Serbian Plant Physiology Society

Institute for Biological Research „Siniša Stanković", University of Belgrade

2nd International Conference on Plant Biology

21th Symposium of the Serbian Plant Physiology Society

COST ACTION FA1106 QUALITYFRUIT Workshop

Petnica Science Center, June 17-20, 2015

2st International Conference on Plant Biology • 21th Symposium of the Serbian Plant Physiology Society • COST ACTION FA1106 QUALITYFRUIT Workshop

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PROGRAMME

2st International Conference on Plant Biology • 21th Symposium of the Serbian Plant Physiology Society • COST ACTION FA1106 QUALITYFRUIT Workshop

Petnica Science Center 17-20 june, 2015

Wednesday 17th June, 2015

09:00-14:00	*Registration*	
14:00-15:00	*Lunch*	

Section I: Plant Biotechnology

15:00-15:30	*Opening Ceremony*	
15:30-16:00	(Invited talk) **Alain Tissier**	Systems biology of a plant cell factory, the tomato glandular trichomes
16:00-16:20	(Invited talk) **Jules Beekwilder**	Biotechnological production of plant compounds
16:20-16:40	(Invited talk) **Milen Georgiev**	Metabolomics, lead, discovery and plant biotechnology: perfect holistic match?
16:40-17:00	(Invited talk) **Dragana Božić**	Exploring the secondary metabolism in trichomes of *Salvia fruticosa* and *Rosmarinus officinalis:* the case of carnosic acid
17:00-17:30	*Coffee break*	
17:30-17:45	(Selected talk) **Milica Bogdanović**	Problems in detecting activity of fluorescent reporter genes – case of DsRED and GFP
17:45-18:00	(Selected talk) **Stevan Jeknić**	Alteration of flower color in *Solanum lycopersicum* through ectopic expression of a gene for capsanthin--capsorubin synthase from *Lilium lancifolium*
18:00-18:15	(Selected talk) **Miloš Prokopijević**	Characterization of soybean hull peroxidase immobilized on glycidyl methacrylate copolymers
18:30-19:30	Poster session: *Plant Biotechnology*	
20:00-21:00	*Dinner*	
21:00-	Wine tasting	

Wednesday 17th June, 2015

08:00-09:00	*Breakfast*

Section II: Plant Growth, Development, Metabolism and Nutrition

09:00-09:30	(Invited talk) **James Giovannoni**	Harnessing genetic diversity to better understand regulation of tomato fruit ripening and nutritional quality
09:30-09:50	(Invited talk) **Christian Fankhasuer**	Photosensory receptor-mediated growth responses in Arabidopsis
09:50-10:10	(Invited talk) **David Honys**	Male germline development: lesson from the -omics
10:10-10:30	(Invited talk) **Dragan Vinterhalter**	Acid growth theory, auxin and potato phototropism
10:30-10:50	(Invited talk) **Bojana Banović**	How to avoid self-fertilization in plants- a buckwheat story
10:50-11:20	*Coffee break*	

11:20-11:50	(Invited talk) **Hrvoje Fulgosi**	Revisiting alternative electron partitioning pathways in photosynthesis
11:50-12:10	(Invited talk) **Miroslav Nikolić**	The rhizosphere: perspective and challenges for plant nutrition
12:10-12:30	(Invited talk) **Jelena Samardžić**	Silicon alleviates oxidative stress in cucumber plants grown under copper excess
12:30-12:45	(Selected talk) **Lidija Begović**	Lignin deposition and synthesis in the internodes during barley (*Hordeum vulgare L.*) development
12:45-13:00	(Selected talk) **Milan Dragićević**	DUF1070 is a conserved signature domain of some arabinogalactan peptides
13:00-13:15	(Selected talk) **Jan Fíla**	Phosphoproteomics profiling of tobacco mature pollen and pollen activated *in vitro*
13:15-13:30	(Selected talk) **Václav Motyka**	New findings about the role of *cis*-zeatin-type cytokinins in plant physiology and evolution
14:00-15:00	*Lunch*	

Section III: Plant and Fungal Natural Products in Human Nutrition and Medicine

15:00-15:30	(Invited talk) **Autar Mattoo**	Functional Foods & Nutrition: Facts, Fiction, and Needs
15:30-15:50	(Invited talk) **Nataša Simin**	Wild-growing *Allium* species (sect. *Codonoprasum*) as promising sources of novel herbal drugs
15:50-16:10	(Invited talk) **Marina Soković**	Alternative sources of natural products: mystery of mushrooms and beyond
16:10-16:25	(Selected talk) **Miloš Đorđević**	*Centaurium erythraea* extract improves redox-status and antioxidant enzyme activity of STZ-treated pancreatic β-cells and diabetic rat liver and kidney
16:25-16:40	(Selected talk) **Bojan Jevtić**	Effects of cucumber extracts on cytokine production in encephalitogenic cells
16:40-16:55	(Selected talk) **Filis Morina**	Quercetin 7-*O*-glucoside inhibits the formation of dinitrosocatechins and their quinones in catechin/ nitrite systems under stomach simulating conditions
16:55-17:10	(Selected talk) **Milica Pešić**	Development of natural product drugs in a sustainable manner
17:10-17:30	*Coffee break*	

Section IV: Phytochemistry

17:30-18:00	(Invited talk) **Roque Bru Martínez**	Early and late molecular mechanisms involved in the biosynthesis and accumulation of stilbenoids in elicited grapevine cell cultures established from berries
18:00-18:20	(Invited talk) **Sokol Abazi**	Chemical analysis of secondary metabolites isolated from endemic Albanian plants with subcritical CO_2
18:20-18:40	(Invited talk) **Vuk Maksimović**	Composition and therapeutic values of berry wines - bitter truth about sweet product
18:40-19:00	(Invited talk) **Maja Natić**	Phenolic profiles of wild fruits grown in Serbia
19:00-19:15	(Selected talk) **Dorisa Cela**	NMR structure elucidation of a new alkaloid isolated from *Gymnospermium maloi*
19:15-19:30	(Selected talk) **Đura Nakarada**	Thapsic acid, a rarely found natural product among bryophyte species
19:30-20:30	Poster sessions: *Plant Growth, Development, Metabolism and Nutrition; Plant and Fungal Natural Products in Human Nutrition and Medicine; Phytochemistry*	

20:30-21:00	*Dinner*	
21:00-21:30	*Presentation of Petnica Science Center*	
21:30-22:30	*Tour around Petnica Science Center*	

Friday 19th June, 2015

08:00-09:00	*Breakfast*	
Section V:	**Biodiversity and Conservation**	
09:00-09:30	(Invited talk) **Goran Anačkov**	Phenotypic plasticity or new taxa?
09:30-09:50	(Invited talk) **Jelena Aleksić**	What does Balkan Peninsula has to offer to conservation biologists?
09:50-10:10	(Invited talk) **Maja Lazarević**	Plant diversity drivers in the Balkans: ploidization, hybridization and cryptic speciation
10:10-10:25	(Selected talk) **Zora Dajić Stevanović**	Conservation of floristic and vegetation diversity in Southeast Europe: sustainable use and ecosystem services approach
10:25-10:40	(Selected talk) **Mihailo Jelić**	Assessment of genetic integrity and diversity of *Populus nigra* in protected areas along the Danube River
10:40-10:55	(Selected talk) **Marko Sabovljević**	Conservation biology of European bryophytes
11:10-11:30	*Coffee break*	
Section VI:	**Evolutionary Plant Biology**	
11:30-12:00	(Invited talk) **Petr Smýkal**	Past legume crop domestication and agriculture of tommorow
12:00-12:20	(Invited talk) **Stevan Avramov**	Comparative approach in evolutionary ecology of plants
12:20-12:40	(Invited talk) **Yuval Sapir**	Population divergence and speciation within a species: ecology and the Royal Irises
12:40-12:55	(Selected talk) **Aleksej Tarasjev**	Population scale multi-year monitoring of *Iris pumila* in Deliblato Sand: flowering phenology
12:55-13:10	(Selected talk) **Vukica Vujić**	Light induces variation in size and shape of *Iris pumila* flower parts in two natural habitats
13:10-13:25	(Selected talk) **Sanja Manitašević Jovanović**	How do *Iris pumila* plants respond to photo-oxidative stress in the wild: the variation of leaf functional traits?
13:30-13:45	*Group photo*	
14:00-15:00	*Lunch*	
Section VII:	**Molecular mechanisms underlaying health compounds biosynthesis in fruits (COST ACTION FA1106)**	
115:00-15:40	(Invited talk) **Angelos Kanellis**	Introduction to Session Genetic improvement of fruits and vegetables for health
15:40-16:10	(Invited talk) **Mondher Bouzayen**	Cross-talk between multiple hormone signaling pathways associated with the ripening of tomato fruit
16:10-16:40	(Invited talk) **Julia T Vrebalov**	The role of transcription factors in regulation of tomato fruit ripening and quality

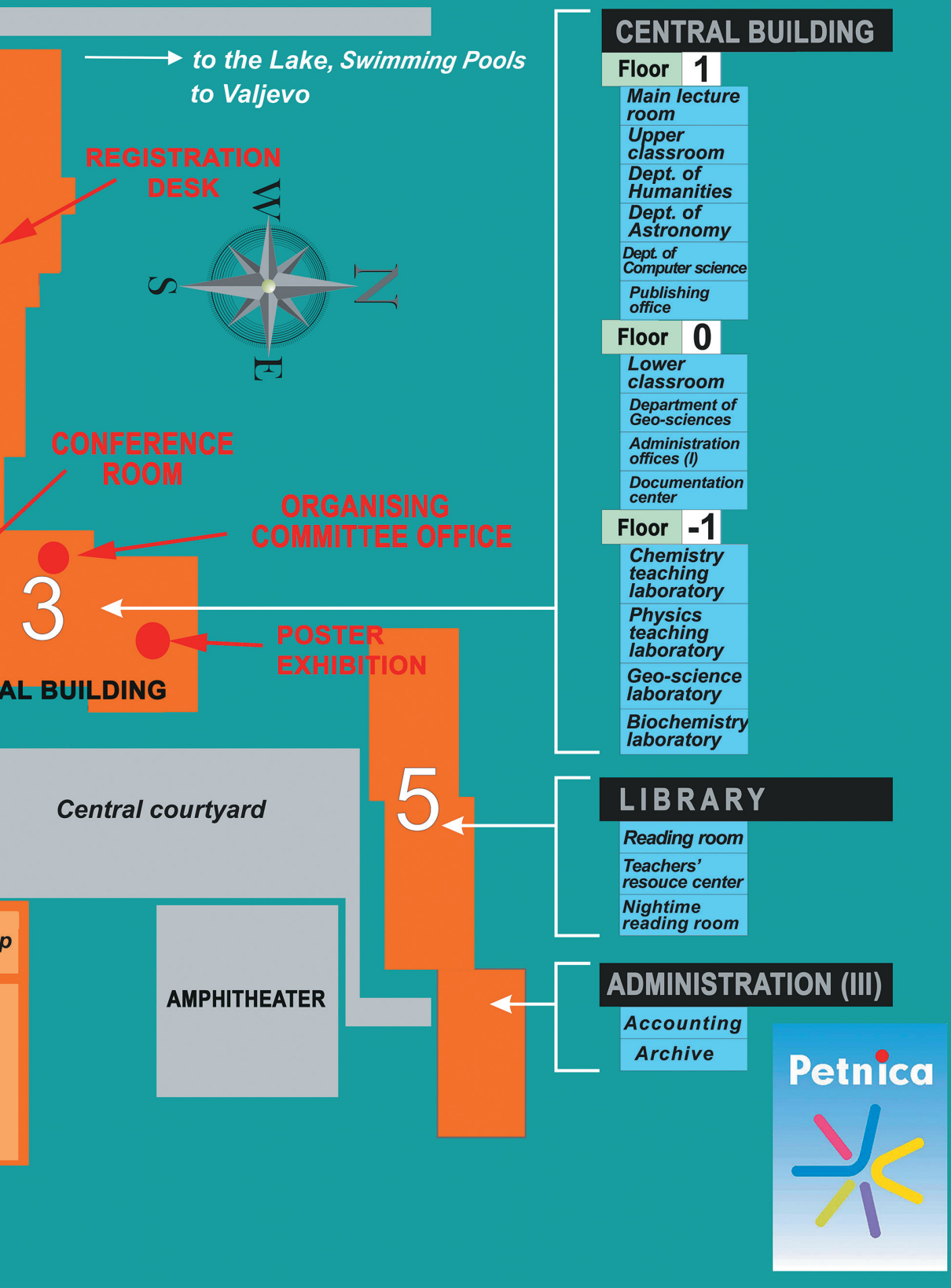
16:40-17:10	(Invited talk) **Cathie Martin**	Engineering the production of health-promoting metabolites in tomato for studies of comparative nutrition
17:10-17:40	(Invited talk) **Giovanni Giuliano**	Tomato fruit carotenoid biosynthesis: regulation and evolutionary aspects
17:40-18:10	(Invited talk) **Panagiotis Kalaitzis**	Suppression of a tomato prolyl 4 hydroxylase results in multiple alterations on fruit development, ripening and health components
18:10-18:30	*Coffee break*	
18:30-19:30	Poster sessions: *Biodiversity and Conservation; Evolutionary Plant Biology*	
21:00-	*Gala dinner*	

Saturday 20th June

08:00-09:00	*Breakfast*	
Section VIII:	**Abiotic and Biotic Stress and Ecophysiology**	
09:00-09:30	(Invited talk) **Harro Bouwmeester**	Strigolactones. Key players in the adaptation of plants to the abiotic environment
09:30-09:50	(Invited talk) **Miroslav Lisjak**	H_2S and NO signalling in plants
09:50-10:10	(Invited talk) **Jelena Savić**	Essential oils elicit defense genes in potato: Can volatiles released from damaged plants prime defense in their undamaged neighbours?
10:10-10:30	(Invited talk) **Živko Jovanović**	*Alyssum markgrafii* as a model organism to study metal hyperaccumulation
10:30-10:45	*Coffee break*	
10:45-11:00	(Selected talk) **Dejana Panković**	The influence of *Trichoderma* spp. treatment on water regime, ABA content and gene expression in leaves and roots of tomato in drought conditions
11:00-11:15	(Selected talk) **Zorana Katanić**	Effect of dynamic changes of vegetative compatibility types in *Cryphonectria parasitica* populations on biological control of chestnut blight in Croatia
11:15-11:30	(Selected talk) **Nevena Nagl**	Effect of *in vitro* induced water deficit on lipid peroxidation intensity and antioxidant capacity of sugar beet
11:30-11:45	(Selected talk) **Marija Vidović**	High PAR and UV-B radiation-induced differential responses in green and white leaf sectors of *Pelargonium zonale* in relation to sugar, antioxidative and phenolic metabolism
12:00-13:00	Poster session: *Abiotic and Biotic Stress and Ecophysiology*	
13:00-13:30	*Closing Ceremony*	
13:30-14:30	*Meeting of the Serbian Plant Physiology Society/Cost Action FA1106*	
14:30-15:30	*Lunch*	
16:00-19:30	*Excursion (Gradac Canyon and "Ćelije" Monastery)*	
19:30	*Departure*	
21:00	*Arrival in Belgrade*	

Map of Petnica Science Center


ROOMS
Floor 2
Halls 8, 9, 10
Floor 1
Halls 5, 6, 7
Floor 0
Reception desk
Hall 3
Computer System Administration
Floor -1
Computer room
Hall 2
Technical services
Dept. of Archaeology
Laboratory on Analytical Archaeology
Archaeological Depot
LABORATORIES
Floor 2
Movie Theater
Floor 1
Chemistry Lab
Floor 0
Biology Lab
Microbiology Lab
Floor -1
Lab of Physics and Electronics
Optics Lab
Rogljević hill
P
1
2
CENTR
to the Cave
Exhibition
Café & Sho
WC
4
Restaurant
P


to the Lake, Swimming Pools
to Valjevo
REGISTRATION DESK
W
S
N
E
CONFERENCE ROOM
ORGANISING COMMITTEE OFFICE
3
POSTER EXHIBITION
AL BUILDING
Central courtyard
5
AMPHITHEATER
CENTRAL BUILDING
Floor 1
Main lecture room
Upper classroom
Dept. of Humanities
Dept. of Astronomy
Dept. of Computer science
Publishing office
Floor 0
Lower classroom
Department of Geo-sciences
Administration offices (I)
Documentation center
Floor -1
Chemistry teaching laboratory
Physics teaching laboratory
Geo-science laboratory
Biochemistry laboratory
LIBRARY
Reading room
Teachers' resouce center
Nightime reading room
ADMINISTRATION (III)
Accounting
Archive
Petnica

I

Plant Biotechnology

INVITED TALKS

Systems biology of a plant cell factory, the tomato glandular trichomes

IT1-1

Alain Tissier
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Department of Cell and Metabolic Biology, Leibniz-Institute of Plant Biochemistry, Halle (Saale), Germany

Tomato glandular trichomes produce a variety of secondary metabolites including terpenoids, phenylpropanoids and fatty acid derivatives. They represent the first line of defense against various pests and wild tomato species such as *Solanum habrochaites* are particularly well equipped in this regard, both in terms of diversity and abundance of the metabolites. Metabolites produced by these structures can represent up to 10% of the leaf dry weight. We are using *S. habrochaites* and *S. lycopersicum* as model systems to elucidate pathways of secondary metabolites, particularly terpenoids, and to understand how these cell factories develop and achieve their huge metabolic flux. For this, we are combining several approaches including genetics, cell biology, transcriptomics, metabolomics and isotope labeling. Our progress in the biosynthesis of various tomato sesquiterpenoids and in the understanding of the connection between primary and secondary metabolism will be presented.

Keywords: tomato, glandular trichomes, sesquiterpenoids, metabolomics, transcriptomics.

Biotechnological production of plant compounds

IT1-2

Jules Beekwilder, Catarina Cankar, Nikolay Outchkourov, Robert Hall, Dirk Bosch
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Business Unit Bioscience, Wageningen University and Research Centre, Wageningen, The Netherlands

Compounds from plants have a wide range of applications in cosmetic, food, agrochemical and pharmaceutical products. We are exploring ways to produce such products via biotechnology in plant as well as microbial systems. Biosynthesis of anthocyanins, carotenoids and terpenes in plants is relatively well understood and they have interesting commercial applications. We use these compounds therefore as models to improve biotechnological production methods of plant metabolites. Examples will be described of transient overexpression of the anthocyanin and terpene biosynthetic pathways. The overexpression of enzymes for the commercially important flavor compounds valencene and nootkatone in *Nicotiana benthamiana* indeed results in formation of the expected compounds, but also of a range of side products and modified end products. Similarly, the overexpression of MYB and bHLH transcription factors regulating anthocyanin biosynthesis in *N. benthamiana* leads to accumulation of a wide range of compounds, including anthocyanins, but also putrescin and nicotine-derived molecules. On the other hand, upon overexpression of the same transcription factor pair in tomato vegetative tissue, we observed accumulation of anthocyanins, but only very few other compounds. Thus, metabolic engineering approaches, combined with broad metabolomics analyses, show the complexity of the consequences of pathway overexpression for the metabolite make-up of a plant.

Keywords: terpene, anthocyanin, flavor, color, biotechnology

Metabolomics, lead discovery and plant biotechnology: perfect holistic match?

IT1-3

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Per definition, metabolomics represents a comprehensive holistic approach, comprising of systematic identification and quantification of all metabolites in an organism, at given conditions. The comprehensive analysis of the chemical fingerprints left by metabolic processes started to play a crucial role in the personalized medicine. Since the term 'metabolome' has been introduced several techniques for high throughput analyses of targeted metabolites have been developed. Nuclear magnetic resonance (NMR) appears very suitable and adequate platform to carry out metabolomics analyses, because it allows simultaneous detection of diverse range of abundant (primary and secondary) metabolites, which opens novel avenues to fully explore the total biochemical machinery of plants. A great advantage of ^{1}H NMR-spectrometry over other analytical platforms is the possibility for quantification and thus direct comparison of concentrations of all compounds present in the sample, as the signal intensity is only dependent on the molar concentration of the solutes. Some case studies of the application of NMR-based metabolomics concept in natural products research, plant biotechnology and lead finding will be discussed.

The authors are thankful to the National Science Fund of Bulgaria and DAAD Germany for their financial support (Grant No DNTS Germany 01/8).

Exploring the secondary metabolism in trichomes of *Salvia fruticosa* and *Rosmarinus officinalis*: the case of carnosic acid

IT1-4

Dragana Božić[1], Antigoni Papanikolaou[2], Dimitra Katsarou[2], David Manzano[3], Ulschan Scheler[4], Kathleen Brückner[4], Albert Ferrer[3], Alain Tissier[4], Angelos K. Kanellis[2]
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Glandular trichomes (GTs) are secretory structures involved in the production and storage of plant secondary metabolites. Carnosic acid (CA) is a phenolic diterpene found in GTs of Cretan sage (*Salvia fruticosa*) and rosemary (*Rosmarinus officinalis*). CA is considered potentially significant for the pharmaceutical industry, but its biosynthetic pathway in plants is only partly explored. Better understanding of the CA biogenesis and metabolism would make production of this valuable compound possible by using advanced biotechnological methods. We have investigated the CA biosynthesis at the molecular level, by isolating and functionally characterizing genes involved in this process. From an existing Cretan sage GT cDNA library, two putative diterpene synthase genes, copalyl diphosphate synthase (*SfCPS)* and kaurene synthase (*SfKSL)*, were identified.

The candidate genes were isolated and functionally characterized in *Escherichia coli*, *Saccharomyces cerevisiae* and *Nicotiana benthamiana*. Heterologous expression of these genes has resulted in the synthesis of miltiradiene, which was confirmed by GC-MS analysis and NMR spectroscopy. In order to retrieve the remaining CA biosynthetic genes, total RNA from GTs of Cretan sage and rosemary was sequenced using 454 GS FLX Titanium platform. Sequences have been annotated using Blast2GO, PRIAM and RPS BLAST tools to assign Gene Ontology (GO) terms, Enzyme Commission numbers (EC numbers) and functional domains. Associated Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway maps were also collected. Using the functional characterization assays in yeast and *Nicotiana benthamiana* it has been determined that the Cretan sage enzyme SfFS and rosemary enzymes RoFS1 and RoFS2 exhibit the activity of a ferruginol synthase.

Keywords: *Salvia fruticosa*, diterpene synthases, carnosic acid, miltiradiene, ferruginol, phenolic diterpenes

This work was supported by E.U. grant agreement KBBE-227448 for the TERPMED project (http://www.terpmed.eu)

SELECTED TALKS

Problems in detecting activity of fluorescent reporter genes – case of DsRED and GFP

OP1-1

Milica Bogdanović[1], Slađana Todorović[1], Angelina Subotić[1], Milan Dragićević[1], Katarina Cankar[2, 3], Jules Beekwilder[2, 3], Ana Simonović[1]

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Reporter genes are indispensible tools in plant biotechnology, used to assess transformation efficiency, select transgenic tissues, study promoters, etc. Advantages of fluorescent markers include non-destructive visualization, simplicity of use, non-toxicity, and stability in most cellular contexts. GFP and DsRED are widely used fluorescent reporters and are considered compatible. Unlike animal systems, plant tissues are notoriously known for autofluorescence, spanning most of the visible spectrum. This limits the application of fluorescent proteins in plants and requires special equipment and a set of control samples, to discern autofluorescence from transgene expression. In the course of study of guaianolide biosynthesis in chicory, several vectors were constructed containing *DsRED* as co-transformation reporter and *GFP* as a reporter fused to promoters of the studied genes. To observe DsRED and GFP fluorescence in transgenic chicory tissues, we have used: a system for macro visualization consisting of LED light sources and emission filters, fluorescent stereobinocular, fluorescent microscope and confocal microscope. These methods revealed strong green autofluorescence in newly formed roots, calli, lignified cell walls and parenchyma cells. The intensity of autofluorescence was especially high at high magnification and could obstruct GFP visualization. DsRED fluorescence, on the other hand, was easily discerned from any kind of plant autofluorescence. Another problem was signal crossover from DsRED into GFP channel. DsRED has a small emission peak in the green part of the spectrum, originating from its chromophore maturation. Thus it was impossible to separate fluorescent emission of these two markers present in the same tissue without using spectral deconvolution techniques.

Keywords: DsRED, GFP, LED, fluorescent microscopy, confocal microscopy

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173024) and EU FP7 Project acronym: TERPMED, grant agreement no. 227448.

Alteration of flower color in *Solanum lycopersicum* through ectopic expression of a gene for capsanthin-capsorubin synthase from *Lilium lancifolium*

OP1-2

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Red irises flowers do not exist naturally and to produce them efforts using classical breeding have failed. Genetic engineering provides a potential avenue to create novel flower hues, but irises are difficult to transform and take a long time to reach flowering. For these reasons, we used tomato flowers (*Solanum lycopersicum*) as a model system to investigate flower color modification by alteration of the carotenoid biosynthetic pathway. *S. lycopersicum* is a useful model system due to its ease of transformation and short time from seed to flowering. We expressed a capsanthin-capsorubin synthase gene from tiger lily (*Lilium lancifolium*) under the control of a petunia chalcone synthase gene promoter fused to an enhancer sequence of the cauliflower mosaic virus 35S promoter. Capsanthin-capsorubin synthase (*Llccs*) catalyzes the conversion of antheraxanthin and violaxanthin, two yellow xanthophylls that are produced in tomato flowers, into capsanthin and capsorubin, two red κ-xanthophylls, respectively. All transgenic lines produced flowers with a new light orange pigmentation, as opposed to the natural yellow. UHPLC analysis confirmed that the color change coincided with the accumulation of two novel xanthophylls, capsanthin and a capsanthin-like carotenoid. A more pronounced color change likely could have been achieved using a stronger or more specific promoter; nevertheless, these results indicate that alteration of the carotenoid biosynthetic pathway is a potential approach to altering flower color in ornamental crops.

Keywords: genetic transformation, flower color, carotenoids

This research was sponsored by the Cooley's Gardens Inc., Silverton, OR (Grant: ARF3711) and by the Ministry of Education, Science and Technological Development of the Republic of Serbia (TR 31019).

Characterization of soybean hull peroxidase immobilized on glycidyl methacrylate copolymers

OP1-3

Miloš Prokopijević[1], Olivera Prodanović[1], Dragica Spasojević[1], Mira Stanković[1], Željko Stojanović[2], Ksenija Radotić[1], Radivoje Prodanović[3]
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Toxic aromatic pollutants that are found in various industrial wastewaters pose a serious environmental threat. Current methods for phenol removal have certain disadvantages, such as low efficiency, high cost or generation of even more toxic products. On the other hand enzyme-based treatments are highly selec-

tive and efficient. Soybean hull peroxidase (SHP) as well as other class III peroxidases catalyzes oxidation reaction in the presence of hydrogen peroxide, resulting in phenol polymerization and formation of less hazardous phenolic polymers. As a by-product of the food industry, soybean hulls are inexpensive and readily available source of large quantities of crude peroxidase. The aim of our research was to isolate SHP from soybean hulls and immobilize it onto a glycidyl methacrylate based carriers using glutaraldehyde method and characterize the resulting product. Immobilized SHP showed dependence upon the pore size of the carrier matrix, with the highest obtained specific activity of 22.8 U g^{-1} of carrier. Immobilized enzyme proved as an effective phenol removal alternative method with improved thermal and organic solvent stabilities compared to the free form. It also showed greater stability and tolerance to pH fluctuations, showing higher specific activities over a wider pH range. Operational stability was tested by repeated pyrogallol oxidation cycles in a batch reactor. After three cycles, immobilized SHP retained over 60% of the initial activity.

Keywords: soybean hull peroxidase, immobilization, glycidyl methacrylate

This study was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173017 and OI172049).

POSTER PRESENTATIONS

Germacrene A synthase and oxidase promoter analysis in chicory

PP1-1

Milica Bogdanović[1], Milan Dragićević[1], Katarina Cankar[2, 3], Jules Beekwilder[2, 3], Harro Bouwmeester[2], Angelina Subotić[1], Ana Simonović[1], Slađana Todorović[1]
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Chicory (*Cichorium intybus* L.) is rich in sesquiterpene lactones, bitter compounds whose biosynthesis involves several recently characterized genes, namely germacrene A synthase (GAS), in chicory present as long and short form, and germacrene A oxidase (GAO, CYP71AV8). Promoters of these genes have been studied by cloning promoter regions to drive the expression of eGFP, and obtaining stable chicory transformants carrying promoter constructs. Due to incompatibility of eGFP with another fluorescent marker – DsRED, which was used for selection of transformed clones, promoter activity was detected in transgenic plants by RT-PCR and qRT-PCR techniques. Most of the obtained chicory clones were expressing GFP, in roots, leaves, stems and flowers, suggesting that cloned promoters were functional in different chicory organs. The promoters were characterized by different strengths – GAO and GAS long promoters were stronger than both GAS short promoters, judging by eGFP expression level. The promoters also showed partial tissue specificity – GAS long was active in roots, leaves, stems and flowers, while GAS short promoters were mainly active in chicory roots.

Keywords: germacrene A synthase, germacrene A oxidase, promoter analysis, eGFP

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI 173024) and EU FP7 Project acronym: TERPMED, grant agreement no. 227448.

Efficiency of different molecular markers in detection of Ogu-INRA *cms* and *Rfo* genes in NS rapeseed

PP1-2

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Development of rapeseed hybrids was enabled by the discovery and introduction of *cms* and *Rf* genes. Several cms genes have been discovered so far: *Pol*, *Nap*, *Cam*, *Ogu*, *Nsa*, *Shan 2A* and *Kos*. Ogu-INRA *cms*, derived from *Raphanus sativus*, is one of the most widely analyzed and used gene in rapeseed breeding. This gene, together with compatible *Rfo* gene (derived from line R2000), is currently being introduced in NS breeding material. During the process of development of cms and Rf lines, some deviations from expected segregation ratio were observed. In order to analyze NS breeding material, the efficiency of four markers, two for de-

tection of Ogu-INRA *cms* gene and two for detection of *Rfo* gene, was tested. Both markers used for detection of Ogu-INRA *cms* gene produced similar and comparable results, as was the case with two markers, Bn-RFO-AS2 and BolJon, used for detection of *Rfo* gene.

Keywords: *Brassica napus* L., marker, Ogu-INRA cms system

This work is a part of the project TR31025, supported by Ministry of Education, Science and Technological Development of the Republic of Serbia.

Study of chicory germacrene A synthase function in guaianolide biosynthesis through amiRNA-induced gene silencing

PP1-3

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Germacrene A synthase (GAS) catalyzes an important step in guaianolide biosynthesis converting farnesyl pyrophosphate to germacrene A – universal sesquiterpene precursor in chicory. The function of two genes coding for GAS – *CiGASlo* and *CiGASsh*, was studied by gene silencing. Chicory was transformed with amiRNA constructs designed to specifically silence either of the two genes, and regenerated transgenic plants were tested for changes in gene expression and sesquiterpene lactones content. Silencing was successful in most of the clones, which had reduced gene expression. However, the amiRNA constructs were not specific for *CiGASlo* or *CiGASsh*, so that both genes were silenced in most transgenic plants. To verify that the gene silencing had impact on sesquiterpene lactone levels, three representative guaianolide oxalates (lactucin-15-oxalate, 8-deoxilactucin-15-oxalate and lactucopicrin-15-oxalate) were identified and quantified by UPLC-MS/MS in roots and shoots of transgenic and control plants. Data analyses showed unequal segregation of targeted compounds in the plant tissues, and implied that the synthesis of these compounds highly depends on the expression of GAS genes in the shoots and that these metabolites are pooled in the shoots, while the root guaianolide oxalates content is regulated on a level different from GAS expression. Nevertheless gene silencing reduced the targeted metabolites levels significantly. It was concluded that both genes are involved in guaianolide biosynthesis, and that their complex pattern of expression in different parts of the plant determines the amount of guaianolides and their derivatives.

Keywords: germacrene A synthase, silencing, amiRNA, chicory, guaianolides

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI 173024) and EU FP7 Project acronym: TERPMED, grant agreement no. 227448.

