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ABSTRACTS OF UNKP CONFERENCE (MAY 30, 2018, SZEGED)

Antioxidant mechanisms accompanying physical exercise in estrogen depleted rat model

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Myocardial extracellular matrix (ECM) plays an important role in the homeostasis of cardiovascular system. Changes in the structure of ECM can cause certain pathological processes, including the disregulation of matrix metalloproteinases (MMP).

The aim of our work was to investigate the cardiovascular effects of voluntary physical exercise against the disregulatory and detrimental consequences of MMP-2, linked with the measurement of ischemia/reperfusion injury.

In our experiment female Wistar rats were underwent either ovariectomy surgery (OVX) or sham operation (SO). After 4 weeks of resting period we divided them into subgroups based on the type of diet (CTRL: standard chow, HT: high-triglyceride diet) and exercise (running or non-running). At the end of the 12-week experimental period, the activity of 64 kDa and 72 kDa MMP-2, the level of total glutathione (GSH), the concentrations of nitrotyrosine (3-NT), tissue inhibitor of matrix metalloproteinase (TIMP-2), and type-I collagen were determined. Following 30 min LAD occlusion and 120 min reperfusion, the ratio of infarct size was also evaluated.

Our results clearly show, that estrogen deficiency and HT diet caused a significant increase in type-I collagen accumulation and in the ratio of infarct size, on the other hand the activity of MMP-2 and the level of TIMP-2, GSH, 3-NT was decreased. 12 weeks of physical activity resulted a significant increase in the level of TIMP-2, 3-NT, GSH as well as in MMP-2 activity, also due to the exercise training the collagen content and the infarcted area significantly reduced in each group.

Voluntary physical exercise can be a potential non-pharmacological strategy to ameliorate the adverse cardiovascular effects by enhancing of MMP-2 activity and reducing the accumulation of type-I collagen and the necrotic extension of heart.

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Effects of tunicamycin on photosynthetic activity

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Tunicamycin (Tm) blocks N-linked glycosylation (N-glycans) and induce unfolded protein response (UPR) in plants. The UPR plays an important role in restoring the protein folding capacity of the ER membrane by increasing the levels of molecular chaperones and reducing the protein load into ER. At the same time, Tm can affect the biosynthesis of several components of photosynthetic apparatus. This potential effect can influence the activity of photosynthesis in higher plants. The aims of our work were to study photosynthetic activities after Tm and chemical chaperone treatments in order to reveal their changes upon stress condition.

Tm significantly decreased the maximal (Fv/Fm) and effective [Y(II)] quantum yield of PSII, as well as the effective quantum yield of PSI [Y(I)]. Interestingly, the quantum yield of non-regulated non-photochemical energy dissipation [Y(NO)] did not change significantly upon Tm, but the limitation of the quantum yield of regulated non-photochemical energy dissipation [Y(NPQ)] increased significantly under stress condition. Application of chemical chaperon could modulate the effects of Tm on photosynthetic activity. Not only parameters of photosynthetic activity, but also the product of photosynthesis (sugar and starch contents) were also analyzed, which contents changed significantly upon Tm treatments.

In conclusion, the effects of exogenous Tm application on photosynthetic activity can be significant, which can play a crucial role in UPR and plant defense under stress condition.

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Molecular identification and characterization of mycoviruses of different filamentous fungi

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Detection of double-stranded RNA (dsRNA) elements in fungal isolates suggests the presence of mycoviruses. More than 90 mycoviruses have been described and some of them can cause smaller or greater changes in the phenotype or may cause hypovirulence or hypervirulence. Mycoviruses lack the extracellular route for infection, thus they can be transmitted only intracellularly. All major phyla of fungi contain mycovirus-harboring fungal isolates; however, we have limited information about their distribution and their biological role in the host fungal cells.

