Hungary-Serbia
IPA Cross-border Co-operation Programme

HUSRB/1203/221/173 ″PLANTTRAIN″

Joint development of higher education and training programmes in plant biology in support of knowledge-based society

OPENING CONFERENCE

20-21 April, 2015, Szeged, Hungary

BOOK OF ABSTRACTS

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Opening Conference
of the Hungary-Serbia IPA Cross-border Co-operation Programme

"Joint development of higher education and training programmes in plant biology in support of knowledge-based society"
(PLANTTRAIN, ID: HUSBR/1203/221/173)

20-21 April, 2015, Szeged, Hungary

Clubhouse of the Szeged Committee of the Hungarian Academy of Sciences
Somogyi u. 7., 6720 Szeged, Hungary

Monday, 20 April, 2015

11^{00}-11^{10}: **Opening of the Conference**
Dr. Barnabás Wodala
Department of Plant Biology, University of Szeged

11^{10}-11^{30}: **Dr. Tari Irma**
Hungary-Serbia IPA Cross-border Co-operation Programme – "PLANTTRAIN"
Department of Plant Biology, University of Szeged
Introduction of the Hungarian partner

11^{30}-11^{50}: **Prof Dr. Dubravka Štajner**
Hungary-Serbia IPA Cross-border Co-operation Programme – "PLANTTRAIN"
Faculty of Agriculture, University of Novi Sad
Introduction of the Serbian partner

12^{00}-13^{00}: **Lunch**

Chairs: Dr. Jolán Csiszár and Dr. Irma Tari

13^{00}-13^{20}: **Prof. Dr. Đorđe Malenčić**
Soybean as a remedy: biologically active substances and phytopreparates

13^{20}-13^{40}: **Dr. Dejan Prvulović**
Cherry - rich source of natural antioxidants

13^{40}-14^{00}: **Dr. Alexandra Popović,** Jovana Šućur, Miloš Petrović
Interaction between plants and insects

14^{00}-14^{20}: **Dr. Attila Ördög,** Tímea Körmöczi, Péter Poór
Analysis of essential and toxic elements in Hungarian honeys originated from Zala county

14^{20}-14^{40}: **Dr. Jolán Csiszár,** Krisztina Bela, Edit Horváth, Dr. Ágnes Gallé, Szilvia Brunner, Prof. Dr. László Szabados, Dr. Ferhan Ayaydin, Dr. Irma Tari
Oxidative stress responses - the redox regulated aspect and molecular investigations

14^{40}-15^{00}: **Coffee break**

Chairs: Prof. Dr. Dubravka Štajner and Prof. Dr. Đorđe Malenčić

15^{00}-15^{20}: **Dr. Zsuzsanna Kolbert,** Gábor Feigl, Árpád Molnár, Prof. Dr. László Erdei
Growth responses induced by microelement excess: the role of reactive nitrogen species

15^{20}-15^{40}: **Dr. Ágnes Szepesi,** Péter Borbély, Ágnes Hurton, Zoltán Takács, Szabolcs Tóth, Dr. Izabella Kovács, Prof. Dr. Christian Lindermayr, Dr. Irma Tari
Polyamine catabolism under salt stress: inhibiting diamine oxidase by aminoguanidine

15^{40}-16^{00}: **Zoltán Takács**, Péter Poór, Dr. Ágnes Szepesi, Dr. Irma Tari
Comparison of the time-dependent role of polyamine oxidases under sublethal and lethal salt stress in tomato plants

16^{00}-16^{20}: **László Bakacsy** and Dr. István Bagi
Reproductive and invasive strategies of *Asclepias syriaca*

16^{20}-16^{40}: **Dr. Ágnes Gallé,** Dániel Benyó, Dr. Jolán Csiszár, Dr. Irma Tari and Dr. János Györgyey
Stress induction of plant detoxification system: comparison of *Triticum aestivum* and *Brachypodium distachyon* lines

18^{30}: **Dinner**

Tuesday, 21 April, 2015

10^{00}-11^{00}: **Gábor Feigl, Dr. Ágnes Szepesi**
Introduction of the Botanical Garden of the University of Szeged: research work, scientific and popular programmes. The most important species of the garden.