Production of phenylpropanoids and naphtodianthrones in *Hypericum perforatum* callus cultures elicited with dextran

PP1-4

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Hypericum perforatum L. callus cultures were evaluated for their growth, antioxidant potential and ability to produce phenylpropanoids (phenolics, flavonoids, flavanols and anthocyanins) and naphtodiantrones (hypericin and pseudohypericin) after elicitation with different dextran concentrations (50-200 mg L^{-1}). Non-enzymatic antioxidant properties (NEAOP) and specific activity of antioxidant enzymes such as peroxidase (POD) and catalase (CAT) were observed in callus culture extracts. The activities of two key enzymes of the phenylpropanoid/flavonoid pathways, phenylalanine ammonia lyase (PAL) and chalcone isomerase (CHI) were also monitored to estimate channeling in the different metabolic pathways. Treatment with dextran did not affect the biomass production of *H. perforatum* calli after 21 day of elicitation. Exogenously applied dextran induced NEAOP, POD and CAT activities in treated calli suggesting a strong perturbation of the cell redox state leading to the activation of defense responses. The elevated activities of PAL and CHI confirmed an efficient activation of the phenylpropanoid/flavonoid pathway. In general, naphtodianthrone and phenylpropanoid production was globally stimulated upon treatments with all dextran concentrations tested. These findings suggest the involvement of an efficient antioxidant defense system in the adaptive response of calli to dextran elicitation. These results indicated that *H. perforatum* elicited callus cultures represent a promising experimental system for the enhanced production of bioactive compounds in food and pharmaceutical industry.

Keywords: callus cultures, dextran, *Hypericum perforatum* L., naphtodianthrones, phenylpropanoids

Amelanchier alnifolia var. *Cusickii* propagation *in vitro*

PP1-5

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Amelanchier alnifolia var. *Cusickii* is an attractive small fruit species with wide adaptability to various environmental conditions. Its berries contain large amount of bioactive components, vitamins and minerals. Although there are good climatic conditions for its cultivation on the territory of Slovakia and Serbia, the commercial cultivation of this species is not yet spread. One of the reasons is the lack of information about this plant and inaccessibility of planting material for establishment of commercial plantations. In the present paper we focused on elaboration of *in vitro* propagation system for *Amelanchier alnifolia*. Cultures were established using dormant buds collected from mature plants. Shoot initiation was achieved on MS medium with 30 g L^{-1} sucrose, 8 g L^{-1} phyto agar and 1 mg L^{-1} N^6-benzyladenine (BA). Four different culture media variants

were tested for efficient shoot proliferation: 1) MS medium van der Salm Modification with 1 mg L^{-1} BA and 1 mg L^{-1} indole-3-butyric acid (IBA); 2) MS medium with 0.5 mg L^{-1} BA and 0.5 mg L^{-1} IBA; 3) MS medium with 1 mg L^{-1} BA, 0.1 mg L^{-1} IBA and 0.1 mg L^{-1} gibberellic acid (GA_3); 4) MS medium with 0.5 mg L^{-1} BA and 0.5 mg L^{-1} 1-naphthaleneacetic acid (NAA). Out of the tested culture media, variant 3) proved to be the best for shoot proliferation. On this medium, the formation of 9.93 shoots per explant was achieved, which was statistically significantly different from other tested variants.

Keywords: *Amelanchier alnifolia*, micropropagation

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The possibility of growing confectionery sunflower under the conditions of organic production system

PP1-6

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Sunflower belongs to the genus *Helianthus*, which comprises a large number of species. One species is the cultivated *Helianthus annuus* L., with two types - oil and confectionery sunflower type. Confectionery low-oil protein type is used in human nutrition and as bird food. The main aim of confectionery sunflower breeding is the increase in yield, content and quality of protein, decrease in seed oil content and improvement of seed oil quality, with reduction in hull content. In recent past, there has been a steady increase in global demand for organic foods because of the health risks posed by the conventional method of production. The aim of this research is to examine the effect of both organic and conventional production system on the protein content in seed, oil content in seed, 1000 seed weight, and total number of seeds per head of confectionery sunflower. Twenty-two confectionery NS hybrids, created and produced in the breeding program of IFVCNS, were set as parallel trials, under the conditions of the conventional production at the location of Rimski Šančevi, and under conditions of the organic production at the location of Ljutovo, as a randomized block design with three replications. Mean value of seed protein content in the conventional production system (14.5%) was lower compared to the organic production system (15.9%), while the type of production had no effect on mean oil content (31.6% and 31.3% respectively). Mean value of 1000 seed weight and total number of seeds per head were higher in the conventional production system. Testing in different agricultural systems can be useful in identifying sunflower hybrids with broad adaptability.

Keywords: confectionery sunflower, hybrids, organic production

Genetic mapping of a broomrape resistance gene in sunflower line LIV-17

PP1-7

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Broomrape (*Orobanche cumana* Walr.) is becoming the most important limiting factor in sunflower production in Europe. The parasite considerably reduces sunflower yield and reduces seed oil content, leading to great economic losses. While the application of herbicides is limited for health and economic reasons, breeding for resistance is regarded as the most effective and environmentally friendly solution. Still, broomrape race composition constantly changes and new, more aggressive physiological races appear. Therefore, finding new sources of resistance and developing molecular markers for detecting *Orobanche* resistance genes are of immense importance in sunflower breeding. Line LIV-17 is a sunflower inbred line that was found to be resistant to new races of broomrape in heavily infested locations in Spain, Romania and Turkey during trials conducted from 2007 to 2010. In the present study, preliminary molecular analyses with the use of bulked segregant analysis (BSA) were conducted with the aim of identifying the region of the genome that could potentially carry the resistance locus. For this purpose, a mapping F_2 population from a cross LIV-17/HA-26-PR was used. Two contrasting bulks were prepared, each containing DNA from extremely resistant or susceptible F_2 plants. In total 210 SSR markers were selected from 17 linkage groups from the public genetic map of sunflower. Preliminary results indicated that the resistance gene was placed in LG3 of the sunflower genetic map. Identification of closely linked molecular markers which will enable marker-assisted selection is underway.

Keywords: sunflower, broomrape, SSR

Recent advancements in the production of a red *Iris* flower through genetic engineering

PP1-8

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The elusive red iris (*I. germanica*, *I. hollandica*) has been a subject of interest for over a century as it does not exist naturally and efforts with classical breeding methods have failed to produce it. Genetic engineering, on the other hand, offers alternative ways of introducing this desirable trait by expanding the color-determining gene pool that is available. We first studied the effects of ectopic expression of a bacterial phytoene synthase gene (*crtB*) from *Pantoea agglomerans* in the pink *I. germanica* cultivar 'Fire Bride'. This approach aimed to increase the flux of metabolites into the carotenoid biosynthetic pathway, and ultimately lead to elevated accumulation of lycopene, thus generating darker pink or red flowers. *CrtB*-transgenic plants showed prominent color changes in the ovaries (green to orange), flower stalk (green to orange), and anthers (white to pink) when compared to control plants, while the standards and falls showed no significant color change. Next, we studied the feasibility of using a gene for capsanthin-capsorubin synthase (*Llccs*) from tiger lily (*Lilium lancifolium* 'Splendens') in yellow iris flowers to alter their color to red. This approach aimed to produce the flower-specific accumulation of two red κ-xanthophylls, capsanthin and capsorubin. *Llccs*-transgenic iris

calli successfully accumulated the novel carotenoids and changed color from yellow to red-orange, but no plants were regenerated due to the lack of morphogenic response from the cultivar 'Hot Property'. Efforts are currently under way to transform different morphogenic yellow cultivars with *Llccs* to produce red *l. germanica*, as well as *l. hollandica*, flowers.

Keywords: lycopene, capsanthin-capsorubin synthase, tiger lily

This research was sponsored by the Cooley's Gardens Inc., Silverton, OR (Grant: ARF3711) and by the Ministry of Education, Science and Technological Development of the Republic of Serbia (TR 31019).

Removal of apical dominance in *Knautia sarajevensis* shoot cultures

PP1-9

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Knautia sarajevensis (Dipsacaceae) is an endemic perennial plant found on wood margins of Dinaric Alps. In nature, the plants' habitus is characterized by ground rosette and branched shoots bearing apically positioned flower heads. Under *in vitro* conditions the apical dominance was present. Cultivation of *K. sarajevensis* seedlings on solid media containing kinetin at low concentrations did not induce formation of adventive shoots. Decapitation of plantlets resulted in removal of apical dominance and adventive shoot formation was noticed at high as well as low kinetin concentrations. Elevation of kinetin concentration induced formation of one adventive shoot per explant in apical shoot explants. Addition of indole-3-butyric acid during cultivation of nodal explants (decapitated shoots) favoured development of one shoot, resulting in restoration of apical dominance by taking over the role of apical shoot by favouring shoot. Other formed shoots remained underdeveloped. Cultivation of plants in liquid medium resulted in absence of apical dominance in cultivated shoots, and formation of adventive shoots was possible by low kinetin concentration. The absence of apical dominance was the result of agitation, in which manner a disruption of auxin transport was accomplished and formation of adventive shoots was possible without decapitation.

Keywords: apical dominance, *Knautia sarajevensis*, shoot cultures

Adventitious shoot regeneration *in vitro* in *Prunus* sp.

PP1-10

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Plums represent the fruit species that were grown very broadly in Slovakia and Serbia in the past. They are appreciated due to their organoleptic characteristics, nutritional value and suitability for processing. The aim of the study was the optimization of adventitious shoot regeneration of *Prunus cerasifera* – the model object for genetic transformation. As primary explants we used 3-4 leaves and petioles from the upper part of *in vitro* grown plants, which were isolated two weeks after the last subculture on MS medium with 0.5 mg L^{-1} N^6benzyladenine, 0.05 mg L^{-1} indole-

3-butyric acid. The leaf blade was cut by scalpel and cultivated horizontally with adaxial surface on the culture medium. To optimize the system for adventitious shoot regeneration, we tested three types of culture media: QL, WPM and ½MS medium supplemented with full vitamins and growth regulators thidiazuron (2 mg L^{-1}) and 2,4dichlorophenoxyacetic acid (0.2 mg L^{-1}), 30 g L^{-1} sucrose, 8 g L^{-1} phytoagar, pH 5.8. The primary explants - leaves and petioles were compared for their regeneration ability. The percentage of explants with adventitious buds was recorded after 6 weeks of culture and number of shoots/explant was evaluated after 8 weeks. On ½MS medium, the highest percentage of explants forming shoots, as well as the highest number of shoots per explants (petioles) was obtained.

Keywords: *Prunus cerasifera*, *in vitro*, adventitious regeneration

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Efficient plant regeneration of *Viola cornuta* 'Lutea Splendens' L. using seedling explants

PP1-11

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We developed an efficient plant regeneration protocol for a yellow cultivar of *Viola cornuta* 'Lutea Splendens'. Calli and shoots were induced from seedling explants (petiole, leaf, hypocotyls) by one month of culture on ½-strength MS medium supplemented with 2,4-D (0.1 mg L^{-1}) and BA (2.0 mg L^{-1}). The highest frequency of shoot induction (12%) was obtained from petiole explants, while the highest frequency of callus formation (97%) was achieved on hypocotyl explants. The effects of explant origin (top, center, and bottom of hypocotyl) and light/dark conditions on shoot and callus induction in hypocotyl cultures were also investigated. The highest shoot induction in hypocotyl cultures (30%) was achieved when explants from the upper section of the hypocotyls (near the epicotyls) were cultured in light conditions. Shoot multiplication from all explants was achieved by transferring initially formed shoots to ½strength MS medium supplemented with NAA (0.2 mg L^{-1}), GA_3 (2.0 mg L^{-1}), and TDZ (1.0 mg L^{-1}) for four weeks of culture. Developed shoots were then further multiplied on ½strength MS medium supplemented with NAA (0.5 mg L^{-1}) and BA (1.0 mg L^{-1}). Regenerated shoots successfully rooted on ½-strength MS medium without growth regulators, acclimatized in a greenhouse in autumn, and bloomed the following spring. All the regenerated plants exhibited normal morphology and flower color. This regeneration protocol could be useful for mass propagation using *in vitro* cultures and genetic transformation studies using seedling explants.

Keywords: petiole, hypocotyl, *in vitro*, shoot, regeneration

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Associations between SSR markers and multiple important agronomic traits in maize

PP1-12

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Association analysis, a recent approach in studying genetic architecture of quantitative traits, provides higher resolution mapping, time efficient and direct application on breeding material in comparison to biparental QTL mapping. Assuming that markers near stable QTLs across different environments and genetic backgrounds can be useful for marker-assisted selection, we selected SSR markers previously associated with the QTLs for the traits of interest in various mapping studies. To confirm the marker trait associations and evaluate the stability of the QTLs, a set of diverse maize inbred lines developed at the Institute of Field and Vegetable Crops in Novi Sad was chosen. It was genotyped with microsatellite markers near already known QTLs and phenotyped for flowering time, yield and yield components. Association analysis indicated significant correlations between several agronomic traits and three microsatellites on chromosomes 3, 5 and 8, namely umc1025, bnlg1237 and bnlg162. According to different intermated B73 × Mo17 maize genetic maps, these markers coincide with the position of annotated genes within 1 cM to 4 cM. Gene *triose phosphate isomerase 4*, near umc1025, seems to be crucial for efficient energy production, genes *plasma membrane intrinsic protein 1* and *2*, close to bnlg1237, have a significant role in water transport through plasma membrane, whereas *dehydroascorbate reductase like1* gene, near bnlg162, is important for plant growth, as it is responsible for ascorbic acid recycling. Although the results implied possible pleiotropy, additional analyses should be conducted to rule out the linkage and further elucidate the nature of the multiple trait correlations.

Keywords: association mapping, microsatellites, maize, QTL

Molecular tools for NS pumpkin breeding - The beginning

PP1-13

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Since ancient times, pumpkin fruit have been essential in the diet of rural communities. Nowadays, pumpkins are cultivated both for fruit and seeds. Consumers appreciate cold pressed pumpkin seed oil, as it is rich in phytosterols and tocopherols, and fruit due to its sweet and mild taste as well as high nutritive value. The development of molecular tools for use in pumpkin breeding is still in the early stages and marker assisted selection is little-used, compared to other vegetables such as tomato and lettuce. Molecular genotyping and development of markers for marker-assisted selection could be an invaluable way to circumvent the disadvantages of classical pumpkin breeding in NS breeding program. Molecular characterization of existing genotype collection will be performed and the data used for creation of core collection. Making the most of the core collection will be done by exhaustive characterization and association mapping with phenotypic traits

important for cultivation, as well as nutrition. Obtained results will assist in breeding programs aiming to obtain new genotypes with enhanced agronomical, nutritive and health beneficial properties.

Keywords: *Cucurbita* spp., molecular tools, breeding

Nursery production of purple beech (*Fagus sylvatica* 'Purpurea') by grafting

PP1-14

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Fagus sylvatica 'Purpurea' is a purple leaf cultivar of European beech, planted as an ornamental tree in many parks and gardens, but very rare in Serbia. This cultivar changes leaf color during the vegetation period: from red to very dark blackish-purple, that becomes bronze-green and, during the summer, dark green. Five adult purple beech trees were selected from 2 different locations in Belgrade, as sources of scion woods for grafting. Grafting was done in the nursery of Faculty of Forestry in Belgrade. The analysis of survival and vitality was performed, including variability of grafts height and root collar diameter. The data were processed by the software package „Statistica", and the following results are presented: descriptive statistics (min and max values, average value, standard deviation), LSD-test, analysis of variance and cluster analysis. Results of this research can be used to determine the tree that could be appropriate as scion's source for the future mass production of purple beech in Belgrade.

Keywords: purple beech, cultivars, grafting, nursery production

Possibility of SSR and ISSR marker transfer in *Vicia* species

PP1-15

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The genus vetch (*Vicia* L.) includes over 200 herbaceous annual and perennial species, containing food and forage legumes among them, with a prevailing Euro-Asiatic distribution. There are only few studies published considering the assessment of genetic diversity in *Vicia* using DNA markers, except for faba bean, where these were used extensively. The development of novel markers requires high costs and is time consuming. Instead of developing new markers, their transfer within genus is an alternative in less studied species. Having that in mind, the goal of This research was to investigate the possibility of transfer of SSR and ISSR markers within Vicia genus. Cross-species amplifications of the 6 SSR and 4 ISSR primers were carried out, using genomic DNA isolated from three accessions of eight Vicia species. Average transferability for SSR markers was 56.25%. Primers VfG14 and GAI1 displayed the highest, while Vfg24 had the lowest rate of transfer. Primers VfG14 and GATA5 indicated polymorphism among V. narbonensis and V. ervilia accessions. Average percentage of transferability of ISSR primers was very high (96.87%), with three out of four tested primers being 100% transferable. Amplification with primer ISSR8 resulted in different amplification profiles for each investigated Vicia spe-

cies, while polymorphism within species was observed in *V. narbonensis, V. sativa* ssp. nigra and *V. grandiflorum*. Our results suggest that tested SSR and ISSR markers can be transferred and employed within *Vicia* genus.

Keywords: *Vicia*, SSR, ISSR, transferability

Effect of osmotic stress conditions on *BvSTI*-expressing *Lotus corniculatus*

PP1-16

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Plant proteinase inhibitors (PIs) constitute a large and complex widely distributed group of small proteins involved in the regulation of the plant protein turn-over required in multiple physiological processes. Beside the roles regarding development and physiology, PIs are known to act in defense against herbivore insect pests. In recent years, the new intriguing role of plant PIs in abiotic stress tolerance has been implied. Inhibitor of serine type proteinases, *BvSTI* isolated from sugar beet pest resistant genotype was introduced into Bird's foot trefoil (*Lotus corniculatus* L.). To determine the effect of osmotic stress on *BvSTI*-expressing *L. corniculatus, in vitro*-grown shoots were exposed to elevated concentration of sucrose (5%, 7% and 9%). After 15 days of treatment, the growth response of sucrose-treated transformed lines 21, 73 and 109, as well as of nontransformed (NTC) line was determined by measuring the fresh weight and the length increment. Also shoots were scored for visible symptoms of osmotic stress-induced injuries by visual inspection. In order to avoid influence of observed phenotypic differences among lines on parameters analyzed under stress conditions, all values were presented relative to the controls (shoots grown under the same conditions on regular 3% sucrose) of each line. Additionally, the activities of antioxidant enzymes peroxidases (POD) and catalases (CAT) were determined spectrophotometrically.

Keywords: plant proteinase inhibitor**,** sugar, *Lotus corniculatus*

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The effect of antibiotics on the shoot regeneration in apple cultivar "Golden Delicious"

PP1-17

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The effect of antibiotics hygromycin, cefotaxime and meropenem on shoot regeneration in apple cultivar "Golden Delicious" was evaluated to optimize the protocol for *Agrobacterium tumefaciens*-mediated ge-

netic transformation. The careful determination of antibiotics concentration is important step to ensure efficient elimination of *Agrobacterium* and appropriate selection process without escapes and detrimental effect on plant regeneration. Healthy and fully expanded young leaves of *in vitro* grown apple shoots were cut transversely into three pieces that were used as explants for shoot regeneration. Regeneration frequency, the mean number of shoots per explant and the index of shoot-forming capacity were calculated after 8 weeks of culturing on Murashige and Skoog medium containing 5 mg L^{-1} thidiazuron (TDZ) and 0.3 mg L^{-1} indole-3-butyric acid (IBA), supplemented with hygromycin (0, 2, 4, 5, 6, 8 and 10 mg L^{-1}), cefotaxime (0, 100, 200, 300, 400, 500 and 600 mg L^{-1}) or meropenem (0, 25, 50, 75, 100, 125 and 150 mg L^{-1}). Results indicated that the highest index of shoot-forming capacity was obtained at 300 mg L^{-1} cefotaxime (3.67) comparing to control (2.93) and other antibiotic treatments. The increase of hygromycin concentration significantly decreased regeneration efficiency, while at concentrations of 5 mg L^{-1} and higher it completely inhibited shoot regeneration. Therefore, the present study outlines the use of cefotaxime at 300 mg L^{-1} and a stepwise selection procedure with the final hygromycin concentration of 5 mg L^{-1} as a suitable system for *A. tumefaciens*-mediated genetic transformation of apple cultivar "Golden Delicious".

Keywords: cefotaxime, hygromycin, meropenem, transformation

This research was sponsored by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173015).

Effect of growth regulators concentrations in micropropagation of yarrow

PP1-18

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Achillea millefolium is a medicinal, commercially important plant, used in pharmaceutical and cosmetics industry. It is also an ornamental, low-maintenance plant suitable for native gardening. Its propagation using *in vitro* culture enables mass propagation of healthy, pathogen-free plants, during the whole year, and obtained plants contain constant level of secondary metabolites. However, this method still doesn't have significant application in the cultivation of A. millefolium for secondary metabolites production. The aim of this study was to establish an efficient micropropagation system of A. millefolium native plants. Explants (terminal and basal cuttings) were cultured on MS medium supplemented with 0.5, 1, 2 or 3 mg L-1 N^6benzylaminopurine (BAP) and 0.5 or 1 mg L-1 indole-3-butyric acid (IBA). The best results in multiplication stage were achieved in control, on medium without plant hormones, using terminal cuttings as explants. Regeneration rate was 100%, mean number of axillary buds was 9.3, mean length of leaves was 32 mm. Rooting was successful on hormone-free MS medium (100% for terminal cuttings), with mean number of roots per explant 3.9 and mean length of roots 50.6 mm. All rooted microplants were successfully acclimatized in mixture of sand, peat and vermiculite in a ratio of 1: 1: 1.

Keywords: *Achillea millefolium*, BAP, IBA, micropropagation

Effect of sucrose concentration on *in vitro* growth of *Achillea millefolium* L.

PP1-19

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The effect of different sucrose concentrations on *in vitro* growth of *Achillea millefolium* L. in the multiplication stage was investigated. Terminal and basal micro-cuttings were grown on MS medium containing 3 mg L^{-1} N^6benzylaminopurine (BAP) and 0.5 mg L^{-1} indole-3-butyric acid (IBA), supplemented with 1%, 2%, 3%, 4% or 5% sucrose. Regeneration rate of terminal cuttings was high, reaching 100% on media with 1%, 3% and 4% sucrose. Regeneration of basal cuttings was better in hormone-free control (80-100%) than on medium with hormones (66-86%). Mean number of axillary buds ranged from 5.7 to 7.4 for terminal cuttings, and from 3.3 to 5.7 for basal cuttings. Mean length of leaves ranged from 7.3 mm to 24.6 mm (terminal cuttings) and from 8.1 mm to 33.6 mm (basal cuttings). However, the influence of sucrose concentration on mean number of axillary buds and length of leaves wasn't statistically significant. Obtained results show that sucrose concentration does not have a significant influence on *in vitro* growth of *A. millefolium,* indicating that this species could be successfully cultured under photoautotrophic conditions, which should be investigated additionally.

Keywords: *Achillea millefolium*, sucrose, micropropagation

Phytoremediation potential of *Lactuca sativa* L. *hairy roots* culture

PP1-20

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Phytoremediation capacity of transformed and control, untransformed *Lactuca sativa* L. cv. Ljubljanska ledenka root cultures was tested using different phenol concentrations (25, 50, 75, 100 and 125 mg L^{-1}) in liquid MS medium. During 10 days of trial, roots were grown at either constant concentration of phenol (every two days roots were transferred to the fresh medium with the same phenol concentration) or starting concentration of phenol (without changing the medium). While untransformed roots could remove phenol only at lower starting concentrations (25 and 50 mg L^{-1}), *hairy roots* have removed phenol completely at the first three tested concentrations. Possibility of phenol elimination using transformed roots was absent only at starting concentration of 125 mg L^{-1}. Additionally, the *hairy roots* grown at constant concentration of phenol (50 and 75 mg L^{-1}) showed lower phenol removal capacity than those that were grown at starting concentrations and the capability of phenol elimination was decreased with each change of phenol in solution.

Keywords: phenol, *Lactuca sativa*, transformed roots

Effect of phenol on the hydroponically grown lettuce (*Lactuca sativa* L.) varieties Ljubljanska ledenka and Nansen

PP1-21

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The effects of phenol on growth of two lettuce varieties (Ljubljanska ledenka and Nansen) were tested. These varieties have shown the greatest resistance to phenol and maximum percentage of seed germination at high phenol concentrations. The selected phenol concentration, at which 100% of seeds germinated, for both varieties was 200 mg L^{-1}. Two growing series were created: first one with the hydroponics solution containing 200 mg L^{-1} of phenol that was added at the beginning and was not changed for 10 days (called the starting phenol concentration) and the second series, where solution was recovered every two days to reach the starting concentration of phenol during whole period of lettuce growing (called constant phenol concentration). The changes in plant morphology and eventual elimination of phenol from the solution were monitored and measured during next 10 days. For both varieties, distortions of the rosette structure and the necrosis of leaves and roots were observed after four days at phenol concentration of 200 mg L^{-1}. All mentioned morphological changes were more pronounced in plants that were grown at the constant phenol concentration. Elimination of phenol (a decrease of its starting concentration) for both lettuce varieties was linearly dependent, but with Nansen was slower than with Ljubljanska ledenka. Ljubljanska ledenka grown at starting phenol concentration (200 mg L^{-1}) removed phenol to below the detection limit (0.03 mg L^{-1}) after six days. However, at constant phenol concentration, after 10 days of cultivation and five changes of phenol solution, elimination of phenol was absent for both varieties.

Keywords: phenol, lettuce, hydroponic solution

In vitro neuroprotective activity of *Hypericum perforatum* hairy root cultures

PP1-22

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Hypericum perforatum is one of the top-selling herbal medicines used in depression therapy. *In vitro* neuroprotective effect of the methanolic extracts from *H. perforatum* hairy root (HR) lines was investigated through testing against acetylcholinesterase and butyrylcholinesterase linked to Alzheimer's disease and tyrosinase related to Parkinson's disease. The selected HR lines (HR B, HR F, HR H) and non-transformed roots (NTR) showed considerable and dose dependent (250, 150 and 50 μg mL^{-1}) inhibition of the enzyme activities. The inhibition of acetylcholinesterase did not differ significantly among the HR extracts at tested con-

centrations. Among the HR lines, HR F extract exhibited the highest butyrylcholinesterase inhibition (37.83 ± 0.90%), while NTR extract had the lowest enzyme inhibition (27.47 ± 0.50%) at 250 μg mL^{-1}. At dose of 50 μg mL^{-1}, all tested samples showed similar inhibition towards butyrylcholinesterase (about 22 %). With respect to tyrosinase, the highest enzyme inhibition at 250 μg mL^{-1} was found for HR B (70.39 ± 4.98%), followed by NTR (54.12 ± 3.38%), which was almost equal to the value of HR F (53.98 ± 5.00%). At this concentration, only the HR line H was found with a low inhibition of tyrosinase (23.16 ± 5.04%). The HR extracts appears to have significant acetylcholinesterase, butyrylcholinesterase and tyrosinase inhibitory activity, which might be possibly attributed to *A. rhizogenes*-mediated modification of *H. perforatum* secondary metabolites with neuroprotective properties. Therefore, *H. perforatum* HR extracts could be used for treatments of patients with neurodegeneration and cognitive abnormality.

Keywords: hairy root, *Hypericum perforatum* L., neuroprotective activity

Marker assisted selection for conversion of normal maize line to Quality Protein Maize (QPM)

PP1-23

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Maize is a poor source of protein for both humans and monogastric animals due to its deficiency in two essential amino acids, lysine and tryptophan. Quality Protein Maize (QPM) is nutritionally improved and agronomically acceptable *opaque2* maize. Marker assisted selection (MAS) increases reliability and efficiency, reduces time and costs taken to obtain QPM. Three simple sequence repeats (SSR) located as internal repetitive sequences within the *opaque2* gene are being utilized as foreground selection markers for that gene. SSR markers were also effectively employed for selecting *o2* genotypes with the highest proportion of recurrent parent genome. The results presented in this paper are a part of conversion of normal maize line to its QPM version adapted for growing in temperate regions, through marker assisted backcross breeding. The main goal was to achieve high levels of essential amino acids and high grain yield at the same time, in half the time required through conventional breeding. The rapid line conversion strategy included a two-generation backcross program that employs foreground selection for the *opaque2* gene in BC_1, BC_2 i BC_2F_2 generations, background selection in the BC_2, BC_2F_3 and BC_2F_4 generations, and phenotypic selection for biochemical components, kernel modification and other desirable agronomic traits in two subsequent selfed generations. Six genotypes were identified with yield at the higher level (111% to 135%) than the original line, which also had higher tryptophan content (130% to 141%). The results indicate that the integrated molecular and selection strategy considerably improves the efficiency of conventional breeding programs.

Keywords: maize, marker assisted selection, *opaque2*, Quality Protein Maize

II

Plant Growth, Development, Metabolism and Nutrition

INVITED TALKS

Harnessing genetic diversity to better understand regulation of tomato fruit ripening and nutritional quality

IT2-1

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Tomato (*Solanum lycopersicum*) is a tractable and efficient model for fruit development, storage quality and nutrient accumulation, in addition to being a vegetable crop of increasing production, consumption and culinary importance the world over. Investigations undertaken by numerous researchers exploring tomato genetics and biology have elucidated mechanisms of pathogen response, root, leaf, flower and fruit development, ethylene hormone synthesis and perception, and genetic control of fruit ripening. Diverse, painstakingly collected, intricately designed, well characterized and freely available germplasm resources, combined with efficient transformation and a high quality genome sequence, have accelerated the pace of tomato biology with practical implications to crop improvement. Our work has focused on the characterization of ripening transcription factors underlying fruit ripening mutations. Additional regulators have been uncovered via examination of genes associated with ripening based on expression profiles. Other researchers have identified and carefully characterized additional transcriptional regulators that add greatly to our understanding of ripening control. Perhaps not surprisingly, many of these regulators have Arabidopsis counterparts shown to govern silique development. Genome enabled analysis of fruit development further indicates that transcriptional control intersects with changes in the epigenome. An overview of the diverse genetic regulators of fruit ripening in tomato will be presented, with examples of practical value and instances of leveraging tomato discoveries toward insights pertaining to the ripening and shelf-life of other fruit crops.

Photosensory receptor-mediated growth responses in Arabidopsis

IT2-2

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Phototropism is an adaptive response allowing plants to optimize photosynthetic light capture. This is achieved by asymmetric growth between the shaded and lit sides of the stimulated organ. In grass seedlings, the site of phototropin-mediated light perception is distinct from the site of bending; however, in dicotyledonous plants (e.g. *Arabidopsis*), light sensing occurs in the upper hypocotyl coinciding with the site of the growth response. Formation of a lateral gradient of the phytohormone auxin is a key step to trigger asymmetric growth of the shoot leading to phototropic reorientation. To identify important regulators of auxin gradient formation we developed an auxin flux model that enabled us to test *in silico* the impact of different morphological and biophysical parameters on gradient formation, including the contribution of the extracellular space (cell wall) or apoplast. Given that the site of light perception and asymmetric growth coincide in Arabidopsis we simplified the model by considering a two-dimensional hypocotyl section. Our model predicts that cell size, cell dis-

tribution and apoplast thickness are all important factors affecting gradient formation. Among all tested variables regulation of apoplastic pH was the most important to enable the formation of a lateral auxin gradient. To test this prediction we interfered with the activity of plasma membrane H^+ATPases that are required to control apoplastic pH. Our results show that H^+ATPases are indeed important for the establishment of a lateral auxin gradient and phototropism. Moreover, we show that during phototropism H^+ATPase activity is regulated by the phototropin photoreceptors, providing a mechanism by which light influences apoplastic pH.

Male gametophyte development: a lesson from the –omics

IT2-3

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The male gametophyte, a highly organized haploid flower organ, offers a unique opportunity to analyze development and differentiation of single haploid cells, cell-cell interactions and recognition, cellular polarity and pollen tube tip growth. Posttranscriptional control of gene expression plays a vital role during tobacco pollen maturation and tube growth. The need for a high rate of translation during pollen tube growth suggests a demand for a robust storage system that could withstand a long-term storage and transport, ongoing cellular morphogenesis, and yet deliver the message efficiently accompanied with instant translation. A number of pollen genes showed apparent expression discrepancy at mRNA and protein levels and their respective transcripts were shown to be associated with long-term stored ribonucleoprotein particles annotated as EPP complexes. Similarly to the role played in growing mammalian neurons, EPP particles represent pre-loaded complex machinery devoted to mRNA processing, transport, subcellular localization and protein synthesis. We performed a detailed functional, transcriptomic and proteomic characterisation of pollen storage RNP particles in *Nicotiana tabacum* L. In particular, we aimed to integrate our knowledge on the categorization of translationally regulated transcripts in developing pollen and to identify the mode of action of the translational repression and derepression of mRNAs stored in developing pollen and gradually activated during the progamic phase.

