



UNION OF SCIENTISTS IN BULGARIA - BRANCHES
"GENETICS" and "PLANT PHYSIOLOGY AND BIOCHEMISTRY"

THIRD SEMINAR ON GENETICS
WITH INTERNATIONAL PARTICIPATION
**WITH A SPECIAL SESSION ON
PLANT PHYSIOLOGY AND BIOCHEMISTRY**

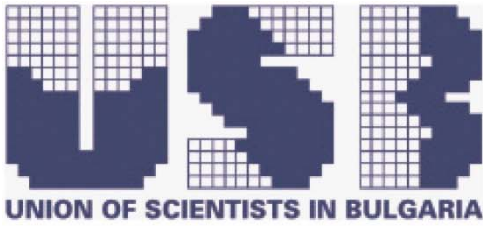
**BOOK OF
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October 2-4, 2019

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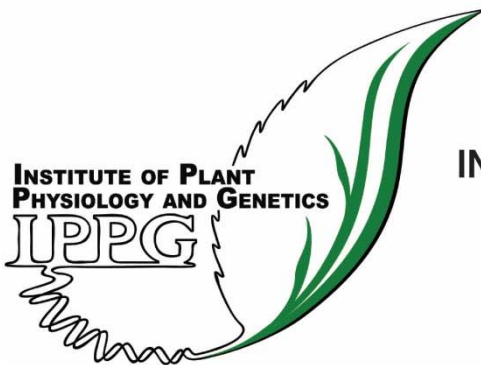
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ORGANIZING BRANCHES:
“GENETICS”
AND
**“PLANT PHYSIOLOGY AND
BIOCHEMISTRY”**

with the support of:



**INSTITUTE OF PLANT PHYSIOLOGY AND GENETICS,
BULGARIAN ACADEMY OF SCIENCES**

**BULGARIAN SOCIETY OF HUMAN
GENETICS AND GENOMICS**

THE SEMINAR IS DEVOTED TO THE 150TH ANNIVERSARY OF THE FOUNDATION OF THE BULGARIAN ACADEMY OF SCIENCES (BAS) AND THE 75TH ANNIVERSARY OF THE FOUNDATION OF THE UNION OF SCIENTISTS IN BULGARIA

LOCATION: THE SGIP 2019 WILL BE HELD IN THE BUILDING OF THE FACULTY OF BIOLOGY, SOFIA UNIVERSITY “ST. KLIMENT OHRIDSKI”, 8, DRAGAN TZANKOV BLVD., SOFIA. THE ORAL PRESENTATIONS AND PLENARY LECTURES WILL BE PRESENTED IN THE AULA ON THE SECOND FLOOR. THE POSTERS WILL BE EXPOSED AT THE LOBBY ON THE THIRD FLOOR.

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ORGANIZING AND SCIENTIFIC COMMITTEES

HONORARY CHAIRMEN:

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WORK PROGRAM

WEDNESDAY - OCTOBER 2, 2019

- 10.00 – 12.30 Registration and poster mounting
- 13.00 – 14.00 Opening and welcome speeches
- Prof. Stoyan Shishkov**, Dean of the Faculty of Biology, Sofia University
“St. Kliment Ohridski”
- Prof. Rumiana Vassilevska-Ivanova**, Director of the Institute of Plant
Physiology and Genetics
- 14.00 – 14.45 “Green biotechnology – from knowledge to innovation” **Acad. Atanas
Atanassov**, Joint Genomic Center Ltd.
- 14.45 – 15.30 “Longevity related genes in the genomes of centenarians” – **Prof. Draga
Toncheva**, Medical University - Sofia
- 15.30 – 15.45 Coffee break
- 15.45 – 17.45 Oral reports session in Genetics I
- Chairman: Dr. Slavica Josifovska*
- GO1: “Patterns of domestication and evolution in the genus *Hordeum* inferred from
retrotransposon-based and pseudogenes markers” – Georgi Bonchev
- GO2: “Psychosocial and epigenetic correlations in problematic psychoactive substances use” –
Maria Krasteva
- GO3: “Multiplex Ligation-dependent Probe Amplification remains a valuable tool for prenatal
diagnosis” - Kameliya Kercheva
- GO4: “Comparative genetic analysis of subfossil dogs from the Neolithic Age and Late
Antiquity” - Iskra Yankova
- 17.30 – 19.30 Welcome cocktail along the posters

THURSDAY - OCTOBER 3, 2019

- 9.30 – 10.00 “ZEISS Research Solutions: Discover New Possibilities for Genetic
Research” – **Ing. Anton Tonchev**, CARL ZEISS regional manager for
Bulgaria and N. Macedonia
- 10.00 – 11.30 Poster session
- 11.30 – 12.30 “New horizons in cancer immunotherapy” – **Assoc. Prof. Velizar Shivarov**,
Faculty of Biology, Sofia University “St. Kliment Ohridski”

- 13.00 – 14.00 *“Current understanding of the underlying genetic causality in Cardiovascular diseases” – Dr. Slavica Josifovska, Faculty of Natural Sciences and Mathematics, SS Cyril and Methodius University in Skopje*
- 14.00 – 14.15 Coffee break
- 14.15 – 16.45 Oral reports session in Plant Physiology and Biochemistry
Chairman: *Assoc. Prof. Kalina Ananieva*
- 14.15 – 14.40 PPBO1: *“The constitutive ethylene signaling mutation ctr1-1 confers salt stress tolerance in Arabidopsis”* - Irina Vaseva
- 14.40 – 15.05 PPBO2: *“Novel small-molecule effectors of the intracellular transport in plants”* - Kiril Mishev
- 15.05 – 15.30 PPBO3: *“Recovery of photosynthetic activity of Haberlea rhodopensis after freezing-induced desiccation”* - Katya Georgieva
- 15.30 – 15.55 PPBO4: *“JIP-test as a tool to evaluate light recipes for plant growth and flowering”* - Miroslava Zhiponova
- 15.55 – 16.20 PPBO5: *“Physiological aspects of natural regeneration in beech forests”* – Tereza Gerashka
- 16.30 – 17.00 Closing ceremony and best poster awarding

FRIDAY - OCTOBER 4, 2019

- 9.30 – 15.30 WORKSHOP:
“DIFFERENT METHODS FOR PREPARATION OF COMPETENT CELLS FOR USE IN MOLECULAR CLONING EXPERIMENTS”
Tutor: Dr. Slavic Peykov
NB: The workshop will take place at Sofia Tech Park!

ORAL PRESENTATIONS

GENETICS

PLENARY LECTURE: “NEW HORIZONS IN CANCER IMMUNOTHERAPY”

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Cancer remains a global healthcare challenge with overall 5-year overall survival rate hardly reaching 70% in Western countries. Recent breakthroughs in immunology are about to revolutionize patients’ management and clinical outcomes in a number of entities. Here, we provide a brief overview of the rationale and development of the immune checkpoint inhibitors as a novel approach for cancer immunotherapy. The development of novel specific predictive biomarkers and future approaches combinatorial personalized therapy are also discussed.

PLENARY LECTURE: “CURRENT UNDERSTANDING OF THE UNDERLYING GENETIC CAUSALITY IN CARDIOVASCULAR DISEASES”

Josifovska, S. ^{1,2}, Vazharova, R. ³, Balabanski, L. ², Malinov, M. ², Kaneva, A. ⁴, Panov, S. ¹,
Toncheva, D. ⁵

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The increased availability of data from sequencing of thousands of people around the world provides essential information for narrowing the list of rare variants and determine potential genetic causality in many diseases. In monogenic diseases, including some Cardiovascular diseases (CVD), the mutations have a large effect on the phenotype and very low carrier frequency in the general population (<1%). They are necessary and sufficient to trigger the expression of the disease. In contrast, more complex forms of CVD tend to derive from the integrated effects of more common genetic variants, known as single nucleotide polymorphisms. Most adults with coronary artery disease or arrhythmias do not have a single gene causality that would fully explain their presentation. Although family history is important in these cases, the milieu of low-impact genes often forms the basis for susceptibility and in combination of exposure to additional non-genetic and lifestyle factors, the disease manifests itself. Better characterization of the genes and gene variants associated with the symptoms in patients with Cardiovascular diseases may enable the introduction of a personalized targeted therapy, a concept that is relatively new to this group of diseases compared to the use of this type of therapy in different types of cancer treatments.

GO-1. PATTERNS OF DOMESTICATION AND EVOLUTION IN THE GENUS *HORDEUM* INFERRED FROM RETROTRANSPOSON-BASED AND PSEUDOGENES MARKERS

Bonchev, G. ¹, Švec, M. ², Dušínský, R. ², Hauptvogel, P. ³

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The cultivated barley (*Hordeum vulgare* L.) is one of the earliest domesticated crop plants and the available worldwide germplasm resources provide an excellent system to study genomic changes in this species associated with human-driven selection. Despite the intensive recent studies dealing with the evolutionary history of barley, the taxonomic status, places and routes of its domestication are still far from comprehensively resolved. The development of novel DNA molecular markers has been a perspective approach to speed up and to increase the resolution of studies addressing the genetic background of barley evolution. In this study, DNA markers based on retrotransposons and pseudogenes were used to assess the genetic divergence of a large collection of barley specimens from different geographical places of origin and domestication. We show that the utilized DNA marker techniques, upon optimization, are informative and relevant to discriminate barley diversity at inter- and within-species level and allow to draw patterns of genome evolution. The obtained data are expected to contribute to a more comprehensive description and optionally to a revision of the current knowledge about the stages of domestication and evolution within the genus *Hordeum*.