11^{00}-12^{00}: **Walk around the garden.**

12^{00}-13^{00}: **Lunch**

13^{00}-16^{00}: **Preparation of the Workshop held in June in Novi Sad**

Abstracts of Opening Conference

Dr. Irma Tari

Introduction of the Hungarian partner

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The Hungary-Serbia IPA Cross-border Co-operation Programme belongs to the "new generation" of cross-border co-operation programmes. It is implemented within the 2007 – 2013 European Union financial framework under the Instrument for Pre-accession Assistance (IPA).

The program has been started in 2003 by the guidance of the Hungary-Serbia Pilot Small Projects Fund (PSPF). The PSPF aimed at supporting people-to-people actions and at facilitating institution building projects of non-profit organisations along the Hungarian-Serbian border.

Our programme, "Joint development of higher education and training programmes in plant biology in support of knowledge-based society" will join forces of the partner universities, University of Novi Sad and University of Szeged in the development of higher education and training of experts on the field of basic and applied plant biology.

In this lecture a short introduction of the educational system and the main research groups of the Department of Plant Biology (University of Szeged) will be presented. The complementing feature of the education and research work at our institutions opens the door to common lectures at master and PhD level, as well as to common research programmes in the future.

Prof. Dr. Dubravka Štajner

Introduction of the Serbian partner

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The Faculty of Agriculture was founded in 1954. and is one of the oldest within the University of Novi Sad. The first lectures for the first generation of students were started on 22nd November, 1954. Alongside the Faculty of Philosophy, the Faculty of Agriculture was one of merely two higher education institutions in Vojvodina Province.

The Faculty of Agriculture was founded so as to create agricultural experts of higher education. The faculty has put all of its resources in fulfilling these demanding tasks over the past years, thus trying to justify its own existence. Nowadays, nearly six decades since its foundation, the faculty is reputed to be one of the most prestigious scientific institutions in the country. It is acclaimed nationally and internationally for the results of its work and for the enormous contribution to the development of agricultural production in Vojvodina Province and the Republic of Serbia as a whole.

The faculty has grown into a reputable and recognizable educational and scientific institution. Many prominent scientists have worked at the faculty, nineteen of whom have become members of the Serbian Academy of Sciences and Arts, the Vojvodinian Academy of Sciences and Arts and other academies; some of them are holders of international academic titles.

The faculty attend approximately 3340 students per year, number of teaching staff is 246 and non-teaching staff 135. So far at Faculty of Agriculture graduated more than 12015 students. Number of PhD thesis is more than 700.

Students are taught in 30 lecture rooms which are provided with up to date teaching equipment. Apart from library and laboratories for student practical there are five experimental stations where students are involved in research programmes and/or e.g. in the process of wine and brandy production from pruning and pest/disease control to fermentation and ageing. Part of the fruit tree gene bank is maintained by students too. There are also stations for field and vegetable crop production and animal breeding.

At the moment faculty participate in approximately 70 national and 42 international projects. Faculty of Agriculture has developed an intensive international cooperation with numerous faculties worldwide. The mentioned cooperation is based on: exchange of students, teaching staff and scientists, joint research work, the publications of scientific papers, magazines and textbooks, etc.

Prof. Dr. Đorđe Malenčić

SOYBEAN AS A REMEDY: BIOLOGICALLY ACTIVE SUBSTANCES AND PHYTOPREPARATES

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Naturally occuring plant phenolics include several groups of compounds that have healthpromoting properties. Phenolics may act as antioxidants, thereby reducing the risk of atherosclerosis and coronary heart disease, which can be caused by oxidation of low-density lipoproteins. They also may protect against some forms of cancer. This is of importance for humans using them as vitamins and/or protectants against oxidative stress. Phenolic compounds play also an important role in plant resistance and defence against microbial infections which are intimately connected with reactive oxygen species (ROS).

