СЪЮЗ НА УЧЕНИТЕ В БЪЛГАРИЯ - СЕКЦИЯ „ГЕНЕТИКА“
UNION OF SCIENTISTS IN BULGARIA - BRANCH “GENETICS”

ПЪРВИ МЛАДЕЖКИ СЕМИНАР ПО ГЕНЕТИКА

FIRST YOUNG SCIENTISTS SEMINAR ON GENETICS

Сборник с резюмета

Book of abstracts

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The seminar is organized by the Branch “Genetics” of the Union of the scientists in Bulgaria *with the cooperation of the Faculty of Biology – Sofia University “St. Kliment Ohridski”, the Institute of plant physiology and genetics – Bulgarian academy of sciences and the Agrobioinstitute - Academy of agricultural sciences*

Място на провеждане: Фоайе III етаж в сградата на Биологически факултет на СУ „Св. Климент Охридски“, бул. Драган Цанков № 8, гр. София

Location: the lobby of III floor of the building of the Faculty of Biology, Sofia University “St. Kliment Ohridski”, 8, Dragan Tzankov blvd., Sofia

Съдържание / Content

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Работна програма

9:00 – 11:00	Регистрация	Фоайе III етаж
9:00 – 11:00	Монтиране на постерните табла	Фоайе III етаж
11:00 – 11:30	Откриване – доц. д-р Светослав Димов, председател на организационния комитет	Фоайе III етаж
11:00 – 15:00	Постерна сесия – дискусии на чаша кафе	Фоайе III етаж
15:00 – 15:30	Награждаване на „Най-добър постер“ Закриване на семинара	Фоайе III етаж

Work program

9:00 – 11:00	Registration	Lobby III floor
9:00 – 11:00	Posters mounting	Lobby III floor
11:00 – 11:30	Opening – Assoc. Prof. Svetoslav Dimov, chairman of the Organizing committee	Lobby III floor
11:00 – 15:00	Poster session – discussions with a coffee	Lobby III floor
15:00 – 15:30	Best posters awards Seminar closing	Lobby III floor

Резюмета / Abstracts

БАКТЕРИАЛНА ГЕНЕТИКА / BACTERIAL GENETICS

BG-1. ЕНДЕМИЧЕН КЛОН ОТ НОЗОКОМИАЛНИ ИЗОЛАТИ *ENTEROCOCCUS FAECIUM* С ГЛИКОПЕПТИДНА РЕЗИСТЕНТНОСТ В БЪЛГАРСКИ БОЛНИЦИ – МОЛЕКУЛЯРНО-ГЕНЕТИЧНИ ПРОУЧВАНИЯ

ТРИФОНОВА Ангелина[1], ДИМОВ Светослав[2], СТРАТЕВА Таня[3]
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За периода февруари 2013 – юни 2017 г., с цел установяване на степента на филогенетично родство, бяха събрани 52 клинични щама *Enterococcus faecium*, резистентни на vancomycin (Минимална потискаща концентрация (МПК)>256 mg/L) и teicoplanin (МПК, 4-16 mg/L), изолирани от пациенти в три големи болници в България: Военномедицинска Академия – София (n=44), УМБАЛ „Св. Иван Рилски” – София (n=5) и МБАЛ „Д-р Тота Венкова” – Габрово (n=2). Видова идентификация на щамовете беше извършена с автоматизираната система VITEK 2 (bioMérieux) и потвърдена чрез полимеразно-верижна реакция (PCR) за откриване на sodA гена, кодиращ ензима манган-зависима супероксид дисмутаза. За епидемиологично типизиране беше използван методът на случайно амплифициране на полиморфна ДНК (RAPD-PCR) с E1 праймер, а получените полиморфни профили бяха подложени на UPGMA анализ чрез софтуер GeneTools 4.01 (Syngene). Бяха установени общо 30 RAPD профили, които образуваха два големи клъстера. Един от проучените щамове генерира уникален RAPD профил и не попадна в тях. Между двата клъстера беше доказано високо клонално сходство (коефициент на Dice около 81%). В заключение, в мониторираните болници беше установено персистиране на ендемичен клон, включващ щамове *E. faecium* с проблемна антибиотична резистентност, за период от 4½ години. Това налага упражняване на строг контрол върху разпространението на нозокомиалните инфекциии, както и непрекъснат надзор на антимикробната резистентност на *E. faecium*.

Благодарност: Проучването е финансирано по Проект с вх. № 8328/06.12.2016 г., Договор № Д-57/2017 г. от Конкурса „Грант 2017” на СМН, Медицински университет – София.

BG-2. MOLECULAR IDENTIFICATION ON FIVE *BACILLUS* STRAINS WITH PLANT GROWTH POTENTIAL ABILITIES

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Plant growth promoting rhizobacteria (PGPR) are group of bacteria typically isolated from rhizosphere where can enhance the growth of plant direct or indirect. The aim of this study was to identify five PGPR isolates using 16S ribosomal RNA gene sequencing techniques. The results of morphologically and biochemically characteristics on these strains shown that they refer to genus Bacillus. The results obtained of PCR method were compared to database in GenBank and five strains were determined. Analyses results acquired from GenBank shown that five isolates are close related species to genus Bacillus.