GO-2. PSYCHOSOCIAL AND EPIGENETIC CORRELATIONS IN PROBLEMATIC PSYCHOACTIVE SUBSTANCES USE

Krasteva, M. E. ¹, Taseva, T. K. ¹, Koycheva, Y. M. ¹, Racheva, R. Zh. ², Raycheva, T. N. ³, Simeonova, S. V. ⁴

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Many environmental factors such as temperature, diet, infectious pathogens, pollutants and others, may cause epigenetic modifications in the DNA or the associated proteins leading to changes in chromatin structure and organization. Such observations may be related to many complex diseases, including asthma, obesity, cardiovascular disease, neurodegenerative disorders, and cancer. Recently, in addition to physical stimuli, emotional stress and traumatic experience was also shown to cause epigenetic changes with long-term consequences on gene expression. The assumption that psychosocial and epigenetic determinants are involved in the etiology of problematic psychoactive substances use, and that there is a link between these parameters, is the core around which the present research was constructed.

Alcohol and drug addiction is a problem of social importance, which may have serious consequences on the mental and physical health of users. A complex analysis of psychosocial and epigenetic characteristics is done in three subject groups: 17 users who enter programs for opium agonists treatment; 22 users in detoxification and psychosocial rehabilitation programs, and 12 healthy controls. All participants gave an informed consent and the study was approved by the local ethical committee. SLESQ questionnaire was utilized to screen for stressful events in person's history. The quality of live was examined by the WHOQOL- BREF generic questionnaire of the World Health Organization. Gene expression is studied by qRT-PCR analysis. The following genes are under investigation: genes for receptors and transporters of neurotransmitters; genes involved in metabolic pathways; genes with a role in DNA methylation, and others. Bisulfite sequencing will be applied for establishment of methylation pattern in selected genes. A correlation between the studied parameters will be explored.

The results will contribute to elucidation of the mechanisms underlying psychoactive substances addictions and will shed some light on the nature of relationship between psycho-emotional stress and epigenetics.

Acknowledgement: This work was supported by the National Science Fund of Bulgaria, Ministry of Education and Science, Grant KP-06-H25/5 - 13.12.2018 "Correlation between psychosocial and epigenetic characteristics in problematic psychoactive substances use"

GO-3. MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION REMAINS A VALUABLE TOOL FOR PRENATAL DIAGNOSIS

Kercheva, K., Bichev, S., Raynova, R., Andonova, S., Bradinova, I., Savov, A.

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Background:

Invasive prenatal procedures are offered to pregnant women with a variety of indications. Different techniques for genetic analyses are used for prenatal diagnosis – karyotyping, QF-PCR, MLPA, aCGH, etc. and these methods are characterized with different detection rate. Multiplex ligation-dependent probe amplification (MLPA) technique can reveal submicroscopic abnormalities that may remain undetected with standard G-banding. The present retrospective study aims to reevaluate the prenatal indications for performing MLPA for the most common microdeletion and/or subtelomeric deletion syndromes for a period of 18 months.

Materials and methods:

A total of 641 prenatal samples was referred for genetic analysis – QF-PCR or/and karyotyping. Additionally, MLPA technique was used in 72 of them because of isolated cardiac anomalies (27), structural abnormalities on US (21), increased nuchal translucency (11) or other indications (13).

Results:

Pathological results were found in 6 samples out of 72. Submicroscopic deletions were detected in 4 pregnancies with isolated ultrasonographic findings: (1) two del16p13.3 (Rubinstein-Taybi syndrome): one fetus with choroid plexus cysts and the other with flat facial profile; (2) del17q21.31 (Koolen de Vries syndrome) in a pregnancy with an increased NT; (3) del1p36.33 in a fetus with a large ventricular septal defect. A 10q subtelomeric deletion was confirmed in a pregnancy with no ultrasonographic abnormalities and a positive NIPT. Duplication of Xq22 was detected in a pregnancy with a structural chromosomal rearrangement found by karyotype.

Conclusion:

The ultrasonographic findings in microdeletion syndromes are usually non-specific and achieving normal karyotype result may not reveal the genetic status in these cases.

GO-4. COMPARATIVE GENETIC ANALYSIS OF SUBFOSSIL DOGS FROM THE NEOLITHIC AGE AND LATE ANTIQUITY

Yankova, I. ¹, Marinov, M. ¹, Spasov, N. ², Hristov, P. ¹, Radoslavov, G. ¹

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The history of dog domestication is still under debate, but doubtless it is a process of an ancient partnership between dogs (*Canis lupus familiaris*) and humans. Although there have been numerous investigations of dog phylogeny and origin, genetic data of dogs in the region of the Balkan Peninsula (South-Eastern Europe) are still scarce.

Aim: In this study we have presented for the first time mitochondrial data about the ancient dogs from Bulgaria.

Materials and methods: A total of 25 samples were analyzed using the mitochondrial D-loop region (HVRI). The methodology includes: isolation of ancient DNA from bones and teeth, amplification of fragments of mitochondrial DNA (D-loop, hypervariable region I, HVR I), sequencing and phylogenetic analysis.

Results: The results show the predomination of A1 and B1 mitochondrial haplogroup. So far two haplogroups have been spread with high frequency in recent Bulgarian and European dog population. This data revealed a similarity of the Bulgarian dog structure to ancient Italian dogs (A, B, and C clades), which suggests a new prehistoric and historic Mediterranean dog population.

Conclusion: All these data are interpreted in terms of the contemporary view of dog domestication and dissemination in Eurasia during the Neolithic period. Our results, though preliminary, can provide interesting information about the wild ancestors and the origin of domestic dogs. This data is directly associated to the understanding of migration and the cultural-historical processes in our region.

Acknowledgements: The experiments were carried out under permissions and the guidelines of the Bulgarian Academy of Sciences and the Bulgarian Ministry of Environment and Waters (no. 627/30.03.2015).

Keywords: Neolithic Age, dog domestication, ancient DNA, haplogroups, subfossil dogs

PLANT PHYSIOLOGY AND BIOCHEMISTRY

PPBO-1. THE CONSTITUTATIVE ETHYLENE SIGNALING MUTATION CTR1-1 CONFERS SALT STRESS TOLERANCE IN *ARABIDOPSIS*

Vaseva, I. I. ¹, Kirova, E. ¹, Simova-Stoilova, L. ¹, Mishev, K. ¹, Van Der Straeten, D. ², Vassileva, V. ¹

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Plants adapt to environmental challenges through anatomical, metabolic, and morphological changes and the gaseous plant hormone ethylene modulates many of these growth-related physiological processes. Among the other plant hormones it distinguishes as a fairly simple hydrophobic molecule which can freely enter cells via diffusion. The ethylene signalling cascade is suppressed when the hormone is absent via CTR1 (a Raf kinase) which inhibits the membrane transporter EIN2 by direct phosphorylation. Ethylene binding to the receptors inactivates CTR1 releasing the repression of EIN2. C-end of EIN2 is cleaved off and moves to the nucleus where it activates the master ethylene transcription factors EIN3 and EIN3 like1 (EIL1).

The induction of ethylene production as a response to high salinity has been previously documented. It has been also proven that salt-induced stabilization of EIN3 and EIL1 confers salinity tolerance by deterring ROS accumulation. To elucidate the involvement of the major regulators of ethylene signaling CTR1 and EIN2 in salt stress response we performed an extensive comparative study of wild type Columbia-0, *ein2-1* (ethylene insensitive) and *ctr1-1* (constitutive) signalling mutants. The performed analyses provide insights into the molecular mechanism of salt stress response which is related to ethylene signals. Our results demonstrate that ethylene insensitivity is linked to increased vulnerability to salt stress while the constitutive *ctr1-1* mutation increases *Arabidopsis* salt tolerance.

In addition, we explored the implication of CTR1 and EIN2 in hormonal crosstalk regulatory mechanisms in the course of salt tolerance acquisition.

PPBO-2. NOVEL SMALL-MOLECULE EFFECTORS OF THE INTRACELLULAR TRANSPORT IN PLANTS

Mishev, K.

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The endomembrane trafficking logistics in eukaryotic cells comprises membrane-delimited organelles and numerous protein regulators that ensure cargo sorting, vesicle formation and targeting to the acceptor membrane. A distinctive feature of plants is the high degree of diversification within protein families engaged in vesicle trafficking regulation. In many cases, the members of trafficking-related protein families have overlapping functions and can complement each other. Since the classical genetic approaches are often inapplicable when dealing with gene redundancy, the use of small-molecule modifiers discovered through large-scale screens of chemical libraries poses an alternative for studying the protein functions. In spite of the large number of chemical effectors of the endomembrane transport that are currently available for use, the mode of action of most of them is still unknown. Recently, we identified and characterized new small-molecule tools that conditionally alter the secretion, endocytosis and recycling pathways in plant cells. Genetic and biochemical evidences revealed the specific protein targets of the chemical inhibitors which in turn allowed us to dissect the biological roles of those proteins in the intracellular transport. Since endomembrane trafficking mediates the distribution of several plant hormone receptors, the newly identified compounds enabled better understanding of the involvement of the targeted proteins in hormone signal transduction.