The contents and antioxidant ability of various classes of phenolic compounds present in the seeds of 14 soybean cultivars and 20 F_1 hybrids of different origin were evaluated. Total phenolics, tannins, flavonoids and proantho-cyanidins were determined spectrophotometrically, after extraction of seeds with 70% aqueous acetone. The antioxidant activity of aqueous acetone extracts was evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay. The isoflavone contents were analysed using HPLC. Aglycons were quantified from three five-point regression curves ($R \geq 0.9998$) obtained using the corresponding standards (daidzein, glycitein and genistein).

The highest contents of total phenolics were found in Serbian genotype 1511 and Chinese genotype LN92-7369, which also displayed the highest total antioxidant activity. Conversely, genotypes poor in phenolics also showed low levels of DPPH-radical scavenging activity. The results suggested that besides protein and oil contents, the phenolic contents should be also considered as an important characteristic feature of soybean seeds, and as a potential selection criterion for antioxidant activity in soybean.

Dejan Prvulović

CHERRY – RICH SOURCE OF NATURAL ANTIOXIDANTS

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The cherry is the fleshy stone fruit of many plants of the genus *Prunus*. Eating cherries are derived from either *Prunus avium* L. (sweet cherry), or from *Prunus cerasus* L. (sour or tart cherry). Sweet cherry is one of the most popular of the temperate fruits. It is mainly consumed as a fresh fruit and in many areas comes as a first fresh fruit of the season. Serbia and Hungary are one of the largest producers of the sour cherry in the world.

Because of the health benefits attributed to cherries numerous studies have been conducted in recent years to evaluate their properties in terms of quality and bioactivity. The consumption of sweet or sour cherries reduce the risk of cancer, pain from arthritis and inflammation, oxidative stress in different tissues, symptoms of exercise-induced muscle damage, and offer protection against neurodegenerative diseases. Apart from several essential dietary components, such as sugars, minerals and vitamins, cherries also contain other phytonutrients that may provide benefits beyond the prevention of dietary deficiencies. Cherries are a good source of natural antioxidant substances, namely polyphenols. Cherry polyphenols include flavonoids (anthocyanins, flavan-3-ols and flavonols), hydroxycinnamic acids and hydroxybenzoic acids. Among these compounds, especial interest has been focused on anthocyanins, which are the polyphenols responsible for the red skin and flesh colour of fruits, due to their strong antioxidant and anti-inflammatory activities.

The genetic background is the first parameter with the potential to influence the antioxidant content in a commodity. Significant inter-cultivar variation in the phenolic content and antioxidant capacity has been documented in cherries. Climatic and agronomic parameters and degree of ripening also exert an important role in the phenolic content of fruit tissues.