The aims of this research were the screening of different fungal isolates for the presence of dsRNA molecules and for the precise identification and characterization of the isolated viruses. Our long-term goal is the examination of the effect of their presence on the host phenotype. We have screened different isolates belonging to *Umbelopsis*, *Mortierella*, *Dissophora*, *Lecanicillium*, *Mycogone* and *Armillaria* genera and we have detected dsRNA elements in 12-23% of more than 200 investigated isolates. We have found a high variability in the numbers and sizes of the detected dsRNA fragments between the genera as well as the isolates of the same genus. We have started the molecular analysis of the detected dsRNA fragments. Four dsRNA fragments were purified from *U. ramanniana* SZMC 11078 and one 6,1 kb size fragment from *Mucor hiemalis* SZMC 12056 strain. The sequence analysis revealed that the identified dsRNA fragments consist of two open reading frames, which are presumably encodes an RNA-dependent RNA polymerase and a capsid protein.

The purification of the virus-like particles (VLPs), polyacrylamide gel electrophoresis and transmission electron microscopy analysis of the purified VLPs are also in progress as well as Northern blot analysis to determine which dsRNA fragment encodes the identified genes.

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Signal interaction between nitric oxide (NO) and Rho GTPases during plant growth

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Nitric oxide (NO) is a gaseous signal molecule which in cooperation with auxin regulates root developmental processes like primary root or root hair elongation and lateral root formation. Some of these physiological processes take place with the participation of plant-specific regulator Rho of Plants (ROP) proteins which are known to interact with auxin. ROPs act as molecular switches due to conformational changes upon GTP binding and hydrolysis facilitating transient interactions with effector proteins. Although, NO and ROPs as regulators share common interacting partner (auxin) and physiological process (root growth), their crosstalk has not been proven so far. My study aims therefore to examine a suspected ROPs-NO signal interplay in plants. I developed an experimental system, in which the NO sensing of different ROP mutant and reporter *Arabidopsis* lines were evaluated.

Compared to the wild-type (*Col-0*), *rop2-1* and *rop2-2* roots showed significant NO insensitivity, while *rop6* responded to the presence of NO donors similarly to *Col-0* (NO-induced root meristem shortening). In agreement with this, neither the rate nor the pattern of ROP6 *in situ* expression was affected by NO supplementation. The *in situ* expression of ROP2, however, decreased in the presence of NO, as well as the PIN-dependent auxin transport and auxin maximum in the root tip. Moreover, both *rop2* mutants possess elevated endogenous NO level in their root tip compared to *Col-0*, which further support the connection between NO and ROP2 signaling the root meristem.

Based on the results we can strongly suspect that exogenous NO negatively influences ROP2 action thus inhibits polar auxin transport and consequently the generation of auxin maximum leading to root meristem shortening. To confirm this conclusion, my further experiments will focus on providing direct evidence for the involvement of ROP2 in NO-induced root meristem shortening.

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Tool use in the ant subfamily Myrmicinae

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The study of tool-using behavior is a rapidly developing part of behavioral ecological studies; however, most of these studies focus on vertebrate tool users. Our aim is, therefore, to widen our knowledge about tool use in insects, particularly in ants. Members of the genus *Aphaenogaster*, and a few other myrmicine species (*e.g., Messor*, *Pogonomyrmex*) use various objects as tools during foraging to transport liquid food sources. In our studies, we investigated the tool-using behavior of various myrmicine species. First, we aimed to detect foraging tool use in species where such behavior is unknown. Second, we aimed to gain more information about the basic mechanisms and possible flexibility of this behavior.

In our research, we successfully identified the following species as novel tool-users: *Aphaenogaster feae, Aphaenogaster beccari, Aphaenogaster iberica, Messor nondentata* and *Messor valentinae*. On the other hand, we failed to detect tool use in *Pheidole noda*. Species of *Aphaenogaster* and *Messor* all belong to a basal myrmicine clade, indicating that foraging tool use might have evolved very early in myrmicine ants. Since the tool-using behavior of *Messor* species seems to be different from that of *Aphaenogaster* species, we aim to compare these species to clarify the differences in their behavior. Furthermore, we are also planning to convey comparative phylogenetic analyses by investigating the foraging behavior of species from other related and unrelated myrmicine genera. In the other part of our study, we investigated in detail the tool-using behavior of *Aphaenogaster subterranea* by examining, how tool-using workers deal with various foraging challenges. We used different liquid baits and different types of tools with varying distances between the baits and the tools piled around them. Our results confirm that tool use in *A. subterranea* exhibits a high degree of flexibility. Ants seem to be able not only to optimize their foraging effort by selecting tools that are best matched to the particular foraging environment, but also to learn how to improve the use of certain tools by modifying them.