Acknowledgement: This work is kindly supported by Advanced Nutrients and ROMB Ltd.

BG-3. MOLECULAR GENETIC STUDY OF POTENTIALLY BACTERIOCINOGENIC AND VIRULENT *ENTEROCOCCUS* SPP. ISOLATES FROM BEEHIVES FROM BULGARIA

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Enterococci are lactic acid bacteria (LAB) of significance in medical microbiology, industrial microbiology and public health. *Enterococci* have been regarded traditionally as low-grade pathogens. Therefore, enterococcal infections could arise as a complication after surgery and invasive procedures. Also, *enterococci* have important implications in the dairy industry. They occur as nonstarter LAB in a variety of cheeses. The positive influence of *enterococci* on cheese seems due to specific biochemical traits such as lipolytic and proteolytic activities, their capacity to use citrate and pyruvate as carbon sources, and production of aromatic volatile compounds. A total of 7 *Enterococcus spp.* isolates from beehives were studied in order to evaluate the incidence of bacteriocin genetic determinants and genes for antibiotic resistance and virulence factors. Structural genes coding for enterocin A (entA), enterocin P (entP), enterocin B (entB), enterocin AS-48 (as-48) , enterolysin A (entlA), collagen-binding protein (ace), hyl , gelE and vancomycin-resistant genes were detected by polymerase chain reaction (PCR) with specific primers.

ФУНДАМЕНТАЛНА ГЕНЕТИКА / FUNDAMENTAL GENETICS

FG-1. PREMATURE CHROMOSOME CONDENSATION (PCC)

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Eukaryote chromosomes condense during mitosis. The condensation is strictly regulated by cellular elaborated machinery but the mechanism is still unknown. Occasionally, chromosomes condense at outside of mitosis under several circumstances. This phenomenon is known as premature chromosome condensation (PCC) or premature mitosis (PM)

The first report for the PCC phenomenon was described in 1967 from Kato and Sandberg. They reported that the several kind of virus infected cells showed something strange - "pulverized" chromosomes.

These "pulverized appearance" of chromosomes is selectively observed in S-phase nuclei. In addition, either G1 or G2 cells do not show "pulverized" chromosomes but showed condensed form of univalent or bivalent chromosomes, respectively. These findings suggested that the "pulverized chromosome" did not due to the actual breakage of chromosomes.

They concluded, therefore, that observed chromosome structures are equivalent to that of the chromosomes of individual cell cycle stage at the time of cell fusion. They named this phenomenon as premature chromosome condensation (PCC). They also named the material which increased in mitotic cells and promotes chromosome condensation as mitosis promoting factor (MPF).

Two mechanisms for PCC are known: mechanism of fusion-mediated PCC and mechanism for chemical-mediated PCC.

PCC is a very interesting phenomenon not only of a biological point of view but also as useful tools for cytogenetic.

Key words: PCC, chromosome, genes,

FG-2. ANALYSIS OF THE ANTIOXIDANT PROPERTIES OF BULGARIAN PROPOLIS

BLAJEV Georgi[1], DIMITROV Martin[1].

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Honey and propolis are among the most studied bee products with respect to their antioxidant properties (Alvarez-Suarez et al.,2009; Kortenska-Kancheva & Bankova, 2006; Nakajima et al., 2009, Beretta et al., 2005). Their biological activities are attributed to the phenolic compounds such as flavonoids (Nakajima et al., 2009).

New methods will be developed and applied to study in vivo the antioxidant activity of laboratory substances and foods, including bee products (honey, propolis and royal jelly). This new original method has the advantage to measure the antioxidant activity in live cells, in a state similar to the one of neoplastic differentiation of cells as a result of the action of carcinogen (Stamenova et al., 2008; Stoycheva et al., 2010).

Ty1 is a retrotransposon in yeast Saccharomyces cerevisiae with structure, function and life cycle very similar to oncoviruses. Its transposition to a new location in the genome is induced by carcinogens and causes reorganizations comparable to those in carcinogenesis. The specific activation of Ty1 mobility by carcinogens is due to increased synthesis of ROS and DNA damages caused by the oxidative stress. A linear relationship between increased level of ROS in cells and the activation of Ty1 transposition was found (Pesheva et al., 2005; 2008; Garfinkel, 2005).

Key words: Biology, Genetics, Molecular Yeast genetics, Carcinogenesis, Biomonitoring

FG-3. NONINVASIVE GENOTYPING WITH APPLICATION TO BULGARIAN POPULATION OF BROWN BEAR, WOLF, WILD CAT AND LYNX

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Noninvasive genetic techniques are getting more and more popular trough the last years, since their founding as a method for investigation of wild animals and estimation of their population by size and structure, 20 years ago. The use of DNA from hair, urine or faeces as an individual genetic tag can provide useful information for populations of wild animals. The amplification of DNA extracted from noninvasive samples is often problematic, because such DNA is usually in low quantity and degraded. This can lead to incorrect genotypes due to allelic dropout or false alleles. These problems are known and different methods have been proposed to limit genotyping errors.

Here we combined already developed methods for determination of brown bear, wolf, wild cat and lynx to the level of species, sex and individual using conventional PCR technique and Applied Biosystems sequencer chemistry for sequencyng and fragment analyses and transfering the conventional PCR to the Real-time PCR SYBR Green chemystry and sequencing and fragment analyses to the GenomLab GeXP (SCIEX, USA) chemistry.

