions of accelerated aging of the seed. Obtained results indicate that the contents of oleic (C18:1), arachidic (20:0) and erucic (22:1) acids insignificantly increased in seeds subjected to accelerated ageing. These results indicate that enzymes engaged in biosynthesis of lipids were stimulated by high temperatures. After accelerated ageing treatment, total tocopherol content (α-, and γ-tocopherol) was significantly lower (from 8 mg/kg to 150 mg/kg) in seeds exposed to accelerated aging compared to control seeds.

OILSEED RAPE, SEED AGEING, FATTY ACID COMPOSITION, TOCOPHEROL

06 – 15 Poster

ASSESSMENT OF STABILITY OF SEED YIELD AND SEED OIL CONTENT IN CONFECTIONERY HYBRIDS USING AMMI ANALYSIS

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High seed yield and low oil content are the most important criteria for the introduction of high protein confectionary hybrids into production. An important part of breeding programs is testing new hybrids and detection of hybrids distinguished by stability of tested traits under different agro-ecological conditions. To evaluate stability of NS confectionery sunflower hybrids under different environmental conditions, a trial was conducted using 10 confectionery sunflower hybrids developed at the Institute of Field and Vegetable Crops, Novi Sad. The trial was set up as a randomized block design with three repetitions, at the location of Rimski Šančevi in 2011, 2012, 2014, 2016, 2017, and 2018. The AMMI analysis was applied as one of the most important and widely used multivariational analyses for determining interaction between genotypes (hybrids) and environment (year). Comparing the environments, the highest average seed yield and seed oil content were obtained in 2011. Hybrid NS 2 had the highest average seed yield in all the examined years (4.21 tha-1), while the lowest was obtained from hybrid NS 9 (3.31 tha-1). The highest average seed oil content was obtained from hybrid NS 9 (41.85%) in all the examined years, whereas hybrid NS 7 had the lowest average seed oil content (30.50%). AMMI analysis showed that the genotypes NS 5 and NS 8 were the most stable when it comes to high seed yields, while genotype NS 2 had the most stable seed oil content in the analyzed period. These genotypes had the lowest values of interaction and are characterized by wide adaptability. Evaluation of NS confectionary hybrids using the AMMI analysis will be continued in order to assess the impact of genotypes, environment and their interaction on seed yield and seed oil content, and provide precise recommendations for growing sunflower hybrids in different regions and under different production systems.

AMMI BIPLOT, SEED YIELD, SEED OIL CONTENT, STABILITY, SUNFLOWER

06 – 16 Poster

ASSESSMENT OF HEMP SEED VIGOUR

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The seed vigour can be explained and described as the theoretical capacity of the seed which allows it to accomplish its substantial function under different environmental conditions. The key purpose of testing seed vigour as an underlying physiological seed characteristic is to establish more susceptible and precise seed quality parameters than a germination test. In seeds of oilseed species such as hemp seed (*Cannabis sativa*), the vigour can be a limiting factor due to the low chemical stability of the lipids compared to starch. The aim of this study was to evaluate the vigour of hemp seed and to make the difference between the tested hemp varieties in terms of vigour capacity. In addition to that, the aim was to identify and standardize the methods for assessing seed vigour and storability of hemp seed. The experimental materials include five varieties of hemp (Helena, Bacalmas, Hu×Uso, Monoica×Uso, Monoica) obtained from the Institute of Field and Vegetable Crops. The seed vigour evaluation was done using a laboratory germination test (LGT), cold test (CT) and different methods of accelerating aging test (AAT). Using the vigour tests, hemp seed was exposed to unfavorable low-temperature conditions (CT), and double stress conditions of high temperature and high relative humidity (41°C-72h, 43°C-72h, 43°C-48h) (AAT). The result found that hemp varieties, the combination of high temperatures and high relative humidity, and the interaction between them significantly affect hemp seed germination, while low temperatures do not show an impact on a significant reduction in germination. Different conditions of the accelerated aging test indicate that the decrease in the hemp seed vigour depends on both the applied temperature and the length of exposure to unfavorable conditions.

HEMP, SEED VIGOUR, VIGOUR TEST, ACCELERATED AGEING

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EVALUATION OF AGRONOMIC AND SENSORY CHARACTERISTICS OF SWEET CORN

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Sweet corn is considered as tasty and quality food. Its kernels contain sugars that are in good balance with amino acids, minerals and vitamin B. It is used as fresh product right after the harvest, but also for further industrial processing, freezing and canning. In breeding of sweet corn equally attention is paid to the production high and stabile yielding hybrids, and to the enhancement of technological and sensory properties of the ear and kernel. The aim of this research was to establish agronomic and sensory properties of 12 sweet corn hybrids, 3 commercial and 9 experimental. The trial was set up according to the RCBD on two locations and in three replicates. The evaluation of agronomic parameters encompassed: fresh ear yield without husk, ear length, number of kernel rows and shelling percentage. Also, sensory characteristics were analyzed: appearance, color, smell, sweetness, juiciness, crispiness, pericarp hardness. Sensory characteristics were evaluated by voluntary panelists and scaled with 1 – 9 point hedonic scale. Fresh ear yield significantly varied over locations and hybrids. The highest fresh ear yield had experimental hybrid ZP 471su – 13.327 t/ha, while the lowest was 7.144 t/ha (ZP 504su). The difference between sensory characteristics among hybrids was also noticed, although all hybrids had satisfactory sensory characteristics with the average sensory marks above 7.3. The best sweet corn hybrid according to sensory characteristics was ZP 483su (8.29), while the highest yielding one ZP 471 had an average sensory mark 7.70.

SWEET CORN, FRESH EAR YIELD, SENSORY CHARACTERISTICS

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THE TWO-LEVEL MARKER ASSISTED SELECTION IN BC2 GENERATION OF THE CONVERSION OF STANDARD MAIZE LINES TO THEIR QPM VERSION

Marija Kostadinović, Danijela Ristić, Anika Kovinčić, Marijana Simić, Vesna Perić, Dragana Ignjatović-Micić, Jelena Vančetović

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Quality Protein Maize (QPM) is nutritionally and agronomically improved maize. In order to shorten the period required for development of QPM hybrids through the conventional method of backcrossing, marker assisted selection (MAS) is being used. After successful conversion of one commercial maize inbred line to its QPM counterpart for growing in temperate climate, this breeding program was continued at Maize Research Institute (MRI) with larger number of maize lines. Four commercial MRI inbreds, chosen for marker assisted introgression of the quality protein trait, and their BC2 progenies were subjected to two-level selection procedure. First, BC2 plants were analyzed with opaque2 (o2) specific molecular markers to identify heterozygotes. Second, the selected heterozygotes were screened with SSR markers distributed throughout the genome to identify genotypes with the highest recovery of recurrent parent's genome (RPG). The specific markers identified 100 out of 192 plants (52%) as heterozygous. Genetic similarity values between parental lines and their BC2 heterozygous progenies were in the range from 0.77 to 0.99 (77-99% RPG). The highest proportion of RPG was found in L1 (93-99%) and the lowest in L3 progenies (77-89%). Average values for the RPG content ranged from 83.9 to 95.8%. Progenies with RPG above 95% were selfed to produce BC2F2 plants which will be subjected to foreground selection. This time selection will be focused on homozygous recessive individuals, given that the presence of opaque2 gene in the homozygous recessive state is the aim of the QPM selection. Finally, those o2o2 genotypes will be screened for biochemical and phenotypic traits to confirm their nutritional and agronomical superiority.

MAIZE, MARKER ASSISTED SELECTION, OPAQUE2, QPM

06 – 19 Poster

ASSESSMENT OF DURUM WHEAT CULTIVARS BASED ON MORPHOLOGICAL TRAITS

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Understanding genetic diversity of durum wheat germplasm is essential for its identification and efficient use in breeding programs. The goal of this study was to assess genetic diversity of durum wheat cultivars from the Institute of Field and Vegetable Crops, Serbia, with 26 morphological characteristics based on International Union for Protection of New Varieties of Plants guidelines. The Shannon diversity index was used as an indicator of morphological diversity and it varies from 0.283, for glaucosity of lower side of the flag leaf blade and density of hairiness of uppermost node of the culm, to 0.950, for the ear color, with the mean value of 0.616, indicating a medium to high level of morphological diversity. On average, the diversity was higher for traits relating to generative organs than for those associated with vegetative organs. The 21 morphological characteristics were sufficient to distinguish unique profiles of all durum wheat varieties. The estimation of varietal diversity and identification of morphological characteristics with the highest discriminative power were done by multiple correspondence analysis. The traits that contributed the most to the distinction of varieties were the ear coloration, length of beak of the lower glume, lower glume shape ear length of awns at tip relative to ear length and color of awns. Morphological characterization using the traits with the highest discriminative power could be a useful complementary method for durum wheat germplasm classification and diversity analysis.

CATEGORICAL DATA, DIVERSITY, DISCRIMINATIVE POWER, MULTIPLE CORRESPONDENCE ANALYSIS, *TRITICUM TURGIDUM* SUBSP. *DURUM*

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06 – 20 Poster

THE CHEMICAL AND PROTEIN COMPOSITION OF GRAINS OF TWELVE DIFFERENT SWEET MAIZE GENOTYPES (*ZEA MAYS* L. *SACCHARATA*)

Milica Radosavljević [1], Marija Milašinović-Šeremešić [2], Zorica Pajić [1], Jelena Srdić [1], Dušanka Terzić [1], Valentina Nikolić [2]

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Sweet maize grains are used in human nutrition in the endosperm milk stage. The quality of these tender, succulent and sweet grains is determined by genes which distinguish sweet maize from common maize. In recent years, the practice of using sweet maize has been increasing both in our country and worldwide, while its production has therefore been growing. The largest part of sweet maize produced in our country is processed and exported as frozen grains. The most important quality parameters of sweet maize grains are taste,composition of the endosperm, and tenderness of the pericarp.

The paper presents the results of chemical and protein compositions of grains of 12 different sweet maize genotypes in the endosperm milk stage. Since sweet maize grain is used for human consumption, this stage is also a stage of grain harvest maturity. The chemical composition of grains of selected sweet maize genotypes was analysed by determining the content of non-structural carbohydrates (NFC - Non-Fibre Carbohydrate), protein, oil, crude fibre, and NDF (Neutral Detergent Fibre – fibres insoluble in neutral detergent made of hemicellulose, cellulose and lignin). The protein composition of grains of selected sweet maize genotypes was analysed by determining the contents of albumin, globulin, α-zein and glutelin. The dry matter content was established refractometrically and it ranged from 20.1 to 26.0% in observed sweet maize genotypes. The results obtained on the chemical composition of grains of the selected sweet maize genotypes showed that contents of NFC, protein, oil, crude fibre, ash and NDF ranged from 69.64 to 77.29%; 10.26 to 11.98%; 3.81 to 6.89%; 1.36 to 2.28%; 1.73 to 2.39%, and from 6.75 to 9.32%, respectively. The contents of albumin, globulin, α-zein and glutelin ranged from 22.59 to 37.10%, 4.88 to 7.37%, 16.97 to 24.71%, and from 18.18 to 22.55%, respectively.

SWEET MAIZE, CHEMICAL AND PROTEIN COMPOSITION

06 – 21 Poster

MOLECULAR CHARACTERISATION OF SOYBEAN VARIETIES BY SSR MARKERS

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Soybean (*Glycine max* (L.) Merr.) is one of the most economically important legume. As the source of plant protein and vegetable oil it is used as food and industrial crop in many regions of the world. The genetic base of soybean cultivars is highly narrow, corresponding to the fact that it is largely self-pollinated species. Twelve soyabean varieties were evaluated with SSR (Simple Sequence Repeat) markers selected based on their distribution on the 20 genetic linkage groups. Out of 36 SSR markers, 33 markers were found polymorphic among analyzed genotypes. Total number of alleles was 88, ranging between two to four with an average of 2.67 alleles per marker. The polymorphic information content (PIC) ranged from 0.153 to 0.775 for the primers Satt186 and Satt276, respectively. Jaccard's similarity coefficient was calculated using NTSYSpc2 program package. The average genetic similarity coefficient for all pairwise was 0.36, with highest value (0.65) between Galina and Lela, while the lowest value (0.18) was found between Bosa and Nena. Dendrogram by the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method was constructed on the basis of genetic similarity matrix. Genotypes were distributed in two groups and one branch, mostly in accordance with their pedigree.

GENETIC SIMILARITY, SSR, *GLYCINE MAX*

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VARIABILITY OF MAIZE LINES IN ABILITY TO USE NITROGEN

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Nitrogen is important macro-nutrient that influences various physiological processes in plants. Nevertheless, nitrogen could be loosed from the soil by leaching and evaporation. Thus, low nitrogen inputs are required together with strategy to improve its utilization by crops. Maize genotypes exhibit various susceptibility to low soil nitrogen. From that reason, variability in reaction of 32 maize lines to growing in conditions with optimal (fertilization with urea), and with low nitrogen (without fertilization) was examined during 2017 and 2018. All other growing measures and fertilization with other elements was applied at the same manner on whole experimental plot. 2017 was drier season, with higher average temperatures, particularly during anthesis and grain filling period.

High variability among genotypes and seasons was present. The values of maize grain yield and 1000 grain weight were slightly lower in the field without nitrogen fertilization. Some lines under the low nitrogen conditions reached even higher grain yields (efficacy of yielding was 139.7% and 156.7% respectively, for 2017 and 2018), than in conditions with optimal nitrogen in soil, declaring them as genotypes with high nitrogen using efficiency. However, these lines achieved moderate yields (in both fields and years) in regard to other lines. Among tested lines, two had relatively higher grain yields indicating them as prominent for further research, i.e. breeding of maize hybrids with better nitrogen usage from soil, even in the conditions with low nitrogen.

MAIZE LINES, NITROGEN USING EFFICIENCY, GRAIN YIELD

06 – 23 Poster

IDENTIFICATION OF eEF1A AND EF-Tu PROTEIN SYNTHESIS IN WHEAT AND OAT GENOTYPE UNDER HEAT CONDITION

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Expression elongation factors eEF1A and EF-Tu, in grain filling stage when the cereal most sensitive in the field conditions, can be an important determinant of heat tolerance. The aim of our work was to examine the expression and accumulation of EF-Tu and eEF1A in grain filling stage of five genotypes of winter wheat and one oat genotype in conditions of heat stress. In addition, the correlation between accumulation of elongation factors eEF1A and EF-Tu, and yield components of cereals in the field was investigated. Flag leaf samples of small grains were analyzed by immunoblotting. Samples were collected in conditions of moderate, control air temperature (23 °C; CT) and high temperature (38 °C; HS) in a field experiment. After the harvest, grain yield was determined. The yield components, the weight of dry seed and grains number per spike, were also assessed in the stage of full physiological maturity of investigated cultivars. Obtained results revealed a difference in the level of EF-Tu accumulation both under conditions of moderate air temperatures and conditions of heat stress among investigated cultivars. Cultivar Zvezdana was the only one that showed increase in EF-Tu accumulation under HS (25%) compared to CT. Immunoblot analysis indicated the increase of eEF1A accumulation in conditions of heat stress in cultivars Talas, Zvezdana, Pudarka and Carica. The highest increase of 43% in relation to control was detected in cultivar Talas. A significant, positive, linear correlation was found between the expression of eEF1A and cereals productivity under heat-stress conditions. Investigation of the molecular mechanisms aims to develop agronomic strategies to improve tolerance against heat stress.

ELONGATION FACTORS, HEAT STRESS, WHEAT, OAT

06 – 24 Poster

VARIABILITY OF STARCH CONTENT IN GENETICALLY DIVERGENT VARIETIES OF WHEAT

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The reliable indicator of wheat flour quality is content of starch of seed, together with content of protein, fat and dietary fiber. The aim of this work is analysis of starch content of wheat seed and identification of variety desirable for wheat breeding. Seeds of ten genetically divergent bread wheat varieties (Ilina, Obala, Futura, Renesansa, Ratarica, Vlajna, Mila, Apache, Salasar, Hyfi) were used for research. Starch content was determined using the spectrophotometric method according to Hansen and Moller (1975). In the investigated varieties, the content of starch ranged from 46.2% to 65%. The highest content of starch was found in variety Apache (65 %) and the least starch content was in variety Vlajna (46.2 %). Besides Apache variety, high starch content is found in varieties Futura, Mila and Hyfi. Varieties in which the lower starch content is determined are the varieties of wheat Vlajna, Obala and Ilina. The results showed differences among analyzed wheat varieties according to the content of starch and that the Apache variety can be used in crossbreeding for creating new wheat genotype with high starch content and improved nutritive quality.