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Development of molecular tools to study the virulence of the human fungal pathogen *Candida parapsilosis*

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Candida species are responsible for the majority of systemic fungal infections in humans. This is a life-threatening condition associated with high mortality rate affecting predominantly immunocompromised individuals. *Candida albicans* is considered as the predominant and therefore the most studied *Candida* species. Although other members of the genus also gained importance in the last decade, still much less effort has been made to study their patomechanism mostly due to the lack of molecular tools and genome editing approaches.

One of these emerging species is *Candida parapsilosis* that endangers low birth weight neonates in the first place. To gain insights into the factors making capable this species of causing such serious infections, we aimed to develop and optimize a set of molecular techniques. We adopted the state of the art Crispr Cas9 approach that has been described as the "bacterial adaptive immune system". Cas9 is an endonuclease that is capable of, introducing double stranded breaks (DSB) into specific sites of the DNA. The exact target site is appointed by an RNA compound called the guide RNA. DSB is a critical damage and therefore it must be repaired in one of two main ways. When an intact template is not present non-homologous end joining (NHEJ) occurs, in the presence of a template though, homology driven repair takes place that provides a more precise genome alteration than NHEJ.

By using this approach, we created deletion, reintegrant and overexpression mutants. It was also suitable for generating a GFP expressing strain. We found this system strain independent as we could create deletion and reintegrant mutants in all the six strains we subjected to genome alterations. Our plan is to use this technique for HIS- and GFP- tagging of proteins.

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Investigation of dark modulated ER stress

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Light is one of the most important environmental factors, which is required for optimal growth and development or during the stress responses of plants. Dark can alter the light-dependent activation of plant developmental or defense responses, it can induce new signaling and regulation pathways modulated by various signaling molecules such as reactive oxygen species (ROS). Tunicamycin (Tm) can induce unfolded protein response (UPR) in plants by blocking N-linked glycosylation (N-glycans). At the same time, Tm can induce the production of ROS and cause several changes in the activity of antioxidant enzymes, but ROS production can be dependent on the presence or absence of light in plants. The aims of our work were to study the effects of Tm and chemical chaperone treatments in the leaves of tomato plants in the light and dark.

Based on our result, Tm induced superoxide production was higher in those plants, which were kept in dark. In contrast to this, levels of H_2O_2 were higher in the illuminated leaves of tomato plants after treatment with Tm. The application of the chemical chaperon reduced the H_2O_2 production both in the light and dark environments. The expression of ER stress marker gene, *BiP* was significantly elevated by Tm in the light, but reduced by the application of chemical chaperon and dark. Expression levels of *IRE1α* and *β* were also elevated by the treatment with Tm in the light but did not change under darkness suggesting a specific role of the presence of the light in ER stress response.

In conclusion, the effects of exogenous Tm application on ROS production can be significant, which can be dependent on the presence or absence of light and can play a crucial role in UPR and plant defense under diverse environmental stimuli.

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The role of exercise training on cardiovascular system in aging animal model

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Aging is a major risk factor for cardiovascular diseases, which can be associated with oxidative stress, inflammation, cardiomyocyte death and cardiac morphological changes.

Echocardiography (ECHO), as a non-invasive imaging technique, provides insight into changes in cardiac structure and function related to aging-associated cardiovascular diseases. Exercise is a diagnostic and therapeutic tool, which may improve aging-influenced cardiac physiology and function.

The aim of our work was to study the potential use of voluntary wheel running exercise to prevent or treat the cardiac morphology and function in aged rats.

12-month-old female and male Wistar rats were divided into running and non-running groups.

At the end of 12-week period, echocardiographic measurements of rats were carried out under *i.m.* anesthesia. Following the ECHO examination, infarct size was also evaluated after 45 min LAD occlusion and 120 min reperfusion.