In the frame of the project following noninvasive samples were collected: hair from bear, wild cat and lynx by hair traps (suitable for each species) and excrements from bear and wolf by transect method. The collection and storage of samples followed the methodologies described within the project. DNA was extrarcted using commercial kits. The determination of the species and haplotype was done by sequencing of the mitochondrial DNA. The determination of the individual and sex was done by fragment analisis of microsatellite loci.

FG-4. LONG-LASTING EFFECT OF ETHANOL EXPOSURE ON THE EMBRYOGENESIS AND *IGF2/H19* EXPRESSION

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Ethanol is a strong teratogen that negatively affects embryo and placental growth and development. However, studies show that it may have a stimulating effect on the embryogenesis if administered *in vitro* during preimplantation and within a certain range of concentrations. Our previous study established that the dose of 0.2% ethanol supplied in the culture medium stimulated the development of blastocysts and increased the expression levels of the imprinted gene *Igf2* (Taseva, 2016). We have now examined whether the stimulating effect and altered gene expression during preimplantation are retained in the middle of gestation. Blastocysts from C57BL/6 mice were exposed to 0.2% (v:v) ethanol for 4 hours at 3.5dpc and transferred to pseudopregnant females. On 11.5 dpc we observed a statistically insignificant trend for embryo and placental weight reduction. In embryos, the expression from the three P0, P2, and P3 *Igf2* promoters was up-regulated. The *H19* expression was not affected. In placentas, the expression level of the *Igf2* promoters was not changed, except for P3, where we measured a reduction of 0.8 ± 0.14 ($p < 0.05$). *H19* expression remained unchanged. Our results indicate that the stimulatory effect of ethanol observed during preimplantation is not retained through the middle of gestation, while the altered expression of *Igf2* persists. The opposing effects of prenatal ethanol exposure over the gene expression observed in embryos and placentas indicates that the mechanisms of alcohol action are tissue specific.

FG-5. SEXUAL DIMORPHISM AND GENETICS OF Y CHROMOSOME IN PLANTS AND *DROSOPHILA MELANOGASTER*

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Sexual dimorphism is the phenotypic expression of a multi-stage procedure at chromosomal, hormonal and behavioral level. Genetic sexual dimorphism is due to the presence of two identical (XX) or two different (XY) chromosomes in females and males.
The Y chromosome is one of the smallest chromosomes within the human genome. In 1959, when Jacob was studied Klinefelter syndrome (XXY) and Ford studied Turner syndrome (X0) concluded that Y-chromosome is determined by sexes. Recent studies of sex chromosome in humans and other primates, as well as in plants and Drosophila, throw light on its structure, origin and fate.
After the sequencing of the human genome, scientists have found discrete sequence classes, which they called a male specific region (MSY). This region is predominantly responsible for male sex determination differentiating between the male and female sexes.
Many groups of organisms even mammals have Y chromosomes, but these Y chromosomes do not share common ancestry with *mammalian* Y chromosomes. Such groups include *Drosophila*, some other insects, some fish, some reptiles, and some plants.
While the presence of a Y chromosome is sufficient for male determination in mammals, it does not directly participate in sex determination in fruit flies. Instead sex determination in *D. melanogaster* is regulated by an X chromosome counting mechanism.
Plant sex chromosomes are particularly interesting because they evolved much more than those of mammals or *Drosophila* – most plants with separate sexes seem to have evolved recently from ancestors with both sex functions. Plant sex chromosomes may tell us about the first stages of the evolutionary process that has led to the massive gene loss in Y chromosomes.

Keywords: Y-chromosome, genes, plants, *Drosophila melanogaster*

МОЛЕКУЛНО КЛОНИРАНЕ / MOLECULAR CLONING

MC-1. OPTIMIZED PROCEDURE FOR COMPETENT CELLS PREPARATION COMPATIBLE WITH THE STANDARD BIOBRICK PROTOCOLS

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The primary goal of synthetic biology is to make the process of engineering biological systems easier. One of the biggest steps in this direction was the development of the BioBrick registry. It is open and contains the biggest collection of standard and well characterized genetic parts worldwide. Here we present an optimized procedure for preparation of chemical competent cells that can be used with different BioBrick parts from this registry. We intensively optimized all transformation conditions for the widely used general cloning strain *E. coli* DH5alfa. Our efforts resulted in a very simple, quick and cost efficient protocol that gives reliable and reproducible results with the BioBrick distribution kits. Our cells were tested in a variety of application like plasmid transformations, BioBrick assembly procedures and Aqua cloning of complex constructs.

MC-2. CONSTRUCTION OF TWO CLONING VECTORS WITH POSITIVE SELECTION USING RF CLONING

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Two novel plasmid cloning vectors, pBlue and pYellow, were constructed by substituting the *lacZ'* gene of pUC19 with amilCP and amilGFP coding sequences obtained from the coral *Acropora millepora*. The sequences were codon optimized for expression in *Escherihia coli*, and when expressed, produce blue and yellow coloring respectively. The substitution of the *lacZ'* gene eliminates the need for adding X-Gal to the growth medium while keeping the same mode for positive transformants selection as the one used by the ancestral vector.