PPBO-3. RECOVERY OF PHOTOSYNTHETIC ACTIVITY OF HABERLEA RHODOPENSIS AFTER FREEZING-INDUCED DESICCATION

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Haberlea rhodopensis belongs to the group of resurrection plants which possess the unique ability to survive desiccation to air-dry state. Upon watering the plants rapidly revive and are restored to their former state. In contrast to the other resurrection plants, most of them residing in South Africa, *H. rhodopensis* can withstand freezing temperatures during winter. Similarly to drought, freezing stress cause also dehydration of plants and they survive the harsh winter conditions in a dry state. In the present study, the alterations in photosynthetic activity upon plant acclimation to low temperatures, freezing stress, freezing-induced desiccation and following rehydration were estimated by chlorophyll fluorescence measurements. The quantum yield of PSII electron transport (Φ_{PSII}) measured in the beginning of November was closed to that in May. However, photochemical activity of PSII declined upon cold acclimation. Exposure of plants to freezing stress caused additional reduction of Φ_{PSII} values and they were completely inhibited as a result of freezing-induced desiccation in the middle of December. This down-regulation of photosynthesis was accompanied by increased thermal energy dissipation, thus keeping the balance between the light absorption and its utilization via carbon metabolism.

Plants slowly increased their water content in the early hours of rehydration and their relative water content was 20% after 9 h, when some enhancement of PSII activity appeared. In fact, photochemical activity of PSI recovered faster compared to PSII, reaching maximum after 15 h of rehydration. The quantum efficiency of PSII was significantly increased after 24 h and completely recovered after 7 days of rehydration, when plants regain their water content.

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PPBO-4. JIP-TEST AS A TOOL TO EVALUATE LIGHT RECIPES FOR PLANT GROWTH AND FLOWERING

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Light is a major factor controlling plant growth and development. The present study aimed at investigating the effect of a blue:red:far red LED light formula for improved plant performance (flowering) recommended by OSRAM Opto Semiconductors. High (BR) and low (BRS) light intensity variants were compared to normal (W) and "shadowed" (WS) white fluorescent controls. We utilized the JIP-test for efficient assessment of the physiological state during early growth of pea plants subjected to different light treatments. The obtained results were in correlation with additional physiological analyses including changes in morphometric and photosynthetic parameters (thermal stability of the thylakoid membranes, chlorophyll content, CO₂ assimilation, transpiration). The data pointed the start of growth inhibition under BR and WS light, while BRS stimulated plants to reach flowering stage similarly to the W control. We concluded that early photosynthesis diagnostics could help to set up an optimal recipe for plant growth and flowering.

PPBO-5. PHYSIOLOGICAL ASPECTS OF NATURAL REGENERATION IN BEECH FORESTS

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Forests in which beech (*Fagus sylvatica*, L.) are widespread, are common in Bulgaria in the mountain belt and have been relatively successful despite the late succession nature of the species. The growth and development of beech undergrowth depend on many factors, the most important, which is the light mode. The potential for adaptation of a young tree individual to the abrupt change of the microclimate conditions is realized in several stages, which are crucial for the participation in the future composition of the forest.

The main aim of the project is to study and analyze the adaptation changes in beech forests after logging or natural disturbance. In this regard we plan to investigate: the concentration of enzyme and non-enzyme antioxidants, antimicrobial activity, the intensity and balance of the elements of gas exchange and the concentration of chlorophyll in the leaves of common beech and some of its main competitors; changes in species composition and structure of plant communities in the grassy phytocenotic horizon; absorption changes and the mineralization of N and C; biometric indicators such as height, diameter and leaf index surface.

The investigated sample areas in plantations with a different renewal history in the territory of Petrohan „Training and Experimental Forest Range“, were compared with a neighboring plantation in which no logging has been carried out for the last 20 years. Common beech responds adequately to changed conditions after logging. It acclimated the fastest in its optimal altitude (1000 m) and the slowest – the highest altitude (1450 m). In the lower sub-belt (at around 700 m), its' main competitor (the common hornbeam) acquired rapid dominance in composition, but some advantages of the Common Beech, related to its antioxidant strategy and water and carbon balance, allow it to be, in long term, the main tree species in the future composition of the forest. The established damages from biotic and abiotic factors are more pronounced in the clear-cut, rather than in the forest, but they are preserved to the extent that they either do not significantly change their vital signs or even slightly stimulate them.

This research was supported by grant № НИС-Б-1010/2019/ЛТУ, University of Forestry, Sofia

POSTER PRESENTATIONS

GENETICS

GP-1. NGS STUDY OF POTENTIALLY PROBIOTIC *ENTEROCOCCUS DURANS* EDD2 ISOLATED FROM A BEEHIVE

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Enterococci are Gram-positive, non-spore-forming facultative anaerobes and obligatory fermentative chemoorganotrophs with various habitats. They can be isolated from animal hosts, as well as plants, soil, water, and man-made products. The genus includes antibiotic-resistant strains and strains causing hospital-acquired infections. However, some *enterococci* possess many positive traits leading to their use as probiotics in human and veterinary medicine as well as in food fermentation and preservation processes. The probiotic properties of some *enterococci* strains consist of their ability to produce antimicrobial peptides, called bacteriocins, which inhibit the growth of various pathogens.

The current study focuses on the assessment of potentially probiotic properties of one isolate from a beehive. The analysis of *Enterococcus durans* EDD2 antimicrobial activity were performed using the agar well-diffusion method. The strain showed clear inhibitory activity against the bee pathogen *Paenibacillus larvae*. The strain was previously determined by genus- and species-specific PCR, along with Sanger sequencing of the 16S rRNA gene. Because of its potentially probiotic properties the entire genome was subjected to next generation sequencing. The data obtained were used to perform multiple locus sequence typing (MLST), as well for the search of bacteriocin gene clusters which were found and presumably confirmed the bacteriocin nature of the antimicrobial activity of the strain.

The findings so far allow us to speculate that there might be *Enterococcus* strains within the beehives which are unconsciously selected and transferred by the bees and which serve as protective tools against pathogens.

This research was supported by the National Science Fund of the Ministry of Education and Science, Grant № KII-06-H26/8 from 17.12.2018.

GP-2. REEVALUATION OF TUMOR SUPPRESSOR GENE VARIANTS BY EXAMINING DATA FROM WHOLE EXOME SEQUENCING OF TWO POOLS OF BULGARIAN INDIVIDUALS

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The aim of the present study was to evaluate the clinical relevance of mutations in tumor-suppressor genes by using whole-exome sequencing data (WES) from centenarians and young healthy individuals. Two pools, one of centenarians and one of young individuals, were constructed and WES was performed.

We examined the WES data of Bulgarian subjects for the carriership of tumor suppressor gene variants. Of all variants annotated in both pools, 5080 (0.06%) are variants in tumor suppressor genes but only 46 show significant difference in allele frequencies between the two studied groups. Four variants (0.004%) are pathogenic/risk factors according to dbSNP database: rs1566734 in PTPRJ, rs861539 in XRCC3, rs203462 in AKAP10 and rs486907 in RNASEL. Based on their high minor allele frequencies and presence in the centenarian group, we could reclassify them from pathogenic/risk factors to benign. Our study shows that centenarian exomes can be used for re-evaluating the clinically uncertain variants.

GP-3. ASSOCIATION OF C677T POLYMORPHISM IN MTHFR GENE WITH THE PULMONARY EMBOLISM

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Pulmonary embolism is a relatively common clinical entity accompanied with a high mortality and is a perplexing diagnostic and therapeutic problem. Current research indicates that pulmonary embolism has a genetic association, including with polymorphisms in the gene coding for methylenetetrahydrofolate reductase (MTHFR).

The aim of this prospective, observational, genetic association, case-control study is to investigate the association of the rs1801133 (677 C>T) polymorphism in the MTHFR gene with the pulmonary embolism.

The demographic, clinical, laboratory, as well as genetic data are analyzed from 31 patients with pulmonary embolism, as well as from 24 control subjects without history of previous thrombotic entities.

The genetic analysis revealed that there is a statistically significant association of homozygous TT genotype and allele T with the pulmonary embolism. The carriers of this genotype and allele have 4.4 fold and about 2.5 folds higher odds for pulmonary embolism, respectively, regarding to the carriers of the homozygous CC or heterozygous CT genotypes and allele C.

These results are concordant with the results of some of the similar previously published studies and indicate the possible applicability of this genetic marker in the prediction of the risk of pulmonary embolism and disease progression.