STARCH, WHEAT, VARIETY, GENETIC DIVERGENCE

06 – 25 Poster

CEREAL GENOTYPES COMPARED FOR THE CONTENT OF TOTAL AND FREE LYSINE AS PRECURSORS IN FORMATION OF FUROSINE AND HARMFUL DIETARY ADVANCED GLYCATION END PRODUCTS DURING MAILLARD REACTION

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Maillard reaction generates a multiplicity of sensory active volatiles and nonvolatiles that are the key compounds responsible for the development of aromas, colors and flavors, and hence improve food palatability. A high antioxidant capacity of Maillard reaction products is one of their main beneficial properties. However, some MRPs have the potential toxic, mutagenic or carcinogenic activity on animals and humans. Cereal based foods are one of the major source of MRPs in diet. Free amino acids and reducing sugars are considered as the main precursors in the formation of these heat induced compounds. In order to determine genetic resource of cereals with reduced potential for the formation of furosine and harmful dietary advanced glycation end products (AGE), such as Nε-carboxymethyllysine (CML) and Nε-carboxyethyllysine (CEL), the content of free and total lysine was analyzed in a total of 13 genotypes, which belonged to nine varieties and six species, grown at the same location in the 2018 growing season. Our results provided the evidence of differences in the content of free and total lysine between cereal species, as well as their varieties. Hull-less oat and rye, with total lysine content of 10670.3 and 6231.5 mg/kg, respectively, were followed by blue popping maize and hard wheat. The content of total lysine in triticale, durum wheat, soft wheat and red standard maize ranged from 2940.9 to 3978.8 mg/kg. The third group, with the content of total lysine from 1844.5 to 2739.4 mg/kg, consisted of white standard maize, bread wheat, yellow standard maize, and hull-less barley. The correlation between content of total and free lysine in cereals has not been established. According to results, the lowest furosine, CML and CEL formation potential was found in bread wheat and blue popping maize genotypes. Given that lysine is an essential amino acid, breeding of cereals should go in both directions (low and high lysine content) depending on the purpose of the genotype in food production.

CEREALS, SPECIES, GENOTYPES, LYSINE, MAILLARD REACTION PRODUCTS FORMATION POTENTIAL

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06 – 26 Poster

EFFECT OF GENOTYPES ON BIOACTIVE COMPOUNDS PROFILE OF WHEAT BRAN AS FUNCTIONAL FOOD ADDITIVES

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The bran fraction is a by-product of milling, and has food and nonfood applications. Globally, the number of wheat bran-incorporated food products increased from 52 in 2001 to approximately 800 in 2011. Bran plays the key role in the overall health benefits of whole grains, probably due to phytochemicals and fibres embedded in the bran. However, these bioactive compounds in wheat bran tissues vary because of cultivar, genetic and environmental factors. Hence, this study aimed to investigate the content of bioactive compounds (total phenolic, flavonoids, phenolic acid, carotenoids and fibres) in bran of 10 different bread and durum wheat genotypes grown at same location in the same growing season. The total phenolic contents of bread and durum wheat bran ranged from 7505.27 to 10823.29 mg GAE/kg and 7746.54 to 12384.55 mg GAE/kg with the average value of 9208.52 and 9798.52 mg GAE/kg, respectively. About 99% of total phenolic compounds were localized in the bran of bread and durum wheat. Flavonoids and phenolic acids represent the most common form of phenolic compounds found in wheat bran where they are among the major and most complex groups of phytochemicals. Flavonoids varied among wheat genotypes as evidenced by a wide range from 173.59 mg CE/kg (the bread wheat genotype ZP 7/I) to 321.86 mg CE/kg (the durum wheat genotype ZP 120/I). Ferulic acid was the predominant phenolic acid in the brans of bread and durum wheat, with its concentration ranging between 5502.05 to 9160.92 mg/kg. The significant correlation (r2 = 0.95**) between total phenolic contents and ABTS radical scavenging activities point to their high contribution to antioxidant capacity. Since varieties showed differences in their content of phenolic compounds, as well as carotenoids, our results can be useful in breeding programs for the development of wheat varieties with higher concentration of health-beneficial phytochemicals in bran.

WHEAT GENOTYPES, WHEAT BRAN, BIOACTIVE COMPOUNDS

06 – 27 Poster

INSTRUMENTAL AND SENSORY PROPERTIES OF BREAD. DIFFERENCES BETWEEN DURUM AND BREAD WHEAT GENOTYPES

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Wheat is one of the most important cereal crops worldwide in terms of production and utilization. Currently, about 95% of the wheat grown worldwide is hexaploid bread wheat, with most of the remaining 5% being tetraploid durum wheat. In order to determine effects of the genotype on instrumental and sensory properties of bread, two bread and two durum wheat genotypes cultivated in two growing seasons were used. Bread loaf volumes and specific volumes were determined by VolScan profiler and results showed that the loaf volume ranged from 186.90 ml to 327.50 ml and 160.10 ml to 228.10 ml in bread and durum wheat bread samples, respectively. Comparison of bread volume from bread and durum wheat genotypes revealed that the bread volume of durum wheat genotypes with the best performance was significantly lower than that of bread wheat genotypes. Bread samples made from bread and durum wheat genotypes with the smallest specific volume were distinguished by the highest bread crumb firmness and chewiness. The firmness, chewiness, cohesiveness, resilience and springiness of bread crumb were instrumentally recorded on a texture analyzer TA.XT plus. The smallest crumb firmness had a bread made from genotype ZP Zemunska rosa (395.6 g) grown in the rainy season, while the bread made of the same genotype cultivated during the dry season had 5.4-fold firmer bread crumb. Bread samples made from bread wheat genotype ZP 87/I (213.3g) and durum wheat genotype DSP/01 (211.3g) had a smallest chewiness. Results of the sensory evaluation revealed that the sensory properties shape, crumb pore uniformity and structure, did vary greatly among the investigated bread samples made from bread wheat and durum wheat genotypes. It can be concluded that investigated bread and durum genotypes have quite different physical and sensory characteristics which could allow various possibilities of their use.

BREAD WHEAT, DURUM WHEAT, INSTRUMENTAL PROPERTIES, SENSORY PROPERTIES

06 – 28 Poster

OIL CROPS FOR 21ST CENTURY – NEW TOOLS FOR TACKLING CHANGING ENVIRONMENT

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Creation of new oil crop varieties using classical breeding methods is a long-term process, sometimes not efficient enough to meet demands of changing environment and market demands of 21st century. The development and introduction into breeding programs of new techniques, such as phenotyping, genomic selection and genome editing, opened the way for more efficient introduction of desired traits into commercial varieties. Although all these new techniques have their shortcomings and demand some time for optimization and validation on the given crop/system, it is expected that in the future they will facilitate the identification of target genes and markers for complex traits, as well as adaptation of oil crops to permanent changes in the environment and the market. Researchers from Institute of Field and Vegetable Crops (IFVCNS) have already started to work on creation of oil crops for 21st century and the introduction of new techniques of genotyping and phenotyping for more efficient data collection to identify quantitative properties and explain the genetic basis of agronomically important traits. An overview of the projects and research activities of IFVCNS related to introduction of new breeding techniques and their application in IFVCNS oil crops breeding programs is presented in this paper.

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OIL CROPS, PLANT BREEDING, NEW BREEDING TECHNIQUES, PHENOTYPING

06 – 29 Poster

DIFFERENCES IN YIELD AND STABILITY OF MAIZE HYBRIDS PRESENTED BY THE AMMI ANALYSIS

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This study presents three-years (2011-2013) research on 36 commercial hybrids from different FAO maturity groups (300-700). Trial was set up according to the Randomized Complete Block Design (RCBD) in three replications at 8 different locations in Serbia. Analysis of variance showed significant impact of genotype, environment and their GXE interaction on the grain yield (p>0,01). An average grain yield in 2011 ranged from 10.38 t/ha (H1) to 13.32 t/ha (H36), in 2012 it varied from 5.70 t/ha (H3) to 7.86 t/ha (H14) and in 2013 from 8.79 t/ha (H5) to 12.01 t/ha (H36). Based on the AMMI analysis, yield and stability of the maize genotypes were evaluated. In the total sum of squares, environment accounted for 72.4%, 73.3%, 69.13% (2011, 2012, 2013, respectively), genotype 6.22%, 2.81%, 6.15% (2011, 2012, 2013, respectively) and interaction between genotype and environment accounted for 9.09%, 10.06%, 11.87% (2011, 2012, 2013, respectively). Due to the fact that IPC1 and IPC2 encompassed 62.7%, 62.6%, 60.0% (2011, 2012, 2013, respectively) of total sum of squares of interactions, AMMI2 model was also considered. Grain yield of hybrids varied in dependance of location and the prodution year. In 2011, the most stable hybrids were H21, H9 and H24; in 2012: H13, H21 and H6; while in 2013 those were H21, H29 and H22. The lowest variation in average grain yield was recorded at locations Sombor and Kikinda (2011), Svilajnac (2012) and Pančevo (2013) where the grain yield was above the average, while location Loznica proved to be the most yielding in all production years. Based on the results from AMMI analysis, more precise reccomendations could be given for the hybrid production at certain locations, aiming to achieve the highest yield.

MAIZE HYBRIDS, GRAIN YELD, AMMI ANALYSIS, GXE INTERACTION

06 – 30 Poster

IMPORTANT AGRONOMICAL TRAITS ASSOCIATED WITH THE NORMALIZED DIFFERENCE VEGETATION INDEX IN TWO AND SIX-ROWED BARLEY ELITE BREEDING LINES

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Recent advances in agriculture phenotyping gave rise to a plethora of speedy and non-destructive screening tools, one of which is the Normalized Difference Vegetation Index (NDVI) technology, widely used for estimation of yield components, biomass and other important physiological traits for crop production. The objective of this study was to evaluate NDVI values and relationship with agronomic characteristics in a diverse set of barley advanced lines. The selected plant material consisted of 24 six-rowed and 23 two-rowed elite barley lines developed at the Institute of Field and Vegetable Crops from Novi Sad. The trial was conducted in a complete randomized block design with four replications at the experimental field Rimski šančevi in 2017/18. The NDVI parameters were collected using the handheld Green Seeker sensor at the anthesis vegetation stage (Zadoks 65), while the following agronomic traits were measured during the growing season: stem height, spike length, hectoliter mass, thousand grain weight and grain yield. Significant correlations between NDVI and grain yield were detected in two-rowed (R2=0.363) and six-rowed (R2=0.268) barley genotypes. The observed positive correlations with stem height were stronger in two-rowed (R2=0.459) than in six-rowed (R2=0.257) genotypes, whereas the relationship between NDVI and thousand grain weight was significant only in six-rowed barley lines. Genotype variation in NDVI values and, especially, its positive correlations with yield indicate that NDVI technology could be used as an additional tool for selection of early maturing genotypes with better adaptation traits, which could accelerate development of better performing cultivars resilient to future unpredictable changing climate.

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HORDEUM VULGARE, NDVI, YIELD COMPONENTS, INDIRECT SELECTION

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YEAR RELATED INTERACTIONS FOR SEED YIELD AND OIL CONTENT IN RAPESEED AGRONOMIC MANAGEMENT TRIALS INTERPRETED USING CLIMATIC VARIABLES

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Interaction in agronomic trials seriously complicates the identification and recommendation of the highest yielding cultivars for specific environmental conditions. As the part of the study aimed to investigate the influence of climatic variables through the rapeseed development stages, the objective of the presented study was to identify the most important climatic variables in cultivar × year interactions for seed yield and oil content of four commercial rapeseed cultivars. The cultivars were grown during the four cropping seasons in two independent trials: the first one investigated the effect of five nitrogen rates, and the second effect of three sowing dates. Throughout rapeseed developmental stages, thirty climatic variables were derived from the original meteorological records and used for data interpretation. Experimental data were processed by mixed model analysis and individual factorial regression model. All interactions had highly significant impact on seed yield and oil content in both trials, except of year × cultivar interaction in sowing date trial. Individual factorial regression model identified that the considerable number of climatic variables had impact on these interactions. In conclusion, the results of this study showed that more complex factorial regression models are needed to be constructed in order to give us a more robust interpretation of data.

VARIABILITY, SOWING DATE, NITROGEN DOSAGE, CLIMATIC FACTORS, *BRASSICA NAPUS*

06 – 32 Poster

CHANGES IN CAROTENOIDS AND TOCOPHEROL CONTENT IN MAIZE GRAIN IN POPULATION, INBRED LINES, AND THEIR CROSSES

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Maize grain is with the highest content of bioavailable micronutrients within cereals, which makes this crop the most appropriate for biofortification. Since genetic variability for this trait is high, genotypes with enhanced grain content could be used for commercial hybrids improvement. The maize kernel contains two fat-soluble vitamins: provitamin A carotenoids and vitamin E (tocopherols). This work was performed to estimate differences in tocopherol and carotenoid content in F1, F2, F3, BC1 and BC2 crosses of orange grain landrace and five commercial inbred lines, by high-performance liquid chromatography (HPLC). Significant variations were obtained: α, γ and δ tocopherol were higher in all crosses of population and inbred L1, as well as compared to line L1 (16.47 µg/g DW, 23.26 µg/g DW and 0.94 µg/g DW, respectively) and population (16.02 µg/g DW, 31.7 µg/g DW and 0.42 µg/g DW, respectively) per se. Crosses of population and inbred lines L3 and L5 showed higher grain content in tocopherol γ and tocopherol δ. Content of tocopherol γ in crosses of line L3 were within range of 65.03 µg/g DW in F1 cross to 60.34 µg/g DW in BC2 cross, and for L5 content vary from 67.77 µg/g DW in F1 cross to 51.48 µg/g DW in BC2 cross. Considering carotenoids content, lutein+zeaxantin were higher in all crosses between population and inbred lines L3 and L4, and higher than population (36.53 µg/g DW) and lines (17.75 µg/g DW and 24.87 µg/g DW for L3 and L4, respectively) per se. The highest contents of β-carotene, (lower than in population-14.15 µg/g DW), were detected in populations' crosses with inbred line L2 and were in range from 13,38 µg/g DW in F1 cross to 10,52 µg/g DW in BC2 cross. Tested population and inbred L3 could be recommended for further use in breeding programs for improvement of grain nutritive value.

GRAIN, MAIZE, MICRONUTRIENTS, BIOFORTIFICATION

06 – 33 Poster

VARIABILITY OF GRAIN MICROELEMENTS IN MAIZE HYBRIDS AND THEIR PARENTAL LINES

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Maize grain has the highest natural variation in micronutrient content, that imply multiple uses for food, feed and industrial processing. Using of existing variability provide the basis for development of new hybrids with improved grain quality for different purposes. The objective of this work was to compare microelements content (inorganic phosphorous, phytate, Ca, Mg, Fe, Mn and Zn) in grain of eight hybrids and their parental lines. The concentrations of inorganic phosphorous vary from 0.32-0.56 g/kg, with the value higher in hybrids ZP 600, ZP 606 and ZP 666, than in their parental lines. Grain phytate content was in range from 3.06-4.44 g/kg, with the highest level in hybrid ZP 666. Ca content varied from 56.53-151.56 mg/kg and in hybrids ZP 427, ZP 666 and ZP 684 was higher than in their components. Those hybrids have positive mid- and better parent heterosis for grain Ca content. The same results for heterosis was obtained for Fe grain content, and its concentrations vary from 10.22-38.06 mg/kg. Content of Zn was in the range from 17.47-46.00 mg/kg, and positive mid and better parent heterosis were for hybrids ZP427, ZP 560, ZP 666 and ZP 684. Mn content was higher in hybrids ZP 555 and ZP 606 than in parental lines and varied from 1.78-2.91 mg/kg, and positive heterosis was obtained only in ZP 555. Negative heterosis and microelements content lower than in parental lines were detected in hybrid ZP 341. Hybrid ZP 666 has higher content of all tested microelements, except Mg and Mn, than their parental lines, and the highest positive heterosis for those traits. Results of that study showed that none of the hybrids had potential for all the minerals, suggesting the need for multiple crossing breeding works to increase the mineral content without decreasing its vigor advantage for yield and other economic characteristics.

GRAIN, HYBRIDS, INBREDS, MAIZE, MICROELEMENTS

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EXPRESSION VALIDATION OF TWO GENES IMPORTANT FOR PHOTOSYNTHESIS UNDER LOW TEMPERATURE CONDITIONS IN DIFFERENT MAIZE INBRED LINES

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As one of the most important metabolic processes in all plants, photosynthesis is necessary for their survival and ensuring enough energy is created for successful execution of all physiological processes, including nutrient intake and transport, interactions with the environment, growth and development, as well as processes important for crops and their production such as flowering, reproduction, grain filling etc. Many current strategies of avoiding yield loss in crop production include earlier sowing, as to avoid increasingly higher temperatures during late spring and summer, the consequences of climate change. Because of that, the knowledge of the impact of the cold on the process of photosynthesis is of crucial importance. In this research, we focused on the light dependent phase of photosynthesis and chose two genes related both to this phase and stress tolerance – photosystem II cytochrome b559 α-subunit and ATP synthase CF0 A subunit. These genes were chosen for further research from a set of differentially expressed genes (DEGs) detected between Lancaster and non-Lancaster maize lines. Their expression was validated on four maize inbred lines that belonged to different heterotic groups (Lancaster and non-Lancaster). The experiment was performed under optimal and low temperatures, 22° and 6° respectively, during the V4 growth stage. Samples for RNA extraction, cDNA synthesis and qPCR expression validation were taken after 6h and 24h of exposure to experimental temperatures, as well as after 48h in recovery. Results showed significant changes in the expression of these genes, both between different time points of stress treatment, as well as between different inbred lines. These preliminary results give a good base for further studies of gene expression in important maize lines, particularly abiotic stress related genes, as a tool for establishing successful breeding programs and defining pathways involved in maize response to cold stress.

MAIZE, COLD STRESS, QPCR, GENE EXPRESSION VALIDATION

06 – 35 Poster

VARIABILITY OF MACRONUTRIENTS IN MAIZE INBRED LINES CAUSED BY APPLICATION OF ORGANIC PEROXIDES

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Exogenous application of chemical elicitors can be used to reduce damage of plants caused by abiotic stresses and consequently to enhance productivity. Different organic peroxides (mixed with DMSO) were used in this study with the aim to examine variations in grain yield and macronutrient status, i.e. protein, starch and oil contents, of four maize inbred lines. Peroxides in combination with DMSO were applied foliarly. Results showed that two genotypes reacted positively on applied treatments, achieving higher grain yields than control group, with difference up to 2-3 t ha-1. In total, one of the applied substances expressed the highest impact on yield enhance. In terms of nutritive quality, the same treatment mostly increased the starch content. In regard to protein content, higher value was achieved by the genotype which also had higher grain yield, and for oil content, variations in results among treatments were insignificant and irregular. This indicates that various elicitors, such as organic peroxides, could be used not only for increase in grain yield, but also in modification of grain nutritional quality in regard to genotype variability.