Acknowledgement: This research was supported by Sofia University Research Grant № 80-10-99/20.04.2017

MC-3. ENGINEERING A BIOBRICK COMPATIBLE GRNA VECTOR FOR USE WITH CAS9 AND DCAS9

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CRISPR-Cas9 is arguably the most used technology in the modern genetics field. It has dozens of different applications like genome editing, transcriptional silencing/activation, nucleic acid visualization and gene therapy. A key property of this system is its ability for multiplexing. In order to target new DNA regions, one needs to clone only a short 20 bp gRNA compound. In order to simplify this process we designed a novel gRNA cloning vector. New gRNAs can be cloned into it using a simple restriction/ligation based cloning procedure. Moreover, a gRNA arrays composed of many individual gRNAs can be easily constructed using the standard rules of the BioBrick assembly. This process was further simplified by the creation of versions with different antibiotic resistance markers that can be used with the 3A assembly protocol. Our vector has modular design and can be easily modified to fit individual requirements.

МЕДИЦИНСКА И ЧОВЕШКА ГЕНЕТИКА / MEDICAL AND HUMAN GENETICS

MHG-1. MUTATIONS IN *ACTA2* GENE DETECTED BY NEXT GENERATION SEQUENCING IN PATIENTS WITH PATHOLOGY OF GREAT VESSELS

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The *ACTA2* gene provides instructions for making a protein called smooth muscle alpha (α)-2 actin, found in smooth muscle cells that line the layers of the walls of the arteries. It contributes to the ability of these muscles to contract, which allows the arteries to maintain their shape instead of stretching out as blood is pumped through them.
The purpose was genetic profiling of patients with phenotype determined by cardio vascular diseases with pathology of great vessels.
Clinical diagnosis of patients was according to standard hospital procedures. Sequencing of the DNA samples was performed on a MiSeq System by targeted next generation sequencing of 174 genes connected to cardiovascular diseases included in TruSight Cardio gene panel (Illumina). Sequencing data was analyzed by Softgenetics NextGene Software (2.3.3). Alignment was to the human reference sequence (GRCh37/hg19). The variants were confirmed by Sanger sequencing
Heterozygous variants in gene *ACTA2* (10q23.31) were detected in two patients. In one patient, 15 years old boy diagnosed with aortic dissection type III, a heterozygote form of the variant *ACTA2*:p.Arg258Cys was found. The mutation is known and detected in autosomal dominant inheritance of familial form of thoracic aortic aneurysms. The second patient, 40 years old woman with aneurysm of abdominal aorta and familial history of father who died of thoracic aortic aneurysm, a heterozygous *ACTA2*:p.Lys52Glu variant was detected.
In conclusion, the results underline the importance of this method in determining mutation status in correlation to clinical phenotype and has large implication on treatment and prognosis of patients and their families in cases with uncertain clinical diagnosis.

Key words: *ACTA2*; great vessels; next generation sequencing; aortic dissection; aneurysm.

MHG-2. MICA ASSOCIATIONS WITH ORAL SQUAMOUS CELL CARCINOMA

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Major histocompatibility complex class I-related chain A (MICA) is a ligand of Natural killer group 2, member D (NKG2D) receptor. Recent studies have shown that MICA is up regulated in tumors from epithelial origin, playing a key role in immunological surveillance and different alleles are associated with diseases related to NK activity. The aim of our study was to analyze associations of MICA polymorphism with oral squamous cell carcinoma (OSSC). Twenty seven patients with histologically proven OSSC were included in the study. The majority of patients had G2-G3 tumors according to Anneroth's classification. The control group included healthy subjects from the Bulgarian population. MICA genotyping was performed by PCR-SSO kit (LABType SSO MICA, OneLambda) and PCR-SBT. Our results showed statistically significant protective association for MICA*12:01 allele (Pc<0.05, OR-0.07), encoding a full length protein. Interestingly this allele had a higher frequency in the healthy Bulgarian population compared to other European populations. With the highest frequency in patients with OSSC was observed MICA*08:01 allele, encoding truncated protein. However the difference with the control group was with a borderline significance (Pc=0.053). Although our data are preliminary considering the small number of patients analyzed, the associations observed support the model that alleles encoding truncated, ectopic and soluble MICA molecules play an important role in OSSC by down regulation of NKG2D on NK and CD8+ T cells leading to aberrant immunological surveillance.

MHG-3. НОВИ ПЕРСПЕКТИВИ В ПРОИЗХОДА НА БЪЛГАРИТЕ ПОСРЕДСТВОМ ИЗСЛЕДВАНЕ НА ТРАКИ И ПРАБЪЛГРИ

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Траките и прабългарите, които са съществували в различни периоди на миналото, предизвикват голям интерес сред учените. За първи път са изследвани антични ДНКи от траки и прабългари. Тракийските проби са датирани от III хилядолетие Пр. Хр., а прабългарските проби са датирани от VIII–X век. Те са взети от различни некрополи от страната. С цел да се изясни генетичното разнообразие на траките и прабългарите, бяха анализирани 43 проби от 7 различни гробници. Два основни фундаментални метода са използвани за митохондриален ДНК анализ– Класически и NGS платформа Illumina.