Keywords: pollen development, transcriptomics, proteomics, *Nicotiana tabacum*

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Potato phototropism, auxin and Acid Growth Theory

IT2-4

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Auxin, indole-3-acetic acid (IAA) is a potent mediator of plant elongation growth attributed also with important roles in plant tropic responses. To evaluate the auxin effects and related Acid Growth Theory postulates on potato phototropism (PT) modifications were made enabling studies to be done on submersed

plantlets and their excised segments. Potato plantlets growing on media in their culture flask completely immersed in water had similar phototropic response as untreated plantlets even after 24 h of submersion. Intact plantlets showed no apparent response to 6 μM IAA. However, plantlets detached from their root system same as shoot segments showed significant (up to 70%) reduction of the PT bending ability which could be improved by addition of water, acid solutions and IAA. At 6 μM concentration IAA almost completely recovered the PT bending response. Although potent in restoring the PT response of detached shoots and shoot explants, IAA had to wait for the initial 15-20 min long lag phase to pass before it could exert its effect. If added after the shoot segment completed the blue light-induced lag phase, then the PT bending response to IAA appeared almost instantly.

Keywords: phototropism, auxin, Acid Growth Theory

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How to avoid self-fertilization in plants - a buckwheat story

IT2-5

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Self-incompatibility (SI) systems are genetically determined mechanisms for avoiding self-fertilization in flowering plants. They act in approximately 70% of angiosperms and involve highly diverse set of genes, which are usually family-specific (although sometimes they may be shared by different plant families). Having in mind the most fundamental way of SI systems' classification, which is based on the flower morphology within a SI species (homomorphic type displays flowers of same morphology, while heteromorphic type displays flowers of different morphology) one may notice that all of characterized SI systems so far (e.g. Roasaceae, *Brassica*eae, *Poaceae*) are of homomorphic type. Nevertheless, in order to fully understand SI systems and their evolution we need to elucidate heteromorphic SI mechanisms as well. Common buckwehat (*Fagopyrum esculentum* Moench) is a heteromorphic SI species that displays two flower morphs: *pin* (long pistils, short anthers, smaller pollen grains) and *thrum* (short pistils, long anthers, larger pollen grains). The place of self-pollen tube growth arrest is different in two morphs: it is placed at 2/3 of style's length in *pin* flower, but in *thrum* flower it is placed at the junction of stigma and style. We started the reasearch by analyzing 50 proteins isolated from non-pollinated pistils (without ovaries) and pollen grains of both flower morphs of common buckwheat, using 2D-PAGE and LCMS/MS. We present obtained results and discuss potential role(s) of detected proteins in buckwheat SI response, with respect to the role(s) these proteins have in other plant species, particularly in other plant SI systems.

Keywords: heteromorphic self-incompatibility, buckwheat, pin, thrum

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Emerging concept of alternative electron partitioning in photosynthesis

IT2-6

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In vascular plants, photosynthetic electron transport (PET) chain produces reductive power that is utilised by diverse acceptors involved in both chloroplast and cellular metabolism. In the last steps of this process, transfer of energy-conserving electrons beyond photosystem I (PSI) is performed by a small iron-sulphur protein ferredoxin (Fd). Fd acts simultaneously as a bottleneck and as a hub which distributes high-energy electrons to a multitude of enzymes. The dominant pathway in chloroplasts is, however, the one that produces NADPH, by the activity of ferredoxin-$NADP^+$-oxidoreductase (FNR). In order to function efficiently Fd/FNR redox chemistry has to be constantly poised. The question remains how is the partitioning of electrons between the various energy-conserving and -dissipating pathways achieved. Thylakoid rhodanase-like protein TROL has been shown to act as *bona fide* membrane attachment point for the FNR, although other membrane associations of FNR have been described. We have proposed a scheme for the dynamic FNR recruitment to TROL. We posit that TROL-FNR interaction presents the branching point between electron-conserving and electron-dissipating pathways. Without the TROL, light-dependent $O_2^{\cdot-}$ generation is reduced, while the generation of other ROS is enhanced. We propose that other Fd-dependent pathways downstream of PSI and different from the linear electron transfer become dominant by the dynamic detachment of FNR form TROL, thus suggesting a novel mechanism of photosynthesis regulation. Alternatively, efficient scavenging of $O_2^{\cdot-}$ by FNR-dependent pathways can be envisaged.

Keywords: photosystem I, redox regulation and signaling, regulation of electron flow

The rhizosphere: perspective and challenges for plant nutrition

IT2-7

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It was the soil microbiologist Lorenz Hiltner, who coined the term rhizosphere in 1904, defining it as the volume of soil surrounding roots considerably influenced by the microbial composition. It was not before the past 30 years that rhizosphere attracted the interest of a broader group of scientists. Besides the various functions (e.g. water and nutrient uptake, anchorage to soil), plant roots are able also to release a wide range of organic and inorganic compounds into the rhizosphere. Soil-chemical changes related to the presence of these compounds and products of their microbial turnover are important factors affecting availability of nutrients, solubility of toxic elements and thereby, the ability of plants to cope with adverse soil conditions. The aim of my keynote is to provide a comprehensive and updated overview of the most recent advances in the rhizosphere research, focusing on the root release of protons and carboxylates and its significance for nutrient availability and acquisition processes. In particular, I will highlight effects of the genotypic differences, plant physiological status and nitrogen fertilization on root exudation and its significance for increasing availability of macro- (e.g. phosphorus) and micronutrients (e.g. iron) in the rhizosphere of various crop species. Finally, the recent achievements of my research group on silicon-based fertilization, as an innovative approach for sustainable rhizosphere management of marginal soils, will be presented.

Keywords: availability of nutrients, carboxylates, rhizosphere, roots

Silicon alleviates oxidative stress in cucumber plants grown under copper excess

IT2-8

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Copper (Cu) is an essential microelement, a redox-active transition metal involved in electron transfer chains including respiration and photosynthesis. Present in excess, Cu initiates oxidative stress in plant tissues. On the other side, silicon (Si) is the only known mineral element with a protective effect for plants growing under different stressful conditions. Although possible mechanisms of Si-mediated tolerance to metal toxicity have been proposed, the molecular bases of these mechanisms are poorly understood. This study considers the mechanism of Si-mediated protective effect on cucumber plants stressed with copper excess. We analyzed the level of oxidative stress, expression of the genes involved in antioxidative defence, phenolics biosynthesis and metal sequestration, as well as phenolic composition and the Cu content in different cell fractions. Taken together our results clearly indicate that Si supply reduces the Cu excess stress in cucumber plants. The proposed mechanisms are attributed presumably to Sistimulated synthesis of phenolics and Cu cross-linking into cell wall together with the formation of CuNA complex, thus decreasing free Cu and preventing harmful concentrations of ROS generated via the Fenton reaction.

Keywords: copper, silicon, cucumber, oxidative stress

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SELECTED TALKS

Lignin deposition and synthesis in the internodes during barley (*Hordeum vulgare* L.) development

OP2-1

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In monocotyledons, especially grasses (*Poaceae*), process of lignification is still unexplored in many of its aspects. Lignin deposition in cell wall is temporally and spatially specific and differs between species and tissues. The aim of this study was to investigate the dynamics of lignin synthesis and deposition, by using anatomical, physiological and molecular approach, in the stem of three spring barley cultivars (*Hordeum vulgare* L.) during four developmental stages: heading, anthesis, grain filling and ripening. Lignin localization and histochemical changes related to lignin deposition, total lignin content and gene expression of cinnamoyl CoA reductase (HvCCR) and cinnamyl alcohol dehydrogenase (HvCAD) were studied. Analyses showed that most of the lignin was deposited in the sclerenchyma ring in all three internodes. Total lignin content increased with stem development, especially in the first internodes and was highest in ripening, but it was also relative to the cultivar and developmental stage. Genes of the lignin biosynthetic pathways HvCCR showed highest expression in anthesis in the third internode of all three cultivars whereas gene HvCAD, in cultivars Astor and Scarlett, showed highest expression in anthesis and in cultivar Jaran in heading stage. Results obtained in this study will contribute to the comprehensive description of the biochemical and molecular changes that accompany lignin deposition in the cell wall during barley development.

Keywords: lignin, barley, CCR, CAD

DUF1070 is a conserved signature domain of some arabinogalactan peptides

OP2-2

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Most proteins are composed of functional domains, of which >20% are "domains of unknown function" (DUFs). We have recently identified an arabinogalactan (AG) peptide (AGN92423) with DUF1070 domain in *Centaurium erythraea* transcriptome. AGPs are ubiquitous plant cell wall hydroxyproline-rich glycoproteins with diverse functions. Since classical AGPs and AG peptides contain no signature motifs, the presence of highly conserved DUF1070 caught our attention. DUF1070 is present in 479 NCBI plant protein sequences, of which

271 are non-redundant: 126 AG peptides and 145 unknown/hypothetical proteins. All DUF1070-containing sequences are short (71 aa on average). With few exceptions, these sequences are comprised of N-terminal signal sequence, followed by an AG-II glycomodule (typically PAPAPT). DUF1070 is up to 37 aa long, encompassing (a part of) AG-II glycomodule and a highly conserved C-terminal stretch of 26 aa, typically "SDGTSIDQGIAYVLMLVALVLTYLIH". This motif is a textbook example of glycosylphosphatidylinositol (GPI) anchor signal peptide, containing 1) three relatively small aliphatic amino acids at the ω (GPI attachment site), ω+1 and ω+2 positions (SDG in 74% sequences); 2) a relatively polar spacer (often TSIDQG), followed by 3) a hydrophobic domain (typically IAYVLMLVALVLTYLIH). When GPI is attached to ω (S), the C-terminal peptide is cleaved, while the hydrophobic domain remains embedded in the ER membrane. Since most of the DUF1070 is predicted to be cleaved and discarded, it is worth wondering what selective pressure kept it so conserved (unlike other GPIsp sequences) among AG peptides from unrelated plant species. We propose that DUF1070 is a signature of some AG peptides.

Keywords: arabinogalactan proteins, AG peptides, DUF1070, glycosylphosphatidylinositol anchor, GPI signal peptide

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI 173024 and TR 31019).

Phosphoproteomics profiling of tobacco mature pollen and pollen activated *in vitro*

OP2-3

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Angiosperm mature pollen represents an extremely desiccated, metabolically quiescent structure surrounded by a tough cell wall. Upon re-hydration it becomes metabolically active and later on pollen tube growth starts. These changes in cytoplasm hydration and metabolic activity are accompanied by protein phosphorylation, which is one of the most dynamic post-translational modifications. In order to identify the phosphoproteins playing role during tobacco pollen activation *in vitro*, the following experiments were performed. Mature pollen, 5-min-activated pollen, and 30-min-activated pollen were subjected to TCA/acetone protein extraction, trypsin digestion and phosphopeptide enrichment by titanium dioxide. The enriched fraction was then subjected to nLC-MS/MS. We identified 471 phosphopeptides that carried 432 phosphorylation sites, position of which was exactly matched by mass spectrometry. These 471 phosphopeptides were assigned to 301 phosphoproteins, since some proteins carried more than one phosphorylation site. Of the 13 functional groups, the majority of proteins were put into these categories: transcription, protein synthesis, protein destination and storage, and signal transduction. We also presented quantitative data; the identified phosphopeptides were divided into seven groups based on the regulatory trends; the major group comprised mature

pollen-specific phosphopeptides. Several phosphopeptides representing the same phosphoprotein had different regulation, which pinpointed the complexity of protein phosphorylation and its clear functional context. Collectively, we showed the first phosphoproteomics data on activated pollen where phosphorylation sites' position was clearly demonstrated and regulatory kinetics have been considered.

Keywords: phosphoproteomics, male gametophyte, tobacco, protein phosphorylation, pollen activation

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New findings about the role of *cis*-zeatin-type cytokinins in plant physiology and evolution

OP2-4

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Cytokinins (CKs) are evolutionally old and highly conserved low-mass molecules occurring in almost all living organisms. Throughout plant kingdom, CKs evolved into a relevant class of ubiquitous phytohormones controlling numerous plant developmental processes and environmental responses. The most typical representative of CKs in plants is zeatin (Z) occurring in two positional isomers, *trans* or *cis*. While most of biological activity of Z has been for years attributed to the *trans* isomer, *cis*-zeatin (*cis*Z) has been considered an inactive or weakly active CK form. In contrast to this concept, we have recently shown an importance of *cis*Z-type CKs for plants based on their widespread presence in monocots and dicots. A putative role of *cis*Z types has been demonstrated during ontogenesis of higher plants consisting in a subtle regulation of CK levels, especially in states associated with growth-limiting conditions due to internal or external cues (abiotic stress, infection, senescence, seed dormancy) and/or immediately after release from such conditions (early stages of germination). In addition, *cis*Z derivatives have been found to constitute a major component of the endogenous CK pool in evolutionary older organisms such as algae, fungi, liverworts, mosses and ferns. The high abundance of *cis*Z types in these organisms suggests their potential involvement in evolution of phytohormone homeostatic mechanisms, most probably by substituting a CK-*N*-glucosyltransferase pathway deactivating CKs in vascular plants. Our findings indicate a higher distribution and relevance of *cis*Z types to CK biology than previously considered.

Keywords: *cis*-zeatin, cytokinin, phytohormone, plant, zeatin

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POSTER PRESENTATIONS

Proline accumulation in *Impatiens walleriana* shoots after different dehydration treatments

PP2-1

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Impatiens walleriana is an ornamental plant sensitive to dehydration with tendency to wilt without water. Proper tissue dehydration is a necessary step in cryopreservation protocols to avoid formation of intracellular ice crystals which are lethal for plant cells. In order to increase dehydration tolerance of *I. walleriana* shoots, we evaluated proline accumulation, a common osmoprotectant, in *I. walleriana* shoots after two weeks of culture at several dehydration pretreatments: increased concentration of sucrose (3, 4, 5, 6, and 10%), at different temperatures (25 ± 2 °C and 4 °C) and different concentration of abscisic acid (ABA, 0, 0.1, 0.2, 0.5, 1, 5 and 10 mg L^{-1}). We observed that graduated concentration of sucrose in culture media increased proline accumulation in *I. walleriana* shoots grown under standard conditions, as well as at 4 °C. The highest proline concentration was recorded in shoots grown on media with 6% sucrose (100 ± 2.3 μmol g^{-1}) at 25 °C which was more than three times higher than on medium with 3% sucrose (30 ± 0.6 μmol g^{-1}). The highest proline accumulation in shoots grown at 4 °C was observed at 10% sucrose (88 ± 0.9 μmol g^{-1}) in comparison to 37 ± 0.6 μmol g^{-1} at 3% sucrose. Addition of ABA in culture medium increased proline concentration in shoots grown on media with 10 mg L^{-1} ABA (68 ± 1.2 μmol g^{-1}). Our results suggest that applied pretreatments significantly increase accumulation of proline and can be helpful in increasing dehydration tolerance of *I. walleriana* shoots prior to cryopreservation.

Keywords: proline, sucrose, abscisic acid, dehydration tolerance

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Metal content in soil and in selected plants of asbestos tailings (Stragari)

PP2-2

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The aim of this study was to determine the concentrations of 11 metals in the soil and selected plant species of asbestos tailings in Stragari. The concentrations of Ni and Cr in the investigated soil were above remediation values, as well as the maximum allowable concentration of substances in the soil according to regulation of Republic of Serbia, and the concentrations of Cd and Co were above limit values for given metals in

the soil. Concentrations of the metals in plants were variable, depending on the plant species and types of metals. Obtained results present the momentary picture of investigated locality and represent the base for further research.

Keywords: metals, asbestos tailings, plants

Exine characteristics of nine Serbian autochthonous apple cultivars

PP2-3

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The detailed pollen surface ornamentation and the aperture characteristics of 9 Serbian autochthonous apple cultivars were examined by scanning electron microscope - JSM-6390 LV (JEOL, Tokyo, Japan) at 10000×. Exine sculpturing of apple cultivars was striate with 14 pores in 1 μm^2. The diameter of the pores in all cultivars was about 0.1 µm, except in 'Zejtinka' (0.1-0.3 µm). The pores of the tested cultivars differed in shape. According to exine pore characteristics cultivars can be divided into four groups: (1) round pores - 'Budimka', 'Samoniklaja', 'Strekinja'; (2) drawn pores - 'Šarenika', 'Kolačara', 'Kablarka' (3) round to drawn pores - 'Šumatovka', (4) deep round - 'Petrovača', 'Zejtinka'. The exine ridging was generaly longitudinal with more parallel or less parallel ridges. The lateral branching of ridges had three arbitrary levels: low ('Šarenika', 'Samoniklaja', 'Kolačara'), medium ('Budimka', 'Zejtinka', 'Kablarka', 'Strekinja') and high ('Petrovača', 'Šumatovka'). Ridge width (0.18-0.27 µm) and stria width varied (0.10-0.21 µm) from cultivar to cultivar. Each tested autochthonous apple cultivar seemed to have a specific pattern which was distinctive enough to permit the cultivar identification. On the surface of exine some appurtenances were noticed. Further studies are necessary to understand the development and functions of ring-like, bubble-like and tape-like appurtenances on the surface of pollen grains.

Keywords: apple, exine ornamentation, ridge of exine, stria width

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Pollen quality of Serbian autochthonous apple cultivars

PP2-4

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Pollen grains of the nine autochthonous apple cultivars were elliptical, tricolpate with three germinal furrows. The mean size of the pollen grains ranged from 30.31 µm to 51.56 µm in length and from 17.69 µm to 25.97 µm in width. The highest mean pollen length was found in 'Petrovača', while the lowest average pollen length was found in 'Strekinja'. The length/width ratios were from 1.62 to 2.30, depending on the cultivar. Determining pollen viability and production has great importance for assessing pollen quality and for establishing fertilization potential when selecting cultivars. Pollen viability depended on the cultivar and var-

ied from 58% to 93%. The best viability was found for the pollen of 'Petrovača'. Unviable (dead) pollen grains, which were unstained, were distinguished from viable ones, which turned dark after staining with fluorescein diacetate and acetocarmine. The percentage of deformed pollen grains was variable and ranged from 7% to 42%. The lowest frequency of deformed pollen was found in pollen samples of 'Petrovača'. The number of pollen grains per anther ranged from 1260 to 3680 in tested cultivars. However, number of pollen grains per flower ranged from 20160 to 80960. The 'Petrovača' apple had the greatest pollen production. Also, it had the largest pollen with the best viability. Cryopreserving 'Petrovača' pollen, which had the greatest fertilization potential, is important for the conservation of this important Serbian cultivar.

Keywords: *Malus domestica,* pollen production, pollen size, pollen viability

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Effect of exogenously applied cytokinins on expression of genes involved in cell cycle control during *de novo* shoot organogenesis in kohlrabi

PP2-5

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Intensive cell divisions represent the basis of *in vitro* plant regeneration. They are controlled by complexes of cyclins (CYCs) and cyclin-dependent kinases (CDKs), whose activity during cell cycle and organ development can be affected by plant hormones, like cytokinins (CKs). The effect of exogenously applied CKs: *trans*-zeatin (*trans*Z), *cis*-zeatin (*cis*Z), N^6-benzyladenine (BA) and thidiazuron (TDZ) on expression of three genes encoding CDKs and CYCs during *de novo* shoot organogenesis in kohlrabi (*Brassica oleracea* var. *gongylodes* cv. Vienna Purple), was evaluated. The activity of *CDKB2;1*, *CYCB2;4* and *CYCH;1* genes was determined by quantitative RT-PCR in kohlrabi seedlings used as explants after 7, 10, 14, 21, 28, and 35 days of CKs treatment, and presented relative to the control seedlings maintained on media without CKs. Expression patterns of all three genes in terms of time and different treatments were similar, with the highest values recorded for TDZ treatment in almost all time points. The dinamics of adventitious shoots development were accompanied by expression of these genes. A great increase in the expression of all three genes was observed after 7 days of culture. When the first calli were observed, expression of these genes was similar to control. With development of regenerated shoots, the activity of genes was elevated again up to seventh day level, and higher. Our results suggested the role of applied CKs in cell cycle control by influencing the expression of analyzed genes, and that activity of these genes was correlated with distinct phases of *de novo* shoot organogenesis in kohlrabi.

Keywords: kohlrabi, cytokinin, cyclins, cyclin-dependent kinases, *de novo* shoot organogenesis

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Shoot multiplication and rooting of *in vitro* cultured *Tanacetum vulgare* L.

PP2-6

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Tanacetum vulgare L. (Asteraceae), known by the common name of Tansy is an aromatic perennial plant, widely spread in the northern hemisphere. As an herbal remedy, Tansy has traditionally been used in balsams, cosmetics, dyes, insecticides, medicines, and preservatives and as anthelmintic, for migraine, neuralgia, rheumatism and loss of appetite, though the effectiveness for these uses has not been documented. Shoot multiplication was achieved from Tansy seedlings cultivated on MS solid medium supplemented with 0, 0.1, 0.2, 0.5 and 1.0 mg L^{-1} benzyl adenine (BA). Shoot multiplication was satisfactory on media enriched with BA. The highest multiplication was achieved on 0.5 mg L^{-1} and 1.0 mg L^{-1} BA (2.25 and 2.10, respectively), with no shoot-tip necrosis. Individual shoots were elongated when cultured on medium with 0.1 mg L^{-1} BA for 4 weeks. For rooting, each elongated shoot was cultured on solid MS medium with 0, 0.2 or 0.5 mg L^{-1} indole-3-butyric acid (IBA) for 4 weeks. The frequency of rooting was still unsatisfactory (7.98%) and further optimisation of root-inducing phase is needed. However, the highest root elongation (94.68 mm) was obtained on hormone-free medium. Efficient regeneration system can play an important role in genetic transformation experiments and agroindustrial purposes of this medicinal plant.

Keywords: shoot multiplication, rooting, shoots, Tansy

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Analysis of Pyridine Dinucleotides in apoplastic fluid from maize roots by UHPLC-MS/MS

PP2-7

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The apoplast is a complex plant compartment, delimited from the symplast by the plasma membrane. Pyridine Dinucleotides: nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP) and their reduced forms NADH and NADPH, respectively, in apoplastic fluid have not been analyzed yet, except in enzyme assays. Apoplastic fluid was extracted from maize (*Zea mays* L.) roots using two procedures: collection from the surface of intact plant roots by filter paper strips (AF) or vacuum infiltration and centrifugation from excised root segments (AWF). High performance liquid chromatography (HPLC) coupled to a diode array detector spectroscopic detection of NAD metabolites does not offer the specificity and sensitivity necessary for their robust quantification in complex samples. Thus, we developed a targeted, quantitative assay of the NAD and NAD biosynthetic intermediates using UHPLC coupled to tandem mass spectrometry (MS/MS). Our results from the UHPLC-MS/MS analysis show the presence of nicotinic acid adenine dinucleotide (NaAD), precursor in the NAD biosynthetic pathway, in AWF but not in AF. High sensitivity and

selectivity towards Pyridine Dinucleotides suggest our method as a suitable tool for the analysis of Pyridine Dinucleotides in complex matrices.

Keywords: UHPLC-MS/MS, Pyridine Dinucleotides, NaAD, apoplastic fluid

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Study of inter- and intra-molecular OH-bonds and cellulose crystallinity in the cell walls of different plant species by FTIR spectroscopy

PP2-8

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The major biopolymers in cell wall of plants are held together by a combination of covalent and non-covalent bonds to form a highly complex structure. We compared cell walls of three different species, spruce (*Picea omorika* (Pančić) Purkiňe) as an example of softwood, maple (*Acer platanoides* L.) - a hardwood and maize (*Zea mays* L.) - a herbaceous plant from the grass family and widely used agricultural plant. We investigated chemical composition of isolated cell walls and hydrogen-bonds interaction between macromolecules by using Fourier transform infrared spectroscopy (FTIR). The highest content of intra-molecular hydrogen bonds was found in maize (70%), while in maple and spruce this content is similar, 60% and 58% respectively. The inter-molecular hydrogen bonds in cell walls were most abundant in spruce (about 50%), while in maple and maize was 36% and 29% respectively. Free OH (6) and OH (2) are present in very low amount in all samples. The A_{1375}/A_{2900} ratio of band intensities in the FTIR spectra, as a measure of sample crystallinity, was highest for maize (1.5) and lower for maple (0.7) and spruce (0.6). The highest percentage of intra-molecular hydrogen bonds and the highest crystallinity in maize may reflect more compact structure of cellulose in this species. The results of this work provide new data for comparison of the cell wall properties that may be important for selection of appropriate plant as a source of biomass.

Keywords: cell wall, FTIR, OH-bonds, spectral deconvolution

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Irregular growth of pollen tubes inside the ovary of plum

PP2-9

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Fluorescent microscopy upon staining with aniline blue was applied in order to follow certain growth patterns of pollen tubes inside the ovary of the 'Pozna Plava' plum cultivar. Growth of pollen tubes was analyzed in open, self- and cross-pollination variants. Besides the pollen tubes characterized by growth directed towards the micropyle and into the nucellus, the observation also revealed those with irregular growth patterns. Specific growth of pollen tubes was observed in different regions of the ovary, from the obturator zone, to the zone above and in the micropyle, as well as in the embryo sac. This specific growth of pollen tubes was analyzed from the aspect of either presence or absence of penetration of pollen tubes in the nucellus in these ovules. In the obturator zone, the observed specific growth of pollen tubes is characterized by larger or smaller branching, while in the zone above and in the micropyle it is characterized by the formation of a smaller or larger bundle. Pollen tubes rotated by 180° have been observed in all parts of the ovary. Except for the open pollination, in all other pollination variants it was observed that in the ovules with no penetration of pollen tubes in nucellus, the percentage of specific growth of pollen tubes was more frequent in the obturator zone. After the penetration of pollen tubes in the nucellus, in all pollination variants the percentage of pollen tubes with specific growth was more frequent in the micropyle zone.

Keywords: plum, ovary, pollen tube growth

Histological and immunohistochemical studies of *in vitro* morphogenesis induced in leaf culture of centaury (*Centaurium erythraea* Rafn)

PP2-10

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Arabinogalactan proteins (AGPs) are a class of cell surface proteoglycans containing over 90% (w/w) carbohydrate. AGPs are located at the plasma membrane and plant cell wall, where they are thought to have important roles in plant growth and development. The carbohydrate moiety of AGPs, which consists mainly of arabinose and galactose with minor amounts of uronic acids, can undergo specific tissue degradation during differentiation and morphogenesis. The aim of this study was to investigate the origin of somatic embryos and adventitious buds in leaf culture of centaury and also to investigate the precise distribution of AGPs with specific epitopes recognized by several monoclonal antibodies, LM2, JIM4 and JIM13. Histological studies revealed that both morphogenetic paths were indirect and asynchronous. Immunohistochemical studies have shown that the expression of specific epitopes of AGPs recognised by LM2, JIM4 and JIM13 monoclonal antibodies, was developmentally regulated during somatic embryogenesis. The AGP epitope recognised by the LM2 antibody was detected with strong intensity in the cell walls of meristematic cells from which somatic embryogenesis was initiated as well as in globular somatic embryos. During further development of somatic embryos the occurrence of JIM4 epitopes, at early cotyledonary stage, and JIM13, at late cotyledonary stage,

was observed. The results showed that AGPs are clearly involved in induction of morphogenesis *in vitro*, development of somatic embryos and adventitious buds in leaf cultures of C. erythraea Rafn.

Keywords: arabinogalactan proteins, morphogenesis, histology, monoclonal antibodies, *Centaurium erythraea* Rafn.

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Investigation of the role of arabinogalactan proteins during *in vitro* morphogenesis in *Centaurium erythraea* leaf culture using β-D-glucosyl Yariv reagent

PP2-11

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Arabinogalactan proteins (AGPs) constitute a diverse family of hydroxyproline-rich cell wall glycoproteins implicated in plant growth and development. Hereby, the role of AGPs was comparatively studied in four developmental pathways induced from centaury leaf explants: direct shoot development (DSD), induced on hormone-free media in light; direct root development (DRD), occurring in the absence of growth regulators in darkness; indirect shoot development (ISD), induced by combination of CPPU and 2,4-D in light, and indirect somatic embryogenesis (ISE), induced by CPPU and 2,4-D under both light regimes. The addition of β-D glucosyl Yariv (βGlcY) reagent, a synthetic phenylglycoside that specifically crosslinks and inactivates AGPs, in increasing concentrations to the growth medium reduced the number of somatic embryos and adventitious buds formed per explant. No somatic embryos developed in direct contact with βGlcY-containing medium, and even 5 μM βGlcY significantly reduced the number of formed somatic embryos. The morphogenetic paths can be arranged in order of increasing sensitivity to βGlcY as: DRD (insensitive)<ISD<DSD<<ISE, suggesting an important role of AGPs during somatic embryogenesis. The content of AGPs in regenerating explants, as determined by radial diffusion based on AGP-βGlcY interaction, increased during all four processes, but the accumulation of AGPs was the most rapid during ISE and when ISE and ISD occurred simultaneously. Finally, βGlcY was also used for the crossed electrophoresis assay, showing that explants undergoing different developmental changes accumulate sets of AGPs with different electrophoretic profiles. This work emphasizes the versatility of βGlcY applications in studying the role of AGPs in plant morphogenesis.

Keywords: arabinogalactan proteins, crossed electrophoresis, radial diffusion, β-D-glucosyl Yariv, morphogenesis

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Photoblastic response of seed germination of Japanese katsura tree (*Cercidiphyllum japonicum* Siebold & Zucc)

PP2-12

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Radiant energy, besides initiating numerous photobiological processes, is a key factor which regulates seed dormancy and germination of positive photoblastic types of seeds. Morphological character of the seed, as its small size and minimal content of nutritive tissues, clearly indicates potentially positive photoblastism. The aim of the investigation was to find out photoblastic response of Japanese katsura tree seeds (*Cercidiphyllum japonicum* Siebold & Zucc) which are very minute about 5 mm (1,577,456 seeds per kg). The red and far red filter treatments, as well as control treatments were applied two hours after imbibition. There were three treatments with filters: (1) red light (5 min), (2) red light + far red (5 min), and (3) red light + far red + red (5 min); and two controls: (1) sample in light and (2) sample in darkness. Seeds (4×100 grains per treatment) were placed in a cabinet germinator in Petri dishes on germination paper. The testing lasted 21 days at 20 °C. The result was presented by means of: germinative capacity (GC), real germination (RG), germinative energy (GE), mean germination period (MGP), and germination intensity (GI). It was found that indicators of germination in darkness showed strongly photoblastic seed response, i.e. last treatment with red light caused high values of the indicators (GC 72-74%) which are not significantly different from results of germination in light (GC 74%). At the same time, impulse of far red, as the last treatment, provoked low germination (GC 5%) that was not significantly different from germination in darkness (GC 0%).

Keywords: photoblastism, katsura tree seeds, germination, red/far-red effect

Effects of growth medium on antioxidant activity, total phenolics and flavonoid content in *Ocimum basilicum* L. *in vitro*

PP2-13

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Ocimum basilicum L., sweet basil, is a widely used plant from mint family. This medicinal plant and culinary herb is a rich source of natural antioxidants due to high content of phenolics. In this study, the effect of growth media on antioxidant activity, total phenolics and flavonoids was determined using comparative analysis of plants cultured in four different media. Plants, obtained from the seeds, were maintained *in vitro* on full-strength Murashige and Skoog (MS) medium, half-strength Murashige and Skoog (1/2MS) medium, full-strength Gamborg (B5) medium and half-strength Gamborg (1/2B5) medium without plant growth regulators. The cultures were maintained in a 16/8h photoperiod, 24 ± 1 °C and after four weeks plants were analyzed. The higher antioxidant activity, evaluated by DPPH assay, was measured in cultures from B5 medium (71.76 μg mL^{-1}) followed by sweet basils from 1/2B5 medium (85.00 μg mL^{-1}), while cultures from MS and 1/2MS media have shown lower antioxidant capacity (MS = 96.05 μg mL^{-1}; 1/2MS = 98.15 μg mL^{-1}). The higher content of total phenolics was measured in full-strength media (B5 = 30.61 mg GA g^{-1}; MS = 28.24 mg GA g^{-1}) without major differences in other media (1/2B5 = 26.39 mg GA g^{-1}; 1/2MS = 27.56 mg GA g^{-1}). B5 medium was the best medium for the production of sweet basil flavonoids (174.39 mg RU g^{-1}) while in all other cases

flavonoid concentration was significantly lower. Full-strength B5 medium may be considered the best medium for secondary metabolite production and high antioxidant activity of *in vitro O. basilicum* cultures.