Key words: pulmonary embolism, thromboembolism, MTHFR C677T, gene polymorphism

GP-4. GENTOTOXIC PROPERTIES OF *COTINUS COGGYGRIA* SCOP. ON HUMAN MALIGNANT MELANOMA A375 CELLS

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Cutaneous melanoma is a highly metastatic and lethal cancer form. Primary and acquired drug resistance are major obstacles to the treatment of the disease as they strongly limit the effectiveness of chemotherapy. *Cotinus coggygia* Scop. is a medicinal plant which has been recognized as a valuable plant species with significant pharmacological potential. The anticancer properties of *C. coggygia* have so far been relatively poorly studied. At present, no data concerning the effect of extracts or fractions of the medicinal plant on in vitro melanoma model system are available except our recent study which revealed considerable antiproliferative activity of crude aqueous ethanolic leaf extract from *C. coggygia* and its chloroformic and aqueous fractions on human melanoma cell line A375. The aim of the present research was to investigate genotoxic potential of the aqueous fraction of *C. coggygia* extract in A375 cells by alkaline comet assay. The obtained results showed that after a 72-hour treatment period in a range of concentrations from 20 to 60 µg/ml the fraction causes statistically significant genotoxicity effects in A375 cells. Future investigations will involve more detailed elucidation of the molecular mechanisms and targets underlying anti-melanoma activity of the aqueous fraction.

Key words: *Cotinus coggygia* Scop., malignant melanoma, in vitro genotoxicity

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GP-5. STUDY OF THE CYTOTOXIC POTENTIAL OF AGRICULTURAL BIOSTIMULANT NATURAMIN-WSP ON HUMAN CELL LINE BJ AND MOUSE EMBRYONIC CELL LINE BALB/3T3

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Although the wide application of biostimulant products in the last years due to their role in the development and production of valuable agricultural cultivars a need for a more in-depth study on their safety on human health is required. The objective of the present study was to assess the potential of biostimulant Naturamin-WSP to influence cell viability of two fibroblast cell lines, BJ and BALB/3T3, of human and mouse origin, respectively. Cell viability was determined by MTT assay after 24 h treatment period in a range of concentrations (from 0.01% to 15%) in parallel with light microscopy examination of cell morphological characteristics. The obtained results showed that the applied biostimulant has minor effect on the viability of the both cell lines at doses up to 5% and more considerable cytotoxic impact only at the highest tested concentrations of 10% and 15% which exceeds substantially the doses applied in agriculture. Further analysis will be directed to elucidation of the possible mechanism of action.

Keywords: biostimulants, cell viability assessment, in vitro safety analysis

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GP-6. IN VITRO ANTIDIABETIC ACTIVITY OF HYPERICUM PERFORATUM L. TRANSGENIC SHOOT CULTURES

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Three transgenic shoot clones (TS B, TS F and TS H) of *Hypericum perforatum* L. were evaluated for phenolic compounds composition and in vitro antidiabetic activity. The TS clones were spontaneously regenerated from the corresponding hairy root cultures induced by *Agrobacterium rhizogenes* A4-mediated transformation. Phenolic compounds in TS clones and non-transformed shoots (NTS) were analyzed using high-performance liquid chromatography (HPLC) coupled with diode-array detection (DAD) and tandem mass spectrometry (MSn) with electrospray ionization (ESI). In vitro antidiabetic activity was performed through the capability of TS extracts to inhibit enzymes such as α -amylase (α -AMYL) and α -glucosidase (α -GLUC) related to diabetes disease. The chromatographic analysis of TS and NTS extracts revealed the presence of phenolic acids, flavan-3-ols and flavonols. Among transgenic shoots, TS F was selected as the best-producing clone for chlorogenic acid, epicatechin, procyanidin derivatives and hyperoside, while TS B was shown as superior for the accumulation of rutin and quercitrin. With respect to in vitro antidiabetic properties, TS and NTS extracts showed considerable and dose dependent inhibition of α -AMYL and α -GLUC activities. Statistical analysis demonstrated that phenolic acids significantly contributed to the antidiabetic activity of TS extracts through α -AMYL and α -GLUC inhibition. Present data suggested that *H. perforatum* transgenic shoot extracts represent a promising source of phenolics with antidiabetic properties that could be used as hypoglycemic compounds for regulation of postprandial glucose level.

GP-7. CELL ABNORMALITIES IN *ALLIUM CEPA* L. ASSOCIATED WITH CERTAIN CONCENTRATIONS OF SYSTHANE 12E

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Allium cepa L. is a plant with a great sensitivity to chemical agents and can be used as a model plant for detection of cytotoxic and genotoxic effects induced by exposure to certain agents used in agriculture. The use of fungicides if not properly dosed may result in alternation of cell processes and morphology, so a proper concentration that will influence only the pathogens that may harm the plant cycle and not the plant itself, is of great importance.

We examined certain cytogenetic and physiological parameters in *Allium cepa* L in terms of treatment with different concentrations of the fungicide Systhane 12E.

After a treatment with different concentrations of the fungicide, all the groups of control and treated plants were analyzed. Every phase of the cell cycle was inspected for irregularities and the mitotic index (MI) was calculated. Depending on the concentration, many abnormalities were found including conjugated chromosomes, polynuclear cells, micronucleouses etc. In the severe cases, cells with complete erosion of the nucleus were detected. The higher concentrations had impact on the physiology of the plant, resulting in reduced production of chloroplast pigments.

In conclusion, a correlation between the concentration of the fungicide and the amount of abnormalities in the cells was found, indicating the importance of its careful dosage.

Key words: Systhane 12E, *Allium cepa* L., mitotic index, aberration of chromosomes, chloroplast pigments.

GP-8. APPLIED ANIMAL GENETICS IN BULGARIA

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The theoretical knowledge about the inheritance of traits and diseases can have a huge application in a veterinary context. By using molecular genetic tests, crossbreeding between carriers of autosomal recessive mutations can be avoided. Identification of inborn predispositions can help to prevent or at least delay the development of a disease. Finally, in cases of phenotypic ambiguity, the genetic sex of an individual can be determined. Here we resume our first genetic testing experience of animals, more precisely companion animals and birds. So far, we have implemented molecular genetic tests for variations with well-known correlation to a pathological condition and thus with a high practical value. Our first target has been the canine Multi-Drug Resistance Gene (MDR1, ABCB1). We have successfully performed genetic testing for identification of the c.227_230delATAG mutation in a control group and identified the ABCB1-1Δ genotype in a pair of Australian Shepherd dogs. Concerning the feline genetics, we have focused our work on the Polycystic Kidney Disease (PKD). Optimization of the molecular genetics assay and application of the protocol to a control group of cats of different breeds has been executed. The c.10063C>A variant in the PKD1 gene was as expected detected in Persian cats. So far, we have also implemented some breed specific tests in dogs, for example: in Lagotto romagnolo – for Lagotto Romagnolo Storage Disease; In Shar-Pei – for Shar-Pei Autoinflammatory Disease (MTBP gene), in golden retriever – for PNPLA1-related Ichthyosis and Progressive Retinal Atrophy 1 and 2, in Mini Schnauzer – for Myotonia Congenita. Lastly but not least, we have determined the sex of different species of birds: domestic chicken (*Gallus gallus domesticus*) and parrots (*Serinus canaria*, *Nymphicus hollandicus*, *Melopsittacus undulatus*, *Psittacus erithacus*). For the future, we plan expanding our activities in the canine and feline genetics by identifying typical for certain breeds mutations and maybe focusing also on other species.

GP-9. DEREGULATED LEVELS OF RUVBL1 PROTEIN INDUCE TRANSCRIPTION DEPENDENT REPLICATION STRESS

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The frequent occurrence of transcription and DNA replication in cells results in many encounters, and thus conflicts, between the transcription and replication machineries. These conflicts constitute a major intrinsic source of genome instability, which is a hallmark of cancer cells. The INO80 family members RUVBL1 and RUVBL2 are overexpressed in cancers from many different tissue types. They belong to a large family of ATPases known as AAA+ proteins (ATPases associated with diverse cellular activities) including nucleic acid-processing enzymes, chaperones, and proteases. Their functional diversity is likely due to participation in different cellular complexes or complex sub-assemblies, including INO80, TRRAP-TIP60 and SRCAP chromatin remodeling complexes. RUVBL proteins regulate the expression of p21 and interact with c-myc, RPA which could play a significant role in cancer cell growth. Previously, we have shown that the knock-down of Ino80 and Arp8 subunits of INO80 remodeler led to deficient replication elongation and destabilized stalled replication forks, implying a similar requirement for RUVBL proteins. In line with this expectation, knock-down of RUVBL1 like their overexpression slowed S-phase progression and led to reduced rate of replication elongation as found by DNA fibre labelling and analysis with EdU incorporation. Reduced levels of RUVBL proteins and their overexpression also lead to increased replication stress and genome instability. We hypothesize the possible involvement of RUVBL in resolution of transcription-replication conflicts in the nucleus.

GP-10. CYTOSTATIC CAPACITY OF FRACTIONS FROM LEAF EXTRACT OF *COTINUS COGGYGRIA* AGAINST A431 HUMAN EPIDERMAL CARCINOMA CELLS

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Medicinal plants represent highly promising source for development of new advanced oncotherapeutic agents for a variety of malignancies. Non-melanoma skin cancer is the most common type of cancer in Caucasians with about more than 2 million patients diagnosed each year. The aim of the present research was to study cell growth inhibition potential of chloroformic and aqueous fractions from crude leaf aqueous ethanolic extract of *Cotinus coggygia* Scop. on human cutaneous squamous cell carcinoma cell line A431 and to compare it to the antiproliferative properties of the fractions on human normal skin cell line BJ. In parallel, the cytostatic effect of gallic acid on A431 cells was also investigated in order to compare it to the effects observed for the two *C. coggygia* fractions. The assessment of the cell growth reduction properties was performed through MTT cell proliferation assay after treatment for 72 hours in a set of concentrations. The obtained results showed that chloroformic and aqueous fractions of the herb extract possess a dose-dependent antiproliferative activity on epidermoid carcinoma cell line with a stronger effect registered for the aqueous fraction. Gallic acid was detected to reduce A431 cell proliferation in a highest degree. The cell growth of the normal control BJ line was influenced by the tested substances in a considerably weaker degree in comparison to the cancer cells which is an indicator for antiproliferative selectivity. Future investigations will be focused on examination of some molecular mechanisms of the anti-skin cancer effect of the *C. coggygia* fractions.