Fractional shortening (FS) and ejection fraction (EF) of running male and female animals were significantly as compared to matched aged control groups. Left ventricle systolic function was also evaluated measuring mitral annular plane systolic excursions (MAPSE), which showed a significant increase in running female rats. As a result of physical exercise, the E/A ratios were significantly elevated in both running genders, while the isovolumic relaxation time (IVRT) and Tei-index were significantly decreased in running males. The ratio of septal E'/A' and septal E/E' revealed a significant increase in running male groups in comparison to aged male rats. Right ventricle diastolic function was improved in running female animals. Moreover, 12 weeks of physical exercise reduced the infarct size in both female and male rats.

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The effects of voluntary physical exercise on the consequences of isoproterenol-induced myocardial infarction in experimental menopause

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The incidence and progression of coronary artery diseases in premenopausal women are lower as compared to age-matched men but increases rapidly after the onset of menopause.

Fertile Wistar females (CTRL) and pharmacologically ovariectomized (POVX, 750 μg/kg triptorelin, *i.m.*, every 4th week) rats were used in our experiment. CTRL and POVX animals were randomly assigned to receive injection of 0.1 g isoproterenol (ISO)/kg. ISO is a synthetic β-adrenocepor agonist, which induces myocardial infarction (MI) in rats. At 24th hours after ISO injection serum markers of myocardial injury, such as LDH and myoglobin were measured. After a 3-week resting period ISO-treated and untreated animals were further divided based on the physical exercise. During a 6-week period, the running animals were placed into cages fitted with a running-wheel. At the end of the experiment the cardiac activity of antioxidative heme oxygenase (HO) enzyme, the expression of HO-1, the level of glutathione (GSH) and the activity of myeloperoxidase (MPO) were detected.

ISO treatment significantly increased the serum levels of LDH and myoglobin, which were more marked in POVX animals. At the end of the experimental period, MI resulted the lowest HO activity and HO-1expression in the heart of POVX animals, whereas 6 weeks of physical exercise significantly improved the HO and GSH values in both CTRL and POVX rats. Furthermore, our training protocol significantly reduced the pathological activity of MPO.

Our findings clearly demonstrate that 6 weeks of voluntary physical exercise is a potential non-pharmacological therapeutic strategy to ameliorate the antioxidative status and inflammatory parameters in women post-MI.

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Characterization of bioactive secondary metabolites produced by microorganisms

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The natural products are naturally derived compounds playing a very important role in health care and prevention of diseases. Moreover, it is well established that natural products have been the sources for the development of many of the most effective drugs currently available for the treatment of a variety of human diseases. Natural products could be presented as metabolites from broad range of organisms including fungi and bacteria, which are almost infinite resources for drug discovery to provide new medicinal agents to the human health care and therapy.

In our research work, both the targeted and the activity-guided metabolic seeking were applied. In the case of targeted identifications, firstly unknown members of the known molecular families were identified including peptaibols and surfactins, which are produced mainly by *Trichoderma* and *Bacillus* species, respectively. On the other hand, endophytic fungi were isolated from medicinal plants possessing production of known, already clinically used compounds and production abilities of the host metabolites were tested in fermentation conditions. In these experiments, the taxol, hypericin and emodin were involved as the host metabolites of *Taxus baccata* and *Hypericum perforatum.* In the case of non-targeted screening, endophytic fungi were also isolated from the medicinal plants (*Artemisia asiatica, A. annua, Ononis spinosa, Juniperus communis, Ephedra distachya, Glycyrrhiza glabra, Asclepias syriaca, Calendula officianalis, Ricinus communis*) collected from the Hungarian areas, and bioactivity assays were applied for their crude extracts to find novel molecules showing anti-bacterial or antifungal effects.

Detailed knowledge on the properties of microbial secondary metabolites or discovery of new ones may provide valuable information about their potential applicability as lead molecules in the drug developments.

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The joint development of oral health literacy and critical thinking in the teaching of Biology

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Critical thinking is one of the essential elements of scientific thinking. However, the analysis of Biology textbooks highlighted the lack of those tasks which improve critical thinking. Numerous biological topics are suitable for this purpose, especially the topic of the human anatomy and health. Within the confines of it, the development of students' health literacy is also possible. One of the specific areas of health literacy is the oral health literacy, whose state of development has an influence on oral hygiene and general health condition. Researches show that the oral hygienic condition of Hungarian population is maleficent, more than half of them have restricted health literacy. The joint development of critical thinking and oral health literacy is likely to happen on Biology classes, whose basis is that their common element is the evaluation as cognitive operation.