Резултатите са уникални от античните HVSI хаплотипове и цялостни митохондриални геноми от тракийски и прабългарски костни или зъбни останки. От получените резултати на тракийски проби, процента на макро-хаплогрупите е: H- 41.6%;N и JT- 12.5% всеки; K,U, HV и D- 8.3% всеки. От оптимизираните резултати на прабългарите, процента на макро-хаплогрупите е: H- 47%; T-15.8%; J и U4- 10.5% всеки; H5,HV и U3- 5% всеки. Установените хаплогрупи са преобладаващо Евразийски. Те определят възможната позиция и роля на траките и прабългарите във формирането съвременния български генофонд и тяхната генетична връзка с други Западноевразийски популации.

Базирайки се на анализ на основните компоненти (PCA) се наблюдава генетичното разстояние между траки, прабългари и съвременни българи. Траките са генетично най-близки до съвременни словаци, немци и швейцарци. Голямо генетично разстояние съществува между траки и съвременни гърци. Прабългарите са много близки до съвременни българи, унгарци, италианци от централна Италия и словаци. Голямо генетично разстояние съществува между прабългарите и популациите от Волга-Урал региона, татари и тюрки.

MHG-4. PHARMACOGENETIC SUSCEPTIBILITY TO COMMON DRUGS AMONG PREDISPOSING VARIANTS TO FAMILIAL PAPILLARY THYROID CANCER

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Introduction: Sporadic papillary thyroid cancer (PTC) is the most prevalent form of thyroid malignancy. Genetic predisposition to PTC has been widely studied however the genetic causes of familial cases are still unknown or controversial.
So far, FPTC usually presents as multifocal disease at a younger age and is associated with an increased incidence of nodal involvement and recurrence. The aggressive nature of FPTC suggests the existence of genetic aberrations that are yet to be identified.
The identification of new causative variants predisposing to FPTC and their relation to standard therapy tolerance will pave the way to the development of new targeted therapies for this kind of malignancy. Here we report two SNPs defining sensitivity to opioids (MC1R_p.Arg151Cys) and to chemotherapeutics (cisplatin and cyclophasphamide) (MUTYH_p.Val22Met) identified by NGS analysis of four families with FPTC.
Materials and methods: We performed NGS analysis using Illumina® sequencing platform and Trusight Cancer Panel®. Seventeen (17) predisposing variants have been selected among the members of the four families. Subsequently we checked each variant's relationship to commonly used drugs and/or chemotherapeutics using publicly available database PharmGKB (https://www.pharmgkb.org/).
Conclusion: We identified two SNPs (MUTYH_p.Val22Met and MC1R_p.Arg151Cys) that could impact patients' response to opioids and standard chemotherapeutics (cisplatin and cyclophosphamide). Such an approach could be used for drug response' predictions also in other common diseases.

MHG-5. COTINUS COGGYGRIA PROAUTOPHAGIC AND HISTONE ACETYLATION MODULATORY POTENTIAL IN BREAST CANCER CELLS

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Over 60% of chemotherapeutic agents applied in clinical practice are with natural origin or synthetic derivatives of nature sources and the plant kingdom has been the dominant source. At present, more than 3000 plants have been reported to display anticancer properties. At recent years a lot of studies revealed that the medicinal plant *Cotinus coggygria* possesses large pharmacological and therapeutic potential due to its numerous medicinal activities. Our previous investigations established that leaf aqueous ethanolic extract from Bulgarian *C. coggygria* exhibits significant antiproliferative activity on human breast, ovarian and cervical cancer cell lines without considerable influence on the growth of non-tumorigenic cell line. The objective of the present research was further to examine some mechanisms of the extract effect on MCF7 breast cancer cells by fluorescence microscopy analysis of autophagic cell death and quantitative RT-PCR analysis of the influence on the transcriptional levels of genes coding for histone acetyltransferases p300 and CBP. The results indicated tendency for induction of autophagic processes and inhibition of *p300* expression after cancer cells extract treatment for 24 hours. Future investigations will be focused on more detailed studies of *C coggygria* effect on the expression of target autophagy-related genes.

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MHG-6. MITOCHNODRIAL DNA AND Y-CHROMOSOME GENETIC LANDSCAPE OF SLAVIC SPEAKING POPULATIONS

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Slavic speaking populations are the most numerous Indo-European ethnolinguistic group in Europe. They speak an assortment of languages, which fall into three groups: West, East and South Slavic languages.

The genetic and genomic makeup of Slavic populations has been the object of many previous studies. However, genetic comparisons only among Slavic populations are few. Therefore, in order to contribute to the understanding of the correlation between Slavic linguistic and genetic affiliation, we have analyzed for the first time the mitochondrial DNA (mtDNA) and Y-chromosome relationships only among Slavic speaking populations from different groups and we have also depicted their position in the European genetic landscape.

The analysis was performed on a collection of published results for the frequencies of mtDNA and Y-chromosome haplogroups. In the data sources, mtDNA haplogroup assignment was based on partial or entire control region sequences and/or coding region markers; and Y-chromosome haplogroup classification was performed by genotyping of informative biallelic markers.

The matrilineal and patrilineal relationships among the populations were illustrated by Principal Component Analysis.