Key words: *Cotinus coggygia* Scop., antiproliferative assay, A431, BJ

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GP-11. APPLICATION OF BIOSTIMULANTS FOR IMPROVEMENT OF SEED PERFORMANCE IN MAIZE

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Biostimulants are innovative products which are becoming increasingly popular in modern agriculture due to their ability to improve plant stress defense mechanisms thus stimulating the growth and productivity of various crop plants. This study was designed to investigate whether biostimulant application might have beneficial effect on the germination potential and early seedling development in maize as well as to look at some of the molecular and physiological aspects of biostimulant's mode of action on this crop. Three brands of commercially available biostimulants, namely Kaishi, Terra-Sorb and Naturamin, were tested for their ability to improve the germination characteristics of maize seeds. Each biostimulant was applied individually at five different concentrations ranging from 0,01% to 10%. Seeds were soaked in the respective solutions for 24 or 4 hours and then germinated at 24°C. Germination characteristics of control and biostimulant-treated seeds were examined after 24, 48 and 72 hours. The results showed that 24h-treatment had rather negative effect on the early stage of seed development. On the other hand, lower biostimulant concentrations applied for 4 hours improved the germination performance of maize seeds. The concentrations showing positive effects differed depending on the biostimulant brand. Application of Kaishi as 0,5% solution stimulated both germination rate and root growth, though the highest concentration of 10% showed clear inhibiting effect. Terra-Sorb was active as a seed stimulant at 0,05% concentration, whereas Naturamin exerted positive effect at three different concentrations 0,01%, 0,05% and 0,5%. Both biostimulants, however, had detrimental influence on seed development as 5% and 10% solution. In conclusion, the results showed that all three biostimulants have the capacity to improve seed germination in maize and thus might be useful to enhance the vigor of low quality seeds. DNA damage defense mechanisms have a primary role during early seed germination and their proper action underlies all physiological processes necessary for successful seedling establishment. Experiments are in progress to study the molecular mechanisms of biostimulant action on maize seeds at the level of gene expression and DNA damage induction.

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GP-12. ISOLATION AND CHARACTERIZATION OF BACTERIA THAT CAUSE TONSILLITIS IN DOG BREEDS ENGLISH BULLDOG AND GERMAN BOXER IN BITOLA REGION

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In this study, 12 samples from clinical cases of dogs of breeds English Bulldog and German boxer in Bitola region for the period from January 2016 till April 2018. Totally 12 strains were isolated and identified based on their growth, colony morphology, Gram stain, catalase and oxidase activity using standard protocols. From the results obtained, it can be concluded that the most common bacteria that cause tonsillitis in dogs from the above-mentioned races are. *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pasteruella spp*, *Klebsiella spp*.

Key words: Dogs, Tonsillitis, Bacterial causes, German Boxer, English Bulldog.

GP-13. WHOLE EXOME DIVERSITY IN BULGARIANS

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Introduction: In order to delineate Bulgarian exome diversity we have performed whole exome sequencing (WES) of DNA pool samples and compared the obtained results with those in gnomAD genome database.

Materials and methods: The analysis was performed on two pool samples comprising 32 Bulgarian centenarians and 61 healthy controls (aged 18 to 30). The samples were WES sequenced at BGI with 250 x coverage. The variants were annotated using wANNOVAR and filtered by stringent criteria. Statistical analyses were performed by using the R package. Bulgarians were compared with gnomAD populations altogether – by PCA (Principal Component Analysis) and pairwise -in allele frequency plots and by Pearson correlation coefficients.

Results: The number of annotated variants passing filtering criteria was 55 519 out of 177 126. In the PCA analysis, Bulgarians fall within the same ancestry group with non-Finish Europeans, Finns and Ashkenazi Jews, apart from Latino-Americans, Africans and East Asians. The values of the Pearson correlation coefficients between Bulgarians are as follows (in decreasing order): Non-Finnish Europeans - 0.9502603; Ashkenazi Jews - 0.9328112; Finnish Europeans - 0.9243355; Americans - 0.8679907, East Asians - 0.7020868, Africans -0.6916458. Despite the high Pearson correlation coefficients between Bulgarians and Europeans, there are variants with more than 30% difference in allele frequency in the lactase gene and in genes involved in the immune system; cell cycle; cell-cell communication; lipid, protein and carbohydrate metabolism.

Conclusions: The present study is a step towards the establishment of a Bulgarian National Reference Genome, necessary for the interpretation of sequencing data. Acknowledgment - DN 03/7 from 18.12.2016 - National Science Fund of Bulgaria.

GP-14. COMPARISON OF DIFFERENT TOOLS FOR THE GENERATION OF DRAFT BACTERIAL GENOME ASSEMBLIES FOR *E. FAECALIS* ISOLATES WITH CLINICAL SIGNIFICANCE

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Multidrug resistant *E. faecalis* isolates have proven to be a serious problem for both medicine and public health as they have been heavily associated with severe life-threatening infections and persistent colonization of the human intestinal tract. Recently, several cases of nosocomial vancomycin-resistant *E. faecalis* isolates have been reported in Bulgaria. The best approach for studying the genetic bases of such resistances is to sequence the genomes of strains of interest using NGS technologies. Unfortunately, the assembly of bacterial genomes from short Illumina reads is not a trivial task. In this work, we compare several different tools for genome assembly in order to find the optimal conditions for this procedure in *E. faecalis* strains.

GP-15. PRELIMINARY ASSESSMENT OF STRAWBERRY TREE (*ARBUTUS UNEDO* L.) CLONES BY SSR MARKERS

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Strawberry tree (*Arbutus unedo* L.) is an underutilized evergreen small fruit tree of the *Ericaceae* family naturally growing in the Mediterranean basin through the north of the Iberian Peninsula until the southwestern France, and in Macaronesia and Ireland.

Although edible and palatable, the usually red and relatively small fruits are fragile and with short shelf life when mature, which hampers their large-scale commercialization.

In Portugal, particularly in the Algarve region, this species is of relative economic importance since, besides the common use for production of jams and preserves, the fruits are used to produce the relatively expensive distilled liquor “aguardente de medronho”.

Traditionally cultivated by small farmers in small orchards in the woods, strawberry tree is progressively being cultivated in larger orchards by modern companies, a new paradigm that requires the substitution of the traditionally seed propagated plant populations by previously selected, even improved, vegetatively propagated clones.

Having into consideration that these new developments will contribute to narrow the genetic variability the company Corte Velada, in collaboration with the LGGI of the University of Algarve (Faro, Portugal), is establishing a publicly accessed germplasm field collection.

In this communication, we report the results of a preliminary genetic assessment of 50 accessions of the Corte Velada field collection by SSR markers. This set of accessions were previously selected for a more detailed genetic, biochemical and agronomic evaluation for later registration of the best performing clones in the national catalogue of plant varieties.

The genomic DNA was isolated from cell nuclei enriched leaf pellets using a protocol previously developed in the laboratory.

The four microsatellite loci (AU32030/KF023875, AU81604/KF024305, AU93953/KF024411, AU1427/KF023647) analyzed in this study were retrieved from a set of 1185 microsatellite loci uploaded to the Gene Bank database (NCBI), identified among 99,786 sequences obtained in the frame of a next generation sequencing project performed by the LGGI, UALg (ncbi.nlm.nih.gov/sra/SRX341237).

The currently obtained results allowed us to establish a binary profile of the examined clones regarding the homozygous/heterozygous state of the assessed microsatellite loci and to obtain a first insight of the genetic diversity of the field collection. The preliminary molecular fingerprint of the assessed strawberry tree genotypes, obtained by agarose gel electrophoresis, will be later confirmed and refined by a more precise analysis by fluorescence capillary electrophoresis.

GP-16. MICRORNAS: NOVEL DIAGNOSTIC AND PROGNOSTIC BIOMARKER FOR MULTIPLE HUMAN DISEASES

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MicroRNAs are a class of small non-coding single-stranded RNAs of ~ 21 nucleotide in length. They regulate gene expression at translational or posttranslational level through repression of translation and degradation of mRNA. They are expressed in various cells and tissue types. MiRNA are involved in regulating many physiological processes such as cell growth, proliferation, differentiation, metabolism, etc. Dysregulation of their expression is associated with different human disorders - different types of cancer, neurogenerative disorders, endometriosis, cardiovascular diseases, diabetes and others. Recent research show that miRNA are differently expressed in healthy individuals and in patients with the aforementioned disorders. That is why miRNA are proposed as diagnostic and prognostic biomarkers for different diseases.