MAIZE INBRED LINES, ORGANIC PEROXIDES, GRAIN, YIELD, NUTRITIONAL QUALITY

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RESPONSE OF TEN MAIZE INBRED LINES TO SEED PRIMING TREATMENTS ANALYZED USING GT BIPLOT METHODOLOGY

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Early emergence and seedling growth is considered as one of the most important yield-contributing factors in maize. Seed priming has been successfully used for improving these traits in different crops. The objective of this study was to determine the effect of seed priming treatments (hydropriming and KNO3) on emergence and seedling growth traits, inbred line's response to seed priming treatments as well as to evaluate the application of GT biplot methodology in this type of analysis. Observation was made on 10 maize inbred lines. Testing was conducted in sterile moistened sand at suboptimal (15° C) and optimal temperature (25°C) for early maize growth traits. Results showed that seedling emergence and growth of maize lines under both temperatures could be improved with seed priming. In most of lines, seed priming improved certain emergence and seedling growth traits; however, in some lines it had adverse effect on seedling emergence traits. Some lines responded better to hydropriming and other to KNO3 priming. There is also the possibility for one genotype to benefit from specific seed priming at one temperature and show adverse effects at another. Generally, seed priming showed greater improvement in studied traits at suboptimal temperature than at optimal one. The highest percentage of variation, in all studied traits, was explained by line main effect, suggesting the existence of high genetic diversity in used maize inbred lines. Observed diversity represents useful information for selection of appropriate maize inbred line and adjusted seed priming treatment for sowing in different temperature conditions. This research also showed that biplot analysis could be used as a quick visual method for identifying the effect of seed treatments such as priming on different germination, emergence and seedling growth traits and evaluation of genotypes response to these treatments.

MULTIVARIATE ANALYSIS, SEEDLING EMERGENCE, SEED TREATMENT, *ZEA MAYS*

06 – 37 Poster

GENE EXPRESSION AND BIOCHEMICAL PARAMETERS DURING GERMINATION OF MAIZE SEEDS UNDER THE LOW TEMPERATURE CONDITIONS

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Maize is one of the most important crops worldwide. During several past decades severe yield losses due to climatic changes have been observed. Avoiding period of high temperatures during the summer, which could have negative effect on flowering and grain filling, could be achieved by earlier sowing. The main concern is how the temperature lower than optimal could affect early phases of maize growing. The object of this research was to test the changes in biochemical parameters and gene expression related to low temperature effect during the imbibition phase in two maize lines, contrasting in the level of tolerance according to breeder's experience. Experiment was set at 8°C as treatment and 22°C as control temperature. Both genotypes were exposed to cold stress for 24h. Germination test performed under treatment/control showed better results for the tolerant genotype. Cell conductivity was significantly higher under cold treatment in the sensitive genotype compared to control conditions. Lipid peroxidation was elevated under the low temperature in both genotypes, but it was more prominent in the sensitive line. Also, expression of some genes proven to be involved in plant response to abiotic stress was tested. Expression of GID1, gibberellin receptor, was three fold higher in the sensitive line. The role of this gene in cold tolerance is not yet completely elucidated. FAD2 (fatty acid desaturase 2) gene was not activated by low temperature, while expression of FAD6 (fatty acid desaturase 6) was much higher in the sensitive line which is in accordance to literature data for some plant species. Results of these analyses confirmed previous presumption about the level of tolerance in tested material which is important for breeding programs and gave some directions for further more profound research in defining pathways involved in maize plant response to low temperatures in germination phase.

MAIZE, IMBIBITION, COLD TOLERANCE

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GRAIN YIELD STABILITY PARAMETERS OF SINGLE CROSS MAIZE HYBRIDS AND THEIR RECIPROCAL CROSSES

Olivera Đorđević Melnik [1], Zoran Čamdžija [1], Sofija Božinović [1], Tomislav Živanović [2], Jelena Vančetović [1]

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Grain yield stability was tested in 10 single cross maize hybrids and their reciprocal crosses in 2016 and 2017, within a total of seven environments. Five were early hybrids with Lancaster ZPL-1 line as a mutual parent (ZPH1 to ZPH5 where ZPL-1 was a father, and ZPH1r to ZPH5r with ZPL-1 as a mother). Other five were late hybrids with mutual ZPL-2 Lancaster line (ZPH6 to ZPH10, and ZPH6r to ZPH10r). Significant difference at $p<0.01$ level was observed between average grain yield of the early *vs*. late hybrids (10.82 vs. 11.04 tha-1). Also, stability parameters were significantly different; bi was 0.946 on average for the early hybrids and 1.054 for the late ones ($p<0.01$), thereby confirming that the early hybrids performed better in poorer environments, and *vice versa*. This is a common finding in Serbia. Regarding S2di, the difference between early and late hybrids was significant at $p<0.1$ level (0.166 and 0.487 for early and late hybrids, respectively). Also, S2di was significantly different at $p<0.1$ level for early hybrids and their reciprocals, as well as all the other hybrids and their reciprocals. Namely, reciprocal hybrids were less stable (Lancaster as a mother). Best-performing hybrid was ZPH6r, yielding 12.06 tha-1 on average (the highest yield in the trial), with bi=1.16, and S2di=0.06. The hybrid performed well in favourable agronomical conditions, as well as maintaining grain yield stability across other environment types.

GRAIN YIELD, MAIZE, STABILITY, RECIPROCALS

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IMPROVING WATERMELON DROUGHT TOLERANCE BY GRAFTING: THE IMPLICATIONS FOR QUALITY AND YIELD

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Drought is a common abiotic constraint affecting world agricultural production. This study was aimed to investigate grafting as a means to improve watermelon response to drought, as well as to assess the effects of different rootstocks on watermelon agronomic and quality traits. The popular vegetable is among species with a rather large water demand. Five scions (*Citrullus lanatus* breeding lines, experimental hybrids and cultivar) were grafted on nine rootstocks (*Citrullus lanatus* var. *citroides*, *Lagenaria siceraria*, *Cucurbita moschata* and *Cucurbita maxima*) in all combinations, without previous knowledge on their compatibility. Plant material originates from the Institute of Field and Vegetable Crops, Novi Sad, Serbia, where the experiments took place. Three quarters of the total number of the scion-rootstock combinations were successfully acclimatized in a growth chamber, whereas in the open field only a half of previously acclimatized combinations completed life cycle. To various extent, irrigation improved yield in non-grafted watermelons, by increasing both fruit number and weight. Total sugars were generally lower, and β-carotene and lycopene were higher in irrigation. The performance of the grafted watermelons depended on scion-rootstock combination and could not be generalized for both irrigated and rainfed plants, as well as for agronomic and quality traits. The scion-rootstock combinations with enhanced yield and/or quality in limited water supply were identified, and were mainly with *Citrullus lanatus* var. *citroides* or *Lagenaria siceraria* rootstocks. The results imply grafting as a promising approach for mitigating the effects of drought in watermelon production.

DROUGHT, GRAFTING, WATERMELON

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THE ANALYSIS OF COMBINING ABILITIES OF MAIZE INBRED LINES OF DIFFERENT CYCLES OF RECURRENT SELECTION FOR THE EAR LENGTH

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The evaluation of combining abilities is an inevitable step in the development of highly productive maize hybrids. The mean values, heterosis, and general (GCA) and specific (SCA) combining abilities of maize inbreds and their hybrids were observed in this study. Inbreds of different cycles of recurrent selection of two maize synthetic populations BSSS and BSCB1 were selected for the study. Hybrids showed higher values of the ear length than inbreds, since inbreeding depression occurs in inbreds as a result of the inbreeding process. The analysis of variance of combining abilities indicates significant positive values of GCA and SCA over both locations and both years of investigation. Since GCA to SCA ratio for inbreds of the BSSS population and parents of an elite hybrid was lower than unit, it can be concluded that a non-additive gene effect might be more important in controlling the expression of this trait. On the other hand, the additive gene effect was the most important for inbreds and hybrids originating from the BSCB1 population. The highest GCA values were recorded in inbreds ZPL1 and ZPL2. Hybrids derived from these two inbreds showed high SCA values in both years of investigation.

MAIZE, COMBINING ABILITY, HYBRIDS, INBREDS, EAR LENGTH

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THE EVALUATION OF DONORS OF FAVOURABLE ALLELES FOR THE IMPROVEMENT OF F1 MAIZE HYBRIDS

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The basic aim of maize breeding programs is the development of new hybrids that will exceed the existing commercial hybrids. The purpose of this study was to estimate which inbreds derived from the BSSS and BSCB1 populations had the highest relative values of favourable alleles for the improvement of the number of kernels per row in an elite maize hybrid. Inbreds B73 (C5), B97 (C9) and B99 (C10) had the highest values of favourable alleles µG* for the improvement of the number of kernels per row. The inbred line B73 (C5) originating from the synthetic population BSSS had a significant and positive difference µG*-(µD* or µF*), hence in the breeding process, the F1 generation should be crossed to an inbred donor and then self-pollinated to develop an improved inbred line. Inbred lines B97 (C9), B99 (C10) and B84 (C7) had high values of µG* but did not have high differences µG*-(µD* or µF*), not significantly higher than null. Ranking of inbreds as donors of favourable alleles over four applied parameters (µG*, UBND, PTC and NI) shows congruence. The best ranked inbred line B73 (C5) was the best over all four parameters, while remaining inbreds expressed identical ranking confirming high and significant rank correlations.

INBRED LINES, MAIZE, DONORS, HYBRID, ALLELES, RANK CORRELATIONS

06 – 42 Poster

SCREENING EARLY SOYBEAN GERMPLASM FOR YIELD AND AGRONOMIC IMPORTANT TRAITS

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The objective of this study was screening of soybean germplasm with reference to yield and other agronomical important traits, as novel source of genetic variability in soybean breeding program. The focus is on early soybean germplasm as a potential genetic source for breeding in the light of global climate changes and to increase diversity of early varieties that can be grown as a second crop in Vojvodina province.

The trial was conducted in 2018 year, established as row-column design, at experimental fields of Institute of filed and vegetable crops, Novi Sad. A 262 genotypes from three maturity groups (MG 000, MG 00, MG 0) were included in this research. Number of soybean genotypes per MG was 85, 93 and 84, respectively. Sowing was conducted in early April while harvesting start from July and lasting till September. The earliest genotype matures in 65 days, while the latest one mature after 125 days. This extremely early genotype can be very useful source in breeding program for earliness. It was identifying several genotypes with 1000 seed weight over 300 grams. This is good genetic source in breeding programs dedicating to food soybean and edamame production. Wide yield variability was observed, in the range 0.3-3.9 t/ha, indicating on potential source of useful germplasm for introducing in elite breeding program.

The results showed a good correlation between maturity group and yield, but also with length of phases VE-R8, VE-R1 and R1-R8, respectively. Genotypes from MG 0 had longer vegetation than genotypes from MG 00 and MG 000, which resulted in higher plants, more nodes, higher 1000 seed weight and after all a higher total yield per plot. Also, number of fertile nods can be used as indicator for productive trials of soybean germplasm. The trial will be repeated for obtaining more precise results in the year of 2019.

SOYBEAN; MATURITY GROUP; YIELD

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BREEDING OF MEDIUM EARLY MATURITY MAIZE HYBRIDS WITH FAST DRYDOWN RATE

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Medium early maturity hybrids, belonging to the FAO 300-400 maturity group have increasing importance in maize production in our country. Although they have lower grain yield potential compared to the hybrids of later maturity groups, they also have a number of advantages which are manifested particularly under unfavorable production conditions. The main advantage medium-early maturity hybrids are lower harvest moisture content, which enables farmers to finish harvest in optimal time and to prepare soil for the next crop. Identification of faster drying inbreds and hybrids is difficult task over time. There are several factors influencing a maize drydown rate, such as timing of physiological maturity, weather conditions following maturity and hybrid characteristics. The most important hybrid characteristics that are influencing drydown rate are husk leaf coverage, husk leaf senescence, ear angle and kernel pericarp characteristics, so breeding for those traits is very effective in order to create inbred lines and produce hybrids with lower moisture content.

One of the recently developed maize hybrids in Maize Research Institute „Zemun Polje“, with good drydown rate is ZP 3536. During the period of official testing, 2017-2018., this hybrid achieved a higher grain yield and had lower moisture content in harvest compared with both check hybrids. On average, during two years period it achieved 12% higher grain yield compared to the average of check hybrids, with a moisture content of 1.63% lower than the average of two standards.

Considering the very good results obtained during the two-year trial, this hybrid can be recommended for wide testing and for intensive growing practices. It can provide high production and low moisture content in harvest, enabling farmers to finish harvest period in time and prepare soil for the next crop.

MAIZE HYBRIDS, GRAIN YIELD, MOISTURE CONTENT, DRYDOWN RATE

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SILAGE MAIZE BREEDING AND CREATION OF MAIZE HYBRIDS WITH INCREASED SILO MASS YIELD FOR PRESENT CLIMATE CONDITIONS

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According to the datas of Annual report of PSS Sombor for attested quantities of seed for 2018/2019, the participance of late maturing hybrids, designated for silage purposes is around 3%. Estimations from Marketing department of Maize Research Institute „Zemun Polje“ (MRIZP) is that in 2019th around 800.000-840.000 ha has been sown under maize, leading to the conclusion of production on around 23000 ha under silage maize in Serbia nowadays. Silage maize is one of the most high-yielding forage crops (NASS 2015, Arvalis 2011) and therefore estimations for future maize production is that area under such usage of maize as silage will be increased.
Breeding of silage maize in Serbian agroecological conditions is becoming more challenging during the instability and unpredictability of climate conditions in different years, where regardless of reduced rainfall on average annual level during the vegetation season, increased yield remains primary goal. For such purpose MRIZP has created new hybrid ZP 707. MRIZP performed silage trials in 2017th (drought stress year) measuring silo mass yield (SM), dry matter content (DMC), yields of dry matter and digestible dry matter of whole plant (YDM and YDDM respectively) of silage forms of ZP 707 compared to the check variety ZP 735. Trials were conducted at two locations with planting density of 65000 plants per ha.
Results show that values ranged from 26.6-28.1 t/ha for SM, 35.06-40.33% for DMC, 9.2-11.2 t/ha for YDM and 5.3-6.7 t/ha for YDDM, with all of mentioned results in favor of ZP 707. As mentioned silo mass yield and related yield parameters correlate to greater genetic potential of newely created hybrids.
Other important parameters as in vitro digestability of dry matter of whole plants (DDM), and based on that digestibility of neutral detergent fibers (NDFD) was also calculated. According to the results values showed 60.67% for ZP 707 for DDM where the same parameter was 57.9 % for ZP 735. NDFD was also in favor of new hybrid ZP 707, but with less difference 19.96% compared to 19.41% for ZP 735. Observing results of NDFD, again, represent the advantage od ZP 707 not only in quantity of reached silomass, but also it’s quality, taking into consideration that not only greater volume is obtained, but also higher digastability of such created silo mass. Obtained results confirmed new hybrid ZP 707 to be very suitable for market request and farmers needs for silage maize.

SILAGE MAIZE, MAIZE HYBRIDS, SILO MASS, DIGESTIBILITY

06 – 45 Poster

PERFORMANCE OF SUNFLOWER HYBRIDS INTERCROPPED WITH LEGUMES REVELED BY AMMI ANALYSIS

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Intercropping, as ecologically designed agricultural system, could help in agriculture advancement for many crops. The aim of research was to evaluate the effects of legume-sunflower intercropping and their interaction on the sunflower seed yield during 2017 and 2018 vegetation seasons. A field experiment was set up by intercropping three sunflower hybrids (Dukat, Rimi PR, and NS Gricko) with three types of legumes (*Vicia sativa* L., *Medicago sativa* L., and *Trifolium pratense* L.), whereas sole crops of sunflower were used as control. The Additive Main Effects and Multiplicative Interaction Analysis (AMMI) was used to test the main effects and interaction of intercrops. Year × crop combinations were examined as 8 environments. Analysis of Variance (ANOVA) tests of seed yield in 8 environments indicated highly significant effects of genotype, environment and their interaction, with the following share: genotype 61.78%, environment 27.69%, and interaction 10.53%. Mean seed yield of the hybrids varied from 3 t/ha (Dukat), 3.5 t/h (Rimi PR), to 4.4 t/ha (NS Gricko). Knowing that hybrid Dukat, which had the smallest seed yield, is a short vegetation hybrid, while NS Gricko, which had the highest seed yield, is a confectionary hybrid, the results were expected. The AMMI ANOVA test showed high significance of IPC1 with the contribution of 94.39%. The interaction was detected using the AMMI1 biplot. The correlation of main effects and IPCA scores can be used in making predictions of yield performance. Small IPCA scores indicate more stable genotypes. Therefore, hybrid NS Gricko had the highest stability of seed yield. Hybrid Rimi PR was the most unstable for the examined trait, but interacted best with red clover in 2017 and alfalfa in 2018, respectively. Hybrid Dukat interacted best with red clover in 2018. The obtained results could be important for the recommendation of sunflower hybrids that are stable and high yielding when intercropped with different legumes.