In the inter-Slavic population comparisons, West and East Slavs are closer between themselves, than with South Slavic populations. Furthermore, in the European context, South Slavic populations are positioned more close to neighboring Balkan non-Slavic and North Italian populations, than to other Slavic populations.

In conclusion, this study points that West-East and South Slavic speaking populations, behave as separate groups based on their mtDNA and Y-chromosome genetic structure, which shows that they do not share substantial common genetic ancestry and that there is great genetic variety in the Slavic linguistic unity.

MHG-7. GENETICS OF CONNECTED WITH FINGERPRINT PATEERNSS IN MONOZYGOTIC TWINS

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Monozygotic twins are mirror images that have identical genotype. In human genetics, the twin method is applied to study the laws of inheritance of qualitative and quantitative traits and their expression as a model system. Although monozygotic twins represent identical genetic copies, they differ in their unique phenotypic fingerprint images. It has been previously reported, that the genes *A, L* and *W*, which respectively determine the images of arcs, loops and whorls on distal phalanges, are inherited independently. Our studies have shown that allele and genotypic frequencies are variable, concerning monozygotic twins.

Key words*:* monozygotic twins, fingerprints, alleles, genotype frequencies.

MHG-8. GENETICS CONNECTED WITH FINGERPRINT PATTERNSS IN THE BULGARIAN POPULATION

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Genetics connected with dermatoglyphics represents a valuable area of science, not only because it studies the unique finger phenotype, but also because it finds practical application in public life.
Three gene loci determine the different fingerprint phenotypes. The main features - arcs, loops and whorls of distal finger phalanges are determined by the genes *A, L* and *W* respectively.
Independent segregation of the alleles and their recombination determines the independent inheritance of the finger phenotypic images.
The purpose of the present research is to study the laws of inheritance, determining papillary finger images on the distal phalanges in the Bulgarian population. Comparing the three ethnic groups (Bulgarians Christians, Bulgarian Mohammedans and Bulgarian Turks) in the Bulgarian population we established differences in their allelic (*A, a; L, l; W, w*) and genotypic frequencies (*AA, Aa, aa; LL, Ll, ll; WW, Ww, ww*).
From the applied morphological - dermatoglyphic analysis it was proven, that the prevalence of dominant alleles (*A, L, W*) was the lowest in all three studied ethnicities.

Key words: *fingerprints, phenotype, allelic and genotypic frequencies.*

MHG-9. CORRELATION OF BRAF MUTATION (V600E) AND CANCER PROGRESSION IN PATIENTS WITH METASTATIC MELANOMA: A SMALL COHORT STUDY

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Malignant melanoma is the most aggressive form of skin neoplasm characterized by rapid clinical progression and high mortality. The normal function of gene *BRAF* (7q34) results in protein involved in RAS/MAPK signaling pathway controlling important cell functions. The pathogenic *BRAF* (V600E) missense mutation (Chr7: 140753336, NM_004333.4:c.1799T>A, NP_004324.2:p.Val600Glu) is one of the most frequent mutations present in patients with metastatic melanoma. Various therapies have been developed targeting this mutation in advanced stages of melanoma.

The aim of this study was to determine correlation between the presence of *BRAF* (V600E) mutation, progression and survival of patients with metastatic melanoma.

16 patients with confirmed metastatic melanoma were analyzed. Clinical assessment was according standard protocols of the hospital. Tissue samples for DNA extractions were taken during surgery scheduled for removal of the tumor. DNA amplification and genotyping was performed on Real-Time PCR platform using TaqMan probes.

The results of the study showed presence of the *BRAF* (V600E) in 11 samples (68.8%). The association of the clinical stage and *BRAF* (V600E) resulted in distribution of 80,00% of patients with clinical stage of primary melanoma IIb and 20.00% with IIIa or IIIc in *BRAF* (V600E) negative patients and 36.36% stage IIa and 63.64% stage IIIa or IIIc in *BRAF* (V600E) positive patients. Furthermore, a correlation between the mutation and survival during the 12-months evaluation period was established, namely the survival rate in *BRAF* (V600E) negative patients was 80.00% versus 63.64% in *BRAF* (V600E) positive patients.

In conclusion, the results are in concordance with literature data and can influence the treatment of such patients.