Dr. Alexandra Popović – Jovana Šućur – Miloš Petrović

INTERACTION BETWEEN PLANTS AND INSECTS

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Stored product pests such as *Tribolium castaneum* (Herbst, 1979) and *Sitophilus oryzae* (Linnaeus, 1763) are a major problem. Adult insects were obtained from laboratory cultures maintained in the dark in incubators at 25± 1C and 70–80% r.h., reared on wheat flour and on grain wheat. Both insects species were fed with flour disks containing a known concentration of essential oil taken from 7 plants, collected in Serbia and Montenegro. The chemical components of essential oil taken from 3 plants (*Calamintha glandulosa* L., *Satureja Montana* L., *Teucrium polium* L.) collected on the area of Montenegro, were identified using GC-MS analysis. The results of insecticidal effect of essential oils were discussed. Also, mortality rate of adult insects was tested after 24h, 48h, 72h. In our research, the essential oils taken from *C. glandulosa* which were rich in monoterpene alcohols carvacrol and contained ketonic component showed strong insecticidal and fumigant activity against adults of *T. castaneum* and *S. oryzae*. Accordingly, it was observed that essential oils from *C. glandulosa* with concentration of 1.14% showed powerful toxic and repellent effect on *T. castaneum* insects, with very high mortality rate after 24h (56,67%). Also, mortality of *Sitophilus oryze* was strong and it was 85%, after 24h by the 1,50% concentration oil. After 48h, the most effective formulation was, again, *C. glandulosa* with 83.33 % mortality on the *T. castaneum* and with 100% on the *S. oryzae*. Less toxic effect showed essential oils of *Satureja montana* which had a lower carvacrol and ketonic content. On the other hand, essential oils of *Teucrium polium* which did not contain ketonic component did not show any activity. Taking into account our results and other references it could be concluded that carvacrol has very broad insecticidal and fumigant activity. Therefore, some essential oil could be used as an alternative method of insect control.

Dr. Attila Ördög – Tímea Körmöczi – Péter Poór

Analysis of essential and toxic elements in Hungarian honeys originated from Zala county

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Honey is a key product of traditional agriculture in Zala County and it is also considered an important export item in Hungarian commerce. The aim of this study was to determine the essential and toxic elements of black locust, rape and chestnut honeys and flowers or leaves of plants from various locations within Zala County and to compare them in terms of botanical origin and origin within the region. The macro- (K and Mg), trace- (Fe, Zn, Cu, Mn, B, Al, Co, Ni, Se and Mo) and toxic elements (As, Pb and Cd) were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

Results show that the elements found in the highest amount in samples collected in the region was K, followed by Mg. As for microelements; Fe, Al, Zn and Cu were present in the largest amounts. Chestnut honeys, flowers and leaves included the significant higher concentrations of Cu in comparison with rape or black locust honey. In contrast, rape honey and plant organs showed the highest B and Co contents, while black locust samples contained the highest value of Fe. The toxic element levels in the unifloral honey samples from this region were low and close to the detection limit.

Trace element analysis showed that the differences in the values found in different unifloral honey samples could be used as an indicator of the quality of honey samples.

This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.1.1.C- 12/1/KONV-2012-0014, „Élelmiszerbiztonság és gasztronómia vonatkozású egyetemi együttműködés, DE-SZTE-EKF-NYME".

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Oxidative stress responses - the redox regulated aspect and molecular investigations

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Reactive oxygen and nitrogen species (ROS, RNS), ROS-producing enzymes, antioxidants and their oxidation/reduction states all contribute to the redox homeostasis in plants. Cells can regulate alterations in redox balance by a number of redox-active compounds, such as NAD(P)H, ascorbate, glutathione (reduced form: GSH, oxidized form: GSSG), phenolics, carotenoids, tocopherols, polyamines and proteins carrying redox-active S-groups. To monitor changes in redox status in living cells, redox-sensitive green fluorescent proteins (roGFPs) have been developed whose fluorescence depend on their oxidative status.

Salicylic acid (SA) applied exogenously, is a potential priming agent and it was reported that SA-triggered changes in the antioxidant mechanism are key factors in the successful salt stress acclimation of plants.

In our experimental system 2- or 3-week-long pre-treatment with 10^{-9}-10^{-4} M SA and subsequent 1-week 100 mM NaCl stress was applied hydroponically on tomato and *Arabidopsis* plants, and the non-enzymatic and enzymatic antioxidant mechanisms were investigated. Our data indicate that changes in the gene expression levels and enzyme activities of glutathione transferases play important role in the successful hardening by pre-treatment with proper SA concentrations. The roGFP technology was adapted to monitor the changes in the redox status during the SA-pre-treatments or salt stress. According to our results the roGFP1 fluorescent probe can be used to follow the acclimation process.