SUNFLOWER, LEGUMES, INTERCROPPING, AMMI

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GENOMIC PREDICTION IN MAIZE BREEDING

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In recent years the availability of cheap genome-wide markers resulted in a novel approach in maize breeding called genomic prediction (GP). Genomic prediction is a special form of marker assisted breeding, in which genetic markers covering the whole genome are used for estimation genomic breeding value of individuals under selection. Genomic estimated breeding value (GEBV) of an individual represents the sum of effects associated with all the marker alleles present in an individual and included in the GP model applied to the population under selection. The GP is based on training (TP) and breeding (BP) populations. The TP is used for training of the GP model and for estimation of the marker effects required for estimation of GEBV of the individuals in the BP. The BP is the population subjected to GP for identification of the superior lines for use as parents for new hybrid combinations. In real maize breeding programs, successful implementation of the GP is based on data from multi-environment trials. Thus, appropriate modeling and statistical approaches are required to deal with the complexity of the multi-environment grain yield data coupled with genomic and environmental data to speed-up maize breeding.

MAIZE BREEDING, GENOMIC PREDICTION, GEBV, TRAINING AND BREEDING POPULATION

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MAIN YIELD COMPONENTS OF SOYBEAN FULL SEEBS DECREASE UNDER DROUGHT STRESS

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Full sib belongs to reccurent selection methodes, where lines developed from same cross combination (sister lines) are crossed, aimed to accumulate genes determined desired characteristics, grain yield and its components. Soybean grain yield and its components are quantitative traits, their expression is strongly influenced by environmental conditions. Progenies of two full sibs made by crossing three chosen soybean sister lines were grown on two locations during two seasons (2011 as near optimal growing season and 2012 as unfavorable growing season with two drought periods). Grain yield per plant and the most important yield components values decreased in less favorable season. The most expressed decrease was found in the number of pod and number of grain per plant, and the smallest with plant height and 1000 grain mass. In spite of the tested material relatedness, significantly different reaction on drought stress were observed, both between progenies of the two sister combinations and within each of them with grain yield reduction of 18-43% and 5-36%, pods number per plant reduction of 19-51% and 2-33% and grain number per plant reduction of 14-38% and 9-30%. Two progenies showed the smallest reduction in grain yield as well as the number of pod and number of grain per plant, indicating their greater tolerance in conditions of abiotic stress compared with other tested full sib progenies. These progenies can be used for developing soybean lines with higher tolerance to drought stress.

SOYBEAN, FULL SEEB, DROUGHT STRESS

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THE BREEDING INFLUENCE ON THE PHENOLOGY OF SOUTHERN PANNONIAN SIX-ROWED WINTER BARLEY

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In order to decrease negative impact of the climate changes on grain yield production, adjustment of crop phenology with changing climate should represent important breeding objective. As result of breeding activities and changes in agro-ecological conditions, phenology of different cereal crops has been notably changed in past century. However, information about changes in duration of developmental phases of winter barley under conditions of southern Pannonian Plain are scarce.
Therefore, we evaluated changes in developmental pattern in historical set of 15 six-rowed winter barley cultivars from four breeding periods, released in past 50 years. The study was conducted during two growing seasons at the experimental field of the Institute of Field and Vegetable Crops, Novi Sad, Serbia.
Results from our study showed that duration of studied developmental phases varied across growing seasons and cultivars. Year of cultivar release were negatively related with duration of tillering period (r = -0.83), emergence-anthesis (r = -0.75) and total crop cycle (r = -0.69). On the other hand, modern cultivars had prolonged duration of stem elongation phase compared to the older ones. Shortening of time to anthesis was the result of decrease in duration of tillering phase, since duration of stem elongation period was prolonged. There was absence of relationship between duration of grain filling period and year of cultivar release.
Considering that duration of studied traits is closely related with grain yield determination, further fine changes in partitioning of main pre- and post-anthesis phases should represent an important goal for further grain yield increase in six-rowed winter barley.

ANTHESIS, DEVELOPMENT; GENETIC GAIN, *HORDEUM VULGARE*

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BREEDING OF LATE MAURITY HYBRID WITH HIGH YIELDING POTENTIAL

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Maize breeding and selection have been performed at the Maize Research Institute "Zemun Polje" over past 70 years. Many commercial hybrids of various maturity groups (FAO 100-700) have been developed. In recent times, late-silage maize hybrids have been derived at the Maize Research Institute, and newly developed hybrid ZP 7920 of FAO 700 is one of results of such a work. This hybrid is characterised with high and stable yields. Morphological traits of this hybrid indicate that this is a hybrid of a greater habitus (higher plant) and it produces a higher amount of leaf mass that makes it suitable for silaging. The broad erect leaves are vertically orientated forming so called erectophilous stand, while upper-ears are positioned low. Ears are cylindrical with 14-16 kernel rows. Kernels are of the semi-dent type. This hybrid was tested in trials of the Commission for the Varietal Approbation of Cultivated Plants in 2015 and 2016. Trials were conducted at six location during 2015, and 8 locations during 2016. with planting density of 60.000 plants per ha. Sowing and harvesting was done mechanicaly. Each plot consisted of two rows, 7 m long with interrow distance of 75 cm. The experimental data were statistically processed by analysis of the variance (ANOVA). In this period, the recorded grain yield of the hybrid ZP 7920 was significantly higher (by 14.3%) than yields of checks NS 6010 and AS 72. At the same time, the grain moisture content at harvest was satisfactory. Considering everything stated, it is expected that the hybrid ZP 7920 will rank high in the markets of hybrids intended for both ear and silaging.

MAIZE, NEW HYBRIDS, YIELD

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RESPONSE OF TWO-ROW AND SIX-ROW BARLEY TO TERMINAL DROUGHT AND HEAT STRESS

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Changing climates expressed through scarce rainfall and rising temperatures during grain development of temperate cereals have already negatively affected yield gains in regions of lower altitudes. Since spike architecture is one of basic footprints of barley domestication it could be hypothesized the importance of spike forms in adaptation to different environments or abiotic stress. Therefore, in order to compare different barley spike types in terms of kernel growth and yield components 15 two- and 10 six-row winter genotypes were tested in eight environments (year-site-treatment combination) where terminal drought was simulated by plants defoliation at seven days after heading, while control plants were grown intact. The ordinary logistic (s-shaped) model was found to be the most appropriate for kernels dry weight accumulation. Four parameters were estimated from the logistic model. Also, for each environment climatic factors were calculated and their effects on mean kernel growth rate were analyzed. To explore genotype × environment interactions for production per spike regression approach was adopted using climatic data as explanatory variables. On average, two-row genotypes out yielded six-rowed by 17% under control and by 33% under simulated late drought. Maximum kernel weight and mean rate of kernel growth (RG) was higher ($P < 0.05$) in two- than in six-row barleys. The number of days with moderately high (between 25 and 30°C) and high (over 30°C) temperatures had a higher negative effect on RG of two-row barley than six-row barley. On the other hand, minimum temperatures were more negative for RG of the six-row barley than two-row barley. In general, two-row barley showed better adaptation to low yielding environments, while six-row barley was more responsive to high yielding environments.

BARLEY TYPE, GRAIN FILLING, DROUGHT, HEAT STRESS

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DROUGHT STRESS INDICES AND THEIR CORRELATIONS WITH YIELD AND GRAIN FILLING PARAMETERS OF BARLEY

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In two-years field trials, set in two locations with two treatments we studied yield and grain filling parameters of 25 two- and six-rowed barley genotypes. One treatment was control (C), while in the other treatment (D) through the inhibition of current photosynthesis (as result of defoliation 7 days after flowering of each genotype) simulation of drought conditions was performed. In order to quantify the drought resistance, several indices that can serve as selection criterion were calculated. These indices, SSI (drought susceptibility index) and STI and TOL (drought tolerance indices) have shown that two rowed barley genotypes had better resistance to drought than six rowed. Of all tested genotypes, Maksa had the highest ranks for all three stress indices STI (2), SSI (3) and TOL (4), combined with very high ranks for the averaged and the yield in both treatments YD (1), YC (3) and YL (3). The highly significant correlation coefficients between yield in CT and DT (0.730***) indicated that the selection under normal conditions would lead to a yield increase in drought conditions and vice versa. The STI index was the most reliable parameter for the yield increase in stress conditions caused by terminal drought, for such conditions the genotypes with the highest yield and the highest values of the STI index should be selected. The factor analysis showed that in the control conditions, genotypes with the highest individual grain weight, average and the maximum grain filling intensity were the least sensitive to drought stress. In the terminal stress conditions, the most tolerant genotypes were filling grains rapidly, achieving the highest individual grain weight and eventually the highest yield.

BARLEY, STRESS INDICES, TERMINAL DROUGHT, GRAIN FILLING PARAMETERS

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HERBICIDE IMPACT ON CONTENT OF PHENOLIC COMPOUNDS IN SWEET MAIZE

Jelena Mesarović, Jelena Srdić, Snežana Mladenović Drinić, Milena Simić, Milan Brankov, Branka Kresović, Vesna Dragičević

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Worldwide consumption of sweet maize, in the past ten years, is significantly increased. Such data indicate that phytochemicals content requires a higher attention in addition to yield. Popularizations of phenolic compounds become worldwide trend due to their benefits to human health. The usage of herbicides is widely present for weed control in sweet maize growing practices. In this study the influence of two herbicides, on the content of ferulic and cinnamic acid as well as on the total phenolic compounds (TPC) in three sweet maize hybrids during two vegetation seasons was evaluated. Meteorological conditions in 2016 were favorable for maize growing, opposite to 2017 which was a drier year. The variability of examined phytochemicals after applied herbicides was genotype-dependent and also influenced by growing seasons. Interestingly, for hybrids ZP355su and ZP553su, a higher content of ferulic acid was found in treatment with nicosulfuron in comparison to mesotrione. The same trend was noticed for cinnamic acid content in all tested hybrids. Opposite trend was achieved for ferulic acid content and TPC in ZP515su. Content of ferulic and cinnamic acid negatively correlated with the maize yield, opposite to TPC, for hybrid ZP355su. Positive correlation of TPC and cinnamic acid to maize yield was found in hybrid ZP515su emphasizing it as promising hybrid among all tested hybrids. Although relatively small number of hybrids was examined, the obtained data revealed a new potential of herbicide, i.e. the enrichment of health promoting compounds in sweet maize grain.

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NICOSULFURON, MESOTRIONE, MAIZE GRAIN, FERULIC ACID

06 – 53 Poster

SEED QUALITY OF SOYBEAN CULTIVARS SUITABLE FOR GROWING IN EUROPE

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Given the potential of soybean (*Glycine max* L. Merr.) as a staple crop for food, feed and pharmaceutical industry, improving the genetic basis of seed quality is one of the main breeding aims on a global scale. The aim of this three-year trial (2010-2012) was to give insight into the soybean seed quality parameters of 22 cultivars suitable for growing in Europe. Field trials were set up as a randomised complete block design with two replicates at the Agricultural Institute Osijek (Osijek, Croatia). The determined seed quality parameters were: protein and oil content, saturated fatty acid content, monounsaturated fatty acid content, polyunsaturated fatty acid content, total saccharide content, total oligosaccharide content, total isoflavone content. Soybean oil and protein contents are the most important seed quality parameters. The usage and value of oil are mainly determined by its fatty acid composition, which affects physical, chemical and nutritional properties. Saccharide composition affects digestibility and nutritional value of soybean seed, affecting the taste and usability while isoflavones are nutraceuticals with many different medical benefits, important for the functional food market and pharmaceutical industry. The results indicate the variability between genotypes for all tested parameters while the influence of environmental conditions was not significant only for total saccharides. The obtained results should be beneficial in determining and planning future breeding strategies, thus enhancing the added value properties of final soybean products for industry and end consumers, making it an integral part of sustainable production.

SOYBEAN, SEED QUALITY, BREEDING, VARIABILITY

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MULTIVARIATE ANALYSIS OF TOMATO GENOTYPES BASED ON MORPHOLOGICAL AND CHEMICAL FRUIT PROPERTIES

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In recent decades the importance of tomato considered as “functional food” is reflected in the rising trend of harvested areas and consumption per capita. During three consecutive years (2010-2012) at experimental fields in Rimski **Šančevi** near Novi Sad, five landraces, four old varieties, eight breeding lines and three commercial cultivars were chosen for the investigation. Following fruit characteristics were analyzed: average weight (g), length (cm), width (cm), pericarp thickness (mm), locule number, moisture content (%), total soluble solids (°Brix), ash content (%), total acidity (%) and pH value. For the most fruit characteristics high variability was determined. Four principal components explained 90,6% of total variance or 36.5%, 24.2%, 19.8% and 10.1, respectively. Along the axis of the first main component, genotypes were classified into three groups. The objective of this study was to classify 20 tomato genotypes based on physical and chemical traits and to segregate perspective genotypes for improvement of tomato quality by breeding programs.

PRINCIPAL COMPONENT ANALYSIS, QUALITY, TOMATO

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ASSESSMENT OF METHOD EFFICIENCY FOR SUNFLOWER INOCULATION WITH *MACROPHMINA PHASEOLINA*: IMPORTANT STEP IN SUNFLOWER TOLERANCE TESTING

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The fungal disease, Charcoal rot, caused by *Macrophomina phaseolina* is becoming one of important pathogen in sunflower production, mostly expressed in areas with high temperatures and dry conditions. Development of tolerant sunflower genotypes is the most reliable way to control this disease. It is important to find proper evaluation method which can provide accurate insight in potential genetic control of tolerance in sunflower. The aim of this work is: to compare two inoculation methods of sunflower with *M. phaseolina*, to compare the tolerance of inbred lines to this pathogen and to determine which method gives better insight into the inheritance of tolerance in sunflower. The experiment was determined under greenhouse conditions and two methods were used. The Unwounded stem base infection method (USBI), is less aggressive approach where the infection is similar to natural conditions. While for the toothpick method (TM) inoculation it is necessary to puncturing the tissue with the toothpick and create the entrance for pathogen infection. Four inbred lines were used in research which was created at the Institute of Field and Vegetable Crops, Novi Sad (IFVCNS). The inoculation method efficacy and sensitivity of inbred lines, was evaluated based on the length of rotted area covered with microsclerotia, after the sunflower stalks were split longitudinally. The results in USBI and TM showed that, USBI successfully infected one inbred line L4 (5.0 cm), while the TM method was more efficient infesting two inbred lines (L1 and L4). Line L4 (5.5 cm) had statistically significantly higher length of rotted area covered with microsclerotia than L1 (1.3 cm). Inbred lines L2 and L3 did not showed any symptoms. It can be concluded that although USBI method is more similar to natural infection, TM is more suitable for giving an insight to tolerance of sunflower inbred lines for Charcoal rot.

CHARCOAL ROT, INBRED LINES, USBI, TOOTHPICK METHOD, FUNGAL DISEASE

SESSION 7

Microbial genetics

VI CONGRESS OF THE SERBIAN GENETIC SOCIETY

MOBILE INTEGRONS: SENSING THE CELL PHYSIOLOGY

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Mobile class 1 integrons have been recognized as genetic elements involved in rapid dissemination of genes encoding for antibiotic resistance. Despite the fact that the dynamics of gene cassette recombination depends on expression of the class 1 integrase (*intI1*) gene; the regulation of *intI1*gene expression has not been sufficiently explained so far. Previously, a binding motif of LexA protein was identified within the promoter of the *intI1* gene. When the SOS response is activated, LexA undergoes autoproteolytic inactivation and the *intI1* gene expression is activated. This control allows a subtle coupling of integron activity with bacterial physiology. However, initially simplistic understanding of the *intI1* gene expression regulation was revised when significant differences in regulation among different bacterial species were revealed: (i) control through the cAMP receptor protein in *Vibrio cholerae*; (ii) nucleoid proteins, Fis, IHF and H-NS affect expression in *E. coli*; (iii) stringent response regulates the *intI1* expression in *E. coli* biofilms independently of the SOS response. Our study was performed on *Pseudomonas* spp. model. We have shown the role of RpoS and PsrA proteins in positive regulation of integrase transcription during the stationary phase of growth. Additionally, we determined that the activity of the *lexA* gene promoter was decreased in *rpoS* and *psrA* mutants. Thus we proposed that PsrA indirectly regulates the *intI1* gene promoter activity through regulation of the *lexA* gene expression in co-operation with additional regulators. Also, Tn*5* transposon mutagenesis and subsequent site-directed mutagenesis enabled identification of a novel two-component system (TCS) regulating *intI1* transcription in *Pseudomonas* spp. The role of this TCS in complex network of the *intI1* expression regulation, as well as in regulation of other cell processes, will be determined by wild-type and TCS mutant transcriptome sequencing.

RESISTANCE TO ANTIBIOTICS; GENE EXPRESSION REGULATION, *PSEUDOMONAS* SPP.

SEQUENCING OF STREPTOMYCES SP. NP10 GENOME – A KEY TO THE TREASURE CHEST

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In post genomic era the whole genome sequencing (WGS) is only a start point that offers an alternative, but complementary approach to standard biodiscovery. In that light, sequencing genome of *Streptomyces* sp. NP10, a strain from our laboratory collection, not only supported the previous research on its secondary metabolites but spurred further discovery of important enzymes. NP10 stands out by its ability to produce exceptionally high amounts of fatty acids (FA) that have characteristics of secondary metabolites, some of them detected in living organisms for the first time. Owing to WGS it was possible to pinpoint synthesis of these FA to an unusual cluster C12-2 whose inactivation almost completely abolished their synthesis. Further, genome mining confirmed the presence of the genetic elements that corresponded to metabolic and physiological features of NP10: DNase and hemolytic activity, cellulase, endoglucanase, and catalase activity, hydrolysis of urea, degradation of arabinose and xylose, high salt and low temperature tolerance. By carefully narrowing our search and utilizing different bioinformatics tools and databases, we were able to isolate a highly active cutinase/lipase. This enzyme, important in biocatalysis, can be repurposed for plastic modification and degradation. Finally, specific search for biosynthetic gene clusters (BGC) revealed over 30 of them spanning from polyketides and non-ribosomal peptides synthesis to terpene and ectoine production. Most of these small molecules cannot be detected in the cultivation medium during standard cultivation, but once identified, BCGs could be activated by biotechnological engineering or heterologously expressed. However, our inability to detect cyclic dipeptide BGC (although we were able to detect cyclic dipeptides in the cultivation broth) shows that in spite of tremendous advances in bioinformatic predictions that make genome mining a reliable approach to enzyme discovery, there are a few shortcomings that still need to be conquered.