Keywords: metastatic melanoma, disease progression, BRAF gene, V600E mutation

РАСТИТЕЛНА ГЕНЕТИКА / PLANT GENETICS

PG-1. EFFECTS ON THE MEIOTIC CYCLE IN *VICIA FABA* L.IN TERMS OF DIFFERENT TIME/DOSAGE X-RAY TREATMENTS

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The alterations of the cell structure and chromosomes caused by short-term exposure to X rays differ greatly and their overall effect on the next generations largely depends on the reproductive material of the first mutant generation.
The purpose of the study was to experimentally induce and detect cytogenetic changes in the plant *Vicia faba* L. (broad bean, faba bean) by exposure to X-rays.
Irradiation by different doses and exposure times (3 min 20 sec/16,2 cGy; 6 min 40 sec/32,4 cGy; 10 min/48,6 cGy, 13 min 20 sec/64, 8 cGy) was conducted by X-ray device "Shimadzu"on seed in the stage of germination. The meiotic cycle was precisely analyzed during all phases on chromosomes dyed by leuco-basic fuchsine method (Darlington and La Cour, 1962) and 2% acetate-orcein. Pollen staining was by 2% acetate-orcein and iodineglycerin method (Petrovic and Vuchenovikj, 1992). Fertility was determined on 3000 pollen grains per group and was expressed compared to 100.
Deviation during the process of cariokinesis within the ten idealized groups was detected, resulting in damage of various degree. Numerous deletions, defects during chromosome shaping, conjugation in bivalents and their separation, lagarde presence, micronucleuses and multinuclear cells were registered depending on the dose, being most numerous in the groups exposed to the highest dose of 64.8 cGy. The deviations caused sterile flowers, empty antero and defected pollen grains, thus decreasing fertility in all treated groups. In conclusion, *Vicia faba* L. is radiosensitive and X-ray irradiation affects the plant development leading to dosage dependent deviations on cytogenetic level and non-dosage dependent decrease in infertility.

Key words: *Vicia faba* L.; X-rays; meiosis; genetic variability; pollen analysis.

PG-2. ANTIOXIDANT ACTIVITY AND PHENOLIC COMPOUNDS PRODUCTION IN *HYPERICUM PERFORATUM* HAIRY ROOTS

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The growth characteristics, production of phenolic compounds and antioxidant activity have been evaluated in fifteen *Hypericum perforatum* hairy root (HR A-HR O) clones in comparison to non-transformed roots (NTR). The HR clones were induced from root segments of *in vitro* grown seedlings from *H. perforatum* after co-cultivation with *Agrobacterium rhizogenes* strain A4. The transgenic nature of HR clones was confirmed by PCR analysis of the presence of *rol*B and *aux2* sequences from TL-DNA of *A. rhizogenes* Ri plasmid. The growth index (GI) of HR clones varied from 5.58 to 16.74 compared to NTR (5.16). The highest value of GI was noticed in HR B clone (3.2-fold) compared to NTR. The total phenolic (TP) contents in HR clones were ranged from 12.10 to 25.46 mg gallic acid (GA)·g^{-1} DW compared to control roots (9.06 mg GA·g^{-1} DW). The HR F and HR B clones showed significantly higher TP production (2.7-fold) compared to NTR cultures. The antioxidant activity of HR clones measured by phosphomolybdenum (PM) assay was ranged from 26.29 to 47.21 mg ascorbic acid (AA)·g^{-1} DW compared to NTR (30.88 mg AA·g^{-1} DW). The highest PM value was noticed for HR F clone (1.5-fold) compared to control roots. Present results indicated that *A. rhizogenes*-mediated transformation triggered defense responses in *H. perforatum* HR through the accumulation of phenolic compounds with antioxidant properties. Taking into account that T-DNA genes have a large impact on diverse biochemical processes, their presence in *H. perforatum* HR makes them a self-sufficient system for the production of secondary metabolites of pharmacological importance.

Key words: Antioxidant activity, Hairy roots, *Hypericum perforatum* L., Phenolic compounds.

PG-3. CHANGED DNA METHYLATION STATUS AFFECTS CELL DIVISION ACTIVITY IN *ARABIDOPSIS* ROOT MERISTEM

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DNA methylation is involved in key biological processes including cell differentiation, organismal development and stress response. In plants, the genomic methylation patterns **are established by DRM2**, **MET1 and CMT3**, the generally recognized *de novo* and *maintenance* DNA methyltransferases. MET1 has a maintenance function that preserves CG methylation during cell division. However, at least at some loci, MET1 shows *de novo* methylation activity, as well. *How and to what extent* methylation patterns determine plant developmental programs is still poorly understood. Phenotypic evaluation of the *Arabidopsis* mutant *met1* showed pleiotropic defects, including greatly reduced primary root length, *agravitropic growth and aberrant* waving. After application of zebularine (inhibitor of DNA methyltransferases that significantly reduces DNA methylation independently of sequence context), more severe inhibition of root growth and development was observed. The mitotic activity in root meristem is tightly linked with the transcriptional regulation of cell cycle regulatory genes, such as B-type cyclins. We used a line containing a fusion construct between the *CYCB1;1* promoter and a bacterial β-glucuronidase marker gene (*uidA*) encoding the enzyme *β-glucuronidase* (*GUS*). The *met1* mutants and zebularine-treated wild-type plants were screened for *CYCB1;1-GUS* expression patterns. We observed rather asymmetric and stronger accumulation of the signal in the root meristem of *met1* compared to the wild type. Zebularine treatment triggered even a *stronger CYCB1;1-GUS* activation. Since the root growth rate is determined by meristematic activity and cell expansion, the shorter root phenotype of plants with disturbed DNA methylation could not be associated with the reduced cell division activity but rather with reduced rate of cell expansion.