Keywords: *Ocimum basilicum*, phenolics, flavonoids, antioxidants

This investigation was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (III41010).

Biostimulants effect on enzyme antioxidative response in leaves of different tomato (*Lycopersicum esculentum*) hybrids exposed to reduced mineral nutrition

PP2-14

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The study investigated impact of two different biostimulants (Viva and Kendal) on photosynthetic pigment concentration, activities of superoxide dismutase (SOD, EC 1.15.1.1) and peroxidase (POD, EC 1.11.1.7) in the leaves of four different hybrids of tomato (Bostina, Ombelina, Gravitet and Minaret) subjected to 50% reduced mineral nutrition. Results have shown that reduced nutrition decreases the concentration of total chlorophyll in all hybrids (42--50%), and that contrary to that biostimulants insignificantly increase their content (Viva by 58% and Kendal by 27%). Apart from that, all plants with reduced mineral nutrition had a slight decline of total chlorophyll when biostimulants were applied. On the gel for native electrophoresis four peroxidase isoforms were detected in all hybrids (Rf_{POD1} = 0.28; Rf_{POD2} = 0.37; Rf_{POD3} = 0.52; Rf_{POD4} = 0.58). Reduced nutrition also leads to an increase in POD activity in all hybrids. In all hybrids subjected to reduced nutrition in the presence of biostimulants, peroxidase activity and isoenzyme profile did not differ from those in control plants. In all control and treated plants two SOD isoforms were detected (Rf_{SOD1} = 0.49; Rf_{SOD2} = 0.58). Growth under the conditions of reduced mineral nutrition led to a decrease in SOD activity in Bostina and Ombelina hybrid lines, while in Gravitet and Minaret SOD activity increased. Obtained results for POD and SOD activity, together with changes in the content of photosynthetic pigments indicate that both Viva and Kendal can decrease oxidative stress level in the conditions of decreased mineral availability.

Keywords: tomato *(Lycopersicum esculentum)*, superoxide dismutase (SOD), peroxidase (POD), reduced nutrition

Anatomical characteristics of seed coat of the field pea (*Pisum sativum* L.) genotypes in relation to seed cracks and damage

PP2-15

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In this paper we analysed morphological characteristics of seed and micro-morphological, anatomical and chemical characteristics of seed coat of the pea genotypes Jezero, Javor and NS Junior. The aim was to investigate whether these genotypes can be differentiated on the basis of seed coat morpho-anatomical characteristics, depending on the harvest of treatment. For the purpose of anatomical analysis, parts of the seed coats, laterally from the hilum, were separated. Cross-sections of those parts were obtained using a Leica CM 1850 cryostat. All observations and measurements were performed using an Image analyzing System Motic 2000. The surface of seed coat was observed using SEM. Tuberculate surface of seed coat characterized all examined pea genotypes, and the average diameter of tubercule was about 12 μm. Statistical analysis showed that NS Junior genotype had the smallest seeds, thinnest seed coat and the highest number of macrosclereids per mm^2. Also, genotype NS Junior stands out as a genotype with the highest percentage of crude fiber within seed coat. The lowest percentage of seed coat damage and cracks in the NS Junior genotype may be related to the characteristics of the above mentioned micro-morphological, anatomical and chemical characteristics. There is a marked difference between the genotypes with regard to morpho-anatomical characteristics of seed coat, which is confirmed by the results of Multivariate discriminant function analysis.

Keywords: anatomy, seed micromorphology, seed coat

Genetic variability of fatty acid content in rapeseed (*Brassica napus* L.) as basis of breeding for oil quality

PP2-16

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Rapeseed is not only the third largest source of vegetable oil in the world, but is also considered as a source of high quality oil used for various purposes. Since 1991, rapeseed production has shifted to "00" rapeseed cultivars with low content of erucic acid and glucosinolates, achieved through intensive selection which resulted in quite a narrow genetic basis of modern lines and cultivars. The variability of seed tocopherol and fatty acid content in rapeseed cultivars and their F1 generation were the objectives of this study.The material consisted of five distinct rapeseed cultivars, including Orkan, which is not a "00" cultivar. Cultivars were grown in the field together with ten F1 hybrid combinations in three repetitions. The seed tocopherol content was quite variable. Alpha tocopherol content ranged from 176 mg kg^{-1} to 334 mg kg^{-1} in cultivars and 200 mg kg^{-1} to 384 mg kg^{-1} in hybrids, while gamma tocopherol content also had wider range in F1 (316-440 mg kg^{-1}) than

in parents (334-444 mg kg^{-1}). Beta and delta tocopherol content ranged from 0 to 1.4 mg kg^{-1} and 1.3 mg kg^{-1} respectively, but with lower content in F1. Presence of strong antioxidants, such as beta and delta tocopherol, is significant not only for oil stability from the analysed genotypes, but also as a valuable source of variability for breeding. Further efforts should include a wider assessment of variability in the available gene pool for quality-affecting parameters. Similarly to the presented results on oil quality, such assessments should help breeders to use genetic resources more efficiently and produce cultivars of desired quality.

Keywords: rapeseed, breeding, oil quality, fatty acids, tocopherols

Uptake, translocation and accumulation of zinc and copper in strawberries

PP2-17

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The term heavy metal, when related to its impact on the life of the plant and human health, almost always implies negative connotations. However, certain heavy metals are essential for physiological processes in the plant, and without them the plant would not be able to successfully complete its life cycle. Cu and Zn are particularly significant for the life of the plant since the plant needs them in a slightly higher quantity than other heavy metals. The aim of this study was to determine the contents of Zn and Cu in the examined soils, to determine their accumulation in the leaves and fruits of strawberries, and to get a fuller insight into the dynamics of Zn and Cu in the system 'soil - leaf - fruit' on the examined site. The content of Zn and Cu in the soil, leaves and fruits of strawberries was determined by atomic absorption spectrophotometry. The average Zn and Cu contents were 42.06 mg kg-1 and 8.45 mg kg-1 dry matter of soil; 100.34 mg kg-1 and 0.11 mg kg-1 dry matter of leaves, and 91.72 mg kg-1and 0.42 mg kg-1 dry matter of fruits. The degree of uptake and accumulation of Zn in the leaves and fruits of strawberries was at a satisfactory level in accordance with the plant's needs for this element, which was not the case when Cu was studied. The reasons are: a low copper content in the examined soil, low mobility of copper in the plant, and the antagonistic relationship between zinc and copper in the soil.

Keywords: soil, leaf, fruit

The influence of seed size on sunflower protein content

PP2-18

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Seed quality is a collection of seed attributes which are considered to be of significance for the value of the seeds used for sowing purposes. Seed size is one of the seed quality components which affect the performance of the crop. A commercial seed lot is rarely uniform in seed size, and seeds of various sizes within a seed lot can have different quality properties. In this research, the influence of seed size on seed protein content was investigated. Sunflower hybrid seeds were classified into two categories by seed size: large (seeds retained on a > 4.5 mm screen) and small (seeds that passed through a 3.5-4.5 mm screen), and then each category was separated in the gravity desk by specific mass. Field experiments with six seed samples of sun-

flower hybrid were conducted in 2010 and 2011 in Rimski šančevi and Zrenjanin, Serbia. Data were analyzed using three-way ANOVA for a split-slit-plot design. Based on obtained results it can be concluded that the influence of locality and year on seed protein content was statistically highly significant, while the influence of seed size was not statistically significant. On average, the highest protein content was observed on the locality Zrenjanin (19.31%), and during the first year of examination (18.77%).

Keywords: seed size, protein content, sunflower

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Morphological characteristics and variability of sycamore maple (*Acer pseudoplatanus* L.) seedlings

PP2-19

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The comparative morphological analysis of 11 half-sib lines of sycamore maple showed main characteristics and individual, inter and intra-line variability of the seedlings. Seeds for analysis of seedlings' characteristics and variability were collected from micro-populations in the area of Belgrade. The trial was set up in the nursery of the Institute of forestry in Belgrade. When 30 seedlings per each half-sib line (that were taken as a sample) were 30 days old, the following characteristics were measured: root, epicotyl, hypocotyl and cotyledon length, cotyledon width, mass of the seedlings, root collar diameter and the number of cotyledons. The obtained results contribute to the understanding of analyzed characteristics, the preliminary assessment of the genetic variability of the studied half-sib lines and they represent a good basis for the adequate use of genetic potential of the species.

Keywords: sycamore maple, seedling, half-sib line, variability

Effect of fertilizers on concentration of photosynthetic pigments in leaves of one-year-old seedlings of walnut (*Juglans regia* L.)

PP2-20

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The effect of three different fertilizers on concentration of photosynthetic pigments in leaves of one-year-old seedlings of walnut (*Juglans regia* L.) has been studied in the nursery conditions. Three types of pigments have been examined: chlorophyll a, chlorophyll b and carotenoids as well as the total concentration of chlorophyll a and b. The trial was set up in a random block system with three types of fertilizers and the control in three replications in the nursery of Institute of forestry in Belgrade in 2013. The leaf sampling was carried out in the middle of the growing season. The highest concentration of photosynthetic pigments was found in the leaves of seedlings treated with the preparation Bactofil B 10 and the lowest in the seedlings treated

with mineral fertilizer NPK 15:15:15. The highest mean value was recorded for chlorophyll b (0.486 mg g^{-1}) after treatment with Bactofil B 10 and the lowest mean value was recorded for carotenoids (0.165 mg g^{-1}) after treatment with NPK 15:15:15. Research showed that the concentration of photosynthetic pigments in leaves of walnut seedlings varied depending on the fertilizer that was applied. Based on the obtained results it can be concluded that the proper fertilizer can increase the concentration of photosynthetic pigments in leaves and therefore the intensity of photosynthesis which contributes to increasing the biomass production.

Keywords: walnut, fertilizer, photosynthetic pigments, leaves

Plant growth promoting characteristics of soil yeasts and effects on red clover and wheat growth

PP2-21

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Modern agricultural practice strives for obtaining high yields and focuses on the health and quality of foods. In efforts to lower down chemical inputs in food production, modern agriculture is moving to eco-friendly approaches. Inoculation of plants with beneficial microorganizms has important place in these new tendencies. While bacteria and fungi inoculatns are widely used, the yeasts as plant growth promoting agents have been under-exploited. We researched the ability of the soil yeasts (*Candida* sp., *Cyberlindnera* sp., *Schwanniomyces* sp.) to stimulate red clover and wheat growth. Laboratory culture experiments were used for determination of the mechanisms by which plant-yeast interaction may occur (production of indole-3-acetic acid (IAA), solubilization of inorganic phosphates and antagonistic activity). Seeds inoculated with single yeast strain were planted into overburden (coal mine Kolubara) and its effects on seedling (root and shoot) length and dry biomass were recorded two weeks after sowing. The best effect on growth of both plant species was achieved by isolate which is the member of *Candida* sp. Inoculation with this isolate caused an increase in dry biomass by 42% in both plant species compared to control. The results that we obtained in this research idicate that soil yeasts have the potential for plant growth promotion and validate their application in sustainable agricultural practice.

Keywords: yeasts, plant growth promotion, red clover, wheat

Contracted stem anatomy in resurrection *Ramonda serbica* and *Ramonda nathaliae* plants

PP2-22

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Resurrection plants *Ramonda serbica* and *R. nathaliae* are tertiary relicts and endemics of the Balkan Peninsula. They thrive in sheltered, the north exposed sides of canyons and gorges in the Southern Balkans. The rosette of these hasmophytic plants species have a vertical contractile stem that is positioned largely underground and maintains the plant's rosette close to the ground surface that is covered with dense carpet of mosses. Although the age of investigated plant specimens was estimated to be more than 5 years, all vascu-

lar bundles remained individual and extended radially during the secondary growth, without unifying laterally. The specific net-like organization of vascular tissue is adjusted to the dimensions of the contracted stem, being convoluted and without disruption.
The pith consists of thin-walled and unequally tapered parenchymatous cells, elongated in different directions and with no visible intercellular spaces. Thus, the cells differ in size in the transverse plane, resulting in tessellation. Hence, there is no clear alignment of parenchymatous cells in either transverse or sagittal plane. Several layers of parenchyma cells that rest directly against the vascular bundles are strikingly pressed or even collapsed.

Keywords: contraction, stem, xylem, radial extension

Expression of four arabinogalactan genes during *in vitro* morphogenesis from *Centaurium erythraea* leaf explants

PP2-23

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Arabinogalactan proteins (AGPs) are ubiquitous plant cell wall hydroxyproline-rich glycoproteins. Being involved in cell division and differentiation, as well as in growth and pattern formation, AGPs are implicated in diverse morphogenetic processes. We have identified four AGP sequences in centaury transcriptome, of which CeAGP1, CeAGP2 and CeAGP4 contain fasciclin domains and thus are classified as fasciclin-like AGPs (FLAs), whereas CeAGP3 is an AG peptide characterized with DUF1070 domain. In addition, CeAGP1 contains a short Lys-rich motif. CeAGP1, CeAGP 3 and CeAGP 4 are predicted to be GPI-anchored proteins. To elucidate the role of these proteins, we have monitored their expression using RT-qPCR during indirect somatic embryogenesis (ISE), direct and indirect shoot development (DSD and ISD) and direct root development (DRD) induced by different hormonal and light regimes from centaury leaf explants. While *CeAGP1* is induced, and thus probably involved, only in indirect processes (ISD and ISE) that include both cell dedifferentiation and differentiation, CeAGP3 is also induced, although to a lesser extent, during DRD and DSD. The highest induction of CeAGP3 of 36.6 fold relative to control was recorded on CPPU and 2,4-D containing media in darkness after 20 days in culture, whereby ISE is promoted, while the highest *CeAGP1* induction of 26.7 fold was after 10 days on inductive media in light, where ISE and ISD occur simultaneously. Circumstantial evidence suggests that none of the CeAGPs is specifically induced during ISD. CeAGP1 can be considered as a potential marker of ISE, while CeAGP3 is nonspecifically involved in morphogenesis.

Keywords: AGP, FLA, AG peptides, somatic embryogenesis, organogenesis, expression

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Orientation of cell wall polymers in needles of Serbian spruce (*Picea omorika* (Panč) *Purkyne)*

PP2-24

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Cell wall can be considered as a nano-composite in which cellulose, lignin and hemicelluloses are interconnected in a specific manner. It is well recognised that the cell wall development or cell wall expansion and deposition implies an anisotropic arrangement of the cell wall components. Structural organisation of the cell wall and related polymers is important for both mechanical properties of a plant and chemical reactions occurring in the wall space, especially in a response to stress. Understanding the arrangement and anisotropy of the polymers in the cell wall is important for understanding the mechanical properties of a plant, which has implications in plant response to stress, but also in possible application of needles as a source of new biomaterials. By using imaging FT-IR microscopy, run in transmission mode and at different polarisation modes (from 0° to 90°), it is possible to follow the chemical variability and the orientation of the cell wall polymers. The orientation of cellulose, xylan and lignin, as essential components of the plants, was analysed by iFTIR with regard to the sample axis. It has been demonstrated that xylan is oriented in parallel to the cellulose and more or less parallel to the axis of the cell wall, in isolated cell wall fragments from Omorika needles. There was also a clear indication of lignin being orientated parallel to the longitudinal CW axis.

Keywords: Omorika, needles, orientation, polymers

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Correlation analysis of expression profiles between microRNA 398 and 408 and their targets: Cu/Zn superoxide dismutase and laccase 3 in Fe-deficient cucumber plants

PP2-25

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Iron is an essential microelement for all living organisms, with numerous important physiological functions such as respiration and photosynthesis. Iron deficiency is considered as one of the major limiting factors for crop production. Recently, it was shown that silicon alleviates iron deficiency in plants by promoting mobilization of iron in the root apoplast. In order to explain molecular mechanisms of anti-stress role of silicon, we investigated the correlation between expression profiles of microRNAs 398 and 408 and their targets: copper superoxide dismutase and laccase 3 in Fe-deficient cucumber plants grown with or without Si. The expression of miRNAs and target genes was analyzed in roots, stems and leaves. Plants under Fe stress showed dramatic decrease of miRNA expression comparing to control plants. Although Fe-deficient plants in the presence or without Si did not show significant changes in the expression of analyzed miRNAs, lower level of Cu-SOD transcripts in Fedeficient plants grown in presence of silicon was detected. Only miR408 expression in

the roots was increased in Fe-deficient plants, particularly those treated with silicon. In this study we investigated expression level of another potential target gene – laccase 3 and noticed significant down-regulation in Fe deficiency in roots. The fact that this enzyme is involved in the process of lignification and strong negative correlation between expression of miR408 and laccase 3 indicate that miR408 could regulate delignification in Fedeficient cucumber plants on the posttranscriptional level. In addition, we noticed that potential regulatory effect is more prominent in the presence of silicon.

Keywords: microRNA, iron deficiency, silicon, CuSOD, laccase

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Micropropagation of *Micromeria croatica*

PP2-26

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Micromeria croatica (Pers.) Schott is an endemic species of Illyrian-Balkan region, and is widespread in Croatia, Bosnia and Herzegovina, Montenegro and western Serbia. In Serbia, *M. croatica* inhabits relatively small area of Mokra Gora Mt. This dwarf shrub grows in the crevices of limestone rocks at altitudes of 1502000 m. Plants belonging to the Lamiaceae family and genus *Micromeria* are known as rich natural source of essential oils and antioxidant compounds. Biotechnological methods based on plant tissue culture can provide rapid and mass multiplication of plants for different purposes, such as studies of the production, accumulation and metabolism of important secondary metabolites. The aim of this study was to elaborate the protocol for plant regeneration through axillary buds on nodal explants of *M. croatica* by using different growth regulators. The highest propagation rate was achieved with 0.3 μM kinetin. The rooting of regenerated shoots was obtained on media supplemented with indole-3-acetic acid or α-naphthylacetic acid. Auxin at all tested concentrations stimulated rooting of *M. croatica* shoots. Rooted shoots were acclimatized under greenhouse conditions.

Keywords: axillary buds, *Micromeria*, shoot culture

Dynamics of chlorophyll degradation in leaves of *AtCKX* transformed centaury (*Centaurium erythraea* Rafn) plants grown *in vitro*

PP2-27

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Leaf senescence is a complex, genetically controlled process preceding abscission. The earliest events of leaf senescence include decline of the photosynthetic function, loss of chloroplast structures and degradation of pigments, proteins, membrane lipids and nucleic acids. The total darkness, as well as other environmental stress factors, can induce leaf senescence. Cytokinins (CKs) are the major senescence-inhibiting hormones. Decline in CK levels correlates with leaf senescence progression. However, the effect of cytokinin oxidase/dehydrogenase (CKX), a key enzyme in CK catabolism, on the senescence and chlorophyll degradation still remains rather unclear and ambiguous. In this study, we have investigated changes in chlorophyll and carotenoid content in centaury leaves during their dark incubation in water. The leaf segments for analysis were excised from *in vitro* grown control and transformed plants overexpressing *AtCKX1* and *AtCKX2* and exhibiting increased CKX activity, reduced bioactive CK contents and changed CK profiles. Pigment contents were determined spectrophotometrically in 10 time points during period of 30 days. Chlorophylls, especially chlorophyll *a*, showed very little degradation, with final level reaching not less than 63% of the initial value. Chlorophyll *b* was less stable, up to 73% being lost during incubation in control and transformed *AtCKX* lines. Carotenoid degradation was most evident in control, with final content declining to 15% of the initial value, while in *AtCKX* lines the decline was slower and final carotenoid concentration reached 33-57% of the corresponding initial value.

Keywords: cytokinins, *AtCKX*, chlorophyll retention, centaury

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Short glandular trichomes of *Nicotiana tabacum* leaves: morphology, ultrastructural organization and secondary metabolite accumulation under *in vitro* conditions

PP2-28

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The short glandular trichomes of *in vitro* grown *Nicotiana tabacum* plants were analyzed using a combination of light and electron microscopy. Short glandular trichomes arise as epidermal protuberances that di-

vide asymmetrically to produce a more vacuolated basal cell, and a more meristematic apical cell. This apical initial gives rise to a multicellular gland, atop a single, commonly curved, stalk cell. The glandular cells of the short trichomes are tightly linked with numerous plasmodesmata and display structural changes related to trichome maturity and presence of the secretory product. In the early secretory stage, ultrastructural organization of the glandular cells is characterized by large nuclei in the electron dense cytoplasm rich in organelles, and with little vacuolation. The secretory stage coincides with the formation of the extraplasmic space, where densely stained droplets of the secretory product are observed. Prominent electron dense globular inclusions are frequently observed within the vacuoles. Abundant rough endoplasmic reticulum remains parallel to the plasma membrane and the nucleus throughout the secretory phase. Plastids with little lamellar development also contain electron dense inclusions; starch accumulation is observed with maturation. The limited number of dictyosomes in short trichome glandular cells and the absence from the cytoplasm of the vesicles containing secretory product, suggests a non-vesiculate mode of secretion.

Keywords: glandular trichomes, tobacco, ultrastructure

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Somatic embryogenesis in *Gentiana utriculosa* L.

PP2-29

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Somatic embryos (SEs) in *G. utriculosa* were obtained from various explant types including immature seeds as well as leaves and roots of *in vitro* grown plants. For SE production, immature embryos and leaves were cultured for two months on MS medium with 1.0 mg L^{-1} 2,4-D while excised roots were grown on ½MS medium with 0.1 mg L^{-1} NAA. Then, all three types of explants were transferred to plant growth regulator-free ½MS medium, where SEs appeared indirectly from callus. Although the highest callus proliferation was registered on root explants (85.6%), followed by the leaf explants that were placed with abaxial side in contact with the medium (36.4%) and immature seeds (14.0%), the highest SE production rate was achieved from leaf explants (82.0%) compared to roots (14.6%) and immature seeds (42.8%). SEs that originated from immature seeds displayed the best conversion rate (68.5%) while root- and leaf-derived SEs had significantly lower conversion potential (17.95% and 6.35%, respectively). Histological examination confirmed SEs in all three types of explants. Meristem centers of future SEs appeared on callus surface or deeper and could be easily distinguished from the surrounding callus tissue due to their dense packing, dark stained cytoplasm and large conspicuous nuclei. All SE developmental stages were registered including globular, heart shaped, torpedo and cotyledonary SEs.

Keywords: somatic embryos, *Gentiana utriculosa*, callus, histological analysis

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173015).

Influence of plant growth regulators on the morphogenesis of the rare moss *Hennediella heimii*

PP2-30

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The moss *Hennediella heimii* is a rare and threatened species in Europe. It is considered as a facultative moss halophyte that inhabits the salty environment. *H. heimii* is a specialized, mainly moss of bare, salty and brackish soil, such as in saltmarshes, saline fields and muddy depressions. The influence of exogenously added growth regulators on the morphogenesis of this species was studied. The plants were cultured in the basic type of medium, BCD, supplemented with indole-3-butyric acid (IBA) and N^6-benzyladenine (BA) at different concentrations (0.0310 μM), under a 16-h photoperiod. For this purpose, auxin and cytokinin were used separately or combined with each other. The influence of growth regulators on gametophores multiplication *in vitro* as well as on protonemal diameter was observed. Based on multiplication index *in vitro*, maximum development of gametophores was determined on BCD medium supplemented with 3 μM IBA and 0.03 μM BA. The production of the secondary protonema was highest on BCD medium containing the combination of 0.1 μM IBA and 0.3 μM BA. Similar values for the protonema growth were also observed in the control group. Interestingly, when comparing the effect of individual hormones on gametophore multiplication, IBA had a stronger stimulatory effect than BA at all concentrations. Successfully applied micropropagation of this threatened moss species enables better knowledge of its biology and is of great value for the conservation and stress physiology research.

Keywords: IBA, BA, bryophyte

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173024 and OI173030).

Some aspects of mode of action of brassinosteroids in maize

PP2-31

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In contrast to the mechanism of action, the mode of action of certain chemicals in the plants is defined as the totality of the effects that these chemicals cause the effect on metabolism, growth and development of plants. In this sense, we studied the effect of the concentration range (5.2×10^{-6}–5.2×10^{-15} M) of 24-epibrassinolide on germination of two maize hybrids (ZP 434 and P704), by monitoring the effect of the mentioned brassinosteroid phytohormone on germination, sprouting thermodynamics and sugar content, and a num-

ber of polyphenols in the seeds during germination. Then we exposed plants of two corn hybrids (ZP 434, ZP 505), grown in different light environments (light vs. shadow) or at different volumes (5 L vs. 11 L) of pot in which we cultivated plants, to the effects of 24-epibrassinolide or propiconazole (an inhibitor of the synthesis of brassinosteroids). During trial we registered some parameters of growth and photosynthesis. Finally, we conducted a mini field trial, treating two corn hybrids (ZP 434 and ZP 341) with 24-epibrasinolide at the aforementioned concentration range, as well as with propiconazole at two concentrations ($\approx 10^{-6}$ and $\approx 10^{-7}$ M). We checked the final yield and some characteristics of growth of maize plants in the field, as well as the chemical composition of the grain after harvest. The results are discussed from the perspective of a 24-epibrassinolide effect on maize plants of different ages.

Keywords: mode of action of 24-epibrassinolide, germination, whole plant physiology, crop physiology

III

Plant and Fungal Natural Products in Human Nutrition and Medicine

INVITED TALKS

Functional Foods & Nutrition: Facts, Fiction, & Needs

IT3-1

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Wikipedia defines 'functional foods' as the food that contains an additive with some health-promoting or disease preventive property. Such foods are generally perceived as having a 'potentially' positive effect on health beyond basic nutrition (Mayo Clinic). Thus, research on fruits and vegetables - which are sources of health-promoting and/or disease-preventing nutrients - has intensified as horticultural produce takes the helm for future functional foods. To truly achieve such a status, the health-positive nutrient levels in a produce need to be nearer to the threshold 'required daily allowance' (RDA) levels for their positive effects on human health. Convergence of agriculture with health is a distinct possibility but more scientific base has to be developed to realize such an advance. For instance, nutrient levels in current domesticated germplasm are generally low and in a dynamic state, being influenced by genotypes/cultivars, growth conditions, and developmental stage of the crop. Therefore, unambiguous analysis for nutrient levels, nutriome, of edible crops grown under similar conditions in the field or in a greenhouse is required to determine their robustness as functional foods. Also, it is important to discern the genetic, biochemical, and physiological regulation of pathways, which define nutriome of a crop. Interestingly, genetic engineering of the rate limiting steps in the biosynthesis of a few nutrients *in planta* has demonstrated the power of this technology by achieving phytonutrient levels unattainable thus far by conventional breeding or other conventional methodologies. Robust and repetitive animal/human trials are essential in determining the effectiveness of a nutrient. Such studies should provide feedback on which nutrient(s) need to be enhanced in crops through molecular strategies including modern biotechnology.

Wild-growing *Allium* species (sect. *Codonoprasum*) as promising sources of novel herbal drugs

IT3-2

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Species of the genus *Allium*, especially garlic (*A. sativum*) and onion (*A. cepa*), have had a valuable place in human nutrition and medicine since ancient times. Garlic and onion are well researched species, while data on other *Allium* species are very scarce. Therefore, the aim of This study was to investigate phytochemical profile and biological activities of five wild-growing *Allium* species from section *Codonoprasum* (*A. flavum*, *A. melanantherum*, *A. paniculatum*, *A. rhodopeum* and *A. carinatum*) and to estimate their potential applications. Phytochemical profile of investigated species was determined by headspace GC/MS analysis of fresh

bulbs volatiles and by evaluation of presence and content of 44 phenolic compounds in methanol extracts using LC-MS/MS technique. The assessment of antioxidant activity was done by several assays (total reducing capacity, DPPH, NO, $ABTS^{\bullet}+$, $OH^{\bullet}$ assays and ability to inhibit lipid peroxidation). Anti-inflammatory activity of the extracts was evaluated by measuring the ability to inhibit COX-1 and 12-LOX enzymes. Antiproliferative activity was tested by sulforhodamine B assay in three cancer and one normal cell line, while comet assay was used for estimation of genotoxic activity. Dimethyl-disulphide was the main volatile sulphur compound in the bulbs, while methanol extracts were rich in phenolics – quercetin glycosides and kaempferol 3-O-glucoside were dominant. *A. flavum* and *A. paniculatum* extracts expressed the highest antioxidant and anti-inflammatory activity. The most pronounced inhibition effect on tumor cell growth was obtained with *A. rhodopeum* and *A. paniculatum* extracts, while genotoxic effect was not observed for any tested sample. The results obtained suggest that the species of the genus *Allium* sect. *Codonoprasum* are promising sources of novel herbal drugs.

Keywords: *Allium* sect. *Codonoprasum*, LC-MS/MS, antioxidant, anti-inflammatory, antiproliferative

Alternative sources of natural products: Mystery of mushrooms and beyond

IT3-3

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For over a thousand years mushrooms have been used in folk medicine in Asia to prevent and cure a multitude of diseases of quite different nature. Nowadays there is an increasing public interest in the metabolites from mushrooms in order to discover new drugs or leading compounds. The fungal kingdom possesses certain natural advantages in terms of their dietary supremacy over the rest of the vegetarian platter. They are an excellent source of secondary metabolites, vitamins, minerals, protein, and carbohydrates, as well as high in fiber and low in fat. Mushrooms contain various bioactive molecules and they have been considered as potential source of antioxidant, antitumor, antiviral, antimicrobial, and immunomodulatory agents. Herein, the composition in hydrophilic (free sugars, organic acids and phenolic acids) and lipophilic (ergosterol, tocopherols and fatty acids) compounds was evaluated in a number of mushrooms species from *Ganoderma, Phellinus*, *Coprinus, Cordyceps, Agaricus* and other genera, as well as their antioxidant, antibacterial and antifungal properties, and cytotoxicity in human tumor cell lines. Instead of evaluating individual compounds, the whole methanolic extract as well as different fractions (polysaccharides, glucuronides, triterpenoids and polyphenols) were used, allowing evaluation of potential synergistic activities. Our research showed that polysaccharides are often pointed out as the most bioactive compounds isolated from mushrooms, but other molecules such as triterpenoids, polyphenols or glucuronides, might also be highlighted for their bioactivity. Mushrooms are novel prototype therapeutic agents representing new chemical classes, operating by different modes of action compared to the existing agents and, consequently, lack cross-resistance to chemicals currently used.

Keywords: functional food, polysaccharides, polyphenols, mushrooms

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SELECTED TALKS

Centaurium erythraea extract improves redox-status and antioxidant enzyme activity of STZ-treated pancreatic β-cells and diabetic rat liver and kidney

OP3-1

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Centaurium erythraea Rafn (CE) has long been used in traditional medicine in diabetes treatment. Since oxidative stress plays a major role in development of diabetes and its complications, the main goal of This study was to evaluate antioxidative properties of CE extract and its ameliorating effects in streptozotocin (STZ)-induced diabetes. CE extract displayed strong hydrogen peroxide- and nitric oxide-scavenging activities, as well as high reducing power *in vitro*. Treatment with CE extract improved redox-status of STZ-treated rat pancreatic beta cells (Rin5F) by reducing DNA damage, lipid peroxidation and protein oxidation. This was accompanied by repaired activity of antioxidative enzymes in STZ-treated Rin5F cells. CE-treatment lowered STZ-induced increase in glutathione peroxidase (GPx) and manganese superoxide dismutase (MnSOD) activities and partially restored decrease in catalase (CAT) activity. Improvement of redox-status was accompanied by the increase of Rin5F cell viability and insulin expression. Administration of CE extract (100 mg kg-1 orally, two weeks before and four weeks after diabetes induction) to STZ-induced diabetic rats exerted antioxidant effects in liver and kidney. CE-treatment reduced DNA, lipid and protein damage and restored activity of CAT, Mn/CuZnSOD and GPx that were disturbed in diabetic liver and kidney. This improvement is reflected by the preserved functional integrity of diabetic liver and kidney. CE-treatment reduced the level of blood urea nitrogen and activity of liver transaminases (ALT and AST). According to these results, CE extract has great potential in preventing diabetes and its complications, but additional experiments are needed in order to reveal underlying mechanisms.