GENOME MINING, BIODISCOVERY, BIOINFORMATICS

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SURVIVING OXIDATIVE STRESS - NEW CELLULAR FUNCTIONS INVOLVED IN RECYCLING AND GENOME PROTECTION IN *USTILAGO MAYDIS*

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Ustilago maydis is a unicellular phytopathogenic fungus known for its extreme resistance towards UV and ionizing radiation. This feature selected *U. maydis* as a powerful model system for studying DNA repair and homologous recombination.

As a free-living microorganism and biotrophic fungus infecting maize, ustilago is exposed to various harsh environmental stresses throughout both of its life styles. *U. maydis* cell population subjected to severe stress could be rescued, to a large extent, by efficient DNA repair of harmed DNA. But powerful DNA repair system may not be the only cellular facility underlying genome protection and overall ecological success of *U. maydis*.

We showed that after severe oxidative insult, *U. maydis* cell populations restore viability if kept under starvation conditions. This restitution of viability is not based on intracellular repair of damaged macromolecules but rather on cell multiplication at the expense of the intracellular compounds freed from the damaged cells. Moreover, the capability of the survivors to recycle intracellular compounds depends on the level of treatment-induced damage of cytosolic molecules. The findings indicated that *U. maydis* must possess cellular mechanisms involved not only in reabsorption of the released compounds from external environment but also in contending with their treatment-induced toxicity. Nine genes contributing to the process were identified by mutant hunt, phenotypic characterization and gene cloning (*adr1, did4, kel1, tbp1, snf8, slm1, chk1, snf5* and hypothetical heatschock UMAG11087). Mainly from studies in other organisms, these cellular factors are known to play roles in growth regulation, protein turnover, cytoskeleton organization, transcription, cell cycle regulation, chromatin remodeling and stress response. Importantly, some mutants exhibited sensitivity to different genotoxic agents implying that the gene products are in some overlapping fashion involved in the protection of genome integrity.

OXIDATIVE STRESS, STARVATION, LIQUID HOLDING, GENOME INTEGRITY

SPECIFICITY OF THE PATHOVAR OR UBIQUITY OF THE SPECIES: *THE CASE OF PSEUDOMONAS SYRINGAE PV. APTATA*

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Pseudomonas syringae is complex species and the most frequently emerging group of plant pathogenic bacteria. During 2013, in Vojvodina fields 104 bacterial isolates from sugar beet that showed symptoms of bacterial leaf spot disease was gathered. Isolates were identified as *Pseudomonas syringae* pv. *aptata* using pathovar specific primers. DNA fingerprinting methods revealed high genetic variability represented with 25 groups of profiles. Even more variability was detected when PFGE and MLSA were included in the analysis, clustering the isolates in 3 and 4 major groups, respectively. Partial sequence of the citrate synthase housekeeping gene (*cts*) put all the analyzed isolates into 02d phylogenetic group of the *P. syringae* complex. Also, high diversity in pathogenicity of isolates was detected. Isolates belonging to the same group according to their aggressiveness belonged, in most cases, to the same MLST cluster. Although *P. syringae* pv. *aptata* is denominated as a pathogen specific for sugar beet, the host range analysis conducted for 20 isolates on 16 plant species showed very diverse spectrums. Moreover, two isolates exhibited the capacity to cause leaf spot disease in all tested plants. These results, combined with more extensive analysis of numerous *P. syringae* strains/pathovars pointed out that host range in the whole of *P. syringae* complex is an overlapping continuum of pathogenic potential. In addition, like most pathogenic bacteria, *P. syringae* is not obligatory parasitic; it has the potential to thrive in environment as a saprotroph. To study bacteria beyond plant-pathogen context we isolated *Pseudomonas* spp. from waters of the Sava and Danube rivers. Less than 2% of total *Pseudomonas* isolates were identified as *P. syringae*. To investigate the potential of such a scarce subpopulation of *P. syringae* to cause contamination of agricultural fields as well as to probe the notion that non-agricultural environment could be important reservoirs of new disease outbreaks, analysis of water from the water baring channels conecting large rivers with fields is, thus, necessary.

PSEUDOMONAS SYRINGAE, DNA FINGERPRINTING, MLSA, CTS, PATHOGEN RESERVOIRS

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07 – 05 Invited lectures

BACTERIOPHAGES AND PHAGE-ENCODED DEPOLYMERASES

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Bacteriophages are the most numerous biological entities on the planet, occupying every habitat on Earth. Although they induce high mortality on bacterial realm, phages are seen as the driving forces of bacterial evolution and diversification. As natural killers of bacteria, their potential in treating bacterial infections was acknowledged immediately after their discovery, a century ago. However, with the lack of understanding of phage biology and the emergence of antibiotics, the "phage therapy" was abandoned in the West. The perception changed with modern day rise of antibiotic resistance. Advantages of phage therapy include its potency against bacterial biofilms, structures underlining much of the reported resistance to antibiotics. Biofilm matrix consists of extracellular polymeric substances (EPS) which provide many advantages to bacterial cells especially in terms of resistance to various agents. But as with every other bacterial adaptation, phages evolved ways to subvert it and developed enzymes that degrade EPS polysaccharides, allowing them access to surface receptors. These enzymes, depolymerases, are phage-encoded and usually located on tail fibers of phage particles, but can also be released from bursting bacterial cells as soluble enzymes.

We isolated several phages active against multidrug-resistant *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, three members of the WHO's list of bacteria for which new antibiotics are urgently needed. The phages were isolated from Belgrade wastewaters, purified, screened for host range against laboratory collection and characterized based on plaque and virion morphology. *A. baumannii* phages NOVI and ISTD produced halos around plaques on majority of sensitive strains, indicating the presence of potent depolymerase(s). Their adsorption rate, one step growth curve and SDS–PAGE profiles were determined as well as their antibiofilm activity, while genome sequencing and depolymerase purification are underway.

PHAGES, BIOFILM, ANTIBIOTIC RESISTANCE, DEPOLYMERASES

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07 – 06 Oral

BACILLUS CEREUS NM1 ISOLATED FROM SOIL PRODUCES TWO-COMPONENT LANTIBIOTIC THUSIN, ACTIVE AGAINST MULTIRESISTANT PATHOGENS

Nemanja Mirkovic[1,2], Mina Obradovic[1], Branko Jovcic[1,3], Paula O`Connor[4], David J. Studholme[5], Goran Vukotic[1,3], Paul Cotter[4], Milan Kojic[1]

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Increasing number of multidrug resistant pathogens in the world generates a need for discovering new antimicrobial compounds. Bacteriocins, bacterial antimicrobial peptides, are considered as potential alternatives to antibiotics. Understanding of how these peptides work (mechanism of action) or better characterization of bacteriocins is one of the main goals in the field.

The aim of this study was to (i) isolate cultivable bacteria from soil, (ii) screen for production of antimicrobial peptides (iii) identify bacterial strains with antimicrobial activity against various pathogens and (iv) characterize antimicrobial compound/s.

For isolation of bacteria, serial tenfold dilutions were spread onto LB plates. After incubation at 37°C for 48h, fifty colonies per plate were randomly selected, inoculated into LB broth and incubated overnight at 37°C. Among the randomly selected isolates from soil, only one - NM1 - showed a strong inhibitory activity against *Staphylococcus aureus*, *Listeria monocytogenes* and clinical *Streptococcus agalactiae* strains. It was identified by 16S rRNA gene sequencing as *Bacillus cereus*. Bacteriocin was extracted from the supernatant and purified to homogeneity by C18 solid phase extraction and reversed phase HPLC. Based on the amino acid sequence and molecular mass of the active fraction, the bacteriocin from NM1 was identified as thusin, a two-component lantibiotic isolated previously from *Bacillus thurigiensis* BGSC4BT1. Spontaneously resistant mutants of *S. agalactiae* were isolated on thusin. Whole genome sequencing of Wt and three resistant mutants was performed in order to identify mutations causing resistant phenotype.

In sum, we characterized bacteriocin thusin from *B. cereus* NM1, and showed its broad spectrum of antimicrobial activity. Also, we isolated spontaneous mutants of *S. agalactiae* resistant to thusin, however the localization (mapping) of the mutations is still in progress.

BACTERIOCIN, THUSIN, RESISTANT MUTANT, *STREPTOCOCCUS AGALACTIAE*

07 – 07 Poster

BACTERIAL COMMUNITY STRUCTURE ASSOCIATED WITH *PRUNUS DOMESTICA CULTIVARS*

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European plum (*Prunus domestica*) is significant industrial crop in Serbia with a high share in total fruit production. Distribution and occurrence of bacteria associated with resident plum cultivars are largely unknown. The objective of this study was to identify the culturable bacteria from phyllosphere of four plum cultivars. The leaf and fruit samples from four local Serbian plum cultivars, Požegača, Ranka, Čačanska lepotica and Čačanska rodna were collected from orchard, not treated with pesticides, in two consecutive years (2015-2016) during different leaf and fruit phenological stages (May and July). Partial 16S rDNA gene sequences and reference sequences were used for phylogenetic tree construction using Neighbor-Joining method. Based on 16S rDNA sequences, 17 different genera were identified from all four cultivars. All identified isolates corresponded to genera commonly isolated from plants. The genera *Arthrobacter, Bacillus, Clavibacter, Curtobacterium, Erwinia, Frigoribacterium, Frondihabitans, Labedella, Micrococcus, Oerskovia, Pantoea, Pseudarthrobacter, Pseudomonas, Rahnella, Sanguibacter, Sphingomonas, Staphyllococcus, Therribacillus* have been isolated. The prevalent genus detected was *Pseudomonas*, present in both phenological stages. Important plant pathogen *P. syringae* well known for its capacity to grow epiphytically on diverse plants was detected on every plum cultivar. *Bacillus* and *Sphingomonas* known for their antagonistic activity against pathogens were also present. According to detected genera in two consecutive years there are differences in their occurrence on cultivars and in phenological stages suggesting that the cultivar of the plant contributes to the structure of the bacterial community associated with plum.

BACTERIAL DIVERSITY, PHYLLOSPHERE, *PRUNUS DOMESTICA*, 16S rDNA

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07 – 08 Poster

TRANSFORMATION PROTOCOL OPTIMIZATION FOR *BACILLUS SUBTILIS*, *BACILLUS LICHENIFORMIS* AND *BACILLUS THURINGIENSIS STRAINS*

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Many members of the genus *Bacillus* have the ability to produce a large number of different enzymes, antimicrobial compounds and other secondary metabolites, making them very important for many industries, agriculture and medicine. The natural competence of these strains is the physiological and genetic property expressed under certain conditions or constitutively. However, the incompetent *Bacillus* strains can be brought into the state of competence by the treatment with chemical or physical agents that enable their transformation with exogenous DNA. For *Bacillus* spp. transformation pA13 and pAZIL plasmids were used. Transformation of naturally competent *Bacillus* strains was more efficient if performed at the stage of growth when cells are naturally competent. From four different methods examined on the strain *Bacillus subtilis* 168 we determined that for successful transformation the addition of manganese sulphate in the growth medium was essential. *Bacillus licheniformis* 30.3 was successfully transformed when medium contained maleic acid as buffer and succinate for cell regeneration. The protoplasts regeneration on succinate was a key step in the production of *B. licheniformis* 30.3 transformants. *Bacillus thuringiensis* HD1 was successfully transformed by the method which used Tris-induced competence, and longer incubation in the medium of competence was very significant for the transformation efficiency. All the transformants were obtained only with pA13, a rolling circle replication plasmid. In conclusion, efficiency of *Bacillus* spp. transformation is species specific and depends on the characteristics of the plasmids used.

TRANSFORMATION, *BACILLUS* SPP., PLASMID

07 – 09 Poster

THE ROLE OF INTRACELLULAR TRAFICKING IN RECONSTITUTION OF OXIDATIVE DAMAGED POPULATION OF USTILAGO MAYDIS

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Ustilago maydis is a phytopathogenic fungus exhibiting extreme resistance to UV and ionizing radiation. Even regarding this distinctive feature that marks *U. maydis* out among eukaryotes, the molecular knowledge that would have a direct bearing on our understanding of its ecological success is still fragmentary. Our results show that after heavy exposure of a population of *U. maydis* cells to oxidative damage a great increase in viability is observed if the treated cells are incubated for prolonged period in distilled water (starvation). This restitution of viability is achieved through cell multiplication of survivors by reabsorption of the intracellular compounds released from the killed cells. This finding suggests that *U. maydis* must possess cellular mechanisms involved in recycling (reabsorption, processing and reuse) of the leaked material for cell growth and multiplication. By screening mutants defective in capability to use these compounds we identified four genes (*kel1*, *slm1*, *snf8* and *did4*) that contribute to the process. Interestingly, these mutants are also sensitive to different genotoxic agents implying that the gene products are involved in genome protection. Two of the genes, *kel1* and *slm1*, are involved in cytoskeleton reorganization and vesicle movement. The protein products of the other two genes, *snf8* and *did4*, are parts of ESCRT-II and ESCRT- III complexes, which are known to play roles in vesicular trafficking and in destruction of damaged proteins. Obtained results will have a strong impact on our understanding of the cellular mechanisms that enable *U. maydis* populations to reabsorb and process the leaked, potentially harmful cellular waste.

OXIDATIVE STRESS, RECYCLING, VESICULAR TRAFFICKING

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07 – 10 Poster

CHARACTERIZATION OF A NOVEL CUTINASE FROM *STREPTOMYCES* SP. BV286

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Cutinases are members of the α/β- hydrolase superfamily. They possess an enormous potential for a variety of industrial applications based on their ability to hydrolyze a wide range of substrates. The majority of information regarding the characteristics of cutinases refers to fungal cutinases with significantly less information on bacterial cutinases. The advantages of prokaryotic enzymes in industrial application and the large number of predicted bacterial cutinases opened up possibilities for the discovery of novel cutinases with desirable properties. Therefore, a novel cutinase (designated CUT286) from *Streptomyces* sp. BV286 was identified, expressed and biochemically characterized.

Streptomyces sp. BV286 was isolated from the rhizosphere of *Papaver rhoeas* and displayed cutinase activity in enzyme assays. Cutinase gene that was identified from the genome of this strain showed similarity with the cutinase from *Malbranchea cinnamomea*. pRSET B vector was used for cloning and expression of cutinase in *E. coli* ROSETTA (DE3) cells. CUT286 was partially purified using affinity chromatography and its esterase activity was determined in the standard cutinase assay with para-nitrophenilbutyrate (pNPB) as a substrate. Activity of CUT286 was highest in neutral to slightly acidic environments (pH 5.0 - 7.0) with a sudden drop of activity at pH levels higher than 8.0. CUT286 exhibited optimal activity at pH 5.0 and 60 °C, and high stability at 80 °C retaining 85% of its original activity after two hours. Additionally, CUT286 was stable in 15% v/v of the following organic solvents: dimethyl sulfoxide (DMSO), dimethylformamide (DMF), methanol and acetonitrile. All these properties, mainly thermostability, make cutinase from *Streptomyces* sp. BV286 suitable for potential industrial applications.

CUTINASE, *STREPTOMYCES*, BIOCATALYSIS, CHARACTERIZATION, THERMOSTABILITY

07 – 11 Poster

CHARACTERISATION OF NEWCASTLE DISEASE VIRUS GENOME ISOLATE FROM SERBIA BY NEXT GENERATION SEQUENCING

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Newcastle disease virus (NDV) from *Avulavirus* genus of *Paramyxoviridae* affects many avian species and is transmissible to humans causing conjunctivitis and influenza like disease. NDV appears in about 20 genotypes. Depending on the severity of disease in poultry NDV is classified into three pathotypes, velogenic, mesogenic, and lentogenic, determined by F protein cleavage site. Sequencing of complete NDV genome is the first step in genotyping, determination of origin and transmission paths. In this study we performed detailed analyses of NDV genome sequence obtained by massively parallel sequencing technology (Next-generation sequencing, NGS).

Viral isolation from bird tissue of the Columbidae samples collected in 2007 was performed by inoculation of chicken embryos. After allantoic fluid harvesting, the total RNA extraction was followed by DNAse treatment, reverse transcription of total RNA and dsDNA synthesis using random primers. NGS data was prepared by Illumina Nextera flex library preparation kit on Illumina iSeq 100 platform with a paired read length of 150nt. For sequence quality control, trimming, mapping and variant calling the Bioconda distributed packages were used in the following order: Fastqc, Trimmomatic, Bioconductor-RBowtie2 and Perl-bio-samtools. MEGA v. 7.0 software was used for phylogenetic analyses.