PG-4. IMPACT OF EXCESSIVE NITROGEN ON THE PHOTOSYNTHETIC APPARATUS IN TWO WHEAT GENOTYPES

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The aim of this study was to examine the effect of different levels of nitrogen (N) on the pigment composition, growth parameters and the functions of the photosynthetic apparatus in two Bulgarian bread wheat genotypes (Slomer, an old historic variety, and Katya, a modern one). PAM chlorophyll fluorescence, 77K chlorophyll fluorescence, oxygen evolution (measured with Clark-type and Joliot-type electrodes) and P700 photo-oxidation measurements were used to assess the changes in the functional activity of the photosynthetic apparatus of wheat seedlings grown hydroponically with different levels of nitrogen (5.5, 10 and 20 mM of N). The results revealed that the high nitrogen concentration led to: (i) an inhibition of plant growth parameters; (ii) an increase of the energy transfer from PSII to PSI and a modification of the energy transfer between pigment-protein complexes of PSII; (iii) an inhibition of the photochemical activity of both photosystems, the effect was stronger on PSII in comparison to PSI; (iv) a modification of the kinetic parameters of the oxygen evolving reactions and a delay of the cyclic electron flow around PSI; (v) smaller impact on the acceptor side of PSII in comparison to its donor side. Data revealed stronger influence of the high nitrogen concentration on the photosynthetic apparatus in the old variety Slomer in comparison to the modern one Katya. The reasons for different sensitivity to high nitrogen concentration of the two genotypes are shown.

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PG-5. PLANT *NUDC* GENES CONTRIBUTE TO POST-EMBRYONIC ROOT ORGANOGENESIS IN *ARABIDOPSIS THALIANA*

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This study focuses on investigation of the biological role of genes, encoding for low molecular weight proteins with NudC domain that play an important role in organismal development. The family of NudC proteins have representatives in *all eukaryotes,* including human, which suggest important evolutionary conserved biological functions. The studies of plant NudC components are still at a very early stage. We use *Arabidopsis thaliana*, a well-known model plant of the *Brassicaceae* family, to investigate the biological role of one of the *NudC* members, named *At1*, in post-embryonic root organogenesis. Transgenic lines expressing the GUS reporter under the control of the endogenous *At1* promoter (*pAt1::GUS*) were generated. The expression of GUS was found to be root specific during post-embryonic development. The highest expression levels were detected in root tissues, where *active cell divisions* take place (root tip meristem and lateral root primordia). These observations suggest specific functions of *At1* related to cell division pathways. Overexpression or downregulation of *At1* negatively affected primary root length and lateral root density. When *At1* expression was reduced to about 30-40% of normal levels, we observed a reduction in primary root length and branching. Overexpression of *At1* showed similar tendency of root phenotype changes. These observations suggest that misexpression of *At1* in varying *ways prevents proper development of final root architecture.*

PG-6. VARIABILITY, HERITABILITY AND GENETIC ADVANCE OF SOME GRAIN QUALITY TRAITS AND GRAIN YIELD IN DURUM WHEAT GENOTYPES

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The main priority in durum wheat breeding is the creation of high yielding varieties with improved grain quality. The study were carried out to determine the variability, heritability, genetic advance for the following grain quality traits: protein content (PC), wet gluten (WG), yellow pigment in grain (YPG), SDS sedimentation value, vitreousness (VS) thousand kernel weight (TKW), test weight (TW) and for grain yield (GY) of 24 durum wheat genotypes - varieties and breeding lines of different origin: Bulgaria (FCI – Chirpan, DAI - G. Toshevo), Europe, CYMIT - Mexico and ICARDA - Syria. All genotypes were grown under field conditions in competitive variety trial in three replications during three harvest years (2014 – 2016) in the experimental field of Field Crops Institute – Chirpan, Bulgaria. All parameters were evaluated on whole grains on standard methods. Analysis of variance revealed the presence of highly significant ($P \leq 0.01$) variation among genotypes for all studied traits. The PCV was generally higher than the GCV for all studied traits, indicating the influence of the growing season. The greatest Phenotypic Coefficient of Variation (PCVs) were found for sedimentation value (SDS - 44.54 %) , grain yield (GY - 16.92 %) and yellow pigment in grain (YPC -14.8 %) and the greatest Genotypic Coefficient of Variation (GCV) - for the same traits - SDS (43.45 %), (YPG -14.3 %) and (GY - 10.2 %). The lowest PCVs were for test weight (TW - 1,54 %) and protein content (4.66 %). The estimated values of broad-sense heritability were found between 27.9 % for wet gluten (WG) and 95.2 % for SDS sedimentation value. A high level of heritability was also determined for yellow pigment in grain YPG - 92.4 %, followed by thousand kernel weight (TKW) - 72.4 % and for protein content (PC)- 67.2 %; moderate level - for test weight (TW) - 47.4 % and for grain yield (GY) - 36.3% and relatively low level – for vitreousness (VS) -29.1 %. The highest genetic advance was calculated for SDS sedimentation value. High heritability and considerable genetic advance for SDS indicated the predominance of an additive gene effect in controlling this trait and reveal the possibility to conduct effective selection. For protein content and thousand kernel weight high heritability was associated with low genetic advance, indicating the influence of dominant and epistatic genes in heritability of this traits and reveal slower breeding progress in their improvement.

Key words: durum wheat, grain quality traits, PCV, GCV, heritability, genetic advance

Азбучен указател на участниците

List of the participants

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Thank you for your confidence and participation in the “First young scientists seminar on Genetics”! We will be glad if it was useful and interesting to you, as well if you had some good time! We hope that this was just the beginning and it will develop in the future. Information about how it took place can be found on it Facebook page: https://www.facebook.com/1606970022700438 . We will be pleased of your commentaries and recommendations there. Until next time! We are waiting to see you again!