Keywords: *Centaurium erythraea,* diabetes mellitus, oxidative stress, antioxidant activity

Effects of cucumber extracts on cytokine production in encephalitogenic cells

OP3-2

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Cucumber (*Cucumis sativus*) is a source of anti-inflammatory and anti-oxidative compounds that are of potential interest as immunomodulatory agents. Experimental autoimmune encephalomyelitis (EAE) is an an-

imal model of multiple sclerosis, a chronic, inflammatory and neurodegenerative disease of the central nervous system. Encephalitogenic T cells, particularly $CD4^+$ interferon (IFN)-gamma- and/or interleukin (IL)-17- producing helper T cells, play a dominant role in the pathogenesis of the human disease, likewise in EAE. Our aim was to explore the effects of the crude extracts obtained from cucumber on production of these cytokines in encephalitogenic T cells. The ethanol extracts of the roots, stems and leaves of cucumber were subjected separately to solvent partitioning between hexane and aqueous methanol, and the aqueous methanol fraction was then extracted with methylene chloride. The methylene chloride phases were evaporated under reduced pressure and tested for their biological activities *in vitro*. Concurrently, Dark Agouti rats were immunized with myelin basic protein (MBP) emulsified in complete Freund's adjuvant. On the 6th day after the immunization, draining lymph node cells (DLNCs) were isolated from the rats. The crude extracts were tested on DLNCs re-stimulated with MBP in different concentrations. The production of IFN-γ and IL-17 was measured. All of the used extracts significantly reduced production of IFN-γ and IL-17 in DLNCs, in a dose-dependent manner without significant influence on cell viability. These results imply that the extracts might have beneficial effects in the treatment of EAE. Further research on the effect of cucumber extracts in multiple sclerosis-related experimental settings is warranted.

Keywords: cucumber, EAE, encephalitogenic cells, cytokines

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Quercetin 7-*O*-glucoside inhibits the formation of dinitrosocatechins and their quinones in catechin/nitrite systems under stomach simulating conditions

OP3-3

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Polyphenolics, namely (+)-catechin and quercetin, are abundant constituents of foods and beverages in daily human diet. The favourable effects of polyphenolics on human health have been widely accepted. However, in order to elucidate beneficial and adverse effects, their metabolism once ingested in the human body should be understood. During mastication, catechin and quercetin glycosides are mixed with saliva and swallowed to the stomach where the pH is around 2. Under these conditions, salivary nitrate is present as highly reactive nitrous acid. Reaction products of Cat/nitrous acid systems are nitric oxide ($^{\cdot}NO$) and 6,8-dinitrosocatechins (diNOcat), and diNOcat quinones. The reaction between quercetin (Q) and nitrous acid yields $^{\cdot}NO$ and 2-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxy-3(2*H*)-benzofuranone. Here we investigated the interactions between quercetin and quercetin 7-*O*-β-D-glucopyranoside (Q7G) with catechin (Cat) and nitrous acid under stomach simulating conditions. Both Q and Q7G decreased the content of diNOcat formed in catechin/nitrous acid systems, which resulted in an increase of their oxidation. Moreover, the formation of diNOcat quinones was completely suppressed in the presence of both Q and Q7G. When other Q glycosides were used, the inhibitory effects decreased in the following order: quercetin ≈ Q7G > kaempferol > quercetin 4'-*O*-glucoside > rutin. The results indicate that nitrous acid-induced formation of 6,8-dinitrosocatechins and the *o*-qui-

nones can be suppressed by flavonols in the stomach, and that both hydroxyl group at C3 and *ortho*-hydroxyl groups in the B-ring are required for efficient suppression. Ingestion of catechins with components which reduce the formation of diNOcat quinones may decrease their adverse effects on health.

Keywords: (+)-catechin, quercetin glycosides, quinones, stomach, nitric oxide

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (III 43010) and Grants-in-Aid for Scientific Research (22500790 and 23500986) from the Ministry of Education and Science in Japan.

Development of natural product drugs in a sustainable manner

OP3-4

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For approximately 85% of the world's population, plant materials are a primary source of health care. Knowledge of plants and their medicinal properties that were transmitted from generation to generation is in danger of disappearing. Humankind is not sufficiently aware that natural products drug discovery is important for new generations as a tool for their health care. We know that for the major lethal diseases, there are no truly effective drug treatments. In addition, drug resistance to existing chemotherapeutic regimens for fungal and bacterial infections, AIDS, cancer, and malaria is increasing. Biotechnology may play crucial role in the sustainable development of natural product drugs. Translocation of the genes from slow-growing to fast-growing, large biomass plants or other organisms will enable the large-scale production of medicinal agents. Although the use of transgenic plants is questionable for the preservation of biodiversity, genetic engineering will play an important role in saving the medicinal plants, which are rare or endangered. The imbalance between humans and other species on our planet is a threat to the survival of the humankind. Therefore, plant ecology should be considered in the sustainable development of natural products. According to the World Wildlife Fund, due to the human consumption, 20% of medicinal plants in the world are in threat of disappearing. Therefore, medicinal plants, both endemic and widespread, their resources and knowledge about their usage must be preserved since these plants could be renewable source for new drugs.

Keywords: natural product drugs, biotechnology, sustainable development, medicinal plants

POSTER PRESENTATIONS

Seasonal variation of flavonoid content and antioxidant activity of *Salvia officinalis* of different origin

PP3-1

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Sage (*Salvia officinalis* L., Lamiaceae) is widely known as an important culinary and medicinal plant. This study was aimed to investigate the antioxidant activity and flavonoid content in ethanol extracts of four samples of this species. Plants from Pleš (Eastern Serbia) and Luštica (Montenegro), were transplanted in Belgrade, and cultivated under the same conditions. Aerial parts were harvested during summer and winter season, grounded and extracted by ethanol to obtain crude extracts. Antioxidant activity was evaluated using DPPH assay and results were expressed as IC50 values (μg mL^{-1}). Flavonoid contents (FC) were measured spectrophotometrically and data were presented as mg of quercetin equivalents per gram of dry extract (mg QE g-1). All of the extracts performed DPPH activity ranged from 13.12-20.05 μg mL^{-1}, which was evaluated as good comparing to values obtained for standards BHA (13.37 μg mL^{-1}) and BHT (17.94 μg mL^{-1}). Flavonoid content ranged from 20.08 to 40.72 mg QE g-1. Extracts of plants originated from Pleš showed stronger activity and higher FC than plants from Luštica. As expected, extracts of summer samples exhibited stronger DPPH activity and higher FC than the winter ones. Taking into account that uniform procedures have been applied for all of the plant samples, it could be concluded that flavonoid content and DPPH activity of the extracts depended on the locality of origin and season of the plant material collection.

Keywords: *Salvia officinalis*, ethanol extract, DPPH activity, flavonoid content

Hydrolysis of secoiridoid glycosides from *Centaurium erythraea* Rafn increases their antioxidative potential

PP3-2

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Dominant secondary metabolites of common centaury (*Centaurium erythraea* Rafn) are bitter taste secoiridoid glycosides: swertiamarin, gentiopicrin and sweroside, and their function is proposed to be in plant responses against pathogens and herbivores. It can be assumed that secoiridoid glycosides and hydrolytic enzymes (β-glucosidases) form a dual defense system in common centaury, where β-glucosidase plays an essential role in removing non-reducing terminal glucosyl residues from glycosylated compounds, leading to highly active but unstable aglycones. The present study was designed to evaluate antioxidative and antimicrobial activity of hydrolyzed and non-hydrolyzed methanol extracts of *Centaurium erythraea* aboveground parts and its main components sweroside, swertiamarin and gentiopicrin. To examine the concentrations of secoiridoid glycosides and their aglycones in methanol extracts, before and after hydrolysis, UH-

PLC/DAD/+HESI-MS/MS method was developed and evaluated. Hydrolysis was performed enzymatically using commercial β-glucosidase isolated from almond. Results of FRAP, ABTS and DPPH assays showed higher antioxidative activity of hydrolyzed *C. erythraea* methanol extract and pure compounds than non-hydrolyzed ones. Conversely, hydrolysis of *C. erythraea* methanol extracts led to lower antifungal activity and had weak or no influence on antibacterial activity. Based on this study it can be presumed that biosynthesis of secoiridoid glycosides, and their degradation mediated by β-glucosidases are regulated by various biotic factors, and are involved in defense system against herbivores and pathogens.

Keywords: secoiridoid glycosides, β-glucosidase, *Centaurium erythraea*

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Antibacterial activity of Lady's Mantle

PP3-3

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Lady's Mantle (*Alchemilla vulgaris* L.) belongs to the Rosaceae family. In traditional medicine, it was used as herbal treatment for menstrual disorders. Due to the high content of phenolic compounds, *Alchemilla* species were also shown to possess anti-inflammatory, antioxidant, antiinfluenza and anticarcinogenic activity. The purpose of this work was to evaluate the antibacterial properties of *A. vulgaris*. The methanolic extract of aerial parts of *A. vulgaris* prepared by maceration has been used to estimate the antibacterial activity against nine bacterial strains. The *in vitro* antibacterial activity was performed by microdilution method. Minimal inhibitory concentrations (MIC) were evaluated based on the color change of resazurin. The most sensitive bacterial strain was *Micrococcus lysodeikticus* (MIC 0.156 mg mL^{-1}). The methanolic extract of *A. vulgaris* also showed remarkable antibacterial potential against both ATCC and clinically isolated strains of *Enterococcus faecalis* (0.312 mg mL^{-1} and 0.156 mg mL^{-1}, respectively). *Pseudomonas aeruginosa* was the most resistant species with MIC values 20 mg mL^{-1}. MIC values for chloramphenicol, used as standard, were in the range of 2.5-10 mg mL^{-1}. The results of the present investigation suggest that *A. vulgaris* possesses strong antibacterial activity against tested bacterial species, with MIC values ranging from 0.156 mg mL^{-1} to 20 mg mL^{-1}. Based on these results, further chemical and pharmacological investigation, as well as isolation of bioactive compounds may be recommended.

Keywords: *Alchemilla vulgaris*, antibacterial activity, phenolic compounds

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Antioxidant potential of inflorescences and stems of selected *Brassica* vegetables determined by different single electron transfer assays

PP3-4

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High nutritional value of *Brassica* vegetables as well as effectiveness against free radicals and LDL oxidation make them particularly important part of the quotidian diet. High intake of these plants should be encouraged to overcome bad dietary habits observed recently. The aim of this study was to evaluate antioxidant potential of edible parts, inflorescences and stems of broccoli, cauliflower and kohlrabi grown under organic (-Org) and conventional (-Conv) cropping systems and to establish the differences between them. Scavenging activity of fresh juices extracted from edible parts of investigated plants was determined by ABTS and DPPH assays, while reducing power was measured by reaction with CUPRAC reagent. Several times higher antioxidant potential of edible parts juices of investigated plants was obtained by ABTS than by CUPRAC and DPPH assays (2.51-7.03 mM Trolox equivalents (TE), 1.31-3.96 mM TE and 0.36-0.76 mM TE, respectively). The juice of cauliflower-Conv was the most effective scavenger of ABTS+ radicals (7.03 mM TE), while broccoli-Conv was the most successful reducing agent of CUPRAC reagent (3.96 mM TE) and scavenger of DPPH radicals (0.76 mM TE). The way of growing had influence on antioxidant activity. Significant increase in the scavenging ability towards DPPH radicals was noticed in the case of cauliflower-Org in comparison to -Conv ($P<0.01$). Furthermore, kohlrabi-Org showed higher ABTS•+ scavenging capacity compared to kohlrabi–Conv ($P<0.01$). On the other hand, conventionally grown broccoli and cauliflower expressed significantly higher reducing power than the corresponding samples grown under organic conditions ($P<0.01$).
In conclusion, *Brassica* vegetables possess high antioxidant potential, strongly dependent on growing conditions.

Keywords: broccoli, cauliflower, kohlrabi, antioxidant potential, SET

Antioxidant and cytotoxic properties of Tansy extracts

PP3-5

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Antioxidant properties of Tansy (*Tanacetum vulgare* L.) methanol extracts from flower, leaf, stalk and root were evaluated. Total phenolic content in these extracts ranged from 83.6221.7 mg gallic acid equivalents g^{-1}. Free radical scavenging activity of extracts expressed as IC_{50} values varied between17.46 μg g^{-1} and 44 μg g^{-1}. Antiproliferative activity of the investigated extracts and essential oils was evaluated for 72 h of continuous action, using colorimetric SRB (sulforhodamine B) assay. Study was performed in human cervical adenocarcinoma cells (HeLa) and human fetal lung fibroblast cells (MRC-5), used as non-cancerous model for *in vitro* toxicity evaluation. Results are summarized in terms of IC_{50} values, defined as the concentration of drug producing 50% inhibition of cell survival. All tested extracts inhibited the growth of both cancerous and non-cancerous cell lines in dose dependent manner. The best results in HeLa cells were shown for leaf extracts

(IC_{50} (mg) = 0.04339 ± 0.003). Also flower extracts had prominent activity, with IC_{50} (mg) = 0.07059 ± 0.02. The morphology of the HeLa and MDA-MB-453 cells was monitored using an inverted microscope (Carl Zeiss, Jena, Germany) equipped with digital camera (Olympus, USA). Morphological changes of MRC-5 cells, such as cell shrinkage and detachment, were notable following treatment with cisplatin. Cisplatin was used as a standard cytotoxic agent. Results obtained suggested cytoselective potential of tested extracts toward neoplastic HeLa cells. This is the first report on cytotoxic effect of Tansy extract on HeLa cells.

Keywords: antioxidant activity, antitumor effect, total phenolic content, Tansy

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Chemoprotective and regenerative properties of diarylheptanoids from the *Alnus glutinosa* bark in human normal keratinocytes

PP3-6

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Medicinal plants have long been recognized as a source of diverse therapeutic agents and many are used as dietary supplements. Comprehensive approaches are needed to identify bioactive components with evident activity against specific indications and provide a better link between science (ethno-botany, chemistry, biology and pharmacology) and market. Recently, food supplements originating from the bark of black alder (*Alnus glutinosa*) appeared on the market for treatment of different skin conditions. The aim of this study was to evaluate protective effects of two diarylheptanoids isolated from the black alder bark towards doxorubicin damaging activity: platyphylloside - 5(S)-1,7-di(4-hydroxyphenyl)-3-heptanone-5-O-β-D-glucopyranoside (1) and 5(S)-1,7-di(4-hydroxyphenyl)-5-O-β-D-[6-(E-p-coumaroylglucopyranosyl)]heptane-3-one (2), its newly discovered analog. To this end, we employed HaCaT cells, non-cancerous human keratinocytes commonly used for skin regenerative studies. Both diarylheptanoids significantly antagonized doxorubicin action by lowering the sensitivity of HaCaT cells to this drug. Compound 2 prevented doxorubicin-induced cell death by activating autophagy. Both compounds showed protective effect in HaCaT cells against doxorubicin-induced DNA damage. They significantly promoted cell migration and Factin redistribution. The obtained results indicate that chemoprotective effects of diarylheptanoids may occur at multiple subcellular levels. Therefore, diarylheptanoids found in the black alder bark could be considered as protective agents for non-cancerous dividing cells during chemotherapy. In addition, their influence on skin protection and regeneration may find application in pharmacy based cosmetics.

Keywords: diarylheptanoids, chemoprotection, black alder, human normal keratinocytes

Evaluation of antioxidant, antibacterial and cytotoxic activities of Libyan *Salvia fruticosa* ethanol extract

PP3-7

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Greek sage (*Salvia fruticosa* Mill., Lamiaceae), native to the Mediterranean, is well-known as culinary and medicinal plant. The aim of the present study was to examine antioxidant, antibacterial and cytotoxic activity of ethanol extract of this species.Aerial parts of wild-growing plants were collected in Libya, grounded and extracted with 96% ethanol. Extract was evaporated under vacuum to obtain crude extract. Antioxidant activity was evaluated using DPPH assay and results were expressed as IC_{50} value (μg mL^{-1}). Antibacterial activity was tested against six Gram-negative and five Gram-positive bacteria using microdillution assay, presenting data as minimum inhibitory concentrations, MIC (mg mL^{-1}). Cytotoxic activity, studied by MTT assay against HCT-116 human colon carcinoma cell line, was shown as IC_{50} value (μg mL^{-1}). The extract showed DPPH scavenging activity of 29.55 μg mL^{-1}, which is evaluated as good comparing to those obtained for standard BHT (17.94 μg mL^{-1}). The extract performed inhibiting activities against all of the tested bacteria, but lower than streptomycin (0.0050.016 mg mL^{-1}). Gram-positive bacteria were more sensitive (1015 mg mL^{-1}) than Gram-negative ones (1525 mg mL^{-1}), probably due to the lack of outer membrane. The most sensitive bacteria were *Staphylococcus aureus*, *Bacillus subtilis* and *Listeria monocytogenes* (10 mg mL^{-1}), while *Pseudomonas aeruginosa* was found as the most resistant (25 mg mL^{-1}). Extract also showed moderate, but not insignificant activity against HCT-116 cancer cells, especially after 72h of the treatment (375.96 μg mL^{-1}).
Considering that *S. fruticosa* ethanol extract performed strong antioxidant and moderate antibacterial and cytotoxic activities, results of the present study could justify traditional use of this herb.

Keywords: *Salvia fruticosa*, ethanol extract, antioxidant, antibacterial, cytotoxic activity

Radical scavenging capacity and total phenolic content of *Satureja kitaibelii* deodorized extracts

PP3-8

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Satureja kitaibelii Wierzb. ex Heuff. (Lamiaceae) is semi-woody, perennial subshrub species, native in north-eastern part of the Balkan Peninsula (eastern Serbia and southwestern Romania). This species is commonly used as herbal tea and in traditional medicine for immune system strengthening, against cold and for hypotension. The objective of this study was to analyze antioxidant capacities of *S. kitaibelii* deodorized extracts (DEs). The

antioxidant potential of the DEs was evaluated by means of the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging method and determined spectrophotometrically. Among tested extracts, the highest scavenging activity was exerted by deodorized aqueous extract (DAE) (EC50 = 0.141 mg mL-1 of solution) followed by deodorized methanol extract (DME) (EC50 = 0.363 mg mL-1) and deodorized ethyl acetate extract (DEE) (EC50 = 4.159 mg mL-1 of solution). Results were compared to the effects of control substances trolox and butylated hydroxytoluene (BHT) (EC50 = 0.064 and 0.328 mg mL-1, respectively). Total phenolic contents of DEs were determined by Folin-Ciocalteau (FC) assay and gallic acid was used as a standard. DME of *S. kitaibelii* showed high phenolic content (109.22 mg gallic acid equivalents (GAE) g1 of dry extract) in concentration of 1 mg mL-1, and DAE 78.40 mg GAE g-1 of dry extract, in concentration of 0.33 mg mL-1. High total phenol contents of DME and DAE of *S. kitaibelii* explained their high reducing properties. Results presented here may suggest that the deodorized extracts from *Satureja kitaibelii* could be recognized as potential source of antioxidant ingredients for the food and pharmaceutical industry.

Keywords: *Satureja kitaibelii*, deodorized extracts, antioxidant activity, DPPH, phenols

Cytotoxic activity of *Nepeta rtanjensis* Diklić & Milojević essential oil against human tumor cell lines

PP3-9

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Potential cytotoxic activity of *Nepeta rtanjensis* Diklić & Milojević, endemic and endangered plant with various biological activities was investigated. Essential oil obtained from field cultivated *N. rtanjensis* plants was rich in nepetalactone stereoisomers, especially in 4aα,7α,7aβ-nepetalactone (*trans,cis*-nepetalactone). This essential oil was tested for cytotoxic activity against four tumor cell lines (HeLa - human cervix carcinoma cells, A549 - lung adenocarcinoma cells, LS-174 - human colon cancer cells, K562 - human myelogenous leukemia cells) and one normal cell line (MRC-5 - human fetal lung fibroblast cells) using MTT assay. Also, changes in cellular morphology were monitored using an inverted microscope equipped with a digital camera. Results indicated that after 72h treatment the essential oil exhibited cytotoxic activity against all investigated cell lines with IC_{50} values reaching 0.043 μL mL^{-1} for HeLa, 0.061 μL mL^{-1} for A549, 0.087 μL mL^{-1} for LS-174 and 0.054 μL mL^{-1} for K562 cells. Normal cell line (MRC-5) was the least sensitive to the treatment. IC_{50} value for this cell line was not reached in the tested range of concentrations (up to 0.1 μL mL^{-1}). Analysis of morphological changes of treated cells confirmed higher sensitivity of tumor cells (HeLa, A549, LS-174) than normal cells (MRC-5) to the tested essential oil. Due to the determined cytotoxic potential of the examined essential oil, further research will be directed towards the investigation of its mechanism of action against tumor cells including analysis of expression of genes involved in apoptotic signaling cascades.

Keywords: *Nepeta rtanjensis,* MTT assay, nepetalactone, cytotoxic activity

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An insight into antiradical activity and total phenolic content of the lignicolous fungus *Fomes fomentarius* (L.:Fr.) Fr.

PP3-10

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The work presented herein describes *in vitro* determination of antiradical activity and total phenolic content of the lignicolous fungus *Fomes fomentarius* (L.:Fr.) Fr. extracts (obtained by hydrodistillation) performed from the biological material collected at sites with difference in air quality (polluted *vs.* unpolluted habitat). This preliminary screening included four free radical species (DPPH$^{\bullet}$, ABTS$^{\bullet}$, $^{\bullet}$NO and Asc$^{\bullet}$) and two instrumental techniques (EPR and UV-VIS). The observed antiradical activity was correlated with *F. fomentarius* total phenolic content. Indeed, a positive correlation was found between phenolics and antiradical activity towards majority of screened free radical species (DPPH$^{\bullet}$, ABTS$^{\bullet}$ and $^{\bullet}$NO), i.e. against all the free radicals except Asc$^{\bullet}$ (inverse dependence). On the other hand, a negative trend was noticed for the impact of air pollution on the antiradical potential of the examined organism. The obtained experimental data indicate that the fungus *F. fomentarius* may be considered as a promising resource of novel antioxidants of natural origin (1568 mg gallic acid equivalents per g dried extract) with potential use in medicine. In order to provide an optimal quantity of the biological material for the isolation and identification of bioactive secondary metabolites of phenolic type among which some might be new compounds, *in vitro* culture of *F. fomentarius* should be established, putting attention both at the whole fungal organism and its microsymbionts.

Keywords: lignicolous fungus, *Fomes fomentarius,* natural phenolics

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The lignicolous fungus *Schizophyllum commune* Fr.:Fr. may offer novel bioactive substances showing ascorbyl radical scavenging activity

PP3-11

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This work was focused on *in vitro* evaluation of antiradical activity (DPPH$^{\bullet}$, ABTS$^{\bullet}$, $^{\bullet}$NO and Asc$^{\bullet}$) and total phenolic content of the lignicolous fungus *Schizophyllum commune* Fr.:Fr. hydrodistilled extracts prepared from the biomaterial collected at air polluted and unpolluted habitats, respectively. Generally speaking, a pos-

itive trend was noticed for the impact of air pollution on the examined antiradical activity. Interestingly, both hydrodistilled extracts showed to be a potent resource of novel natural products (antioxidants) capable to scavenge ascorbyl (Asc$^{\bullet}$) radical (77.80% and 70.40%, polluted vs. unpolluted habitat, respectively). Such practically unexpected findings are even more important for the reason of using very modern and fully justified methodology – electron paramagnetic resonance spectroscopy (EPR). In addition, it's noteworthy to mention that negative correlation was observed between *S. commune* phenolic content (678 and 1383 mg gallic acid equivalents per g dried extract, polluted vs. unpolluted habitat, respectively) and its anti-ascorbyl radical activity. In comparison with other free radicals, Asc$^{\bullet}$ is relatively stable and unreactive, but may have role in disease progress (e.g. sepsis) as well. According to the best of our knowledge, this is the first record of anti-ascorbyl radical activity of *S. commune*. Taken together, it is reasonable to suppose that this fungal species may offer novel bioactive substances with ascorbyl radical scavenging activity not strictly belonging to the phenolic structural class.

Keywords: lignicolous fungus, *Schizophyllum commune,* Asc$^{\bullet}$

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Thymus praecox subsp. *polytrichus* extracts - antioxidant properties

PP3-12

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Medicinal properties of *Thymus* species are well known for centuries and have been thoroughly described in the literature. *Thymus praecox* subsp. *polytrichus* (A.Kern. ex Borbás) Jalas is a wild growing plant distributed in South-East Serbia. This study was carried out to determine *in vitro* antioxidant capacity of ethanol extracts of this species. Total phenol and flavonoid content of 70% and 96% ethanol extracts, as well as their free radical and scavenging activity were measured. For that purpose, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonate) (ABTS) test were used. The total phenol content (TPC) in the extracts was determined using Folin-Ciocalteu (FC) reagent. Results are expressed as gallic acid equivalent per g of dry weight (mg GA g-1 dw). The concentration of flavonoids was determined by spectrophotometric method, expressed as quercetin hydrate equivalent per g of dry weight (QE g-1 dw). Synthetic antioxidant butylated hydroxyanisole (BHA) and Vitamin C, were used as a control. The values of TPC ranged between 131.00 mg GA g^{-1} dw and 141.00 mg GA g^{-1} dw. The amounts of flavonoids were similar (39.0-41.0 mg QE g-1 dw). Free radical scavenging activity measured by DPPH assay (expressed as EC50) of 70% ethanol was EC50 = 0.0088 mg mL^{-1}, and for ethanol 96% extracts it was EC50 = 0.0186 mg mL^{-1}. The results obtained by ABTS assay indicated that 70% alcoholic extract possesses higher scavenging ability (0.509 EqVitC mg^{-1}) than 96% ethanol extract. Results showed that 70% ethanol extract of *T. praecox* has higher antioxidant potential, and should be recognized as good source of natural antioxidants.

Hypoglycemic and hypolipidemic effects of *Aronia melanocarpa* fruit juice in normal rats

PP3-13

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The aim of this study was to assess how the unlimited drinking of *Aronia melanocarpa* fruit juice, rich in phenolic substances (1.177 g L^{-1} gallic acid equivalents), may affect the levels of several biochemical markers in the rat plasma: glucose (Glu), total cholesterol (ChT) and its fractions (HDL and LDL), triglycerides (TG) and transaminases (ALT and AST). Young male rats were being supplied with 3 combinations of juice solutions in drinking water for 34 days, and their blood samples were collected for analysis after animal sacrifices on day 35. The experimental groups contained 20% juice solutions in tap water of: (ARO) 100% master aronia juice, (MIX) 25% master aronia juice + 75% juice reconstruct (without flavonoids), and (PLC) 100% juice reconstruct, and they were compared with a (CTL) control group on pure tap water. Biochemical analyses of plasma on Cobas c-111 analyzer showed a significant 20% decrease of Glu in ARO group in comparison to CTL. ChT was significantly higher in MIX and PLC in relation to both CTL and ARO groups, which arose mainly from LDL elevations. Also, there was a certain (30%), but insignificant increase of TG in MIX and PLC vs. CTL. The effects of aronia on transaminases were registered only for ALT, whose level in ARO was about 3-fold of that in CTL group. In conclusion, everyday free drinking of flavonoid-rich aronia juice solutions appeared to have global hypoglycemic effect in normal rats and, also, certain hypolipidemic effects, when hyperlipidemia was induced probably with higher sugar consumption.

Keywords: *Aronia melanocarpa*, plasma glucose, plasma lipids, transaminases, rats

The stimulant behavioral effects of anthocyanin-rich juice of *Aronia melanocarpa* in rats

PP3-14

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The fruits of *Aronia melanocarpa* are rich in anthocyanins, plant pigments with a number of registered beneficial effects on human health (e.g. antioxidative, anti-inflammatory, immunomodulatory). Some limited studies have also shown their effects on behavior and cognitive functions in experimental animals. This study was designed to explore the effects of unlimited consumption of diluted aronia juice on rat behavior. Young male Wistar rats were divided into control group (CTL; N=12) with tap water provided *ad libitum*, and 3 experimental groups (N=8) supplied with drinking solutions of 20% mixtures (m/m) in tap water: (ARO) 100% master aronia juice; (MIX) 25% master aronia juice + 75% placebo solution (juice reconstruct without flavonoids); (PLC) 100% placebo. Rats were being allowed to drink these solutions without limits for 34 days, where on

days 31-34 three behavioral tests were performed. These tests revealed that only ARO group exhibited significant behavioral changes: augmented locomotion in an open field test, anxiolytic-like action in elevated plus maze, as well as an increase in active swimming in forced swimming test (which is relevant to antidepressant-like action). These results suggest that everyday drinking of diluted aronia juice (between 6 and 12 g of master aronia juice daily per rat) with high flavonoid content (about 0.12%) for about a month, may induce several stimulant behavioral actions in experimental animals.

Keywords: *Aronia melanocarpa*, anthocyanins, behavioral tests, behavioral stimulant

Antibacterial activity screening of two inedible fungal species *Bjerkandera adusta* (Willd. ex Fr.) P. Karst. and *Trametes* versicolor (L.) Lloyd (1920)

PP3-15

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Due to the growing resistance of pathogenic bacteria to common antibiotics, detection of alternative sources of antimicrobial substances is very important in microbiological investigations, among which the fungi have been recently documented as one of the most potent agents. Since the selected species *Bjerkandera adusta* (Willd. ex Fr.) and *Trametes versicolor* (L.) Lloyd (1920) have not been previously tested against human pathogens, the aim of This study was to preliminarily screen their *in vitro* antibacterial activity at the extract level. The extracts were performed using 70% methanol (rotary shaker, 100 rpm; 72 h). The mentioned bioactivity was screened against seven human vaginal bacterial strains, five of which were Gram-negative (*Escherichia coli* I & II, *Proteus mirabilis*, *Proteus vulgaris* and *Pseudomonas aeruginosa*), while the remaining two were Gram-positive ones (*Staphylococcus aureus* I & II). The antibacterial activity was determined under *in vitro* conditions using microdilution assay in 96-well microplates, according to CLSI procedures. The extracts showed to be active against the majority of the screened bacterial strains, with the MIC values in the range from 5 mg mL^{-1} to 20 mg mL^{-1}. The highest activity was actually observed against *S. aureus* I & II (MIC 5 mg mL^{-1}). Taken all together, it should be emphasized that the obtained experimental data are in a good accordance with the existing literature records. The further research work will be focused on *in vitro* screening of antibacterial activity of the examined fungal species at the extract level of various polarities.

Keywords: antibacterial activity, *Bjerkandera adusta, Trametes versicolor,* human pathogens

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In vitro evaluation of antioxidant activity and total phenol content of the medicinal mushroom S*tereum hirsutum* (Willd.) Pers.

PP3-16

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Bearing in mind that previous studies have clearly indicated the mushrooms as a good resource of natural antioxidants, the aim of this work was to investigate antioxidant (AO) activity and total phenol content of a selected autochthonous fungal species, the medicinal mushroom S*tereum hirsutum* (Willd.) Pers. (fam. Stereaceae), originating from the mountain Fruška Gora (Vojvodina, Serbia). The performed AO screening included its methanol extract, obtained after maceration in 70% solvent, followed by shaking on a rotary shaker (100 rpm) for 24 h. The antioxidant activity was evaluated under *in vitro* conditions using DPPH test and hydroxyl radical scavenging assay, while the total phenol content was determined by Folin-Ciocalteu reagent. The examined methanol extract showed a good scavenging activity against both DPPH and hydroxyl radicals (IC_{50} 67.73 μg mL^{-1} and 16.45 μg mL^{-1}, respectively). On the other hand, the total phenol content of the extract (134.97±14.21 mg g^{-1}, GAE equivalents) might be responsible for the observed activity. These preliminary findings actually support the consumption of *S. hirsutum* as a functional food. The future research work will be focused on the AO screening of *S. hirsutum* aqueous extract under *in vitro* conditions as well as on the identification of the chemical composition of both extracts, namely the methanol and aqueous ones.