GP-17. PHENOTYPIC CHARACTERISTICS OF BULGARIAN LOCAL PEPPER (*CAPSICUM ANNUUM* VAR. *ANNUUM*) ACCESSIONS

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Pepper (*Capsicum annuum* var. *annuum*) is one of the main vegetable crops in Bulgaria. Our country is appeared as a second centre of its formation. A lot of genotypes have been created as a result of long breeding process, which are valuable resource for preservation the genetic diversity of this crop. The group of pungent peppers is of limited production and is not an object of intensive investigation. A big collection of local pepper landraces has been maintained at the Institute of Plant Physiology and Genetics (IPPG). The purpose of the present study was to characterize phenotypically a part of this germplasm. Twelve *C. annuum* accessions of 3 fruit types whose representatives are commonly pungent: shipka, horns (roga) and fishes (ribki) were used as an initial material. Phenological observation, morphological characterization and biometric measurements were performed according to the Descriptors for *Capsicum* of the International Plant Genetic Resources Institute (IPGRI) and the requirements of Bulgarian Executive Agency of Variety Testing, Field Inspection and Seed Control. The features with best distinguishability within every of the studied groups as well as these of economic importance were chosen. The accessions belonging to the horn-like pepper were more uniform. Variations were registered mainly in the fruit colour in technical and botanical maturity. Fish-type group varied in plant height and fruit shape, surface and size. No 60 had unusual longitudinally ribbed large rotund fruits. Shipka-type accessions showed significant variability in plant height, habit, nodal anthocyanin, anther colour, fruit shape, size, colour, position, weight, locule number, corrugation, as well as in productivity and maturity period. Within the No 25-2 population single anthocyaninless plants were found. A stable line with recessive *al* gene, responsible for anthocyanin absence in nodes and anthers was obtained from their progeny and included in this study. Capsaicin was present or absent in representatives of all studied groups. The obtained results are a part of genetic and breeding investigations on pepper.

Key words: local pepper accessions, fruit types, morphological variation

GP-18. PROTEIN AMADORIASE ACTIVITY OF THE GLYCOLYTIC ENZYME PHOSPHOGLUCOSE ISOMERASE

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The Maillard reaction, known as glycation, is a spontaneous non-enzymatic reaction between reducing sugars (such as glucose-6-phosphate (G6P)) and organic amines. In the early stage of this reaction, the nucleophilic amino group attacks the electrophilic oxygen of the sugar carbonyl group, leading to the formation of reversible aldimines (Schiff's bases (SB)), followed by rearrangement of the SBs to less reversible ketoamines (Amadori products (APs)). Further spontaneous rearrangements in the later stages of the glycation reaction result in the formation of stable advanced glycation end products (AGEs), which compromise the structure and function of both proteins and DNA. Nearly two decades ago enzymes for repair of APs-modified proteins were discovered and called amadoriases. However, only recently we have described a similar enzyme for repair of glycation damaged DNA. We have shown that the glycolytic enzyme phosphoglucose isomerase (PGI) of the intestinal bacterium *Escherichia coli* catalyzes in vitro the removal of G6P-derived APs from DNA and an *E. coli* strain with a deleted *pgi* gene exhibits increased spontaneous mutation rate. These data revealed PGI as a novel DNA repair enzyme (DNA amadoriase). In the present study we explored PGI for broader substrate specificity. Using as a model protein G6P-modified chicken lysozyme here we demonstrate that besides as DNA amadoriase PGI functions also as a protein amadoriase. Kinetic studies further revealed that the catalytic efficiency of PGI with glycated lysozyme is about three times lower than that with glycated DNA as a substrate.

GP-19. ENGINEERING AN OPTIMIZED GRNA CASSETTE THAT UTILIZES CHROMOPROTEIN EXPRESSION AS A SELECTION MARKER

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CRISPR Cas9 is arguably the most used technology in modern genetics with numerous different applications like genome editing, base editing, transcriptional control and many others. A key property of this tool is its ability to be easily re-targeted to genomic regions of interest simply via cloning a novel 20-base pair gRNA compound. Many different gRNA expression vectors exist to date, but the cloning of the short targeting compound is inevitably associated with a significant number of negative clones due to self-circularization of the empty plasmid. In this work we present an optimized gRNA expression cassette design that utilizes a red chromoprotein as a selection tool for distinguishing these exact empty colonies and, thus, increasing the fidelity of this step.

GP-20. AN AQUA CLONING-BASED APPROACH FOR INTRODUCING NOVEL GRNAS GENERATED BY GENE SYNTHESIS INTO EXPRESSION VECTORS

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The rapid decrease in prices for gene synthesis services makes them a promising alternative for the conventional cloning approaches that use complementary oligonucleotides. The freedom guaranteed by gene synthesis allows the user to easily manipulate the elements of the gRNA expression cassette, like promoters, terminators, etc. In this work we describe an Aqua cloning-based procedure for cloning such gRNA cassettes generated by gene synthesis without the need for restriction endonucleases and/or ligase.

GP-21. ENGINEERING A BIOBRICK-COMPATIBLE EXPRESSION VECTOR FOR PRODUCTION AND PURIFICATION OF ANTIMICROBIAL PEPTIDES

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Multidrug resistant (MDR) bacteria are one of the worst threats to public health. It bears high economical and sociological burdens expected to significantly rise in the near future. One of the most promising alternatives to the conventional antibiotics for fighting against such strains are antimicrobial peptides (AMPs). They are naturally expressed in all domains of life, but their purification and characterization from natural sources is usually a challenging task. To overcome this, modern biotechnology allows us to perform heterologous expression in order to obtain the needed quantities of the AMPs of interest. This work is concentrated on the development of a new expression system for heterologous production of these new possible alternatives to antibiotics.

GP-22. SPERM DNA MATURITY IS ASSOCIATED WITH TIME-LAPSE VARIABLES OF HUMAN EMBRYOS

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BACKGROUND: Sperm DNA fragmentation and maturity has been associated with delayed natural conception, reduced fertilization rates, lower embryo quality, decreased pregnancy rates and increased miscarriage rates. However, little is known about the associations between sperm DNA fragmentation and certain time-lapse parameters of human embryos.

OBJECTIVE: The aim of this study was to evaluate the associations between sperm DNA fragmentation and certain time-lapse variables of the obtained human embryos.

MATERIALS AND METHODS: Participants were 124 couples undergoing intracytoplasmic sperm injection (ICSI). Sperm DNA fragmentation was assessed by the chromatin structure assay (SCSA). Two DNA fragmentation parameters were analysed: (1) DNA fragmentation index (DFI) and (2) high DNA stainability index (HDS). The obtained embryos were cultured in a single-step Global® culture medium and a time-lapse system (EmbryoScope, Vitrolife, Sweden). Fifteen time-lapse parameters were analysed: time of pronuclei appearance (tPNa), time of pronuclei fading (tPNf), cleavage times (t2, t3, t4, t5, t6, t7, t8, t9), morulae formation time (tM), starting blastulation (tSB), full blastocyst stage (tB), expansion and hatching timing). The relationship between sperm DNA fragmentation and embryo time-lapse variables were analyzed with the Spearman correlation coefficient. Spearman rank correlation analysis was carried out by using SPSS v.21. P<0.05 was considered statistically significant.

RESULTS: Sperm DFI did not show significant association with the studied embryo morphokinetic parameters. However, time-lapse variables from the initial embryo development - time of pronuclei fading (tPNf) and time for two cells (t2), and time for three cells (t3) were significantly correlated with HDS ($r = - 0.32$, $r = - 0.33$, $r = - 0.28$, respectively).

CONCLUSIONS: The study confirmed the hypothesis that early embryo development is influenced by sperm DNA maturity. Higher percentage of immature sperm (HDS population) can slow down certain critical steps of early embryo development that have an essential impact on implantation success and live birth rates.

PLANT PHYSIOLOGY AND BIOCHEMISTRY

PPBP-1. ISOLATION AND OPTIMIZATION OF PRODUCTIVITY OF BIOLOGICALLY ACTIVE SUBSTANCES FROM CYANOPROKARYOTA

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The biotechnology of microalgae and cyanoprokaryota has gained considerable importance in past decades. Applications range from simple biomass production for nutrition to valuable products for ecological and pharmaceutical applications. *Chroococcus* R-10, isolated from a hot spring in Rupite, south-west Bulgaria (28 °C), was introduced to laboratory cultivation for the first time.

In this research we tried to estimate the optimal conditions for growth and accumulation of high amounts of its extracellular polysaccharide. Our results showed that high yield was observed when the alga was cultivated in the range 26 - 35 °C, but the light intensity had an unexpected effect. High light intensity (16 000 lux) stimulated the growth at 29 °C, yet affected it negatively at 26 and 35 °C. Lower temperatures induced the polysaccharide synthesis, while higher ones suppressed it. Using this knowledge, we managed to achieve maximal yield of 10.3 ± 0.3 g L⁻¹ and polysaccharide viscosity of 4.9 ± 0.2 m Pa.cm³g⁻¹ after 336h of cultivation at 26 °C, 8000 Lux, and initial density of 1.1 g L⁻¹. The large quantities of the produced polysaccharide increase the strain's potential for future biomedical studies (anticancer, antibacterial, antifungal, etc.).