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Growth responses induced by microelement excess: the role of reactive nitrogen species

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Excess of microelements can promote development resulting in the phenotype of stress-induced morphogenetic response (SIMR). Alterations in hormonal balance and in reactive oxygen species (ROS) signalling are the known components of the background mechanism of SIMR. Reactive nitrogen species (RNS) are nitric oxide (NO)-related radical and non-radical molecules acting in development and also during abiotic stresses. Thus, the primary goal of our research was to determine the formation of RNS, their interactions with the other components of SIMR induced by microelement excess.

All microelements (Cu, Zn, Se) were able to promote SIMR phenotype in all plant species (*Arabidopsis*, *Brassica juncea, Brassica napus*), which most characteristic symptom was the intensified lateral root generation. Therefore, SIMR can be considered as a general stress response of plants to microelement excess. In cases of all applied microelements, the hormonal balance as well as NO metabolism was disturbed. In *Arabidopsis*, relationship between the elements of the hormonal (auxin, cytokinin, ethylene) and signal system (NO, hydrogen peroxide) was revealed during the microelement excess-induced growth reprogramming. In *Brassica* species NO together with ROS participated in cell death induction through tyrosine nitration leading to nitro-oxidative stress.

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Polyamine catabolism under salt stress: inhibiting diamine oxidase by aminoguanidine

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Polyamines (PAs) have been implicated in many plant physiological processes, such as plant development, or biotic and abiotic stresses such as salt stress. Exogenously applied PAs have positive effect in salt stress tolerance but they can act as pro-oxidants because the catabolism of PAs by diamine oxidase (DAO, EC 1.4.3.6) and polyamine oxidase (PAO, EC 1.5.3.3) produces hydrogen peroxide (H_2O_2) and can induce programmed cell death and senescence. To investigate the role of DAOs in NaCl-induced PA catabolism, exogenous Put (substrate of DAOs, 1 mM) and a specific inhibitor of DAOs, aminoguanidine (AG, 1 mM) was used as treatment for tomato plants. NaCl was applied at two different concentrations (100 and 250 mM) for 1 hour into the nutrient solution. We investigated the contents of some reactive oxygen species and nitric oxide by fluorescent staining in root tips. Enzyme activities (DAO, PAO) were investigated spectrophotometrically, while analysis of free polyamines occurred by HPLC. Moreover, we also detected some changes in nitrosothiol contents by measuring with Sievers Nitric Oxide Analyzer NOA 280i. In conclusion, our experiments support the observation that AG is not only a specific inhibitor of DAOs because this compound also inhibits nitric oxide synthesis at the same time.

This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 'National Excellence Program' and the Hungarian Scientific Research Fund K101243 project.

Zoltán Takács – Péter Poór – Dr. Ágnes Szepesi – Dr. Irma Tari

Comparison of the time-dependent role of polyamine oxidases under sublethal and lethal salt stress in tomato plants

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Polyamines are biogenic polycationics, which have positive effect in salt stress tolerance but they can act as pro-oxidants because the catabolism of polyamines produces hydrogen peroxide (H_2O_2) and can induce programmed cell death (PCD).

In our experiments hydroponically grown tomato plants were treated with sublethal- (100 mM) or lethal (250 mM) NaCl in modified Hoagland solution. Lethal NaCl enchanced the electrolyte leakage from the roots which indicated serious damage to the plasma membrane within the 6-h-long salt exposure. The level of H_2O_2, diamine oxidase (DAO) and polyamine oxidase (PAO) enzyme activity, which catalyzed polyamines oxidation, showed two maximum after 30 min and 2 h after treatment under lethal salt stress. Moreover, 250 mM NaCl concentration induced an increase in the reactive oxygen species (ROS) along with nitric oxide (NO) production after 30 min in the root tips which led to the programmed death of tissues. Lethal NaCl caused significant increase in the expression of DAO and PAO genes at 30 min in roots. Spermidine (Spd) and spermine (Spm) content increased after 100 mM NaCl treatment but the accumulation of these polyamines decreased in leaves exposed to 250 mM NaCl. Lethal NaCl caused significant induction of ethylene production only in the leaves.