Keywords: medicinal mushroom, *Stereum hirsutum,* antioxidant activity, hydroxyl radical

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Antimicrobial activity of *Bupleurum praealtum* and *Bupleurum sibthorpianum* methanol and acetone extracts

PP3-17

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This work presents the antibacterial and antifungal activities of methanol and acetone extracts obtained from aerial parts of *Bupleurum praealtum* L. (City of Sokobanja, Serbia) and *Bupleurum sibthorpianum* Smith var. *diversifolium* (Roch.) Hayek (Mt. Kopaonik, Serbia). Using microdilution technique, minimum inhibitory concentrations (MICs) and bactericidal/fungicidal concentrations (MBCs/MFCs) of extracts were determined. The selected extracts were investigated against four Gram (+), four Gram (–) bacteria and eight fungal strains. Positive

synthetic controls streptomycin and fluconazole were used. Tested *Bupleurum* extracts did not exhibit substantial antimicrobial activity, still, stronger antibacterial activity was observed. Obtained results showed moderate antibacterial (MICs = 0.48 mg mL^{-1}; MBCs = 0.511 mg mL^{-1}) and low antifungal activity (MICs = 1016 mg mL^{-1}; MFCs = 1424 mg mL^{-1}) of both *Bupleurum* species against used pathogens. *B. cereus* and *S. aureus* were proved to be the most sensitive bacteria (MICs = 0.44 mg mL^{-1}; MBCs = 0.55 mg mL^{-1}). The most sensitive fungi were *P. fumigatus* and *A. versicolor* (MICs = 1014 mg mL^{-1}; MFCs = 1418 mg mL^{-1}). Other microorganisms were less susceptible to the inhibitory effects of the extracts and among them *M. flavus*, *L. monocytogenes* (MICs = 28 mg mL^{-1}; MBCs = 410 mg mL^{-1}) and *A. niger* (MICs = 1416 mg mL^{-1}; MFCs = 2124 mg mL^{-1}) were shown to be the most resistant strains. Compared data revealed that *B. sibthorpianum* methanol extract and *B. praealtum* acetone extract demonstrated stronger antimicrobial potency, although, both acetone extracts showed slightly higher inhibition on microbial growth in terms of effective concentrations. In conclusion, *Bupleurum praealtum* and *Bupleurum sibthorpianum* extracts showed similar, moderate antimicrobial activity. Effectiveness of tested extracts was probably affected by polarity nature of the solvent and different extracted compounds.

Keywords: *B. praealtum, B. sibthorpianum*, antibacterial activity, antifungal activity

Potential of *Ruscus aculeatus* L. as source of natural antioxidants

PP3-18

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The aim of this study was to investigate various solvent extracts (ethanolic, acetone and ethyl acetate extracts) of *Ruscus aculeatus* L. (aerial parts) from Serbia to display potent antioxidant activity *in vitro*. The aerial parts of *Ruscus aculeatus* L. (17074 BEOU) were collected from Ovčar - Kablar gorge, near Čačak (Serbia), in late September 2012. The air-dried aerial parts of plant (30 g) were broken into small pieces (2-6 mm) and extracted with 96% ethanol, acetone and ethyl acetate (150 mL) using a Soxhlet apparatus. Antioxidant activity was studied using several different test: DPPH free-radical scavenging activity, total antioxidant activity, metal chelating ability, Fe3+-reducing power, ABTS radical scavenging activity and inhibitory activity against lipid peroxidation. The total phenolic and flavonoid content in each plant extract was also determined. Among three different solvent extracts, ethanolic extract of *R. aculeatus* showed the highest metal chelating ability (IC50 = 150 ± 0.25 µg mL^{-1}), reducing power (IC50 = 209 ± 0.08 µg mL^{-1}) and inhibitory activity against lipid peroxidation (IC50 = 790 ± 0.71 µg mL^{-1}). Acetone extract of *R. aculeatus* had the best total antioxidant activity (23.329 ± 1.85 µg AA g^{-1}) and ABTS radical scavenging activity (IC50 = 342 ± 0.12 µg mL^{-1}) while ethyl acetate extract displayed the highest DPPH radical scavenging activity (IC50 = 182.54 ± 0.21 µg mL^{-1}). In addition, acetone extract of *R. aculeatus* was the richest in phenols (6.510 ± 0.53 mg GAE g^{-1}) and flavonoids (0.136 ± 0.14 mg RU g^{-1}). Based on the obtained results, *R. aculeatus* could be considered as a potential source of natural antioxidants due to their significant antioxidant activity.

Keywords: free radical scavenging, reducing power, chelating ability, lipid peroxidation, total phenolic and flavonoid contents

Ruscus hypoglossum from Serbia as source of new natural antioxidants

PP3-19

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Species from the genus *Ruscus* are used in herbal medicine because of their anti-inflammatory and vein constrictor properties. The aim of this study was designed for the evaluation of antioxidant activity of aerial parts of *Ruscus hypoglossum* L. in order to investigate the relationship between antioxidant properties and total phenolic and flavonoid content. Aerial parts of *R. hypoglossum* (17075 BEOU) were collected from mountain Žeželj (Kragujevac, Serbia), in September 2012. The air-dried aerial parts of plant (30 g) were broken into small pieces (26 mm) and extracted with 96 % ethanol, acetone and ethyl acetate (150 mL) using a Soxhlet apparatus. With each extract, following antioxidant assays were carried out: DPPH and ABTS free-radical scavenging activity, total antioxidant activity, Fe^{2+}chelating ability, Fe^{3+}reducing power and inhibition of lipid peroxidation. Ethanolic extract of *R. hypoglossum* showed the highest total phenolic content (8.569 mg GAE g^{-1}) as well as ABTS radical cation scavenging activity (IC50 = 3.04 μg mL^{-1}) and reducing power (IC50 = 143 μg mL^{-1}). The highest anti-DPPH (IC50 = 278.37 μg mL^{-1}) and ferrous ion chelating ability (IC50 = 110 μg mL^{-1}) were found in ethyl acetate extract. The highest flavonoid content (0.129 mg RU g^{-1}) as well as the highest total antioxidant activity (14.976 μg AA g^{-1}) and inhibitory activity against lipid peroxidation (IC50 = 651 μg mL^{-1}) were found in acetone extract. These data will provide some useful information for healthier living, as well as for the further screening of plants as potential sources of new natural antioxidants.

Keywords: free radical scavenging, reducing power, chelating ability, lipid peroxidation, total phenolic and flavonoid contents

Chemical composition and antioxidant activity of three endemic *Nepeta* species methanol extracts

PP3-20

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Methanol extracts of *in vitro* grown shoots of three *Nepeta* species (*Nepeta rtanjensis* Diklić & Milojević, *N. sibirica* L. and *N. nervosa* Royle ex Bentham) were studied for their main secondary metabolites using UHPLC/DAD/±HESI-MS/MS analysis. Results showed that the major terpene present in the samples was iridoid monoterpene nepetalactone, wherein *trans,cis*- isomer was mostly present in methanol extracts of *N. rtanjensis*, while *cis,trans*-nepetalactone prevailed in *N. sibirica*. *N. nervosa* contained nepetalactone only in traces. Among phenolic acids, rosmarinic acid was predominant in all investigated species, while chlorogenic, neochlorogenic and caffeic acids were present in significantly lower concentrations. The results of ABTS and DPPH assays showed that methanol extracts of *N. rtanjensis, N. sibirica* and especially *N. nervosa* possess considerable antioxidant activities, and the FRAP assay revealed high ferric reducing capacity for all the tested samples.

Significant antioxidant activity was attributed to phenolic acids, particulary to rosmarinic acid. However, nepetalactone did not contribute notably to the antioxidant potential of the methanol extracts.

Keywords: *Nepeta rtanjensis, Nepeta sibirica, Nepeta nervosa,* antioxidant activity

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Chemical composition and antimicrobial activity of selected Apiaceae species against oral microorganisms by TLC bioautography

PP3-21

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Recently, plant essential oils have become one of the most valued source of biologically active substances. Plants from Apiaceae family are known as spices because of their flavour, and have been used as household remedies in traditional medicine, in perfumery and cosmetic industry. The aim of this study was to evaluate chemical composition and antimicrobial activity of *Pimpinella anisum*, *Foeniculum vulgare*, and *Anethum graveolens* essential oils by thin layer chromatography (TLC) based methods as repeatable and cost effective. Phytochemical characterization of oils was done by gas chromatography coupled with mass spectometry (GC/MS) and the presence of dominant components was proved by TLC. Antimicrobial activity against oral microorganisms (*Streptococcus mutans* and *Canidada albicans*) was evaluated using TLC-bioautography method. The results of chemical analysis showed trans-anethole for *P. anisum* and *F. vulgare*, and carvone as dominant components of essential oils. The antimicrobial activity against cariogenic bacteria *S. mutans* and opportunistic pathogen fungi *C. albicans* was also reported. A correlation of the antimicrobial activity of the oils from our study and their chemical composition suggests that the presented activity could be attributed to the presence of the major oil constituents. However, the amount of small compounds should not be neglected, as synergistic effect is often observed that is formed by the compounds acting together. In conclusion, TLC based methods are valuable tools, especially for resource constrained laboratories, as they are simple and compare better with traditional microbiological techniques.

Keywords: *P. anisum, F. vulgare, A. graveolens,* essential oil, TLC

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Anti-quorum sensing activity of *Melaleuca alternifolia*, *Leptospermum petersonii*, and *Pelargonium graveolens* essential oils

PP3-22

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Quorum-sensing (QS) inhibitors are described as the next generation "magic bullet" in treatment of bacterial infections and diseases. This bacterial cell density-dependent communication process regulates the release of virulence factors, antibiotic production and also biofilm formation. Recently, much attention has been devoted to the research of plant essential oils as potential source of quorum-sensing inhibitors. The aim of this study was to evaluate commercial samples of well-known essential oils of *Melaleuca alternifolia*, *Leptospermum petersonii*, and *Pelargonium graveolens* as potential quorum-sensing inhibitors. The QS activities towards the bacterium *Pseudomonas aeruginosa* PAO1 (piocyanin production and twitching and flagella motility) were evaluated *in vitro* for the first time. All the essential oils demonstrated inhibitory activity, reducing its production by 71.70%, 100.00% and 63.59%, respectively. Tested essential oils effectively reduced twitching and flagella motility of *P. aeruginosa* PAO1. From the results obtained in this study, it can be summarized that tested essential oils showed antiquorum-sensing activity. Selected plants play an important role as the antimicrobial agents and this study proves the importance of studying essential oils as potential QS inhibitors.

Keywords: *M. alternifolia, L. petersonii, P. graveolens,* essential oil, quorum-sensing

The authors are grateful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for the financial support (OI173032).

The moss *Rhodobryum ontariense* volatiles may inspire new therapies in the treatment of heart diseases including hypertension

PP3-23

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This work was focused on *in vitro* evaluation of anti-hydroxyl radical activity of the essential oil of the moss species *Rhodobryum ontariense* (Kindb.) Kindb. (Bryaceae), a Traditional Chinese herbal medicine claimed to cure heart disease, still in use in the Yunnan Province. The examined essential oil was previously isolated (using steam distillation) and chemically identified (by GC/GC-MS). Anti-hydroxyl radical activity of the aforementioned oil was estimated using fluorescence spectroscopy (FS) and electron paramagnetic resonance spec-

troscopy (EPR), respectively. This essential oil showed a significant anti-hydroxyl radical activity under *in vitro* conditions, both in the procedures based on FS (81%) and EPR (77%), respectively. Indeed, for the very first time the findings presented herein indicate the potential of the moss *R. ontariense* volatiles (a mixture of natural antioxidants with diterpene alcohol phytol as the main component) in the treatment of a broad range of cardiovascular diseases (including hypertension) whose pathophysiologies are mainly well described and directly linked to the screened free radical species.

Keywords: *Rhodobryum ontariense,* phytol

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Cytotoxic and antimicrobial activities of different plant part extracts of *Inula oculus-christi*

PP3-24

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Inula oculus-christi L. is a perennial species distributed in Asia, Central Europe and the Balkan Peninsula. In this study a potential cytotoxic and antimicrobial activity of ethanol extracts obtained from different parts of *I. oculus-christi* plants was investigated. Cytotoxic activity of extracts was determined on three tumor cell lines (HeLa, FemX and LS-174) by MTT assay. The extracts of leaves and flowers showed significant cytotoxic activity against all investigated cell lines, while rhizome, root and trunk extracts had no cytotoxic activity. The IC_{50} values for flower extract were 68.70 ± 2.79 μg mL^{-1} for HeLa cells, 88.91 ± 1.66 μg mL^{-1} for FemX cells and 81.46 ± 8.45 μg mL^{-1} for LS-174 cells, whereas the IC_{50} values for leaf extract were 73.69 ± 5.35 μg mL^{-1} for HeLa cells, 71.58 ± 4.25 μg mL^{-1} for FemX cells and 96.37 ± 4.92 μg mL^{-1} for LS-174 cells. The observed cytotoxic activity could be attributed to several bioactive sesquiterpenoids and flavonoids which were more abundant in leaf and flower extracts according to LC-DAD/MS non-targeted screening. In the antimicrobial bioassays against eight bacteria and microfungi, all tested extracts exhibited higher antimicrobial activity in comparison with commercial antimicrobial agents used as control. These results indicate the possibility of application of *I. oculus-christi* extracts as valuable natural products in medicine, agronomy and food industry.

Keywords: *Inula oculus-christi*, LC-DAD/MS, cytotoxic and antimicrobial activity

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Anti-cancer activities of jatrophane diterpenoids from *Euphorbia dendroides* in human multi-drug resistant cancer cell lines

PP3-25

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Widely distributed spurge – *Euphorbia dendroides* is a valuable source of new bioactive compounds. Among them, macrocyclic jatrophane diterpenoids, were recognized as new inhibitors of P-glycoprotein (P-gp) leading to modulation of multi-drug resistance (MDR) in cancer cells. In this study, we evaluated the anti-cancer activities of four jatrophane molecules isolated from *Euphorbia dendroides*. The effects of jatrophanes were studied in three different MDR human cancer cell lines developed in our laboratory (NCI-H460/R non-small cell lung carcinoma, DLD1-TxR colorectal carcinoma and U87-TxR glioblastoma cell lines). Tested jatrophanes showed moderate inhibitory effect on cancer cell growth. They significantly increased the accumulation of rhodamine 123 in MDR cancer cells. One of them completely blocked the P-gp pump in DLD1-TxR cells and demonstrated a stronger activity when compared to well-known P-gp inhibitors verapamil and tariquidar. Besides considerably high P-gp inhibiting activity, jatrophane diterpenoids showed significant potential to reverse the paclitaxel resistance in all MDR cancer cell lines. The combinations of jatrophane diterpenoids with low paclitaxel concentrations induced the cell cycle disturbance, which additionally contributed to their chemo-sensitizing activity. Jatrophane diterpenoids isolated from *Euphorbia dendroides* are promising compounds for a potential cancer treatment due to their strong anti-P-gp activity and can be considered as a valuable tool for drug development and the improvement of chemotherapy in MDR cancers.

Keywords: jatrophane diterpenoids, multi-drug resistance, P-glycoprotein, paclitaxel

Drinking of flavonoid-rich juice of *Aronia melanocarpa* may affect fatty acid phospholipid compositions in rat liver

PP3-26

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Aronia melanocarpa fruits are among the richest plant sources of phenolic substances, mainly anthocyanins. Aronia fruit juice and derived flavonoids were evaluated in a number of studies for high antioxidative activity, whose sequels were many beneficial physiological and metabolic effects, like hepato-protective, anti-inflammatory and antitumor. This study was designed to explore the effects of one month intensive consumption of aronia juice on phospholipid compositions in rat liver. Three experimental groups of young male rats were being supplied for 34 days with different drinking solutions *ad libitum*: 1. (ARO group) 20% (m/m) master aronia juice in tap water; 2. (MIX) 5% master aronia juice + 15% juice reconstruct (without flavonoids);

3. (PLC) 20% juice reconstruct. The animals were sacrificed on day 35 and their livers were weighed and used for analysis. The average mass of liver in ARO group was somewhat higher (13-14%) than in other two groups. In comparison with PLC, both experimental groups exhibited significantly lower proportions of stearic acid (18:0) and arachidonic acid (20:4), whilst the level of MUFA was increased only in MIX group on account of vaccenic acid elevation (18:1(7)). Also, there was a certain increase of omega-3 PUFA with a decline of omega-6/omega-3 PUFA ratio in both groups that consumed aronia juice. All these changes in the liver phospholipid compositions, which were induced by everyday drinking of flavonoid-rich aronia juice, appeared to be favorable regarding the healthy ratios of the liver fatty acid contents.

Keywords: *Aronia melanocarpa*, fatty acids, phospholipid compositions, rat liver

The influence of edible mushroom *Laetiporus sulphureus* (Bull.:Fr) Murr. extract on certain quorum-sensing regulated functions

PP3-27

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Quorum sensing (QS) is described as a set of behaviour which related with resistance of pathogenic bacteria. So far, biofilm formation, certain types of bacterial motility and production of pigments are most frequently associated with bacterial pathogenicity and ability to withstand the effect of antibiotics. Having in mind the growing trend of microorganisms resistance to known antimicrobial agents, efforts have been made to develop techniques which would indicate potential anti-QS of natural sources. In the last decade, numerous sources have been subjected to these protocols, including plants, algae, and other, marking them as promising agents. In this study, an edible mushroom, *Laetiporus sulphureus*, was tested for its potential as a QS regulator. This white-rot basidiomycete was previously described regarding its biological activity (including antimicrobial). Its methanolic extract showed dose-dependent antiqourum effect toward *Pseudomonas aeruginosa* PAO1 strain. Effects of the sub-MIC values of *Laetiporus sulphureus* methanol extract regulated production of virulence factor pyocyanin, reducing it by 80.88%, which is in the range of activity of commercial antimicrobial agents (Streptomycin 75.9% and Ampicillin 83.12%). Also, the extract had a significant inhibitory effect on flagella and twitching motility. Results indicate that *L. sulphureus* methanolic extract may be a promising agent for the eradication of pathogenic bacteria.

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Anticandidal activity of Miconazol and *Satureja montana* essential oil

PP3-28

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Opportunistic pathogen *Candida albicans*, is the main causative agent of oral candidiasis. In healthy individuals it is found as commensal organism but in persons that are immunocompromised it can cause serious infections such as the vaginitis in women and oral-pharyngeal thrush in AIDS patients. Bearing in mind all-growing resistance, especially against azole-based mycotics there is much interest in finding an effective and safe antifungal agents. In this study we investigated susceptibility of 48 different *Candida albicans* strains isolated from oral cavity of patients of Department of Pediatric and Preventive Dentistry, Faculty of Dental Medicine, University of Belgrade, Serbia to Miconazole and *Satureja montana* essential oil. Minimal inhibitory (MIC) and minimal fungicidal concentrations (MFC) were determined by microdilution 96-well method. All *Candida albicans* strains were found susceptible to miconazole (MIC < 4 μg mL^{-1}) and *S. montana* (MIC < 1 μg mL^{-1}). The results showed that the isolates susceptibility to essential oils exhibited stronger anticandidal activity. Despite the fact that *in vitro* studies cannot be directly extrapolated to *in vivo* effects, the results suggests that the use of essential oils such as *S. montana* against *C. albicans* could be a viable alternative, alone or combined with antifungal agents, for therapeutic and/or preventive purposes against oral candidiasis.

Keywords: *C. albicans*, miconazole, essential oil, *S. montana,* anticandidal activity

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In vitro screening of antibacterial activity of some edible medicinal mushrooms

PP3-29

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Antibacterial activity of 70% methanol extracts from lyophilised fruiting bodies of four edible mushroom species (*Fistulina hepatica* ((Schaeff.) With. 1801), *Laetiporus sulphureus* ((Bull.) Murrill 1920), *Macrolepiota procera* ((Scop.) Singer 1948) and *Meripilus giganteus* ((Pers.) P. Karst. 1882)) was evaluated by microdilution method under *in vitro* conditions. The bioactivity was screened against five referent bacterial strains (*Bacillus subtilis*, *Enterococcus faecalis*, *Esherichia coli*, *Salmonella enteritidis* and *Staphylococcus aureus*). In general, the examined extracts showed higher antibacterial activity against the Gram-positive bacteria (MIC and MBC values in the range of 0.07810 mg mL^{-1}). Indeed, only the fungus *F. hepatica* exhibited some activity against the Gram-negative bacteria (*E. coli* and *S. enteritidis*) with the lowest MIC and MBC values reached at 5 mg mL^{-1} and 10 mg mL^{-1}, re-

spectively. At the same time, this extract was the most active against *S. aureus* with the both MIC and MBC values of 0.078 mg mL^{-1}, while the *M. giganteus* extract achieved MIC and MBC values at 0.078 mg mL^{-1} and 0.312 mg mL^{-1}, respectively. Finally, the remaining two fungal species, *M. procera* and *L. sulphureus*, showed the lowest antibacterial activity against *S. aureus*, reaching MIC and MBC values at 0.156 mg mL^{-1} and 0.312 mg mL^{-1}, and 0.625 mg mL^{-1}, respectively. According to these experimental data, *F. hepatica* may be considered as a promising resource of novel antibacterials and should be the subject of intensive chemical investigation aiming to isolate and identify its bioactive natural product(s) addressing in such a way the possibility of synergistic effect as well.

Keywords: edible mushrooms, antibacterial activity, *Fistulina hepatica*

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Antioxidant potential of the extracts of the fruiting bodies and mycelia of the fungal species *Coprinus comatus*, *Coprinus micaceus* and *Laetiporus sulphureus*

PP3-30

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The antioxidant potential of ethanol extracts (80% EtOH) obtained from the fruiting bodies and mycelia respectively of the fungal species *Coprinus comatus* ((O.F. Müll.) Pers., 1797), *Coprinus micaceus* ((Bull.) Fr., 1838), and *Laetiporus sulphureus* ((Bull.) Murrill, 1920) respectively was investigated under *in vitro* conditions. Two types of the extracts were obtained: the first one, from the fruiting bodies (F) as a whole; the second one, using 40 days old mycelium (M), cultivated on malt agar. The dried fruiting bodies were ground to fine powder (2 g) and extracted with 80% EtOH to give the corresponding extracts, while the extracts of mycelia were prepared from the dried mycelium (0.05 g) immersed in 80% EtOH during 48 h. Free radical scavenging capacity (FRSC) was evaluated using DPPH test. The highest FRSC value was found for *L. sulphureus* extract (F) (IC_{50} = 118 μg mL^{-1}), while *C. comatus* (F) and *C. micaceus* (F) exhibited slightly lower FRSC activities (IC_{50} 148 μg mL^{-1} and 207 μg mL^{-1}, respectively). On the other hand, the FRSC values of the (M) extracts reached the following order: *C. comatus, C. micaceus* and *L. sulphureus* (IC_{25} 711 μg mL^{-1}, 857 μg mL^{-1} and 1257 μg mL^{-1}, respectively). The weaker total reducing capacity of the (M) extracts may be attributed to the different chemical/biochemical profiles of active substances (mostly, secondary metabolites) and/or the age of mycelium. Therefore, the DPPH test should be repeated using the younger mycelia, i.e. for the (M) extracts, in order to clarify the observed experimental data and draw the right conclusions.

Keywords: *Coprinus comatus, Coprinus micaceus, Laetiporus sulphureus,* antioxidant potential

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Rubus discolor Weihe & Nees - antioxidant activity, total phenol and flavonoid contents

PP3-31

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Rubus species are widely distributed globally as wild and cultivated plant species. Berries with high levels of anthocyanins, flavonoids and phenolic acids are a rich source of natural antioxidants. Antioxidant properties, total phenol and total flavonoid content of *R. discolor* leaf extracts were examined. Antioxidant activity was tested according to two free radical scavenging methods: DPPH and ABTS. The highest antioxidant activity was determined for aqueous (IC_{50} = 0.017 mg mL^{-1}), then methanol, ethanol and the lowest for acetone extract (IC_{50} = 0.03 mg mL^{-1}). Results obtained by ABTS assay, showed that IC_{50} values varied from 0.32 mg mL^{-1} for aqueous to 0.6 mg mL^{-1} for acetone extract.
Phenols and flavonoids are group of compounds isolated from many plant extracts which are often responsible for their antioxidant potential. The richest in phenols (359.19 mg GAE g^{-1}) was aqueous extract, while the highest amount of flavonoids (61.15 mg QE g^{-1}) was determined in acetone extract. The results indicate that antioxidant activity in tested samples could be ascribed to phenolic compounds, but not flavonoids. Additionally, it is more likely that antioxidant activity of *R. discolor* leaves is the result of some other groups of phenolic compounds and their synergistic effects. The highest antioxidant activity of aqueous extract is particularly significant as directive for further investigation, especially for food industry. Our data confirm that *R. discolor* leaves had a good antioxidant capacity and might be considered as a new source of phytochemicals in the nutraceutical and functional food market.

Keywords: *Rubus discolor,* DPPH, ABTS, phenols, flavonoids

Antitumour properties of *Veronica jacquinii* Baumg. and *Veronica teucrium* L. methanolic extracts

PP3-32

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In traditional medicine, several *Veronica* species are used to treat cancer. As currently circumscribed, *Veronica* (Plantaginaceae) is the genus of 450 species found in temperate regions of both hemispheres. Regardless of the widespread use, mostly as diuretics, for their wound-healing properties and in medical treatment of respiratory diseases, there is a scarcity of physiological evidence to support any claim of therapeutic values for *Veronica* species. Therefore, the aim of our study was to evaluate antitumour properties of methanolic extracts of *Veronica jacquinii* Baumg. and *Veronica teucrium* L. flowering aerial parts in Ehrlich ascites carcinoma (EAC) bearing mice. Parameters such as ascites volume, tumour cell count and cell viability were mon-

itored. Tumour was induced in mice by intraperitoneal injection of EAC cells. Pretreatment with dry extracts dissolved in water (5% w/v) at a dose level of 2 mL kg^{-1} body weight showed statistically significant decrease in ascites volume. This parameter is crucial for tumour growth, since it presents a direct nutritional source for tumour cells. In EAC control group the ascitic fluid was found to be 7.24 mL whereas it decreased to 5.33 mL and 5.12 mL in groups pretreated with *V. jacquinii* and *V. teucrium* extracts, respectively. Also, administration of investigated extracts led to slight, but statistically insignificant reduction of tumour cells viability and number of tumour cells compared to control group, demonstrating oncostatic activity. The above results indicate that *V. jacquinii* and *V. teucrium* deserve further research into their potential chemoprevention effects.

Keywords: *Veronica* spp., Ehrlich carcinoma, chemoprevention

IV
Phytochemistry

ANALYSIS
LABORATORY EQUIPMENT

INVITED TALKS

Early and late molecular mechanisms involved in the biosynthesis and accumulation of stilbenoids in elicited grapevine cell cultures established from berries

IT4-1

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The use of cyclodextrins, alone or combined with methyl jasmonate, as elicitors of *Vitis vinifera* suspension-cultured cells (VvSCC), has proved very effective in stimulating the production and extracellular accumulation of stilbenoids, chiefly *trans*-resveratrol (*t*-R). Understanding the molecular mechanisms involved in this process, from signaling to synthesis and transport out of the cell, is of paramount importance in order to design rationale approaches to exploit this system as an effective biotechnological platform for production of bioactive stilbene-family compounds. In this sense, we have undertaken a number of studies to disclose both early and late events. In relation to early events, a pharmacological approach was used to explore the relevance of Ca^{2+} fluxes, H_2O_2, NO and protein phosphorylation/dephosphorylation during the first minutes following elicitation in the elicitor-mediated production of *t*-R at 24 h. Late events have been explored up to 72 h from elicitation using genome-wide transcriptomics and proteomic analysis. All these results suggest that both elicitors provoke a strong activation of secondary metabolism, including shikimate, aromatic amino acid biosynthesis, phenylpropanoid and stilbenoid pathways, in detriment of basic cell processes like the primary metabolism or cell division. Proteomic experiments of time series highlight the co-expression of putative carrier proteins with stilbene synthases, further assessed by qPCR. Its functional role was demonstrated in transformed VvSCC, which were able to produce extracellular *t*-R in the absence of elicitors.

Keywords: *Vitis vinifera*, cell culture, stilbenoids, elicitor, molecular mechanisms

Secondary metabolites isolated from endemic Albanian plants by subcritical liquid CO_2 extraction

IT4-2

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Supercritical fluids have found a widespread use for extraction of secondary metabolites from plants. They have great advantages because they do not leave any traces of solvents into the extract, moderated temperature are used, and wide range of polarity can be achieved by adding polarity modifiers. The use of liquid CO_2 near its critical point appears to have some very special solvent features. Physical parameters of CO_2 at these conditions change very quickly. Such CO_2 has high diffusivity and low viscosity, like gases, and high density, like liquids, in

relatively low pressures. Because of that we have observed some very specific selectivity towards diterpenes, lipids and carotenoids. The yield of rotundifurane, from *Vitex agnus-castus*, is up to three times higher with subcritical CO_2 compared to conventional methods and SFE. Also the ratio of lycopene and β-carotene, extracted from tomato skin using the same method, changes drastically by changing extraction time and temperature conditions. The addition of additives also increases the yield and the composition of such extracts. The reason of this selectivity of subcritical liquid CO_2 towards certain compounds is not yet understood for the time being.

Keywords: subcritical CO2, secondary metabolites, *Vitex*, lycopene

Composition and therapeutic values of berry wines - bitter truth about sweet product

IT4-3

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Although vast amount of data corroborate sanative effects of various berries and their products, it is not easy to find comprehensive review about specific ones for berry and fruit wine. Increased interest in health prevention by proper and optimized nutrition has opened almost unbounded demand for new functional foods such as various fruit products, including different varieties of berry and fruit wines. Broad health benefit effects of berry and fruit wines consumption are given with particular attention conferred to cardiovascular, gastrointestinal and metabolic disorders, antioxidative and anti-cancerogenic protection, as well as sustaining cognitive functions. Polyphenolic constituents of fruits, also present in their wines, are beneficial to human health and contribute to the prevention of degenerative processes caused by different onsets such as aging, unbalanced diet, oxidative stress and even some inherited disorders. Since berries are known as potent source of various bioactive compounds, beneficiary effects of moderate consumption of corresponding wines can be anticipated. Detailed overview of the bioavailability of components with confirmed therapeutic potential is made, with focus on their occurrence and amount, as well as on diversity of their postprandial metabolic transformation. Unfortunately, although significant quantities of various polyphenols are present in the berry wines, they are not proportionally effective, either because they have a lower intrinsic activity or because they are poorly absorbed, highly metabolized, or rapidly eliminated from the body.

Keywords: berry wine, phenolic compounds, antioxidants, bioavailability, health

Phenolic profiles of wild fruits grown in Serbia

IT4-4

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The importance of the presented research lies in the underlining of the nutritional and functional characteristics of wild fruits that are unfairly forgotten and pushed aside in comparison to other cultivated spe-

cies. Our study on *Morus alba* species showed that mulberries are rich in health beneficial secondary metabolites. The characteristic phenolic acids and flavanoids were identified using Ultra High-Performance Liquid Chromatography coupled with Linear Trap Quadrupole and OrbiTrap mass analyzer (UHPLC-LTQ OrbiTrap MS). Quantification of polyphenolics was realized using UHPLC coupled with a diode array detector and triple-quadrupole mass spectrometer (UHPLC DAD-MS/MS). Differences in the contents of anthocyanins, phenolic acids and non-anthocyanins among all the samples were evident. Except for derivatives of chlorogenic acids, this was the first report on the identification of individual hydroxycinnamic acid esters in *Morus alba* L. fruits. Our findings suggested that polyphenols present in mulberry may act as potent superoxide anion radical scavengers and reducing agents, but with a moderate metal chelating ability. Our latest research in the field of phenolic profiling of indigenous fruits from Serbia was conducted on elderberry (*Sambucus nigra*), rose hip (*Rosa* sp.), and cornelian cherry (*Cornus mas*). In order to trace their characteristic phenolics, this work was focused on the quantification using UHPLC DADMS/MS and identification of target compounds using UHPLC-LTQ OrbiTrap MS and thin-layer chromatography coupled with mass spectrometry. Distinctive profiles were obtained. The major polyphenols extracted from the rose hip samples were identified as monomeric and polymeric flavan-3-ols, while anthocyanins were characteristic for elderberry and cornelian cherry.