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PPBP-2. MODULATION OF PHYSIOLOGICAL RESPONSES OF WHEAT SEEDLINGS GROWN UNDER DROUGHT STRESS BY PRE-APPLICATION OF AUXIN TYPE PLANT GROWTH REGULATORS

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Drought is among the abiotic stresses, which considerably affect the agricultural production worldwide. The effects of exogenous auxin-type compounds 1-[2-chloroethoxycarbonyl-methyl]-4-naphthalenesulfonic acid calcium salt (TA-12) and 1-[2-dimethylaminoethoxycarbonylmethyl]naphthalene chlormethylate (TA-14) on the physiological responses to drought stress induced by polyethylene glycol (PEG) in wheat (*Triticum aestivum* L.) plants were investigated. Preliminary application of TA compounds partially restored the normal growth of PEG-treated plants and led to less accumulation of proline, phenolic compounds and low-molecular thiols. The wheat seedlings subjected to drought stress were characterized with reduced total protein content and activity of the antioxidant enzymes catalase and guaiacol peroxidase, while the pretreatment with auxin-type compounds increased these parameters. Taken together these results showed that the preliminary application of TA-12 and TA-14 reduced the negative effects of drought stress in wheat plants.

Keywords: antioxidants, auxin type compounds, drought stress, *Triticum aestivum* (L.).

Acknowledgements: Part of this study was supported by Bulgaria-Egypt joint research program (2019-2021)

PPBP-3. EFFECT OF OSMOTIC STRESS ON PHYSIOLOGICAL STATUS OF WHEAT SEEDLINGS GROWN ON LOW AND HIGH NITROGEN

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Bulgarian wheat variety Slomer was used in the experiments as a representative of old bread wheat germplasm characterized by tall plant stature and non-responsiveness to nitrogen in field conditions. Seedlings were grown as water cultures on two nitrogen levels – full and reduced (1/5 of full nitrogen supply). Stress was imposed by adding 25 % PEG to the nutrient solution for 72 h. Results showed that higher nitrogen supply contributed to the better performance under stress conditions in terms of preservation of higher water content, lower membrane injury and higher antioxidant activity. In addition, levels of MDA and hydrogen peroxide remained considerably lower in stressed plants grown on full nitrogen supply. However, no significant differences in growth parameters were observed regarding seedlings and root length under stress conditions and contrasting nitrogen levels (high and low N supply), which could be attributed to the early growth stage at which the experiment was conducted. All these lead to the conclusion that laboratory stress could provoke quite different reaction in plants than stress in the field.

PPBP-4. OPTIMIZATION OF PHOTOSYNTHETIC PRODUCTIVITY AND ANTIOXIDANT CAPACITY OF *E. SATIVA* BY LIGHT SPECTRUM

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Rocket salad (*Eruca sativa* L.) is one of the popular Mediterranean green leafy vegetables. It is recommended to be included in human diet because it is rich in antioxidants and vitamins but also is a very low-calorie vegetable. *E. sativa* is available in the markets year round. That's why it is often grown in greenhouses and cultivation facilities, where light-emitting diodes (LEDs) are more and more often used as a light source. The technological development in LEDs last two decades have led to new opportunities for protected cultivation. One of the most important advantages of LEDs is the capability of spectral composition control. Numerous processes (e.g. photosynthesis, photomorphogenesis, germination, flowering, biomass accumulation, and phytochemical synthesis) can be controlled and optimized by modulating light wavelengths. It is already known that the reaction to different wavelengths is species specific. Thus the light management should be precisely set according to the cultivated culture and the desired goals.

To assess the effect of changes in light spectrum on rocket salad, three lighting conditions were used: white light (WL) provided by fluorescent lamps, red-blue (RB) and red-green-blue (RGB) provided by LEDs. Plants grown under the three different light treatments were analyzed for growth, photosynthetic performance, biomass production and antioxidant capacity.

The results showed stimulation of photosynthetic productivity in plants grown under LEDs. *E. sativa* grown under RGB light has the lowest biomass yield. Our results show that different light treatments also affect the antioxidant capacity of rocket plants and lead to changes in the accumulation of different phytochemicals with established antioxidant effect for humans.

The conducted experiments confirm the hypothesis that with precise management of light spectrum using LEDs horticultural production quality can be improved so the economical efficiency in vegetable cultivation can be increased.

Acknowledgment: This work was supported by the Bulgarian Ministry of Education and Science under the National Research Program "Young scientists and postdoctoral students" approved by DCM # 577 / 17. 08. 2018

PPBP-5. STRESS-RESISTANCE PROMOTION BY POLYAMINE PRE-TREATMENT IN TWO BULGARIAN WHEAT VARIETIES

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Drought is the most important environmental stress in world agriculture. It is a significant problem for Bulgaria as well, since the agricultural regions are located in areas of extreme continental climate, comprising long periods of drought, excessive quantities of rainfall, extremely cold winters or hot summers. In addition, the global climate changes enhance the severity of the stress on cultivated plants. Wheat seedling stage is a critical period for drought stress, and the time when drought stress often occurs. The development of sustainable ways for plant growth and ultimately grain yield is very important. It has been shown that polyamine biosynthesis and signalling is closely related to plant stress tolerance. In this study two Bulgarian varieties of wheat (*Triticum aestivum* L.) which differ in their tolerance to water deficit have been subjected to osmotic stress after a pre-treatment with putrescine. Our results showed that putrescine pre-treatment increased the photosynthetic rate in both wheat varieties and improved the resistance of photosynthetic apparatus under osmotic stress. The addition of exogenous putrescine and the following osmotic stress exposure provoked changes in polyamine pool. The two wheat varieties demonstrated also different salicylic acid profile under the conditions of the experiment and triggered different mechanisms of regulation of the antioxidant system. The more stress-sensitive variety was more severely damaged, but it also had a beneficial pre-treatment effect. These results suggested that putrescine pre-treatment may alleviate the negative effect of PEG 6000 on growth parameters and photosynthetic activity to a certain extent and affect the biosynthesis of the investigated metabolites.

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PPBP-6. SCREENING OF DROUGHT TOLERANCE OF EIGHT WINTER COMMON WHEAT CULTIVARS

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Drought tolerance of eight winter common wheat cultivars (*Triticum aestivum* L.) was compared by measuring the changes in photochemical activity of PSI and PSII, thermal energy dissipation, specific leaf area, and the extent of electrolyte leakage. The degree of water stress was determined by alterations in the soil moisture and relative water content (RWC) of the leaves. Plants were grown in a climatic chamber (Fytoscope FS-RI-1600, Photon System Instruments, Czech Republic) at 20°C/18°C day/night temperature, the light intensity of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 16/8 h light/dark photoperiod and 55% air humidity until 3rd leaf emerged. The measurements were performed on the second leaf after 3 and 7 days of dehydration and after 4 days of rehydration of the dried plants.

The soil moisture decreased by 50% after three days of dehydration of wheat plants but RWC of leaves was not significantly affected. The quantum efficiency of PSII ($\square\text{PSII}$) slightly decreased only in cultivars Niky and Tsarevets and it was accompanied by 60% enhancement in thermal energy dissipation. The most significant changes were observed after 7 days of dehydration, when RWC was reduced about 50% in all cultivars. The strongest inhibition of PSII activity was observed in cultivars Niky and Tsarevets and it was accompanied by an accumulation of closed PSII reaction centers. In addition, the highest electrolyte leakage from leaves was detected for these cultivars, indicating some membrane damage. The strongest decrease in the specific leaf area was observed in Niky. The values of $\square\text{PSII}$ and electrolyte leakage were the least affected by 7 days of dehydration in cultivars Guinness, Diamant and Sadovska belya. Moreover, significant enhancement in PSI activity was observed in all cultivars except Niky under strong drought stress which could help them to recover upon rehydration. Overall, the results obtained showed that the winter wheat varieties studied could be divided into 3 groups: the most susceptible to drought were the varieties Niky and Tsarevets, and the most resistant - Guinness, Diamant, Sadovska belya, cultivars Bononia, Petya and Lusil showed moderate desiccation tolerance.

This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM # 577 / 17.08.2018".

PPBP-7. CONCENTRATION EFFECT OF DIFFERENT BIOSTIMULANTS TO YOUNG MAIZE PLANTS

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The effects of different biostimulants (the hydrolysates Kaishi, Terra-Sorb and Naturamin) on functional activity and development of young maize plants were analyzed. Our aim was to determine the optimal concentration for each of the studied substances.

Maize seeds were soaked in five concentrations of the commercial biostimulants (0.01%, 0.05%, 0.5%, 5% and 10 %) for 4 h and distilled water was served as a control. The effects of biostimulants were evaluated in 15-day-old plants. Changes in quantum yield of PS II (ϕ PSII), quantum yield of non-regulated heat dissipation and fluorescence emission (ϕ NO) were monitored. An assessment was also made for the growth of roots and shoots (stem and leaves).

A positive effect of Kaishi was observed in plants treated with 0.05% concentration for the quantum yield. The non-regulated heat dissipation was not significantly affected in all of the treated plants compared to the control. Quantum yield of non-regulated heat dissipation and fluorescence emission was the lowest in the same samples. Naturamin had a positive effect on the functional activity of the treated plants. The quantum yield was higher than in control plants in all variations and the optimal concentration was 5 %. The heat losses were lower in the treated plants and they were minimal in 5 % concentration. Favorable effect of Terra-Sorb was also observed on the functional activity in all of the applied concentrations. The optimal concentration was 0.5 % at which the values of quantum yield of PS II were the highest.