Near full genome sequencing identified the presence of NDV in the analyzed samples. The overall number of obtained reads was 423742 in both directions. After the final quality trimming step, based on the reference sequence for NDV genome (NC_ 039223.1), 405114 or 95.60% of paired reads remained while the rest was removed. Phylogenetic analyses clustered our isolate in Class II, genotype VII, with the isolates from Ukraine, Bulgaria and same isolates from China. The sequence obtained for the F gene showed that our NDV strain had a cleavage site motif 112R–R–Q–K–R–F-117, characteristic for highly virulent velogenic strains.

NEWCASTLE DISEASE VIRUS, NEXT-GENERATION SEQUENCING

07 – 12 Poster

IDENTIFICATION AND GENETIC DIVERSITY OF *PSEUDOMONAS PUTIDA* STRAINS ISOLATED FROM SAVA RIVER USING SPECIES-SPECIFIC REP ELEMENTS

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Pseudomonas putida represent a rapidly growing bacterium frequently isolated from most temperate soils and waters. It is a nutritional opportunist par excellence and the paradigm for metabolically versatile microorganisms that recycle organic wastes in aerobic compartments of the environment. Repetitive extragenic palindrome (REP) elements represent 35bp sequences composed of conserved inverted repeats that could be detected as a single independent unit or a part of different clusters in the chromosome. The REP sequence has been extensively characterized in *P. putida*, where they are present in the chromosome more than 800 times with a high degree of conservation and species-specificity. In this regard, the study represents valuable information about identification and intra-species genetic diversity among *P. putida* isolates collected from the Sava River in Serbia, using amplification of specific REP elements. Twenty one isolates from water samples collected from 4 sites at the Sava River were identified as *P. putida*. High degree of genetic diversity among the isolates was distinguished by detection of 8 different REP profiles, where the bands were amplified in a range from 150bp to 4kb, with majority between 1kb - 3kb. *P. putida* has a large potential for application in biotechnology, which includes control of environmental pollution, plant growth promotion and control of plant pathogens. Identification and determination of genetic diversity of *P. putida* offer significant insight into their population structure in the Sava River, representing the first step of characterizing these very potent strains which could play a key role in the maintenance of environmental quality.

PSEUDOMONAS PUTIDA, GENETIC DIVERSITY, REP ELEMENTS

07 – 13 Poster

GENODIVERSITY OF XANTHOMONAS *CAMPESTRIS PV. CAMPESTRIS* ISOLATED FROM LEAVES OF BRASSICACEAE PLANTS

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Brassicas are a significant source of edible vegetables and is the third most important source of oil on the world level. Black rot, caused by the bacterium *Xanthomonas campestris pv. campestris* (XCC) represents the most destructive Brassicas diseases, generally. The bacteria is widespread on all continents, and is present in our country, especially at the places where Brassicas are grown in monoculture. Particularly susceptible are cabbage local genotypes, which are highly appreciated by consumers. Given the importance, frequency and distribution of XCC, there was a need to study the diversity of this pathogen on cabbage, cauliflower, kale, broccoli and oil seed rape. Monitoring was conducted from 2014 to 2017 on the territory of the Republic of Serbia. From different Brassicas 82 bacterial isolates from leaves were collected. All isolates form yellow colonies on the YDC medium and based on biochemical-physiological characteristics were identified preliminarily as XCC. Further identification was done using m-PCR by primer sets Zup 2309/2310 and DLH 120/125, showing amplicons of 370 and 619 bp in all tested isolates, as well as in reference strain NCPPB 1144, and confirmed isolates as XCC. Characterization of isolates by rep-PCR (BOX A1R and (GTG)5 primers) and RAPD (SPH1, OPA8, DJ15 and DJ16 primers) was done. In both cases, all tested XCC isolates and reference strain NCPPB 1144 formed one cluster, while the second cluster contained reference strains belonging to *Xanthomonas* genera (*X. axonopodis pv. phaseoli, X. perforans, X. vesicatoria and X. euvesicatoria*), but other than XCC, showing 56% and 57% differences for BOX and RAPD, respectively. At the 95% level of similarity, 9 different BOX patterns and 14 different RAPD patterns were obtained. Characterization of XCC isolated from leaves in Serbia will enable the comparison of isolates from different origin and facilitate the monitoring in epidemiological studies of this pathogen.

XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS (XCC), REP-PCR, RAPD, M-PCR, BRASSICAS DISEASES

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07 – 14 Poster

EFFECT OF COLD STORAGE ON THE PRESENCE OF FUNGAL SPECIES

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The occurrence of fungal species was examined in four maize grain accessions which were kept for different time under gene bank conditions. Samples were regenerated three times, e.g. in 1985, 2012 and 2016, and afterwards 12 samples in total, were stored in a cold room under medium storage conditions. The aim was to determine the occurrence of fungal species.

The 2012 maize growing season was characterised by extremely hot and dry conditions. A significantly lower amount of precipitation was recorded in this year than in the remaining two years. The recorded drought conditions were favourable for the growth of certain *Aspergillus* species, which resulted in the presence of *Aspergillus* section Flavi in 75% of the examined maize samples, while *Fusarium* species section Liseola, were detected in 33.23% of the samples. The highest amount of precipitation in the 2016 maize growing season in combination with temperatures close to optimum levels resulted in the presence of *Fusarium* species in 65.28% of maize samples, while *Aspergillus* species were not detected. In samples from 1985, the most dominate *Aspergillus* species belonged to the section Niger (44.80%) which were registered in all investigated samples.

The occurrence of fungal species was not affected by storage conditions (temperatures 4°-10°C, relative humidity of 40%). Significant differences in the appearance of fungal species were observed under various weather conditions during all years of investigation. The obtained results indicate that the occurrence of *Fusarium* section Liseola is characteristic for years with high precipitation sums, such was 2016. Maize grain contamination with *Aspergillus* section Flavi was recorded in dry and hot year of 2012.

Maize is continually exposed to the toxigenic fungi whose species can produce highly toxic compounds-mycotoxins. The presence of mycotoxins in maize should be recognised as a significant concern since these contaminants may have negative impact on human and animal health.

FUNGAL SPECIES, COLD STORAGE, MAIZE

07 – 15 Poster

COMPARISON OF TWO GENES, 16S rDNA AND *tuf*, FOR IDENTIFICATION OF *BACILLUS* SPECIES

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The genus *Bacillus* is a genetically diverse group of endospore-forming bacteria with cosmopolite distribution. The discrimination between *Bacillus* species is mainly based on 16S rDNA and housekeeping gene sequences. However, the problem of identifying members of *Bacillus subtilis* and *Bacillus cereus* groups remains. Given that *Bacillus* species can be used in agriculture as crop protective agents, their precise identification as well as the exclusion of the potential human pathogens is of crucial importance.

The objective of this work was to compare the usefulness of the partial 16S rDNA (the hypervariable region) and the *tuf* gene sequence for identification of 33 *Bacillus* spp. strains obtained from mushroom growing substrate.

Amplification of the hypervariable region of 16S rDNA and the *tuf* gene was carried out using 16S-HV-F/16S-HV-R and tufGPF/tufGPR primers, respectively. The PCR products were sequenced and the obtained sequences subjected to multiple alignment and editing in BioEdit program, followed by the Neighbor-joining phylogenetic trees construction using MEGA 6.06 software.

The sequence analysis of the hypervariable region of 16S rDNA showed that 23 *Bacillus* spp. belong to *B. subtilis* species, six isolates were identified as *Bacillus pumilus*, three belonged to *Bacillus amyloliquefaciens* species and one strain was closely related to *Bacillus licheniformis*. In addition, *B. subtilis* and *B. amyloliquefaciens* phylogenetic branches were clearly separated. In contrast, the *tuf* gene sequence analysis failed to achieve a precise distinction between *B. subtilis* group members and species belonging to *B. pumilus* group.

The results indicate that chosen hypervariable region of 16S rDNA represents better genetic marker than the *tuf* gene for identification of closely related *Bacillus* species.

The research was supported by the Ministry of Education, Science and Technological development of the Republic of Serbia (OI 173026 and TR 31043).

BACILLUS, HYPERVARIABLE REGION, *tuf* GENE, PHYLOGENETICS

SESSION 8

Bioinformatics and big data analysis

VI CONGRESS OF THE SERBIAN GENETIC SOCIETY

08 – 01 Invited lectures

EVOLUTION FROM "T" TO π SHAPED PROFILE OF A RESEARCHER IN THE ERA OF miRNOMICS

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Traditional profile of a researcher had to provide both horizontal knowledge encompassing general and cross-disciplinary competences and vertical depth in leading domain specialization (forming a T-shaped profile). However, the rapid growth of genomic data produces an "evolutive pressure" on a modern geneticist toward the new emerging model, denoted as π-shaped, which adds another vertical competence relative to the computational abilities required for integrative interpretation of the data.

MicroRNAs (miRNAs) are tiny genetic rheostats, regulating gene expression by targeting transcripts for cleavage or translational repression. Yet, the growing number of described miRNAs and their complex activity hinders functional miRNome exploration. To tackle this issue, our team adopted the π-shaped approach for systemic, integrative analysis of miRNA activity in complex diseases.

We have integrated transcriptomic signatures of congenital anomalies of the kidney and urinary tract (CAKUT) and miRNA target predictions from multiple prediction algorithms to identify key miRNAs associated with these anomalies. Subsequent experiments confirmed the upregulation of miR-144 in CAKUT. The same method was also applied on publicly available transcriptomic data in atherosclerosis and type 2 diabetes where novel miRNA biomarker candidates were suggested. Furthermore, using network analysis on in-house transcriptomic data, major miRNA regulatory hubs potentially orchestrating pathophysiology in myocardial infarction and chronic otitis media were identified.

The ouroboric loop of understanding *in vivo* miRNAs mechanisms and *in silico* modeling of miRNA activity is making an increasing impact on future research of human physiology and disease. Our results confirm the importance of computational approaches in making foundations of further research in prevention and therapy, based on the growing potential of miRNAs to become clinically applicable biomarkers and therapeutic targets.

MIRNOME, MICRORNA, BIOINFORMATICS, NETWORK

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08 – 02 Invited lecture

EXOME-SCALE VARIATION DATA IN A SERBIAN COHORT STUDY

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Although the importance of population diversity in genomic research has long been recognized, smaller populations, like the Serbian population, are under-represented in genetic data repositories. In order to respond to this need, to fill in the sparse zones of the global genomic map, we analyzed exome data obtained using the next generation sequencing (NGS) platform TruSightOne (Ilumina), in a cohort of unrelated individuals from Serbia, sequenced as part of disease-specific genetic studies. Variant calling was performed by using the GATK (Genome Analysis ToolKit). The over-represented variants are annotated with population allele frequencies and public resources such as GnomAD, 1000G and ClinVar and effect predictions tools, ISTREE and the Ensembl Variant Effect Predictor (VEP). Here we present preliminary findings on genetic variants specific for a Serbian cohort such as those in genes RP1L1, KRT4 and SHANK3. The obtained results will improve the characterization of population-specific, germline and somatic disease associated variants in a wider context. This is the first-ever report on exome-scale data in the thus far under-studied Serbian population. Our study represents the first step towards an ethnically relevant reference dataset, which would be subsequently integrated into precision medical initiatives relevant to individuals of Serbian and Western Balkan descent worldwide.

VARIATION DATA, SERBIAN COHORT, CLINICAL EXOME DATA, ALLELE FREQUENCIES

08 – 03 Invited lecture

LEAF TOTAL TRANSCRIPTOME ANALYSIS ENRICHES INSIGHT INTO MAIZE ABIOTIC STRESS SIGNALING NETWORK

Bojana Banović Đeri [1], Dragana Dudić [2], Manja Božić [3], Pavle Janić [4], Lana Džodanović [4], Jelena Samardžić [1], Dragana Ignjatović-Micić [3], Jelena Vančetović [3], Sofija Božinović [3], Dejan Dodig [3], Nenad Delić [3], Ana Nikolić [3]

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Abiotic stresses cause great losses in crop production worldwide. Due to climatic changes temperatures higher than the average are frequently observed in temperate areas in the course of maize flowering and grain filling period, displaying detrimental effect on maize yield. In order to overcome this problem development of drought tolerant maize lines, but also cold tolerant maize lines that would allow earlier sowing and hence avoidance of extremely high temperatures during sensitive developmental phases in maize, became important goal of maize breeding programs in temperate areas. A set of 46 maize inbred lines was chosen from three main heterotic groups (BSSS, Lancaster, Iowa dent) important for commercial breeding programs. Plants were grown under the optimal conditions in the greenhouse and sampled upon entering V5 growth stage. A total RNA isolated from leaves of three plants of each inbred line was used for DNA library preparation by Illumina TruSeq Stranded RNA LT kit. Pair-end sequencing was performed on MiSeq Illumina sequencer using MiSeq Reagent kit v2 (2x150bp). For data manipulation and analysis a custom made bioinformatics pipeline was used. Differential gene expression analysis revealed a minimum of 21 genes that were strongly statistically supported for differential expression between the inbred lines and also annotated as involved in abiotic stress responses in other plant species. We tested 10 of these genes in maize by using qPCR on the subset of 8 inbred lines grown under optimal, cold and drought conditions. Real-time PCR results revealed significant change in the expression level of tested genes under abiotic stresses, supporting bioinformatics prediction. Verified genes were then submitted to free online gene networking tools and after determining relationships between predicted networks and inbred line phenotypes, possible novel gene candidates for enriching maize abiotic stress signaling network resolution were proposed.

MAIZE, TRANSCRIPTOME, DIFFERENTIAL GENE EXPRESSION, COLD, DROUGHT

08 – 04 Poster

FORWARD-IN-TIME METHOD VS. ARTIFICIAL NEURAL NETWORKING AS A TOOL FOR SIMULATION OF GENETIC DATA

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Simulation studies in population and evolutionary genetics play a significant role in better understanding of different evolution scenarios and effects of different genetic models on genetic diversity. Forward-in-time method is designed in such a way that it starts with an initial population and follows entire evolution under different genetic models within multiple generations. On the other hand, artificial neural networks represent a formidable method for genetic simulation and prediction. In this project, we wanted to compare and corroborate results obtained with forward-in-time simulation which was done in a specialized simulation program with results attained from a specially designed strategy based on cluster analysis/ unsupervised learning using R programming language. As input data, alleles from 13 microsatellite loci from 219 specimens representing autochthonous Adriatic haplotype of *Salmo trutta* L. were used. This species was chosen as a model organism because it has been a center of many previous studies as well as because populations of *Salmo trutta* L. from the Neretva river were under the constant influence of major anthropogenic factors. Finally, genetic variation within these populations was disturbed due to hybridization and introduction of different allochthonous populations in its natural habitat. The main goal of this research is to reconstruct possible genetic structure of *Salmo trutta* L. population before major anthropogenic influence, and then make an assessment of possible deviation in genetic diversity of recent populations with populations from a period before human influence. The second goal was to compare precision and reliability of these two methods for different purposes. Our results are in concordance with other reports from literature which indicate that both of these approaches can be used as a reliable simulation tools. However, it is believed that artificial neural networks can represent more powerful simulation tools.

FORWARD-IN-TIME, ARTIFICIAL NEURAL NETWORK, SIMULATION, MICROSATELLITE

08 – 05 Poster

FUNCTIONAL ANNOTATION OF NOVEL GENES IN *DROSOPHILA MELANOGASTER*

Branislava Gemović [1], Milica Aleksić [1], Milan Đorđević [1], Milica Medić 1, Katarina Stanković [1], Alexandra Jones [2], Zoran Obradović [3], Rob Kulathinal [2], Nevena Veljković [1]

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New genomic sequencing technologies are providing an ever-increasing amount of assembly data that are awaiting functional interpretation. A number of function prediction algorithms have been developed to enable automatic function annotation of genes with the majority of tools based on machine learning classifiers using various features including homology-based scores, secondary structure, amino acid composition, protein-protein interaction networks, and Gene Ontology (GO) terms. Annotation of evolutionary young genes is particularly challenging. In arguably the best annotated model organism, Kondo et al. (2017) described 59 young genes in *Drosophila melanogaster* that are proposed to be essential but for which there was no functional information. We applied state of the art *in silico* methods for functional annotation of the genes of unknown function using a workflow that encompasses two steps: 1) functional annotation with established and well-validated tools, GOLabeler, PANNZER and INGA, and 2) functional predictions based on majority voting among tools. A majority of novel genes (n=37) were functionally annotated using our pipeline with 34 newly predicted functions per gene, on average. Half of these predictions represent unanimous agreement between all three tools. QuickGO slim analysis of terms that were predicted for 5 or more genes demonstrated clusterization of functions related to phosphorylation, microtubule-based movement, protein kinase activity, hydrolase activity, Ras GTPase binding, ion binding, nucleus and cytoskeletal components, which represent essential functions involved in cellular machinery. Our study shows promising achievements of a combined bioinformatics approach in resolving the functional characteristics of novel genes connected to species-specific behaviours.

NOVEL GENES, *DROSOPHILA MELANOGASTER*, FUNCTIONAL ANNOTATION, IN SILICO ANALYSIS

08 – 06 Poster

REML ANALYSIS OF RANDOM MODEL FOR GROWTH AND CARCASS VALUE TRAITS IN GILTS

Mladen Popovac [1], Dragan Radojković [1], Milica Petrović [1], Dragan Stanojević [1],Aleksandar Miletić [2], Radmila Beskorovajni [3]

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The aim of this paper was to assess the ratio of random effects in total phenotypic variability of growth and carcass value traits in gilts in order to obtain necessary parameters for assessing the genetic potential of these animals by means of some reference methods (selection index, BLAP-AM). Following gilt traits were analyzed: daily live-weight gain at the end of the test (ADG), age at the body mass of 100 kg (TT), average loins and back fat thickness (BF), longissimus dorsi muscle depth (MLD) and percentage of meat in carcass (PM). All examined traits were analyzed for two random effects, i.e. the litter in which gilts were born and raised and an animal direct additive genetic effect estimated on the basis of kinship matrix. It was determined that litter in which gilts were born and raised accounts for 45% of total variability for ADG, 13% for TT, 6% for BF, 6% for MLD and 7% PM. An animal direct additive genetic effect, that is, heritability coefficients for ADG, TT, BF, MLD and PM for all traits were h2 = 0.06; 0.24; 0.52; 0.06; 0.51, respectively.