Keywords: polyphenolics, mulberry, elderberry, rose hip, cornelian cherry

SELECTED TALKS

NMR structure elucidation of a new alkaloid isolated from *Gymnospermium maloi*

OP4-1

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Gymnosperium maloi (Tan, Shuka, Maloi) is a new endemic species from the *Gymnospermium* genus identified in the south region of Albania. The members of this genus are poorly studied for what it concern the secondary metabolites in general and the class of alkaloids in particular. To the best to our knowledge, from the genus *Gymnospermium*, there is only one alkaloid characterized, (namely albertramine) isolated from *Gymnospermium albertii*, nearly 40 years ago. Until now, the chemical composition and the structure elucidation of secondary metabolites, especially alkaloids, other than albertramine, remained still largely unknown. Accepting this interesting challenge our group focused its research work on the isolation, characterization and structure elucidation of these compounds. Here we report on the structure, for the very first time, of a new alkaloid isolated from *G. maloi*, designated as maloine, obtained by the use of 2D homonuclear and heteronuclear NMR spectroscopy, FTIR, and HPLC/MS spectra. Further studies are going on in our lab attempting to elucidate the structure of other possible new chemical compounds isolated from *G. maloi*.

Keywords: *Gymnospermium maloi*, NMR spectroscopy, alkaloids, secondary metabolites

Thapsic acid, a rarely found natural product among bryophyte species

OP4-2

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Bryophytes are the second largest group of land plants, after the flowering plants, with about 25,000 species. The phytochemistry of these plants has been neglected for a long time because they are very small and difficult to collect in large amounts as pure samples. Today, bryophytes are known to be a reservoir of interesting and useful chemicals including acetylenic fatty acids. The objective of This study was to preliminarily identify fatty acid pattern of the moss species *Dicranum montanum* Hedw. (Dicranaceae) collected in the winter time in Germany. Air-dried parts of D. montanum were ground (1 g) and extracted three times with the system chloroform:methanol 1:1 for 1 h at room temperature (8.27%, extract yield). The extract was evaporated to dryness and further transesterified with 5% sulphuric acid in methanol (v/v) for 4 h at 80 °C. The resulting methyl esters of fatty acids were analysed by comparing their GC FID chromatograms with the chromatogram of a standard mixture (Supelco 37) obtained under the same conditions, and//or by analysis of GC–MS data using NIST 5 and Wiley 7 libraries. Besides common fatty acids such as palmitic acid, stearic acid, lin-

oleic acid and cis-8,11,14-eicosatrienoic acid, the presence of thapsic acid (synonyms: hexadecanedioic acid and thapsate) was observed (10%). Till date this dicarboxylic acid has been rarely found in mosses (*Hylocomium splendens* and *Pleurozium schreberi*), but such records practically belong to the older ones. However, thapsic acid has been previously reported for the genus *Dicranum*, more specifically for the species *D. elongatum*.

Keywords: bryophytes, *Dicranum montanum*, thapsic acid

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI 172053).

POSTER PRESENTATIONS

Localization of nepetalactone and dehydronepetalactone biosyntehesis and accumulation in *Nepeta rtanjensis* leaves

PP4-1

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Nepeta rtanjensis Diklić & Milojević (Lamiaceae) produces potent bioactive iridoid monoterpenes nepetalactone and dehydronepetalactone. However, the exact production site of these monoterpenes *in planta* has not yet been determined. Generally, glandular trichomes are known to be the main tissue where biosynthesis, accumulation and secretion of volatile secondary metabolites take place. In our work, the tissue localization of nepetalactone and dehydronepetalactone was determined by dichloromethane-dipping of freshly harvested *Nepeta rtanjensis* leaves. Extracts from leaves after organic solvent dipping and the solvent after leaf dipping, which contained trichome-specific compounds, were separately analyzed by UHPLC/DAD/+HESI-MS. The efficiencies of four different dichloromethane-dipping durations (20, 40, 120 and 300 seconds) were evaluated. Relative contents of both nepetalactone and dehydronepetalactone increased in extracts of glandular trichomes concomitant to the increase in dipping duration. On the other hand, we observed a decrease in the relative contents of these compounds in extracts of leaves after dipping with the increase of dipping duration. As confirmed by SEM, the extraction procedure we used has caused a collapse of the subcuticular space of the capitate glands on the leaf surface, whereas no other damage to the leaf surface has been observed. Our data implicate that the leaf glandular trichomes are a major site of storage and accumulation of nepetalactones. This finding is of prime significance for the further elucidation of nepetalactone biosynthetic pathway.

Keywords: *Nepeta rtanjensis*, nepetalactone, dipping, glandular trichomes

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173024).

A new method for increasing antioxidant activity in maize

PP4-2

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Blue kernel maize is popular for human food products. Blue colour originates from anthocyanins that are mainly located in the maize aleurone layer and show high antioxidant and antiradical activities. Compared to other major crops, corn has the highest phenolic content and antioxidant activity. Blue corn germplasm mostly originates from open pollinated varieties showing poor agronomic performances, therefore, development of blue hybrids is necessary to intensify blue maize production. However, more than 20 genes play a role in anthocyanin biosynthesis pathway in maize, making conversion of commercial maize lines to blue colour time and effort demanding.

Top-cross production system can be effective in obtaining blue maize in one year. This system is based on xenia effect caused by cross pollination, with the idea to grow mixture of 80% sterile yellow hybrid and 20% of blue pollinator in a commercial production. In a preliminary research five yellow sterile hybrids were pollinated with blue pop-corn variety. Average total antioxidant capacity and total phenolics for cross-pollinated hybrids were 19.89 mmol Trolox Eq kg^{-1} and 5526.49 mg GAE kg^{-1}, respectively, comparing to 17.58 mmol Trolox Eq kg^{-1} and 5015.65 mg GAE kg^{-1} for yellow self-pollinated hybrids. Blue maize produced in Top-cross system in three locations yielded more than 14 t ha^{-1}, confirming good agronomic performances. Five blue pop-corn varieties and 25 blue common varieties have been found in MRIZP gene bank and are being analysed for antioxidants content. New pollinators for Top-cross system are expected to be found among them.

Keywords: antioxidant activity, blue maize, phenolics, xenia

Preliminary evaluation of autochthonous apple cultivars from Republic of Srpska

PP4-3

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Autochthonous assortment of apples is a source of rich gene pool, which is due to its positive characteristics important for breeding process. Based on pomological analysis and biochemical constitution of fruits, in this research a preliminary evaluation of autochthonous apple cultivars from Republic of Srpska was carried out. Results should be used for the selection of materials for multiplication and raising collection plantations, as well as creating the basis for breeding program. We analyzed twenty-three autochthonous apple cultivars, which are the fruits sampled during 2012. The fruits were taken from trees that grew in free form without the use of agrotechnical measures. For pomological caracteristics, fruit mass and fruit flesh firmness was monitored, and for biochemical parameters, soluble solid content, pH, total dry matter content, content of total organic acids, vitamin C, total phenolics, total flavonoids and antioxidant potential in the cell juice of the fruit flesh were analyzed. The highest content of vitamin C was recorded in the cultivar Šećeruša that also had the highest total phenolics, which is in acordance with good antioxidant activity of this cultivar. Beside Šećeruša, cultivar Litrenjača, whose fruit had the highest friut mass, total flavonoids and total organic acids, can be selected.The cultivar Mirisavka had the lowest fruit flesh firmness and soluble solid content, which indicated poor pre-dispositions of this cultivar for longer storage. High variability of the studied parameters among cultivars should be taken into account for breeding program and conservation.

*Keywords:*x, total phenolics, DPPH, quality

Phenolic profile and antioxidant activity of two Quinoa varieties: combined effect of aging and salt stress

PP4-4

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Quinoa is a dicotyledonous facultative halophytic plant species displaying optimal growth at high salinity levels. We analyzed the relative contribution of enzymatic and non-enzymatic antioxidants in response to salt stress (400 mM NaCl) in leaves of different physiological ages (i.e. leaf positions on the stem) of two Quinoa varieties: salt-tolerant Bolivian Quinoa #10 and salt-sensitive Chilean Quinoa #5206. Higher activity of the major antioxidant enzymes (superoxide dismutase and ascorbate peroxidase) were detected in salt-treated leaves of both varieties. However, total antioxidant activities of salt-tolerant control were higher compared to the treated leaves. At the same time, antioxidant activities were constitutively higher in the sensitive compared to tolerant variety. Total phenolic content (TPC) of salt-sensitive leaves contributed to the overall antioxidant activity in salinity treatment in contrast to the salt-tolerant variety where the influence of both salinity and aging on TPC was not observed. HPLC/MS analysis of individual phenolic compounds revealed increased amount of phenolic acids in salt-sensitive treated leaves and during aging of the same variety as well. Reversed trend was observed in salt-tolerant variety where the concentration of phenolic acids decreased with aging. However, concentrations of particular phenolics were lower after salt treatment of salt-tolerant variety. It could be concluded that salinity induced accumulation of individual and total phenolics, as well as enzyme activity increment in salt-sensitive variety, pointing out the varietal differences in salinity tolerance. Nevertheless, direct link between salinity and aging was not observed in any of the varieties.

Keywords: ascorbate peroxidase, HPLC/MS, superoxide dismutase, total antioxidant capacity, total phenolic content, salinity

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Investigation of phenolic profile of Serbian autochthonous wines

PP4-5

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The aim of this study was to identify and quantify the main phenolic compounds of wine of eight Serbian autochthonous grapevine varieties. The study was conducted on four white wine varieties: Kreaca, Smederevka, Tamjanika bela, Plovdina and two red wine varieties Prokupac and Tamjanika crna. Grape samples of these varieties were collected at harvest and used for monovarietal wines production under the same microvinification conditions. Quantification of 30 phenolics was done using UHPLC coupled with a linear ion trap-OrbiTrap hybrid mass spectrometer. The total phenolic content (TPC) of white wines ranged from 0.141 g GAE L^{-1} wine (Smederevka) to 0.204 g GAE L^{-1} wine (Kreaca), while in red wines it ranged from 0.574 g GAE L^{-1} wine (Crna

Tamjanika) to 1.010 g GAE L^{-1} wine (Prokupac-Blace). Scavenging activity of samples evaluated using DPPH reagent was the highest for red wine Prokupac-Blace (6.358 mmol TE L^{-1} of wine sample) and the lowest for white wine Smederevka (0.535 mmol TE L^{-1} of wine sample). The highest concentration of total anthocyanins was found in Prokupac-Blace (97.242 g mal 3-glu L^{-1}) and the lowest in Crna Tamjanika (36.990 g mal 3-glu L^{-1}). Plovdina wine was characterized with somewhat higher TPC, RSA, and TAC values when compared to other white wines (0.792 g GAE L^{-1}, 4.420 mmol TE L^{-1}, and 3.523 g mal 3-glu L^{-1}, respectively). Finaly, TPC was compared with RSA, and it was well correlated (r = 0.99).

Keywords: Serbian autochthonous wines, UHPLC LTQ OrbiTrap MS, antioxidant activity

Antioxidative potential of mycelia extracts of some wastewater isolated *Penicillium* species

PP4-6

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The genus *Penicillium* is well known for its importance in drug and food production. In the current study, three *Penicillium* species – *P. funiculosum*, *P. chrysogenum* and *P. lividum* were selected for evaluation of their potential antioxidant activity. The fungi were isolated from wastewaters originating from households and flowing directly into the River basin of Great and Western Morava (Serbia). The species were obtained after inoculation and growth in liquid PDA. After five days of incubation at room temperature, the fungal biomasses were separated from the fermentation brought, dried and extracted with ethanol. On mycelia ethanolic extracts of each fungus, screening of antioxidant activity was performed using following assays: DPPH free-radical scavenging activity, total antioxidant activity, Fe^{2+}-chelating ability and Fe^{3+}-reducing power. Also, the total phenolic content was determined by Folin-Ciocalteu method. The ethanolic extract of *P. lividum* had the highest total phenolic content (6.39 mg GAE g^{-1}) followed by *P. chrysogenum* (2.86 mg GAE g^{-1}) and *P. funiculosum* (2.11 mg GAE g^{-1}). Total antioxidant activity was almost unique among fungi as follows: P. *chrysogenum* (3.87 μg AA g^{-1}), *P. funiculosum* (3.17 μg AA g^{-1}) and *P. lividum* (3.09 μg AA g^{-1}). The DPPH free-radical scavenging activity of fungi was 77.40% for *P. lividum*, 51.34% for *P. funiculosum* and 37.42% for *P. chrysogenum*. The ethanolic extract of *P. chrysogenum* showed the best ferrous ion chelating capacity whereas fungus *P. funiculosum* had the best reducing power (A_{700nm} = 0.856). All three species of *Penicillium* can be considered as a promising source of natural antioxidants and a possible preventive agent of some common human health disorders.

Keywords: total antioxidant activity, total phenolic content, DPPH free-radical scavenging activity, Fe^{2+}-chelating ability, Fe^{3+}-reducing power

Antioxidant activities and total phenol contents of *Cuscuta campestris* stem collected from *Beta vulgaris*

PP4-7

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Plants contain high amounts of polyphenols, which are very potent natural antioxidants. *Cuscuta campestris* is a nonspecific parasitic plant of wide range of commercial host species (alfalfa, sugar beet, onions, potato, tomato, legume, *etc.*) and its phytoconstituents vary depending on the type of the host. The present study was designed to evaluate the relative contribution of different polyphenols and their antioxidant activities. For this purpose, the total phenolics, flavonoids and flavonols content of *C. campestris* stem collected from *Beta vulgaris* were determined in the methanol, methanol:H_2O (70:30, w/w), acetone, ethyl acetate and *n*-butanol extracts. Screening of antioxidant activity of each extract was performed using following assays: DPPH and ABTS free-radical scavenging activity, total antioxidant activity, Fe^{2+}-chelating ability, Fe^{3+}-reducing power and inhibition of lipid peroxidation. Maximum level of total phenol content (102 mg GAE g^{-1}), the highest total antioxidant activity (26.59 μg AA g^{-1}), DPPH (IC_{50} = 889.36 μg mL^{-1}) and ABTS (IC_{50} = 185.65 μg mL^{-1}) scavenging activities; reducing power (IC_{50} = 0.615 μg mL^{-1}), chelating activity (IC_{50} = 9.48 μg mL^{-1}) and inhibitory activity against lipid peroxidation (IC_{50} = 71.56 μg mL^{-1}) were found in methanol:H_2O extract. Maximum levels of total flavonoid content (56.10 mg RUE g^{-1}) and flavonols (6.51 mg QUE g^{-1}) were found in ethyl acetate extracts of the plant. The results of the present study revealed that aqueous methanolic extract of *C. campestris* stem exhibited higher antioxidant activities due to its higher phenolics content. The obtained data would certainly help to ascertain the potency of the *Cuscuta* stems as potential source of natural antioxidants for food and pharmaceutical industries.

Keywords: *C. campestris*, antioxidant activity, total phenol content, flavonoids, flavonols

Antimicrobial and antigenotoxic properties of *Digitalis lamarckii* endemic plant from Turkey

PP4-8

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Digitalis lamarckii Ivanina, (gen. *Digitalis*, fam. Plantaginaceae) - dwarf foxglove, is a plant species endemic to the rangelands of Turkey. Members of the genus *Digitalis* are of great medicinal importance as they contain cardiac glycosides, which can increase the force of systolic contractions and regulate heart rhythms. The purpose of This study was to evaluate antimicrobial and antigenotoxic properties of *D. lamarckii* aerial part ex-

tract. The microdilution method for evaluation of antimicrobial activity of *D. lamarckii* extract was performed on the selected strains of bacteria and fungi. The antigenotoxicity of *D. lamarckii* extract (80 mg mL^{-1} of food) against ethyl methanesulphonate (EMS, 1 mM) induced genotoxicity was evaluated *in vivo* in the anterior midgut of *Drosophila melanogaster* using modified alkaline comet assay. The total phenolic and flavonoid contents were also determined. The total phenolic content was 36.60 mg GAEs g^{-1} and total flavonoids content was 40.99 mg REs g^{-1}. The MIC values indicate that *D. lamarckii* exhibited good antibacterial properties against *Pseudomonas fluorescens* (MIC 1.25 mg mL^{-1}), *Escherichia coli* and *Azobacter chroococum* (MIC 10 mg mL^{-1}). The extract exerted excellent antifungal activity against *Phialophora fastigiata* (MIC 2.5 mg mL^{-1}). The most resistant fungi were *Candida albicans*, *Aspergilus niger* and *Doratomyces stemonitis*. High significances in the reduction of % DNA in tail (80.3%) were found in the group simultaneously treated with EMS and extract, with an average frequency of selected parameter of 10.17 ± 0.51 that was similar to that of the negative group (8.42 ± 0.70).

Keywords: *Digitalis lamarckii,* phenolic content, antimicrobial activity, antigenotoxic potential

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (III43004 and III41010).

HPLC analysis and *in vivo* assessment of the genotoxicity and antigenotoxicity of the *Filipendula ulmaria* methanol extract

PP4-9

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Filipendula ulmaria L. is commonly used in traditional folk medicine in the region of the central Balkans to treat fever, the common cold, arthritis and other inflammatory conditions. The aim of the present study was to determine possible *in vivo* genotoxicity of extracts (20, 40 and 80 mg mL^{-1} of food) from the roots and aerial parts in the anterior midgut of *Drosophila melanogaster* using modified alkaline comet assay and protective effect of the highest dose of extract against ethyl methanesulphonate (EMS) induced genotoxicity. HPLC was employed for the identification of the phenolic compounds present in extracts. In both extracts caffeic acid glucosides and procyanidin derivates were identified. In aerial part extract, HPLC analysis showed the presence of catechin, epicatechin, salycilic acid, rutin, hyperozide and several quercetin glucosides. There were no statistically significant differences in total scores between negative and groups treated with *F. ulmaria* root extract, while aerial part extract had weak genotoxic effects depending on the concentration. The percentage reduction in DNA damage was more evident in group treated simultaneously with EMS and root extract (87.5%) and less expressive in group treated with aerial part extract (54.7%). Results of the study provide scientific basis for the use of this plant extract in the future development as antigenotoxic agent.

Keywords: *Filipendula ulmaria,* DNA damage, antigenotoxicity, HPLC profiling

This work was financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia (III43004 and III41010).

The influence of elicitors yeast extract and chitosan on xanthone accumulation in hairy root culture of *Gentiana dinarica* Beck.

PP4-10

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Continuing studies of secondary metabolites in root cultures of *G. dinarica,* we examined the effects of biotic elicitors, yeast extract and chitosan on the xanthone production in A4 M70GUS transformed roots. Hairy roots were maintained in 40 mL liquid ½ MS plant growth regulator-free medium. Hairy roots were treated with 1, 2 and 5 g L^{-1} yeast extract, or 5, 10, 20, 50, 100 and 200 g L^{-1} chitosane. Cultures were harvested after three or seven days of elicitor treatments. Air dried hairy roots were extracted with methanol and analyzed using HPLC-DAD method. Two xanthones, norswertianin-1-*O*-primeveroside and norswertianin were identified and quantified. Both yeast extract and chitosan influenced the growth of the roots, as well as the production and accumulation of xanthones. The root growth index and content of xanthone norswertianin-1-*O*-primeveroside decreased with increasing concentration of both elicitors. On the contrary, increased elicitor concentration stimulated the production of xanthone aglycon norswertianin. At higher concentrations (50, 100 and 200 g L^{-1}), chitosan had a more pronounced effect on growth index and xanthone accumulation than the yeast extract. At highest concentrations, both elicitors induced necrosis in hairy root cultures.

Keywords: *G. dinarica*, elicitors, xanthone, hairy roots

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173015).

The relationship of physicochemical properties and structure to the antioxidative activity of free amino acids in the aqueous Fenton system

PP4-11

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Iron is essential for plant growth, metabolism of mitochondria and chloroplasts, and a wide assortment of enzymes. However, it is also involved in Fenton reaction that generates hydroxyl radical (HO•), the most reactive species in plants. The aim of our study was to examine the effects of amino acids on HO• production. The rank order according to antioxidative activity (AA) of amino acids in Fenton system was: Trp > Phe, Leu > Ile > His > Arg > Val > Lys, Tyr, Pro > Gln, Thr, Ser > Glu, Ala, Gly, Asn, Asp. Sulfur-containing amino acids generated different secondary reactive products, which were discriminated by EPR spin-trapping spectroscopy. AA showed positive correlation with hydrophobicity and negative correlation with polarity. HO•provoked oxidation of amino acids was strongly positively affected by hydrophobic hydration. Group contribution method showed that the reactivity of amino acids with HO• is defined by the properties of side-chains (the contri-

bution of $NH3^{+}CHCOO^{-}$ group and antioxidative activity of Gly were next to zero). Our results might shed a new light on the role of iron in the regulation of free amino acids pool and on protein oxidation in plants. According to our findings, native proteins (with exposed polar residues) might not be particularly susceptible to oxidation by HO•. But once a protein is damaged and partially denatured it exposes hydrophobic side-chains and might become a likely target for HO•, thus potentially acquiring an antioxidative role. Further research on change of redox properties of proteins with denaturation is warranted.

Keywords: amino acids, antioxidant, hydroxyl radical, EPR

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173017, III43010 and OI173014).

Essential oil composition of *Centaurea murbeckii* Hayek - endemic Balkan species

PP4-12

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The composition of essential oil isolated from fresh capitula of *Centaurea murbeckii* Hayek (Asteraceae), an endemic species of the Balkans (Mt. Zelengora, BIH), was analyzed. This is the first report of essential oil composition of this taxon. Essential oil was obtained by simultaneous distillation extraction, using Likens–Nickerson type apparatus. The oil was analyzed by gas chromatography–mass spectrometry (GC-MS). In total, 76 compounds were identified, representing 98.9% of the total oil. The main constituents were: Germacrene D (14.15%), 2-ethylhexyl isohexyl ester phtalic acid (9.83%) and (*E*)-caryophyllene (9.74%). The essential oil was dominated by sesquiterepenes (53.9%): sesquiterpene hydrocarbons and sesquiterpene oxygenated (31.7%, 22.2%, respectively). Other compounds (aliphatic hydrocarbons, aliphatic aldehydes and alcohols, aliphatic acids and their esters and aldehydes, aromatic esters and aliphatic acids, alkyl aromatic alcohols, aryl esters of aromatic acids) represented 35.1% of the essential oil. According to our results and literature data, essential oils of most *Centaurea* species were characterized with Germacrene D as a dominant constituent. According to our previous investigations, this compound is not present in some *Centaurea* species from the Balkan Peninsula. Future investigation of *Centaurea* species is planned to show range of variability in essential oil composition and especially Germacrene D content and their taxonomic utility.

Keywords: *Centaurea murbeckii,* Asteraceae, essential oil, Germacrene D

Phenolic compounds and antioxidant properties of sweet cherry fruits

PP4-13

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Sweet cherry (*Prunus avium* L.) is a species with a great economic importance, due to the commercial, technological, and nutritional value of its fruits. Red fruits are excellent sources of bioactive components such as anthocyanidins, phenolic acids, flavonoids, and other phenolic compounds. Phenolic compounds are mostly concentrated in the skin of sweet cherry fruits and contribute to sensory and organoleptic qualities. In plants, phenolic compounds serve as signaling compounds, pigments, and defense mechanisms in order to survive and prevent molecular damage by reactive oxygen species, and damaging by animals and pathogens. The biological activity of these compounds is associated mostly with their high antioxidant properties. The aim of this work was to determine the content of different phenolic compounds (total phenolics, tannins, anthocyanins and flavonoids) from fresh fruits of three different sweet cherry cultivars (Margit, Linda and Aida) extracted with 70% ethanol and 70% acetone solutions, and to estimate their antioxidant activity with different assays (DPPH, FRAP, ABTS and phosphomolybdenic method). The results of our research show large variability among cultivars in chemical attributes. Our results suggest that the antioxidant capacity of sweet cherries is positively related with measured phenolic compounds. The difference in the sweet cherry genotypes in terms of measured chemical attributes is due to genetic variations, as all genotypes were of the same age and grown under the same ecological and agrotechnical conditions. The results indicated the Aida cultivar as the richest source of bioactive ingredients with highest antioxidant capacity.

Keywords: antioxidant capacity, phenolics, sweet cherry

The influence of fruiting-spur thinning and harvest time on changes in fruit quality of sweet cherries

PP4-14

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The influence of three different intensities of hand fruiting-spur thinning during ripening on physical (fruit weight, fruit diameter, index of fruit shape and length of stem) and chemical fruit properties (soluble solids content – SSC, total acids – TA, total anthocyanin content – TACY, total phenolics – TPH and total antioxidant capacity – TAC) of two sweet cherry cultivars 'Burlat' and 'Stark Hardy Giant' were studied. The following fruiting-spur thinning treatments were performed in this study: a) thinning the first three fruiting spurs located below 1-year-old wood on 2-year-old wood, b) thinning every third fruiting spur on 2-year-old wood and c) thinning every second fruiting spur on 2-year-old wood. Fruit weight and diameter of both studied cultivars were significantly increased by fruiting-spur thinning. During the ripening, slightly before harvest, enhance of fruit weight and diameter was observed in 'Burlat', whereas no significant differences in fruit size across harvests were recorded in 'Stark Hardy Giant'. The highest SSC in fruits of 'Burlat' was obtained in treatments with thinning every second and third fruiting spurs on 2-year-old wood and in the fruits of the last harvest. Conversely, 'Stark Hardy Giant' did not differ in SSC affected by spur thinning and harvest time. Intensity of fruiting-spur thinning had no significant influence on TACY, TPH and TAC of the fruits in either of the studied cul-

tivars. As opposed to that, harvest date significantly influenced TACY and TAC with higher values obtained in fruits harvested later. No differences were observed only in TPH across harvests.

Keywords: sweet cherry, cultivars, intensity of fruiting-spur thinning, harvest date, physical and chemical fruit properties

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173040 and 46008).

Variability of needle essential oil composition of *Juiperus communis* L. var. *communis* from Serbia

PP4-15

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Needle essential oil from four populations of *Juniperus communis* L. var. *communis* in Serbia was obtained by simultaneous distillation extraction using Likens-Nickerson type apparatus. Obtained essential oil was analyzed using GC/MS. In total, 117 compounds were identified in essential oil, of which 75 represent 98% of the total oil. The oil was dominated by monoterpenes (75.2%), a characteristic of species belonging to the section *Juniperus*. Two monoterpenes, αpinene and sabinene, represented, on average, 55% of the total oil. Univariant and multivariant statistical methods were used to determine the diversity of the terpene classes and the common terpenes among analyzed populations. Hierarchical cluster analysis and Discriminant analysis showed existence of three hemotypes exhibiting strong geographic correlation. The comparison of the essential oil compostion obtained in the present study with the literature data, showed strong clinal distribution of essential oil composition from west to east, and simplification of esential oil composition from south to north of Europe. Chemotaxonomic implications of essential oil composition are briefly discussed.

Keywords: *Juniperus communis,* essential oil composition, chemotypes, chemotaxonomy, Serbia

Antioxidant activity of some boraginaceae family plants from Bosnia and Herzegovina

PP4-16

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The aim of this study was to determine antioxidant capacity of leaf and flower extracts for three borage family plants: *Symphytum tuberosum*, *Echium rubrum* and endemic *Halacsya sendtneri*. Many species within this family contain chemical compounds known for both their medicinal and health benefit properties, and are often used in traditional medicine. Plants used in this study were collected from the area around the town of

Žepče, from natural serpentine complex in Central Bosnia and Herzegovina. The antioxidant capacity of the methanol extracts was evaluated by using the DPPH free-radical scavenging assay. Results obtained in this study have revealed that all extracts demonstrate good free-radical scavenging ability, but the extracts of *S. tuberosum* and *E. rubrum* leaves showed remarkably potent activity (IC50 = 0.08 mg mL^{-1} and 0.19 mg mL^{-1}), even higher than the substances used as suitable standards.

Keywords: Boraginaceae, DPPH, *Halacsya*, *Symphytum*, *Echium*

Identification of phenolic compounds in cecidogen and *Linaria vulgaris*. Changes in phenolic metabolism during gall formation induced by *Rhinusa pilosa*

PP4-17

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Rhinusa pilosa (Gyllenhaal) (Coleoptera, Curculionidae) induces galls in *Linaria vulgaris* Mill., Yellow or common toadflax (Plantaginaceae). Gall is defined as atypical plant growth induced by host-specific organisms, and represents larval chamber which provides food and protection during insect development. It has been shown that gall formation is triggered by ovoipositional fluid (cecidogen). In order to determine which cecidogen compounds may have bioactive properties, we performed comparative analysis of phenolics profiles of cecidogen and stem extracts of *L. vulgaris* using UHPLC coupled with OrbiTrap mass analyzer. In addition, we analyzed the effects of feeding, oviposition and early gall development on Class III peroxidase activity (POD, EC 1.11.1.7) and phenolic content in the stems of *L. vulgaris* during seven days. Out of 55 phenolic compounds identified in cecidogen and *L. vulgaris* stem, one unknown phenolic glycoside was found only in cecidogen, which produced an MS^2 base peak at 387 *m/z*, and 327 *m/z* and 267 m/z base peaks at MS^3 and MS^4 fragmentation, respectively. Gall development during seven days was accompanied by decreased concentrations of hydroxybenzoic, hydroxycinnamic acids and flavonoids and by slight lignin deposition. An oscillatory induction of POD activity, with the first peak obtained 3 h after oviposition, was related to oxidative burst during stem wounding and oviposition. Overall results suggested the importance of phenolics in stem structural changes and regulation of plant metabolism induced by *R. pilosa*.

Keywords: cecidogen, gall, *Rhinusa pilosa, Linaria vulgaris,* phenolics

Comparison of antioxidant activity and polyphenol content in peeled and unpeeled apricot cultivars and selections

PP4-18

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Prunus armeniaca L. (Rosaceae), commonly known as "apricot", is one of the most important drupes. Apricot fruits, with velvety skin and flesh, are consumed all over the world. They are enriched with a number of health-promoting phytochemicals. Polyphenols are plant secondary metabolites that exhibit a wide range of biological effects as a consequence of their antioxidant properties. This study investigated antioxidant potential of two apricot cultivars ("NS–6", "NS rodna") and three selections ("SK 3", "SK 13a", "SK 16a") grown in orchard in Maradik, Serbia. Fruits were collected from 6-year-old trees. Total phenolics, flavonoids, tannins and condensed tannins (proanthocyanidins) were determined in 80% ethanol extracts of peeled and unpeeled dried fruits. Total antioxidant activity was assessed with DPPH and FRAP test. Extracts of unpeeled fruits of all examined cultivars and selections showed higher antioxidant activity in both tests. Total phenolic content was in a positive correlation with used antioxidant assays. The content of flavonoids, tannins and condensed tannins differed among cultivars and selections depending on the sample preparation. Peeled fruits of tested selections had higher content of flavonoids and condensed tannins probably due to their genetic characteristics. These results indicate that other compounds, apart from polyphenols, are also responsible for high antioxidant capacity of investigated fruits.

Keywords: apricot, peeling, polyphenols, antioxidants

Seasonal dynamics of *Teucrium chamaedrys* L. (Lamiaceae) phenolic content and antioxidant activity

PP4-19

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In order to determine phenological variability of content and biological activity of secondary metabolites, total phenolics and flavonoid content as well as antioxidant activity of methanolic extracts from different vegetative stages of *Teucrium chamaedrys* L. (Lamiaceae) were investigated. Plant material was sampled from the same population during four different stages of vegetative period: early vegetative stage, before flowering, during flowering and after flowering period. The total phenolic content (expressed in terms of gallic acid equivalent per g extract) varied from 151.78 mg GA g^{-1} to 201.35 mg GA g^{-1}. The results obtained for the quantity of flavonoids only (expressed in terms of rutin equivalent per g extract) were in the range from 66.54 mg RU g^{-1} to 74.31 mg RU g^{-1}. Variability was also found for the antioxidant activity – IC_{50} values were in the range from 10.80 μg mL^{-1} to 19.29 μg mL^{-1}. Based on the results, the dynamics of phenolics indicates a difference in the intensity of synthesis of these compounds during vegetative period. Results of determination of antioxidant activity showed that the intensity of the activity slightly increased from the early vegetative stage until the

beginning of flowering period. The highest value was measured during flowering and post flowering period. The difference in the synthesis and activity of phenolics is caused by the vegetative stage and differences in the regime of the environmental factors, indicating their importance during plant growth and development.

Keywords: *Teucrium chamaedrys,* phenolics, phenological variability

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V

Biodiversity and Conservation

INVITED TALKS

Phenotypic plasticity or new taxa?