Kaishi treatment in 0.5 % concentration stimulated root dry mass accumulation. However, there was negative effect on the shoot dry mass accumulation. Naturamin treatment did not significantly affect the root and shoot growth. Terra-Sorb had positive effect on the dry mass shoot accumulation in all treatments with the exception of 0.01 % and optimal concentration was 5 %. For the root growth there was negative influence in 5 and 10 %.

It was found that three commercial biostimulants (Naturamin, Kaishi and Terra-Sorb) affected differently the development and functional activity of young maize plants. Naturamin (5%) and Terra-Sorb (0.5 %) stimulated the functional activity of the maize plants, while the effect of Kaishi was weaker. Kaishi had a positive effect on root growth. Terra-Sorb had a stimulating effect on leaves and stem growth. There was not significant effect on biomass accumulation in Naturamin treated plants.

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PPBP-8. RESEARCH ON THE EFFECT OF THE PHYTOEFFECTOR VIUSID AGRO® ON GROWTH AND ANTIOXIDANT PARAMETERS OF PEA

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Pea (*Pisum sativum* L.) is a culture used for food all over the world. To improve the growth and the immunity of the plant, we tested a commercial growth promoter VIUSID agro®. Here, we evaluated physiological parameters of pea seedlings grown in several variants of hydroponic cultures: water /H₂O/ and Knop nutrient solution /KNOP/ compared to the respective medium with added VIUSID agro® in concentration recommended by the producer /H₂O+VIS and KNOP+VIS/. Changes in shoot growth and antioxidant performance were followed by measuring morphometric parameters (height and biomass) and determination of the quantity of structural units (reducing sugars and nitrates). As well, non-enzymatic (quantity of phenolics) and enzymatic (peroxidases and catalase) indicators were investigated. The obtained data confirmed best growth in the presence of VIUSID agro® which seems to modulate the distribution of structural units as sugars (in H₂O) and nitrates (in Knop). The general antioxidant potential in shoots was not affected significantly indicating lack of stress for the plants except for the H₂O variant with higher activity of antioxidant enzymes. We conclude that VIUSID agro® indeed acts as a natural bioregulator by increasing plant productivity.

Keywords: phytoeffector, pea seedlings, morphology, phenolics, antioxidant enzymes

PPBP-9. EFFECTS OF VIUSID AGRO® ON HYDROPONIC CULTURES OF *ZEA MAYS* L.

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VIUSID agro® is a commercial growth promoter that acts as a natural bioregulator by promotion and activation of the cellular metabolism of the plants increasing their productivity. The aim of this study was to evaluate the effects of this phytoeffector on hydroponic cultures of *Zea mays* L., which have not been studied so far. Seedlings of *Z. mays* grown in water /H₂O/ and Knop nutrient solution /KNOP/ were compared to plants grown in the respective medium with added VIUSID agro® in concentration recommended by the producer /H₂O+VIS and KNOP+VIS/. Parameters related to growth potential and antioxidant activity were analyzed (shoot and root length, biomass, quantity of nitrates, pigment content, activity of antioxidant enzymes, quantity of phenolic compounds, total antioxidant activity /TAA/). The results showed best parameters for *Z. mays* plants grown in KNOP medium enriched in mineral elements. The addition of VIUSID agro® did not affect significantly the investigated parameters. However, in the variant H₂O+VIS, specific positive effect of the phytoeffector was confirmed on the plant growth potential compared to the H₂O control. In this case, the increased TAA in leaf methanol extracts correlated with the higher quantity of Chl a and carotenoids rather than phenolic compounds. Lower activity of antioxidant enzymes was observed upon growth stimulation. Our data supported the positive impact of VIUSID agro® on *Z. mays* seedlings grown in water. The possible lack of effect in KNOP medium could be due to the low concentration of the bioregulator, or alternative direct shoot application should be tested.

PPBP-10. GIBBERELLINS AS MODULATORS OF GROWTH AND METABOLIC COMPOSITION OF IN-VITRO CULTURED MEDICINAL PLANT *NEPETA NUDA*

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Gibberellins are plant hormones that promote processes including stem elongation, leaf expansion, vegetative-to-reproductive transition, flower development, seed and fruit development, and germination. The effect of gibberellic acid (GA) on medicinal plants is poorly studied. Endogenous levels of GA can be manipulated through application of exogenous GA or inhibitors of GA biosynthesis such as paclobutrazol (PAC). The effect of GA on medicinal plants could be useful to elucidate the role of these hormones in plant metabolism. The aim of this study was to evaluate the effect of GA on growth parameters and metabolite content on in-vitro cultured catnip, *Nepeta nuda*, plants in three different cultivating conditions: with gibberellins (GA), paclobutrazol (PAC), and ethanol as a control. Methanol extracts from dry shoot material were investigated for total content of pigments, reducing sugars, phenols, and antioxidant activity. The results showed that the applied high concentration of GA did not affect plant growth significantly compared to the control. PAC influenced the growth of *N. nuda* seedlings: decreased shoot length, increased the plant biomass and chlorophyll a and b content. Both GA and PAC stimulated the accumulation of reducing sugars. GA lowered the phenols content, while PAC enhanced the levels of this secondary metabolites which correlated with the total antioxidant activity. The current study reveals that treatment with PAC has effect on the growth of the plant and no significant impact was observed after treatment with GA, whereas both of the growth regulators display impact on the levels of secondary metabolites.

Keywords: gibberellic acid, paclobutrazol, in vitro, herb, growth, antioxidants

PPBP-11. NATURAL REGENERATION IN DECIDUOUS FORESTS

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This research aimed to compare physiological changes of two deciduous species – *Fagus sylvatica* L. and *Carpinus betulus* L., in response to their location and exposure. The model trees were grown at three different altitudes, at the Petrohan Experimental Forest Range, Western Balkan Mountains (Bulgaria), in the summer 2018.

Altitude and exposure are among the ecological factors that seriously affect physiological processes in the deciduous trees. We found that *F. sylvatica* and *C. betulus* underwent a number of physiological changes in the antioxidant status, non-enzymatic and enzyme protection system, pigment content, etc., depending on the exposure – full sun or shady habitats. It was measured that the amount of photosynthetic pigments of common beech was increased in shady habitats at 650 m asl and 1450 m asl, while the pigment content in the hornbeam leaves (650 m asl) was slightly affected by the exposure. The beech saplings grown at the forest's patches at the lower and the highest altitude (650 and 1400 m asl) showed significant increase of total phenolic compounds and total antioxidant activity. In the hornbeam growing in the full sun, the highest values of total phenols, flavonoids and total antioxidant activity were measured. The full sun exposure activated antioxidative enzymes – superoxide dismutase, catalase and guaiacol peroxidase in the common beech leaves, contrary to the hornbeam.

In general, the beech saplings showed the fastest adaptation to the changed environmental conditions in the forest patches at 1000 m asl - the optimal growth area for *F. sylvatica*. At the lower altitude (650 m asl) the saplings react positively to the illumination, albeit not as strongly as at 1000 m asl. The main reason is probably due to the increased competition with other tree species (mainly hornbeam), which responded more positively to the high light. At 1450 m asl, the parameters that were studied were insignificantly affected by the exposure, which was probably the result of the harsher conditions.

F. sylvatica L. and *C. betulus* L. responded differently to changes in altitude and light exposure. Therefore, it could be assumed that both species had different physiological mechanisms of stress responses to identical stress factors.

Keywords: *Fagus sylvatica* L., *Carpinus betulus* L., pigments, antioxidative enzymes, phenolic compounds

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PPBP-12. RESPONSE OF EIGHT WINTER COMMON WHEAT VARIETIES TO DROUGHT STRESS

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The aim of the present work was to investigate the response of eight winter common wheat varieties, created in Institute of Plant Genetic Resources in Sadovo, Bulgaria, to dehydration stress (Niky, Tsarevets, Lusil, Bononia, Petya, Guinness, Sadovska belya and Diamant).

The sensitivity of investigated varieties to desiccation and ability to recover after re-watering was evaluated by following the alterations in the pigment content, desiccation-induced lipid peroxidation, generation of reactive oxygen species (H₂O₂) and the level of synthesized protective substances (anthocyanins).

Plants were grown in growth chamber at normal conditions (16/8h day/night photoperiod, illumination 300 μmol photons m⁻² s⁻¹ and 20°/18°C day/night temperature). Watering of plants was stopped at fully developed third leave for 7 days followed by a period of 4 days re-watering to determine the ability of plants to recover after dehydration stress.

Results presented indicate that on the 7th day of dehydration alteration in photosynthetic pigments content occur as indicated by the decrease of chl/car ratio indicating that the amount of total chlorophyll is changed to a higher extent than that of carotenoids. The degree of desiccation-induced lipid peroxidation is highest in Sadovska belya, Petya and Tsarevets. The most prominent dehydration-induced level of H₂O₂ is observed in Guinness. Furthermore, all investigated varieties respond to dehydration stress by increased synthesis of anthocyanins, best expressed in Sadovska belya followed by Bononia and Guinness.

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*We would like to thank you for your participation in the **SGIP 2019!***

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