REML ANALYSIS, HERITABILITY, ADDITIVE GENETIC EFFECT, GILTS

08 – 07 Poster

BIOINFORMATICS PREDICTION OF MITOCHONDRIAL AND CHLOROPLAST TRANSCRIPTS POSSIBLY INVOLVED IN MAIZE ABIOTIC STRESS SIGNALING

Bojana Banović Đeri [1], Lana Džodanović [2], Pavle Janić [2], Jelena Vančetović [3], Dragana Ignjatović-Micić [3], Jelena Samardžić [1], Ana Nikolić [3]

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In plants, the expression of mitochondrial and chloroplast genomes is continuously changing to enable adequate functional adjustments related to overall metabolic changes needed for plant adaptation to fluctuating environmental conditions. In order to identify mitochondrial and chloroplast transcripts expressed prior to stresses, we have analyzed mitochondrial and chloroplast transcriptomes of 46 maize inbred lines chosen from three main heterotic groups (BSSS, Lancaster, Iowa dent) regularly used in elite maize breeding programs. Organelles' data were extracted from transcriptome data obtained by next generation sequencing of total leaf transcriptomes of 46 maize inbred lines grown under the optimal conditions in the greenhouse. All data manipulation and differential gene expression analysis were performed by using Galaxy software (https://usegalaxy.eu/). Detected organellar complex transcripts milieus were expected to contain candidates that modulate responses of their genomes by activating certain signaling pathways upon exposure to abiotic stresses. With the aim to identify such candidates we focused on selecting transcripts that were differentially expressed between 46 maize inbred lines grown under the optimal breeding conditions and also annotated as important for abiotic stresses response in different plant species. Selected transcripts/genes were then subjected to freely available tools for gene networking analysis with the aim to depict possible anterograde and retrograde signaling between nucleus and mitochondria/chloroplasts under the abiotic stresses. Obtained results are promising in terms of planning biological experiments for testing the roles of genes that were bioinformatically predicted as novel members of abiotic stress signaling network between nucleus and organelles in maize.

ORGANELLE, TRANSCRIPTOME, ABIOTIC STRESS, MAIZE

SESSION 9

Miscellaneous topics

VI CONGRESS OF THE SERBIAN GENETIC SOCIETY

09 – 01 Invited lecture (Molecular biotechnology)

ENZYMATIC PRE-TREATMENT OF BIOMASS FOR IMPROVEMENT OF BIOGAS PRODUCTION

Giuliano Degrassi

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Production of biogas from biomasses and organic residues by anaerobic digestion using methanogenic bacteria is an important biotechnological process for sustainable production of biofuel. The yield of biomethane in this process usually ranges only from 40 to 60%. This is mainly due to the difficult metabolism of the plant cell wall components by the microbial consortium present in the digestor, due to the complexity of cellulose, hemicellulose and lignin. Cellulose is very abundant and its full conversion into methane would increase the efficiency of the process. Biogas production from polysaccharides and other biopolymers occurs through four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Hydrolysis is the critical step and very often the bottle neck. It is known that bacterial consortia can degrade cellulose. We identified three bacteria with efficient cellulolytic properties and developed three heterologous expression systems for production of the following enzymes: endoglucanase (*Bacillus pumilus*), cellobiosidase (*Xanthomonas* sp.), β-glucosidase (*Bacillus amyloliquefaciens).*

These three enzymes participate in the depolymerization of cellulose that occurs in three steps: (i) cellulose polymer cleavage and oligomers formation; (ii) release of dimers (cellobiose); (iii) hydrolysis of cellobiose into glucose.

The three genes encoding the above-mentioned enzymes were expressed in *E. coli* and in the yeast *Pichia pastoris.* The endoglucanase was purified to homogeneity and characterized. The final goal of the project is the development of an enzymatic cocktail for pretreatment of feedstock to increase the efficiency of the biogas production process.

BIOGAS PRODUCTION, ENDOGLUCANASE, CELLOBIOSIDASE, β-GLUCOSIDASE

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09 – 02 Invited lectures (Plant genetics)

CRISPR/Cas9 MUTAGENESIS AS A TOOL TO GAIN INSIGHT INTO THE ROLES OF HIGHLY HOMOLOGOUS PLANT DSS1 GENES

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DSS1 is a small protein, highly conserved across different species. As a member of intrinsically disordered protein family, DSS1 interacts with different protein partners, thus forming complexes involved in diverse biological functions. For instance, human DSS1 is a mediator in cellular processes such as: DNA repair; regulation of protein homeostasis; mRNA biogenesis, splicing and export, etc.

Yet, DSS1 involvement in maintenance of genome integrity through homologous recombination is its only function well studied in *Arabidopsis*. None of the other known functions of DSS1 was investigated in plants. Also, the fact that *Arabidopsis thaliana* genome contains two highly homologous AtDSS1(I) i AtDSS1(V) genes, suggests a question of whether or not these gene paralogs share similar functions.

Using CRISPR/Cas9-mediated target mutagenesis, we obtained two stable lines of *Arabidopsis* containing mutations in either DSS1(I) or DSS1(V) gene. Here we present efficient strategies for the selection of targets, design of single-guide RNA, vector construction and analysis of transgenic lines. High resolution melting method followed by Sanger sequencing enabled us to select plants with desired mutations in DSS1 genes. The mutants with 2 nt substitution in AtDSS1(V) and 1nt gene insertion in AtDSS1(I) gene were chosen for further study. The former mutation led to altered ORFs and the latter caused premature stop codon. Morphological analysis of the single dss1 mutant plants reveled differences in rosette shape, stem length and branching pattern. In addition, the dss1(V)-/dss1(V)- lines showed increased sensitivity to oxidative stress as compared to the wild type plants.

In conclusion, as a very precise method for mutagenesis CRISPR technology proved to be a beneficial and supreme method to explore the roles of duplicated genes sharing highly similar DNA sequences in plants.

CRISPR/CAS9, DDS1 GENE, OXIDATIVE STRESS, *ARABIDOPSIS*

09 – 03 Poster (Plant breeding)

RATIO OF DOMINANT AND RECESSIVE GENES OF IMPORTANT QUANTITATIVE TRAITS IN SUNFLOWER

Milan Jocković [1], Slaven Prodanović [2], Sandra Cvejić [1], Siniša Jocić [1], Petar Čanak [1], Ana Marjanović-Jeromela [1], Vladimir Miklič [1], Jelena Ovuka [1]

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For designing efficient breeding procedure adequate information on the genetic structure of the parents and mode of gene action affecting the yield and its related traits are prerequisite for progress in breeding. The objective of this research was to evaluate heritability, components of genetic variance as well as ratio of dominant and recessive genes in parental genotypes of sunflower. The plant material selected for this research consisted of sunflower genotypes distinguished by important characteristics for the production of sunflower. Quantitative traits evaluated in this study were seed yield/plant, oil content, 1000 seed weight, head diameter and plant height. Presented results showed significant variability of evaluated quantitative traits. Phenotypic variance was higher than genotypic demonstrating strong environment effect in expression of evaluated traits. The values of broad sense heritability for evaluated traits were very high for plant height, high for 1000 seed weight, moderate for seed yield/plant and head diameter, while low for oil content. The analysis of components of genetic variance indicated prevalence of dominant component (H) compared to additive (D) and higher concentration of dominant genes (u) compared to recessive (v), in all investigated traits. Position of expected line of regression pointed over dominance in inheritance for seed yield/plant, oil content and head diameter, while for 1000 seed weight and plant height was found that additive gene action played role in inheritance suggesting that selection in early generations for these traits will be effective. Comparing the sequences of the scattering diagrams indicated the presence of interallelic interaction which was discarded after testing the coefficients of regression.

HERITABILITY, PHENOTYPIC VARIANCE, GENOTYPIC VARIANCE, DOMINANT COMPONENT

09 – 04 Poster (Modulation of gene expression: Pharmacological Aspects)

GENE EXPRESSION OF PREFRONTAL DOPAMINE BIOSYNTHETIC ENZYME IN CHRONICALLY STRESSED RATS TREATED WITH LITHIUM

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Our earlier research confirmed that chronic restraint stress (CRS) induced decreased gene expression of tyrosine hydroxylase (TH) a "rate-limiting" enzyme of dopamine biosynthesis and decreased concentrations of dopamine (DA) in the prefrontal cortex (PFC). In addition, we confirm that CRS influenced anxiety and depressive-like behavior in rats. In pathophysiology of mood disorders lithium is known as an effective drug in the long-term stabilization of moods. However, very little is known about gene expression of TH and concentration of DA in the PFC in chronically stressed rats treated with lithium. Therefore, this study aimed to investigate the effects of mood stabilizer lithium on gene expression of TH, as well as concentration of DA in the PFC in animals exposed to CRS (2 hours × 14 days). The investigated parameters were quantified by real-time RT-PCR, Western blot analyses and ELISA kits. In the present study we found that lithium treatment increased levels of TH mRNA by 56% ($p<0.05$), TH protein by 22% ($p<0.01$) and concentration of DA by 72% ($p<0.001$) in chronically stressed rats to the levels found in unstressed animals, which indicates that lithium enabled de novo synthesis of prefrontal DA in chronically stressed rats. Lithium may have induced gene expression of TH in stress condition trough the activator protein-1 (AP-1) transcription factor pathway. The results presented here suggest that lithium treatment may modulate gene expression of prefrontal TH and increase concentration of prefrontal DA in chronically stressed rats to the levels found in unstressed animals.

GENE EXPRESSION, LITHIUM, CHRONIC RESTRAINT STRESS, DOPAMINE

09 – 05 Poster (Plant biology)

THE POLLINATION SCHEME FOR NATIONAL AND INTRODUCED SWEET CHERRY (*PRUNUS AVIUM L.*) CULTIVARS IN GROWING REGIONS OF THE REPUBLIC OF SERBIA

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Sweet cherry (*Prunus avium* L.) is basically a self-sterile fruit species, showing gametophytic self-incompatibility (GSI), controlled by the multiallelic locus S. The knowledge of GSI system and practical application of its main principals have a key role in productivity of commercial sweet cherry orchards. Sufficient overlap in phenophase of full flowering is another crucial factor for effective pollination and fertilization success between compatible cultivars. Production of sweet cherry fruits in the Republic of Serbia is mainly based on the genetic potential of introduced cultivars, which are predominantly represented in the assortment structure. Up to now, three national sweet cherry cultivars have been named and released – Asenova Rana and Čarna (developed by planned hybridization at Fruit Research Institute, Čačak), and Canetova (selected from natural cherry population at Faculty of Agriculture, University of Belgrade). The assessment of an adequate pollenizer composition for both national and introduced sweet cherry cultivars in this research is based on determined S-allele constitution, parameters of pollen tube growth in vivo through the pistil tissue, and perennial average flowering time of pollinated cultivars in relation to the potential pollenizers. The research was conducted in the main sweet cherry growing regions of the Republic of Serbia (Western Serbia/Šumadija and the region of Belgrade), resulting in development of a pollination scheme for a total of 28 national and introduced cultivars. The application of pollination scheme in the plant material production and establishing new orchards could be of great importance for dissemination of commercially significant sweet cherry cultivars with adequate pollenizers. The results could also influence the breeding strategies in developing new sweet cherry genotypes with improved biological and productive characteristics, and application of molecular and reproductive biology methods in this research area.

SWEET CHERRY, CULTIVARS, S-ALLELE CONSTITUTION, FLOWERING, POLLINATION

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09 – 06 Poster (Genetic Resources for Breeding)

S-RNase ALLELE IDENTIFICATION AND INCOMPATIBILITY GROUP ASSIGNMENT IN SWEET CHERRY (PRUNUS AVIUM L.) INDIGENOUS GENOTYPES

Slađana Marić [1], Sanja Radičević [1], Nebojša Milošević [1], Milica Fotirić Akšić [2], Radosav Cerović [3], Milena Đorđević [1], Ivana Glišić [1]

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Sweet cherry (*Prunus avium* L.) is one of the economically most important members of the Rosaceae family. In this fruit species, most genotypes are self-incompatible and certain pairs of genotypes are cross-incompatible. This incompatibility is genetically determined by the multiallelic two linked genes of S-locus (S-RNase and SFB genes), with gametophytic action. Therefore, determination of S-alleles of new sweet cherry genotypes is highly valuable tool for growers to design orchards, and for breeders to choose parents in breeding programmes. This work was undertaken primarily to identify the S-alleles and incompatibility groups in 15 indigenous sweet cherry genotypes collected in orchards of individual growers in the Republic of Serbia, i.e. 11 genotypes in the region of Čačak ('GT-1', 'GT-2' and 'GT-4' to 'GT-12') and four genotypes in the region of Belgrade ('GT-13' to 'GT-16'). The S-alleles of each genotype were determined by PCR amplification of the S-RNase gene, with consensus primers for the first and second introns, as well as with allele-specific primers. The obtained results revealed eight alleles (S1, S2, S3, S4, S5, S6, S9 and S12), that generated the following eight S-genotypes: S1S5 ('GT-1'), S2S3 ('GT-7', 'GT-14', 'GT-15' and 'GT-16'), S3S4 ('GT-10' and 'GT-12'), S3S6 ('GT-4' and 'GT-13'), S3S9 ('GT-8'), S3S12 ('GT-5' and 'GT-6'), S6S9 ('GT-2' and 'GT-11') and S5Sx ('GT-9'). S2S3 was the most frequent allelic combination (27%), which was observed in four assessed genotypes. The S-allelic constitutions allowed assignment of the genotypes to their corresponding incompatibility groups as follows: III ('GT-10' and 'GT-12'), IV ('GT-7', 'GT-14', 'GT-15' and 'GT-16'), VI ('GT-4' and 'GT-13'), X ('GT-2' and 'GT-11'), XIV ('GT-1'), XVI ('GT-8') and XXII ('GT-5' and 'GT-6'). S-genotyping results represent important information on cross-compatibility in these local genotypes and also reveal the S-locus diversity of sweet cherry indigenous material.

PRUNUS AVIUM, AUTOCHTHONOUS GENOTYPE, S-ALLELIC CONSTITUTION, GAMETOPHYTIC SELF-INCOMPATIBILITY

09 – 07 Poster (Plant-microbe interaction)

GENOTYPIC VARIATION IN THE RESPONSE OF SWEET PEPPER ON SEED PRIMING WITH SELECTED *BACILLUS* AND *PSEUDOMONAS* STRAINS

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The worldwide increase in human population followed with industrialization led to environmental changes as a result of water, soil and air pollution. It is believed that one way to address this problem is to change the existing agricultural approaches and to use plant growth promoting bacteria (PGPB) instead of fertilizers and pesticides. The present investigation was aimed to evaluate the influence of PGPB on sweet pepper seed priming. Pepper seeds (*Capsicum annuum* L.) from six different genotype, 115 and 116 (peppers for spice), 261 (sivri type), 133, 258, and 274 (kapia type) were primed with 10-9 CFU/ml of *Bacillus safensis* (SS-2.7), *B. thuringiensis* (SS-29.2), *Pseudomonas putida* (4.3) and *P. protegens* CHA0 strains. Seeds were primed during one hour and after three weeks root and seedlings length, and the total mass of the pepper seedlings were analyzed. One and two-way analysis of variance (ANOVA) and Least Significant Difference test (LSD test) with a 0.05 probability level was used for statistical analysis. Generally, we could not see the difference between these parameters in seeds primed with water as control and any of the strain used (one-way ANOVA). However, there is a different response to the same strain and seeds from different genotype. Seed priming with the *B. thuringiensis* (SS-29.2) gave the highest total weight and shoot length for genotype 274, root length for genotype 116, and plant number for genotypes 133 and 274. Results obtained for the root length after treatment with selected strains grouped genotypes 115 and 116 in one group and 258 and 261 in another group. Genotype 133 had the porrest response after seed priming with selected strains, while genotype 274 showed the best response on seed priming with almost all strains.

GENOTYPIC VARIATION, PEPPER, PLANT GROWTH PROMOTING BACTERIA

09 - 08 Poster (Seed viability)

TESTING OF SEED VIABILITY USING DIFFERENT TESTS

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Tetrazolium test is a biochemical test that distinguishes viable from non-viable seeds based on breathing activity in the seed itself. The test finds application as a backup procedure for identifying a viable, but dormant, i.e. dormant seeds that failed to germinate during the germination test. The tetrazolium test is not an absolute test of the viability of the seed, and to make the conclusions valid, the results obtained by applying this test must be compared with the results of other germination tests for each plant species. This study included seed of two oil type and one confectionery sunflower hybrids, separated by size and weight into six fractions. Testing seed germination was carried out on four replicates of 100 seeds by applied Standard laboratory test and tetrazolium test. Obtained results were statistically analysed by applying analysis of variance. The results of the study show that in the case of oily-type hybrids, higher germination is obtained by using a tetrazolium test. Contrary to these results, in the confectionery hybrid, significant differences were observed that occurred between the seed fractions themselves. Germination in the tetrazolium test was the highest in the smallest fractions, while in the case of large it was lower by about 20%, which resulted in germination was lower in this test, on average. From these results it can be concluded that the genotype itself had a significant influence on the test property, and that it is necessary to know the characteristics of each genotype, especially when it comes to seed of lower quality.