We also analyzed the time dependent effect of specific PAO inhibitor, MDL72527 in leaves and roots of 100 mM and 250 mM NaCl-treated tomato plants in order to reveal, how PAO inhibitor affects polyamine accumulation, ROS and NO production and expression of genes encoding flavoenzyme PAO.

This research was supported by the Hungarian Scientific Research Fund K101243 project.

László Bakacsy – Dr. István Bagi

Reproductive and invasive strategies of *Asclepias syriaca*

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Asclepias syriaca, common milkweed, is one of the most dangerous invasive transformer species in Hungarian vegetation, which primarily endangers psammophilous habitats. Reproduction of milkweed takes place basically vegetatively in seminatural habitats, as its seeds germinate at a low rate even in the most suitable disturbed habitat.

Earlier, in 1995, clonal characteristics of five milkweed genets were recorded, that were embedded in natural, semi-natural psammophilous vegetation in the Kiskunság area (Central Hungary). Later, in 2009 and 2014, the same sampling was repeated. All clones were isolated and far from each other, but they lay inside in a 1-km radius circle. The investigation extended to the localisation of the ramets by measuring their relative positions at 10 cm accuracy, to determine the vitality and fruit-production of ramets, and the supported spatial relations of ramets were analysed. Considering the protection of the territory and the possibility of future investigations, the excavation of rhizomatic root system was not performed, therefore the structure of underground part was excluded from the pattern of aboveground ramets. The supposed underground connections of the ramets are mentioned as spacers.

The main characteristics of the clonal structure of *Asclepias syriaca* can be outlined as follows: besides the solitary ramets there are ramets that form clusters with 2-6 shoots, the ramets remain integrated by root-stolons, the pattern of ramets and their clusters in a genet can be built up by iteration of three basic elementary modules.

Our investigation shows that milkweed is able to spread in unfavourable conditions, the clones of *Asclepias syriaca* are able to grow into the natural vegetation. From the point of view of enviromental protection, it is also important to extirpate the isolated smaller clones. A density autoregulating mechanism is an important component of the clonal plasticity.

Dr. Ágnes Gallé[1] – Dániel Benyó[1] – Dr. Jolán Csiszár[1] – Dr. Irma Tari[1] – Dr. János Györgyey[2]

STRESS INDUCTION OF PLANT DETOXIFICATION SYSTEM: COMPARISON OF *TRITICUM AESTIVUM* AND *BRACHYPODIUM DISTACHYON* LINES

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Brachypodium distachyon (purple false brome) is ubiquitous, temperate grass, which is being developed and exploited as an alternative model to subfamily Pooideae (e.g. wheat). Our aim was to compare the water relations of four *Triticum aestivum* lines (with different drought tolerance ability) with two *Brachypodium distachyon* genotype under osmotic stress conditions, and to define the roles of different glutathione transferase (GST) types in defence. The wheat and purple false brome seedlings were exposed to 400 mOsm polyethylene glycol induced osmotic stress for one week. The osmotic stress caused changes in water relations parameters resulted interesting information about the purple false brome lines: the water potencial showed moderate decrease, which results were rather similar to the water relation of the most resistant isohydric wheat line. The hyperosmolarity of the nutrient solution increased the GST activity in drought tolerant and moderately drought tolerant wheat cultivars and in the purple false brome lines, with one exception: the GST activity and in the GST transcript levels in the drought tolerant Plainsman seedlings were almost unaffected by osmotic stress. There were differences between the water stress induced GSTs in wheat and *Brachypodium* lines: in wheat among the eight group of GSTs tau group sequences were sensitive to hyperosmolarity, while in *Brachypodium* both phi a tau groups were activated by stress.

These results underline the differences between the model organism and the widely used cereal, which reveal to the limits of sweeping generalisations among the subfamily Pooideae.

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