The goal of our research is to get familiar with the lack of knowledge concerning oral health literacy amongst university and high school students and using this information to create activities that help develop both critical thinking and oral health literacy.

Based on our research, for more than two thirds of the university students it is hard to judge the credibility of the dental information gained from the media, for 54% it is a problem to judge the advantages and disadvantages of different dental treatments. Based on the surveys filled out by high school students, it can be said that just like in the case of university students they cannot judge the credibility of the dental information gained from the media, and for 54% it is a problem to judge the advantages and disadvantages of different dental treatments. In 2018 spring, a task made by our research team was tested.

Later in our research, we plan to measure the developed tasks' efficiency using control groups. Moreover, we see the need for a research that focuses on the correlation between oral health literacy and status.

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The effect of salt stress on the maturity and quality of tomato fruits

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Excessive salinity is one of the most important environmental stress factors which greatly affects the growth, nutrition, and productivity of many plant species. Tomato is one of the most important horticultural crops in the world, which contains many health beneficial factors. However, salt stress can reduce the yield and quality of tomatoes. In this study we would like to detect the role of ethylene under salt stress. We have used the ethylene-insensitive *Never ripe* (*Nr*) tomato (*Solanum lycopersicum* L.) mutant for the experiments, which mutation blocks ethylene perception. Results in this mutant were compared to the wild type Ailsa Craig (WT) cultivar subjected to NaCl.

Aims of the present investigation was to study the effects of salt stress on quality and physiological responses of fruits, including ionic-, osmotic- and oxidative stress components. The changes in some growth parameters; ascorbic acid (AsA) and glutathione (GSH)-; H_2O_2-; malondialdehyde (MDA) content were also followed. Furthermore, we analyzed the activity of different antioxidant enzymes in both tomato genotypes.

Reduced fresh weight and diameter of fruits, as well as decreased width of exocarpium were observer upon salinity stress in both genotypes. WT exhibited higher Na^+ accumulation, dehydroascorbic acid (DHA) and total and reduced GSH content under salt stress. It is well known that, ethylene can regulate the metabolism of reactive oxygen species (ROS) by modulating antioxidant enzymes. Fruit cells showed increased H_2O_2 production, MDA content and enhanced guaiacol peroxidase (POD, EC 1.11.1.7) activity under NaCl treatment. Interestingly, activity of catalase- (CAT, EC 1.11.1.6) and superoxide dismutase (SOD, EC 1.15.1.1) did not change in WT fruits after salt exposure.

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Overall, these results indicate that ethylene signaling can modulate the activity of antioxidant enzymes and can influence fruit quality under salt stress.

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Comparison of neutrophil response to *Curvularia* and *Aspergillus* species

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Opportunistic fungal infections represent a continuously increasing problem, because of the growing population with underlying conditions, the difficulties of diagnosis and the high antifungal resistance of certain fungal agents. Members of the genus *Curvularia* (Ascomycota, Pleosporales) are known as plant pathogenic filamentous fungi. Some members of this genus, like *C. lunata*, have been recovered from human infections known as pheaohyphomycoses. These mycotic infections can manifest as fungal keratitis, sinusitis, cutaneous lesions or invasive infections. The aim of this study was to investigate the neutrophil granulocytes' response and killing efficiency to hyphal forms of *Curvularia lunata* in comparison with that to the well-characterized *Aspergillus fumigatus.*

In the present study, *C. lunata* SZMC 23759 and *A. fumigatus* SZMC 23245, both isolated from human eye infection were examined. Release of H_2O_2 from neutrophil granulocytes were measured in the presence and the absence of the supernatant of germinating conidia and after serum treatment. Activation and survival of neutrophils were checked by measuring the myeloperoxidase and LDH release, respectively. After the interaction viability of fungal strains was also measured. Haloperoxidase activity during the infection was gauged as a potential mechanism of fungal defense.

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