SUNFLOWER, SEED, GERMINATION, TESTS

09 - 09 Poster (Plant biology)

EFFECT OF FOLIAR NUTRITION ON THE PRODUCTIVITY OF BUCKWHEAT - *FAGOPYRUM ESCULENTUM* MOENCH

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Buckwheat – *Fagopyrum esculentum* Moench is annual, monocarpus plant from the family *Polygonaceae,* the genus *Fagopyrum*, from the group of alternative cereals. Its origin is Central Asia. Buckwheat is a highly valued food in human nutrition, especially in countries where emphasis is placed on food that is health - safe. In the diet of humans and domestic animals, grain (fruit, nut) is used which has similar nutritious and nutritive value as grain cereals. Above-ground biomass buckwheat is used for feeding domestic animals and is the best quality when plants are in the end of blossom period. Buckwheat can be used as a siderate and is also suitable for bee pasture. Honey has medicinal properties.

On the parcels of the Institute of Field and Vegetable Crops in Backi Petrovac (ϕ N 45° 20', λ E 19° 40', 82 mls), the variety of buckwheat Novosadska in 2018 was tested in two variants: 1. control - variant without nutrition and 2. variant with foliar nutrition. The analysis of variance of the yield indicator showed that there was a significant variability for the studied properties of the buckwheat between the control variant and the variant with foliar nutrition. The variety of buckwheat Novosadska achieved statistically significantly higher yield (2,200 kg ha^{-1}), the height of the plants (150 cm) and the weight of 1000 grains (24.20 g) in the variant with foliar nutrition compared to the control variant ($p < 0.01$).

Based on the results of the research, it is evident that foliar nutrition are desirable in the buckwheat crop, since in the variant with the nutrition, statistically significantly higher productivity of buckwheat was achieved.

Investigations necessary for this paper are part of the project TR 31025 financed by the Ministry of Education, Science and Technology Development of Republic of Serbia and bilateral projects (Montenegro and Serbia; 2019-2020): “Alternative cereals and oil crops as a source of healthcare food and an important raw material for the production of biofuel“.

FAGOPYRUM ESCULENTUM, FOLIAR NUTRITION, 1000 GRAIN MASS, GRAIN YIELD

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09 - 10 Poster (Plant molecular genetics)

ARABIDOPSIS DSS1(V) PROTEIN AS POTENTIAL PARTICIPANT IN RESPONSE TO OXIDATIVE STRESS

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DSS1 gene encodes small and conserved protein which belongs to intrinsically disordered protein class. This protein has unstructured 3D form, where as a partner associates with other protein complexes and play vital roles in various biological processes. It is known that the DSS1 protein is involved in maintenance of genomic integrity and protein homeostasis within the 26S proteasome system. Recent study has proposed another potentially new role which implies specific recognition and binding to the oxidized proteins. Thus marked damaged proteins further promote removal by the ubiquitin-protease system. The mechanism of action is not quite clear. Furthermore, two plant isoforms of DSS1 have been detected in *Arabidopsis thaliana*, AtDSS1(I) and AtDSS1(V). These homologs are generally expressed in all organs at all developmental stages. Of note, as the data related to the DSS1 function in plants is very limited, in this work we examined susceptibility of AtDSS1(V) homozygous line mutants with T-DNA insertion to oxidative stress induced by methyl viologen (MV). In order to demonstrate the role of AtDSS1 in overcoming the effects of oxidative stress, dss1(V) mutant and wild-type (WT) seedlings *Arabidopsis thaliana* were grown on solid medium containing MV. After treatment, increasing trend of lipid peroxidation (LPO) was detected in plants, as an indicator of oxidative stress. Elevated presence of oxidized proteins in Atdss1(V) mutants exposed to MV was shown by OxyBlot methodology. Also, total chlorophyll content in dss1(V) seedlings was lower than in WT Arabidopsis, grown with or without MV. Changes in the expression profile of total AtDSS1 proteins were analyzed by Western blot. The Arabidopsis dss1(V) mutants were slightly more sensitive to the stress and grow more slowly compared to WT. With regard to the new suggested function, the results indicate that AtDSS1(V) protein may have role in defense mechanisms against oxidative stress in plants exposed to abiotic stress.

ATDSS1, OXIDATIVE STRESS, *ARABIDOPSIS THALIANA*

WORKSHOP 1

Personalized medicine: promise and reality

VI CONGRESS OF THE SERBIAN GENETIC SOCIETY

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INTRODUCTION

PERSONALIZED MEDICINE: OLD CONCEPT, NEW ACHIEVEMENTS

PANEL DISCUSSION TOPICS:

PERSONALIZED MEDICINE - THE BEST PATH FORWARD?

ACHIEVEMENTS AND BOTTLENECKS

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PERSONALIZED MEDICINE – WHERE DO WE AND OUR TEAMS STAND?

WORKSHOP 2

The truth is in wine and DNA - applications of molecular methods in viticulture

VI CONGRESS OF THE SERBIAN GENETIC SOCIETY

Workshop 2 Vitis workshop **– 01 Oral**

DISEASE RESISTANCE LOCI IN GRAPEVINE: HAPLOTYPE DIVERSITY AND IMPLICATIONS FOR AN EFFICIENT MARKER ASSISTED SELECTION

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In the past decades, several disease resistance loci have been characterized in domesticated grapevines (Vitis vinifera) and in their wild relatives (Vitis spp.), the most common source of resistance haplotypes. All of them have the commonality of spanning clusters of NB-LRR genes. Haplotype diversity in R gene clusters is usually amplified by molecular events of gene and segmental tandem duplications and invasion of different types of transposable elements, which generate high structural diversity. *Vice versa*, nucleotide sequence diversity in conserved regions within these loci not always reflects species phylogeny, because the crop species retains diversity that predates speciation. Structural diversity and incomplete lineage sorting in the genus Vitis are the main complications for the development of efficient molecular markers to trace resistance haplotypes in natural populations and in breeding material.

VITIS VINIFERA, BREEDING, NB-LRR

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Workshop 2 Vitis workshop **– 02 Oral**

REGISTRATION OF WINE VARIETIES

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At the moment, the number of officially registered Vitis varieties worldwide is 8.383. Each new variety must be different from all varieties which already exist. In the process of registration, following UPOV and CPVO guidelines, a new variety is compared with all other varieties grown in the test field. The problem is that it is impossible to have a test field with all existing varieties, especially with varieties originating from distant areas. Therefore, the possible solution to overcome this obstacle is to employ DNA markers. This procedure is not yet officially recognized and adopted by the official bodies in Europe and worldwide. Nonetheless, this process began, the first proposals have been put forward, and conditions for the official usage of DNA markers in the process of registration of Vitis varieties will be defined in the near future.

REGISTRATION VARIETIES, DNA MARKERS, DUS TRIALS

Workshop 2 Vitis workshop **– 03 Oral**

DNA FINGERPRINTING OF SERBIAN GRAPEVINE VARIETIES BY MEANS OF NUCLEAR MICROSATELLITES

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Grapevine, Vitis vinifera L., cultivated in some regions of Serbia since the Roman Empire, is nowadays grown on c. 20.000 ha. Along with modern vineyards established using varieties with certified identity, old and sometimes abandoned vineyards, which represent a possible reservoir of local and potentially unknown varieties, are also common in Serbia. The Serbian Ministry of Agriculture, Forestry and Water Management has recognized the importance of characterizing local varieties at morphological and molecular levels and safeguarding those that are threatened by extinction. A procedure for grape DNA fingerprinting by means of nuclear microsatellites, established in the frame of several European research projects, has been implemented in Serbia at the Institute of Molecular Genetic and Genetic Engineering (IMGGE) in the frame of the of the EU IPA II Twinning Project "Strengthening capacities of phytosanitary sector in the field of plant varieties registration, including improvement of variety testing authorities". We genotyped 12 Serbian grape varieties grown in the germplasm repository of Sremski Karlovci whose genetic profiles have been compared against 3.888 genotypes available at the Vitis International Variety Catalogue (VIVC, http://www.vivc.de/). We demonstrate that this methodology represents a powerful tool not only for distinguishing Serbian grapevine varieties but also for resolving synonymy/homonymy, which is rather common in grapevines, and in paternity and kinship analysis. We highlight the importance of joint future efforts of molecular geneticists, breeders, vinegrowers and policy makers for the valorization of grapevine varieties available in Serbia, which will help breeders in exploiting local genetic resources and foster the production of quality grapes and wines in Serbia.

GENOTYPING, SIMPLE SEQUENCE REPEATS (SSRS), VITIS VINIFERA

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Workshop 2 Vitis workshop **– 04 Oral**

AUTOCHTHONOUS AND REGIONAL VINE VARIETIES – GENETIC POTENTIAL AND CLONAL SELECTION

Darko Jaksic [1], Ivan Bradic [2], Milos Ristic [3], Radoslav Milic [2], Ivana Mosic [3], Bojan Mandic [4], Jelena Aleksic [5], Bojan Duduk [6], Pierfederico La Notte [7]

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As a country which strives to join the European Union, Serbia is looking forward to the possibility of using the EU CMO measures for development of the viticulture and wine production sector, primarily the measure for restructuring and conversion of vineyards with replacement of vineyards with autochthonous and regional vine varieties. On the other hand, the increasing popularity of wines from international vine varieties and grubbing-up of vineyards in the previous period caused permanent loss of numerous old vineyards with autochthonous and regional vine varieties and disappearance of some rare vine varieties. At this moment there are only about 31 autochthonous and regional wine varieties/group of varieties from the total of 200 commercially cultivated vine varieties in Serbia. Because of these reasons, it is necessary to examine and preserve genetic resources of autochthonous and regional vine varieties and to select the best clones through a detailed research and certification scheme.

The Center for Viticulture and Oenology, through the network of its seven centers in the territory of Serbia, carries out evaluations of vineyards with valuable and endangered genetic material and selection of vineyards according to priorities, and, starting two years ago, genetic and phytosanitary clonal selection of some important autochthonous and regional vine varieties.

This paper presents the results of research conducted in about 2000 vineyards within the Serbian wine-growing areas with the purpose of determining the potential for selection of vine biotypes, as well as research on selection of potential clones of some autochthonous and regional vine varieties.

AUTOCHTHONOUS AND REGIONAL VINE VARIETIES, SERBIA, VINEYARDS, CLONAL SELECTION

CLONAL SELECTION OF Vitis vinifera: THE METHOD FOR CONSERVATION OF AUTOCHTHONOUS GRAPEVINE VARIETIES

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Viticulture is a very specific production where old varieties, that have been cultivated for hundreds and hundreds of years, are still important. Population of the specific variety consists of the individuals that are not identical. These differences among the vines are result of the genetic mutations, pests, variation in soil properties and viticulture practices. Vegetative propagation fix mutations and virus diseases. The probability of the existence of intravarietal variability increases for varieties which are known to be cultivated for a long time. Clonal selection eliminates negative mutations and prevent propagation of the individuals that are virus infected. Moreover, clonal selection is the way for conservation of old autochtonous varieties. There are many approaches to do this. However, the best results showed individual positive selection which is based on the best vines in the field. In Serbia, clonal selection activities have been performed at the Faculty of Agriculture in Novi Sad, Faculty of Agriculture in Belgrade, and at the Center for Viticulture and Wine Production in Niš. Until now, in Novi Sad many clones were released: three clones and three subclones of Riesling italico, four clones of Župljanka and six clones of Seduša. In Belgrade thirteen clones of Prokupac were released. Clonal selections of many old autochtonous varieties are in progress.

VITIS VINIFERA, CLONAL SELECTION, OLD GRAPEVINE VARIETIES

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ACHIEVEMENTS IN THE CREATION OF NEW GRAPEVINE VARIETIES IN SERBIA BY HYBRIDIZATION

Dragan Nikolić [1], Dragoslav Ivanišević [2], Nada Korać 2, Petar Cindrić [2] , Dragoljub Žunić [1], Branislava Sivčev [1], Zorica Ranković-Vasić [1], Aleksandar Tabaković [3], Mira Medić [2], Mladen Kalajdžić [2]

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Hybridization is the most important and widespread method for creating new grapevine varieties. It is very old in its origin, just as old as the phylogenetic development of the grapevine. At first, it was performed spontaneously, without human intervention. Today, within all breeding programs, a planned hybridization is performed where parental partners are consciously chosen. Since the sixties of the last century, planned hybridization in Serbia has been carried out at the Faculty of Agriculture, University of Belgrade, and at the Faculty of Agriculture, University of Novi Sad as well as at the Center for Viticulture and Enology Production in Niš. In this paper, the most important goals, types of crossings and results obtained by planned hybridization in these three institutions are presented. Intraspecific and interspecific hybridizations are highlighted as the dominant ways of crossing. As for interspecific crossings, mainly North-American and East Asian species as well as new interspecies hybrids obtained by complex crossings are used as resistance sources. Using intraspecific hybridization, 9 wine and 15 table varieties were created at the Faculty of Agriculture in Belgrade. In addition, 7 wine varieties were created at the Faculty of Agriculture in Novi Sad. By means of interspecific hybridization, 1 wine and 7 table varieties were created at the Faculty of Agriculture in Belgrade, 14 wine and 3 table varieties at the Faculty of Agriculture in Novi Sad, and 2 wine varieties at the Center for Viticulture and Wine Production in Niš. Therefore, a total of 58 new grapevine varieties in Serbia have been so far created by planned hybridization. Despite its large number, the current fund of Vitis vinifera L. varieties , still does not satisfy the existing economy of grapevine growing nor the modern needs of grape and wine consumption, hence, more attention should be paid to the creation of new grapevine varieties.

GRAPEVINE, BREEDING OBJECTIVES, WAYS OF CROSSING, WINE VARIETY, TABLE VARIETIES

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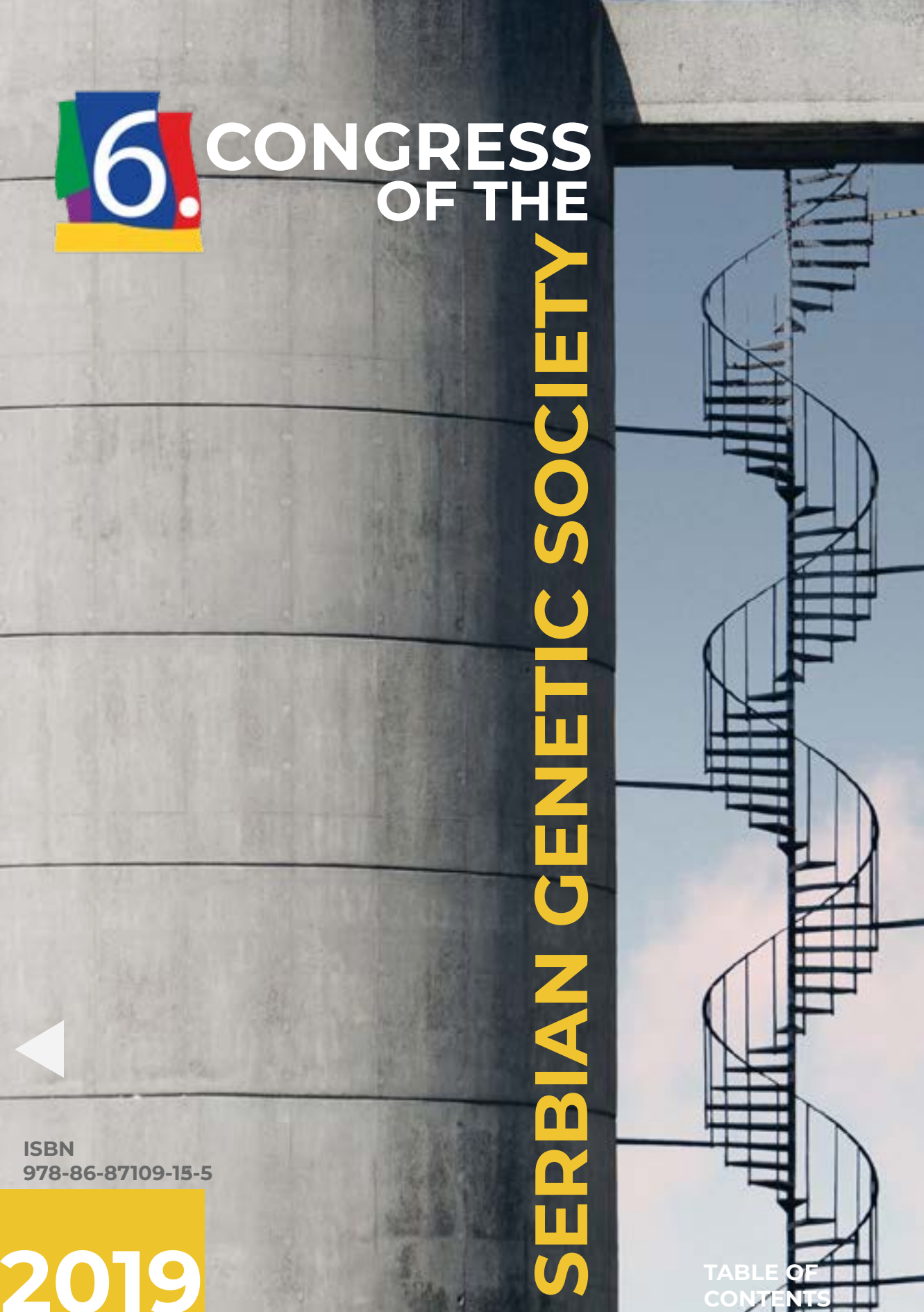

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