IT5-1

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Investigations of infraspecific variability have a long tradition in plant taxonomy. During the 19th century, and especially in the second half of the 20th century, a whole set of specific taxonomic schools appeared and they tend or even favor infraspecific variability. Due to decades of describing a new infraspecific taxa and consequently general increase in their number, local and nationals floras have been loaded with numerous nomenclature and unresolved problems. However, modern tendencies in taxonomy include almost obligatory separation of type subspecies as basic taxa, and quite often beside the other subspecies with restricted distribution, a more of them occur. Nonetheless, every change in vegetative, and notably in generative organs can be a step in a chain of successive changes that lead to bigger changes and hence to specificity that characterizes a new taxonomic status. Though, neglected principle of documenting these changes through infraspecific taxa overcomes the pressures. Regardless of the need for globalisation of taxa, which is favored in regional floras and special taxonomic journals we are witnesses of newly, relevantly described infraspecific taxa. The International Code of Nomenclature for algae, fungi and plants still accepts the concept with greater number of infraspecific categories. On the other side, problem occurs while recognizing new taxa on morphological level. In history of botanical taxonomy and nomenclature, some taxa were incorrectly described because teratology or changes in morphological and meristic characters that deviate from protologs were used as distinctive characters. There are many examples of constraint of infraspecific taxonomy development. Infraspecific variability includes a few phenomena, evolutionary changes, phenocopies, parallel series of linked characters and itself is area in development of botanical nomenclature and taxonomy that represents one new direction known as „deep morphology". Every evolutionary or ecological solution that plant as an organism expresses through changes in its morphology is a unique step towards forming infraspecific taxa and it is unacceptable that those changes remain unknown to the scientific world.

Keywords: variability, phenotype, taxon

What does Balkan Peninsula have to offer to plant conservation biologists? A molecular perspective

IT5-2

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The Balkan Peninsula, currently occupying an area of c. 520000 km^2, arose from an archipelago within the Tethyan ocean and became connected to the southern part of Palaeo-Europe since the late Oligocene (25 Mya). The vast water bodies were present in the Para-Tethys realm in the late Miocene (11–6 Mya), and the Pannonian Sea disappeared from this region during the upper Pliocene (around 3.6 Mya). Such a distinct and rather dynamic palaeogeographic history as compared to the rest of Europe has led to its current exceptional richness in diverse physiographic and climatic conditions. During the turbulent Pleistocene (c. 2.6 Mya–11.2 Kya), the Balkan Penin-

sula, along with the other two southern-European Peninsulas, served as a glacial refugium not only of temperate but also of cold-adapted plant taxa. Along with its specific geographical positioning on the crossroads of several major floras, and high rates of speciation and diversification, the Balkan Peninsula is currently one of floristically the most diverse parts of Europe (c. 7.500 species, of which c. 2.500 are endemic to this region). However, it is still genetically understudied and thus, an array and complexity of genetic/demographic processes within this region, which have led to current distributions of genetic diversity, populations and species, are still poorly known. Recent studies based on molecular data, which provide new insights into patterns of genetic structuring and evolutionary unfolding of both native and agriculturally important plant species, are essential for the conservation of the Balkans' biodiversity at all levels. Here, I present recent findings in pea, faba bean, Serbian spruce and Dalmatian sage.

Keywords: palaeogeographic history, *Pisum sativum, Vicia faba, Picea omorika, Salvia officinalis*

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Plant diversity drivers in the Balkans: ploidization, hybridization and cryptic speciation

IT5-3

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There are many reasons why the Balkan Peninsula is one of the centres of biodiversity in Europe: geographical position, geological, climatic and habitat diversity, existence of refugial habitats where many plant species found rescue from harsh Ice Age conditions. It is estimated that its vascular flora comprises ca. 8000 native taxa, including 2600–2700 endemics. New species can originate in many gradual or abrupt ways, ploidization and hybridization being some of them. Polyploidization is one of the major evolutionary forces in plants as in the case of some genera from the Balkan Peninsula, e.g. *Cerastium*, *Ramonda*, *Sesleria*. These genera were recently extensively researched by flow cytometry indicating the Balkan Peninsula as the centre of their diversity and polyploidization as the main mechanism of their diversification. In some of these investigations, hybridization not easily visible in morphology, but obvious in genome size and chromosome number was discovered, e.g. tetraploid hybrids between diploid *Ramonda nathaliae* and hexaploid *R. serbica*, found only in two sympatric populations of these paleopolyploids in SE Serbia. In other cases, homoploid hybridization takes place, with morphological characters being more valid, e.g. *Edraianthus* × *lakusicii*, hybrid between *E. tenuifolius* and *E. lovcenicus*. Finally, speciation often happens in very fine ways, noticeable in genetic structure, but not easily apparent in morphological characters, leading to formation of cryptic species, as in *Sesleria rigida* s.l. (*S. rigida*, *S. filifolia*), *Campanula pyramidalis* (*C. austroadriatica*, *C. montenegrina*), *Edraianthus serpyllifolius* (*E. pulevicii*), *E. graminifolius* (*E. canescens*). The bryophyte flora undergoes the same processes. However, due to their haploid life form, and much larger ranges, the morphological variability is not easy to interfere with molecular changes or evolutionary drives. Thus, many interesting findings come out once the molecules and morphology are crossed. Some species can be included into known one or the opposite, can be separated. The most problematic are cryptic species, possibly of hybrid or ploidization origin. The first document as such comes in funaroid mosses.

Keywords: chromosome number, genome size, morphology, polyploidy

SELECTED TALKS

Conservation of floristic and vegetation diversity in Southeast Europe: sustainable use and ecosystem services approach

OP5-1

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South East Europe (SEE) i.e. the Balkan Peninsula, is particularly appreciated by quantitative (number of species) and qualitative (endemic – over 2600, relic and internationally important species and habitats) values of biodiversity. In spite of several already ratified international biodiversity conventions and issued biodiversity strategies with action plans, in most SEE countries the enforcement of conservation legislative is rather weak. In some SEE countries, the Important Plant Areas (IPAs) are recognized to support the existing *in situ* conservation programs, including protected areas and their ecological networks, such as Emerald and Natura 2000. In addition, implementation and promotion of High Nature Value Farmlands (HNVF) offer the chance for a long-term protection of natural and semi-natural grasslands and agro-forestry areas of high biodiversity and of high conservational importance. Concerning the *ex situ* conservation achievements, participation in Southeast European Development Network for Plant Genetic Resources (SEEDNet) and European Cooperative Program for Crop Genetic Resources Networks (ECPGR) resulted in establishment and/or enrichment with PGR accessions, respectively. Most recently, comprehensive national vegetation databases were created in most of SEE countries upon participation in the European Vegetation Archive, allowing complete insight in diversity, distribution and ecology of the Balkans' and SEE ecosystems. New conservation concepts address much more attention to socio-economic issues and dependence of local and regional development on sustainable use of biodiversity and preservation of traditional knowledge and practices in use of bio-resources, which also includes the evaluation of complex ecosystem services, such as provisioning, supporting, regulating and cultural and ethic ones.

Keywords: ethnobotany, *in situ, ex situ* conservation, biodiversity, vegetation

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Assessment of genetic integrity and diversity of *Populus nigra* in protected areas along the Danube River

OP5-2

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The European black poplar (*Populus nigra* L.) is a floodplain forest species that has central role in the development of riparian ecosystems. Genetic integrity and diversity of this species has been threatened by urbanization along riversides, drainage of wetlands and controlled river management, but also by cultivated hybrids

with which it intercrosses. Although genetic diversity of this species has been analyzed throughout Western and Central Europe, more detailed analysis was needed along the Danube flow, particularly in SE Europe. We investigated genetic diversity of twelve native *P. nigra* forests in the protected areas along the Danube River flow using highly polymorphic microsatellites and utilized diagnostic microsatellite loci and win3 genetic marker for identification of individuals with hybrid constitution among morphologically determined *P. nigra*. Diagnostic microsatellite loci and win3 marker consistently distinguished *P. nigra* from hybrid individuals. After exclusion of small number of hybrid individuals we showed high genetic diversity in the analyzed populations. Only 2.95% of genetic variance occurred between populations, while the pattern of population differentiation corresponds to isolation by distance. In populations downstream of the Đerdap Gorge, different genetic cluster is dominant compared to other populations. Only one forest has experienced a recent bottleneck; however, it preserved much of its diversity. We showed that genetic integrity and diversity were not compromised in the analyzed forests, which can thus provide suitable genetic material for maintaining and promoting metapopulations in this species.

Keywords: European black poplar, microsatellites, Danube River

This research was supported by South East Europe Transnational Cooperation Programme "DANUBEPARKS STEP 2.0" – "Anchoring the Danube River Network of Protected Areas as Platform for Preservation of Danube Natural Heritage" and by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173024 and OI173012).

Conservation biology of European bryophytes

OP5-3

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Bryophytes are the second largest group of terrestrial plants after angiosperms with ca. 20000 species worldwide. As all other living beings, they face rapidly changing environment and often come to the brink of extinction. Thus, bryophyte conservation biologists are facing significant challenges. A total of approximately 2000 bryophyte species in Europe are neglected/omitted from conservation initiatives at different levels, although they play significant roles in various ecosystems and/or have possible new active chemical constituents. Nowadays, many threaten European species remain in just a few populations (patches). Their biology or ecology was not studied previously. Viability of populations and species can depend as much on genetic factors such as inbreeding and gene flow as on demographic factors such as population growth rate and distribution. Bryophyte Biology Group in Belgrade (BBGB) has a living collection of 131 bryophyte species among which 52% represent red-listed species at the European, regional or national level. BBGB is a co-founder of European Bryophyte *Ex situ* Conservation Network. Bern species and EU habitat directive species are in the focus at present, e.g. a liverwort *Riella helicophylla* and five moss species *Drepanocladus vernicosus*, *Bruchia vogesiaca*, *Buxbaumia viridis*, *Dicranum viride, Pyramidula tetragona* listed in Bern convention and EU habitat directive species like *Sphagnum* spp. and *Leucobryum glaucum*. Many new insights come out during work on specific taxa rescue from extinction. At point when some stable production and propagation in *ex situ*/captivity condition have been achieved, the legislative problems come up. Case studies on active bryophyte conservation, achievements, problems/solutions, further activities will be further presented. Collaboration among scientists with different educational backgrounds and skills leads to good and long-term success in bryophyte active conservation.

Keywords: mosses, liverworts, protection, Europe

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173024 and OI173030)

POSTER PRESENTATIONS

Cryopreservation of *Impatiens walleriana* shoot tips using PVS2 vitrification procedure

PP5-1

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Vitrification with plant vitrification solution 2 (PVS2) is commonly used procedure for chemical dehydration of tissue prior to cryopreservation of the shoot tips. PVS2 contains 30% glycerol (w/v), 15% ethylene glycol (w/v) and 15% dimethylsulfoxide (DMSO; w/v) in basal culture medium containing 0.4 M sucrose. In order to develop PVS2-vitrification based protocol for cryopreservation of *Impatiens walleriana* shoot tips, we tested the effect of different PVS2 concentrations (100, 80, 60, 50, and 40%), and time of shoot tips exposure to PVS2 solution (5, 10, 15, 20, 25 and 30 minutes) on survival and plant regeneration prior and after exposure to liquid nitrogen (LN). Nodal segments of *I. walleriana* were cultured on plant growth regulator-free MS medium for three weeks before the shoot tips were isolated. The results have shown that survival of shoot tips prior to exposure to liquid nitrogen depended on PVS2 concentration and time of exposure to PVS2 solution and ranged from 100% (lower PVS2 concentrations) to 50% (100% of PVS2, 30 min exposure). After exposure to LN, the highest survival (77%) was achieved when shoot tips were exposed 25 min to 50% PVS2 solution, while full strength PVS2 solution with 30 min exposure was harmful when only 26% of cultured shoot tips survived. The highest plant regeneration (10.3%) of the cryopreserved shoot tips was observed when shoot tips were exposed 25 minutes to 50% PVS2 solution prior to immersing in LN. Viability of PVS2 treated shoot tips was determined by staining with fluorescein diacetate.

Keywords: cryopreservation, vitrification, PVS2, DMSO

This research was sponsored by the Ministry of Science and Technological Development of the Republic of Serbia (TR 31019).

Pursuit for transcriptome microsatellites towards estimation of genetic variation, species discrimination and detection of interspecific hybrids within the genus *Centaurium*

PP5-2

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Over the last few decades microsatellites have been the markers of choice for applications in both plant population and conservation genetics. Unassembled 114 bp long transcriptome sequences of *Centaurium erythraea* Rafn, obtained by Illumina GAIIx System, were complementary paired to make short contigs of 170–

200 bp which were used as a starting material for microsatellite mining. Identification and bioinformatics filtering of microsatellite-containing sequences were carried out employing open source and free software components operational on an average performance computer. The designing of primers was performed for 16 selected sequences. Transferability of microsatellite loci was assayed on 22 individual plants originating in the Balkan Peninsula which belong to 7 different taxa of the genus *Centaurium* including three species, two subspecies and five specimens with assumed hybrid constitution. Out of 16 primer pairs tested, 9 were successfully amplified showing high-quality and reproducible bands on 2.5% agarose gels. Seven out of nine functional loci amplified in all taxa accessions, while two loci had the ability to clearly discriminate between the taxa. Further validation of developed microsatellite markers was performed by Lab-on-a-Chip technology using Agilent 2100 Bioanalyzer in order to detect polymorphism of microsatellite loci and to assess their range. Eight loci displayed clearly polymorphic patterns. The number of observed alleles across loci was 3 to 11. Owing to considerable transferability and variability, obtained microsatellite loci are standing for powerful tool for future studies on discrimination of species within the genus *Centaurium*, estimation of their genetic variation, and for tracking interspecific hybridization among them.

Keywords: microsatellites, transferability, *Centaurium*, genetic variation, interspecific hybrids

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173024).

Viability of dimorphic cypselae in *Amphoricarpos autariatus* Blečić & Mayer ssp. *autariatus*

PP5-3

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Amphoricarpos autariatus Blečić & Mayer (Asteraceae), perennial chasmophytic plant, endemic and tertiary relict, grows on scarce soil on mountain rock in the Balkans. In this work micromorphology of dimorphic cypselae and their viability are examined. Cypsealae are analysed using light stereo microscope (LM) and scanning electron microscope (SEM). This taxon produces two types of cypselae in the same capitulum. Inner densely hair-covered cypselae, diverging from central hermaphrodite flowers are tetrangular with long bristles. Outer flathair-covered cypselae, from outer female flowers, have wide lateral wings which end above basal part of the pappus. Hairs on both cypselae types are dual ostensible, forked at the tip. Pappus of both cypselae is barbato–aristate. Tetrazolium test is a simple and fast chemical test for indication of cellular respiration, where 2,3,5-triphenyl-2H-tetrazolium chloride is used as a redox indicator. The results of tetrazolium test of mature cypselae, which were longitudinally cut with razor blade through the middle showed that 25% of the inner cypselae and 50% of the outer cypselae were viable. This is the first report of fruit morphology and seed viability of this taxon. Observations of the morphology and viability of cypselae studied in this work could be relevant for understanding the reproductive biology and dispersal strategy of this taxon, considering that presence of dimorphic achenes was substantially associated with event in unpredictable habitats, which may be an evolutionary adaptation trait.

Keywords: *Amphoricarpos autariatus,* cypselae viability, tetrazolium test, heterocarpy, SEM

Variability source structure in tomato (*L. esculentum* Mill.) germplasm collection

PP5-4

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The purpose of the study was to investigate variability source structure and contribution of particular traits in total variability of tomato (*Lycopersicon esculentum* Mill.) germplasm collection. Plant material, belonging to the collection of the Institute of Field and Vegetable Crops in Novi Sad included: 5 landraces, 10 old varieties, 10 breeding lines and 4 commercial varieties. The trial was set at the experimental fields of the Rimski Šančevi site, during 2010-2012. Observations were recorded on the first truss and following traits were analyzed: fruit length (*FL*) and width (*FW*), locules number (*LN*), pericarp thickness (*PT*), dry matter content (*DM*), average fruit weight (*FWe*), fruit yield per plot (*FY/plot*), number of fruits per plot (*NF/plot*), average fruit number per plant (*FN/plant*), number of days from germination to the beginning of maturity (*ND*). Principal Component Analysis was performed in statistical software *STATISTICA 12*. The Warimax normalized method was used for the factor rotation. Three main components, chosen for further analysis, explained 81.5% of total variability with percentage part of: 49.4%, 19.5% and 12.6%, respectively. The first component was determined by: *FL*, *FW, Fwe, FY/plot*, *NF/plot* and *FN/plant*. The second component was defined by *LN* and *PT*, and the third by *DM* and *ND*. Projection of variables and genotypes on the surface covered by main components was used for visual presentation and data interpretation.

Keywords: Principal Component Analysis, tomato, variability

Pre-breeding for value-added traits in maize

PP5-5

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Pre-breeding represents a link between genetic resources and breeding programs, referring to identification of desirable characteristics and/or genes from unadapted materials, as well as adapted ones that have not been subjected to any kind of modern breeding. The narrow genetic base of maize commercial genotypes has limited variability for value-added traits and gene introgression from landraces could improve grain quality. Maize Research Institute Zemun Polje (MRI) gene bank has one of the largest maize collections in the world with approximately 5500 accessions, including 2217 landraces from the Western Balkans and 3258 populations and inbred lines from 40 countries. Sub-sets of the accessions were subjected to protein and oil content and composition analyses. High oil (up to 7.9%) and high protein (up to 14.29%) accessions were identified. Fatty acid analysis revealed that most of the high oil accessions were also rich in oleic acid content (up to 45.24%). Most of the high protein accessions were rich in tryptophan content (up to 0.089%). Moreover, accessions that concurrently had high contents of protein, oil and tryptophan were identified. Also, a set of 20 coloured popcorn varieties and coloured kernels of 53 populations of common maize were subjected to anthocyanin, total yellow pigment, total phenols and antioxidant activity analyses. Blue coloured popcorns and

common maize genotypes had the highest contents of anthocyanin (up to 1.23 mg g^{-1}). The most interesting genotypes from these researches will be included in the commercial breeding programs.

Keywords: accessions, maize, value-added traits

Contribution to the Bryophyte flora in Douglas-fir forests on Vidlič Mountain (Southeastern Serbia)

PP5-6

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During 2013 and 2014, the survey of bryophyte flora in the area reforested with Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) was done. In total, forty-three species were recorded – nine liverworts and thirty-four mosses. Liverworts belong to 7 families and 8 genera, and mosses to 14 families and 29 genera. Biogeographically, all species belong to six floristic groups. According to species number, boreal group dominated, followed by groups of temporal and atlantic species. *Chamephyte caespitosae* (tufted) growth form is more frequent than crawling (*Chamaphyte reptant*) growth form. This study gives a valuable insight into the bryophyte species composition and diversity in the areas reforested with alien tree species.

Keywords: mosses, liverworts, reforestation

Determination of *ACS1*, *ACO1* and *ETR1* allelic constitutions in autochthonous apple (*Malus* × *domestica* Borkh.) genotypes

PP5-7

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Apple (*Malus* × *domestica* Borkh.) is a typical climacteric fruit, whose ripening is associated with an upsurge in the rate of respiration and ethylene production. Ethylene, the gaseous plant hormone, plays a major role in the ripening of apple fruit. The level of ethylene production is determined by the activity of two enzymes that catalyze the two final reaction steps in the ethylene biosynthetic pathway: 1-aminocyclopropane-1-carboxylate (ACC) synthase (the *ACS* gene) and ACC oxidase (the *ACO* gene). Both genes are encoded by multigene families, although *ACS1* and *ACO1* are predominantly expressed in the apple fruit tissue. Ethylene is perceived by family of endoplasmic reticulum-localized receptors; ETR1 receptor (the *ETR1* gene) is one of the receptors in apple. This work studied the allelic polymorphisms of the *ACS1*, *ACO1* and *ETR1* genes in 48 autochthonous genotypes from the Apple Collection of the Fruit Research Institute in Čačak. Polymorphisms were detected by PCR and by using the restriction enzymes (*Bam*H1 and *Rsa*I for *ACO1*; *Rsa*I, *Alu*I and *Hinf*I for *ETR1*). Two alleles of *ACS1* gene (*ACS1-1* and *ACS1-2*), three alleles of *ACO1* gene (*a*, *b* and *c*) and four alleles of *ETR1* gene (*a*, *b*, *c* and *d*) were detected. Based on allelic constitutions, the autochthonous genotypes were grouped into two classes for *ACS1* gene (*ACS1-1/1* – 43 and *ACS1-1/2* – 5 genotypes), four classes for *ACO1* gene (*aa* – 31, *cc* – 2, *ab* – 14 and *ac* – 1 genotype) and four classes for *ETR1* gene (*aa* – 27, *b,a/c* – 9, *c,a/c* – 3 and *d,a/d* – 9 genotypes).

Keywords: apple, autochthonous genotype, ethylene, genotyping

Cryopreservation and cryotherapy of grapevine (*Vitis vinifera* L.)

PP5-8

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This study aimed at establishing a cryopreservation protocol for grapevine shoot tips and at testing the efficiency of cryotherapy. *In vitro* cultures of healthy genotypes of eight Croatian autochthonous grapevine cultivars and of virus-infected genotypes of Plavac mali exhibited a high genotype effect and lower reactivity of infected genotypes. A PVS2-based cryopreservation protocol established led to approximately 50% recovery with cultivar Portan and three out of four international cultivars tested. By contrast, none or very low recovery was noted with the Croatian cultivars tested. ELISA tests revealed the elimination of the GFLV virus (77.8%) in cultivar Chardonnay and the GLRaV-3 virus (100%) in cultivar Cabernet Sauvignon in cryopreserved samples, respectively. Elimination was possible before cryopreservation. Immunolocalisation revealed GFLV-infected particles in meristematic tissues and GLRaV-3-infected particles in sieve elements. Genetic stability was tested with eight AFLP primer combinations, and polymorphic fragments were observed with increasing durations of exposure to PVS2 solution.

Keywords: grapevine, cryopreservation, cryotherapy, genetic stability

Peat mosses of Serbia: *Sphagnum* flora revision, survival threats and reassessments of IUCN status

PP5-9

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Accrodring to the current Bryophyte Red List of Serbia and Montenegro, all *Sphagnum* species known for Serbia are categorized in the VU (vulnerable) threat category. *Sphagnum* species are listed on the European Union Habitats and Species Directives as well. Based on the recent field work, revision of old herbarium collections and literature chorological data, threat status of the species has been reevaluated and updated. Out of 24 *Sphagnum* species known for the country of Serbia, six are shown to be critically endangered (CR), ten endangered (EN), six vulnerable (VU) and for two the information is lacking hence data deficient (DD). Thus, *S. cuspidatum*, *S. fuscum*, *S. obtusum*, *S. rubellum*, *S. russowii* and *S. warnstorfii* are considered as CR in Serbia; *S. angustifolium*, *S. auriculatum*, *S. cpillifolium*, *S. contortum*, *S. flexuosum*, *S. inundatum*, *S. magellanicum*, *S. platyphyllum*, *S. subnitens* and *S. teres*are are considered as EN in Serbia and *S. centrale*, *S. fallax*, *S. girgensohnii*, *S. palustre*, *S. squarrosum*, *S. subsecundum* are considered as VU in Serbia. *S. molle* and *S. papillosum* are cited for Serbia without precise localities. Recent investigations did not bring any new findings, and these two species cannot be listed into any threat category and thus should be considered as threatened but data deficient (DD).

Destruction of specific mire habitats has been observed as the main peat-moss decline and extinction risk factors, and for many sphagna the old records were not confirmed during recent field studies. Main threats for peat bog habitats are changed in water regime due to the irrigation measurements, turism, construction, succession of vegetation, and deforestation.

Keywords: sphagna diversity, conservation, red list

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173024 and OI173030).

Preservation and characterization of Croatian grapevine (*V. vinifera* L.) varieties

PP5-10

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In the last two decades, many efforts in the preservation of grapevine biodiversity in Croatia have been undertaken. A detailed inventory of vine-growing regions in Croatia has shown that a lot of autochthonous cultivars can still be found, but many of them are endangered and for a long time neglected. To preserve them against the extinguishing, we have established since 2001 national *ex situ* collection at the Faculty of Agriculture in Zagreb, where we have collected more than 130 grapevine accessions so far. Besides, we started to build few regional collections, to save duplicates and enable their economic evaluation in appropriate climatic conditions. After identification many synonyms and homonyms were detected and unique genotypes were selected. Basic data on them are shown: description (according to OIV descriptors) and genetic profiles (9 SSR loci), population level and status of vulnerability. Also, genetic relationship based on the shared alleles distance is computed from SSR data. Results show certain level of similarity among varieties, and classify Croatia as an important gene-pool in Europe. Some additional accessions which were detected and introduced into collections recently are still being investigated and characterized so that here presented list of Croatian native grapevine cultivars number is not final.

Keywords: grapevine germplasm, Croatian autochthonous cultivars, SSR, characterization, ampelography

Vascular Flora of the Klek Peninsula (Bosnia and Herzegovina)

PP5-11

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In Bosnia and Herzegovina there is only one marine aquatorium, which includes part of the Neum bay and half of the Mali Ston channel, with a coastline of about 12.2 km^2. Klek peninsula has a total length of about 7 km and with Neum it is enclosing the Neum-Klek bay. Klek peninsula has noticeable diversity of the vascular flora, which is predominantly caused by the strong influence of the Mediterranean climate and relief plasticity. Diversity of the floristic composition is influenced also by the unequal degradation process. During the period from 2000 to 2014 botanical investigations of the Klek peninsula were carried out only in the late spring time and about 40 families with about 150 plant species were recognized. Strong anthropogenic impact has led to the fact that vascular flora diversity of investigated region significantly decreased over the last 50 years. In Bosnia and Herzegovina, so far only 2.6% of the territory has been protected and habitat fragmentation with excessive exploitation continuously leads to a dramatic increase in environmental degradation and decline in biodiversity. According to the ecological network Natura 2000 for Bosnia and Herzegovina, Neum-Klek is recognized as a relevant area which involves several important habitats. This emphasizes the need to establish an efficient system by means of which this specific natural area with a high degree of biodiversity could be protected.

Keywords: Klek peninsula, vascular plant diversity, habitat protection

Improvement in *Prunus* spp. cryopreservation using D- and V-cryoplate techniques

PP5-12

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In vitro grown shoot tips of cherry plum, plum cultivar 'Požegača' and cherry rootstock 'Gisela 5' were successfully cryopreserved using aluminum cryo-plates. Shoot tips precultured for 1 day at 23 °C on MS medium containing 0.3 M sucrose were embedded in alginate gel into cryo-plates wells. Loading was performed using either t LS1 (2 M glycerol + 0.4 M sucrose) or C4 (1.9 M glycerol + 0.5 M sucrose). In the V cryo-plate protocol, explants were dehydrated at room temperature for 30 or 40 min with PVS A3 (37.5% glycerol, 15% dimethylsulfoxide, 15% ethylene glycol and 22.5% sucrose) or with PVS3 (50% glycerol and 50% sucrose) for 60 min. In the D cryo-plate protocol, desiccation for 2, 2.5 or 3 h was performed over silica gel. Unloading was done in MS medium containing 0.8 M sucrose at room temperature for 30 min or 60 min (cherry plum). In the V cryoplate procedure, regrowth of cryopreserved explants loaded with C4 solution and dehydrated with PVS A3 was 33.3% (cherry plum and 'Požegača') and 95.9% ('Gisela 5'), while in those loaded with LS1 solution regrowth was 47.5% (cherry plum) and 59.2% ('Požegača'). Regrowth of cryopreserved 'Gisela 5' explants previously dehydrat-

ed with PVS3 was 85%. As for the D cryo-plate procedure, regrowth of cryopreserved explants ranged between 65.7–75% in cherry plum, 41.9%–75% in 'Požegača' and 45.8–66.7% in 'Gisela 5'.

These results clearly indicate that both cryopreservation procedures can facilitate *Prunus* germplasm cryostorage.

Keywords: aluminum cryo-plates, loading solutions, vitrification solutions, desiccation, liquid nitrogen

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VI

Evolutionary Plant Biology

INVITED TALKS

Past legume crop domestication and agriculture of tommorow: Tracing pea domestication and using wild relatives to widen diversity.

IT6-1

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Pea belongs to the ancient set of the cultivated plants of the Near East domestication centre and is still an important crop. In pea, explosive pod dehiscence and seed dormancy were probably the greatest barriers to domestication that had to be overcome. The classification of *Pisum* genus has changed over time two currently accepted two species, *P. fulvum* Sibth.&Sm. and *P. sativum* L. There are around 98,000 pea accessions world-wide, but only 3% of *ex situ* collections are wild *Pisum.* The set of 150 samples including wild and domesticated peas was subjected to DArT-seq analysis, resulting in 39,000 SNPs per sample. Distance and model-based analysis of genetic distances led to clear separation of *P. fulvum* and wild pea (*P. sativum/elatius*) samples from domesticated gene pool. This study provides the framework for taxonomical species definition, setting up the global *Pisum* germplasm diversity, as well as suggesting a model for the pea domestication. As a result of domestication the genetic basis of the crop has narrowed, making them prone to pests and diseases. To reverse this bottleneck, the incorporation of traits from wild species is desirable. However, the transfer of genes is often accompanied by inevitable genetic drag. To avoid this, the establishment of introgression lines containing molecularly defined chromosome segments from wild species in genetic background of the crop has been applied to make the use of alien genomes more precise and efficient. The results of this approach in pea will be shown and discussed.

Keywords: diversity, domestication, introgression, legumes, pea

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Comparative analysis in plant ecology and evolution

IT6-2

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Some of the most common experimental approaches in studies of plant ecology and evolution include common garden experiments, experimental manipulations, selection experiments, transplant-replant designs as well as comparative approach. Comparative approach involves studies on different taxa which are either related phylogenetically or share similar ecological niches and provide way for addressing questions about common patterns of evolutionary change and testing hypotheses about evolution. Comparative approach is therefore also used in evolutionary ecology and ecological genetic studies of phenotypic plasticity. Plasticity represents the ability of a single genotype to produce different phenotypes under different environmental conditions and can be an important way in which plants can adapt to environmental heterogeneity. Be-

cause plastic traits tend to be highly variable within as well as across species, studies are often conducted at various taxonomic levels - species but also subspecies, populations or ecotypes. This approach allowed our team working on evolutionary ecology and ecological genetics of plants in Department of Evolutionary Biology at the Institute for Biological Research to compare differences in plasticity of species of two genera - *Lamium* and *Iris*. Findings of those studies can be used to demonstrate some advantages of comparative approach in studies of plant ecology and evolution.

Keywords: comparative analysis, *Iris*, *Lamium*, phenotypic plasticity

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Population divergence and speciation within a species: ecology and the Royal Irises

IT6-3

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Speciation is a continuous and a dynamic process, which can be represented by different magnitudes of reproductive isolation among groups in different levels of divergence. Local adaptation, as well as reduced gene-flow and stochastic fixation of alleles may lead to divergence of populations, which will be expressed in partial reproductive isolation among populations along spatial scale. Because new species arise from populations formerly belonging to the same species, studying intraspecific partial reproductive isolation facilitates identifying the mechanism underlying speciation, whether adaptive divergence or random process. We used the Royal Irises (*Iris* section *Oncocyclus*) to partition the relative role of isolation by ecology, accounts for divergence via local adaptation, and isolation by distance, accounts for reduced gene-flow and stochastic divergence. The Royal Irises species complex has been proposed to be in the course of speciation, due to the lack of reproductive barriers among species and morphological and genetic continuums along environmental gradients. We crossed plants from populations of *Iris atropurpurea* and measured post-zygotic reproductive success, using both reproductive isolation index and Aster model for non-independent life history stages. Using ecological factors that are putatively associated with local adaptation, we found an increase in reproductive isolation with spatial distance, but only at the stage of fruit-set. Ecological differentiation revealed linear reduction of offspring fitness with increased ecological distance. These results provide evidence for the dominance of isolation by ecology over spatial distance in shaping the divergence landscape of *I. atropurpurea*.

Keywords: adaptive divergence, plant evolution, partial reproductive isolation, speciation within species

SELECTED TALKS

Population Scale Multi-year Monitoring of *Iris pumila* in Deliblato Sand: Flowering Phenology

OP6-1

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Population scale multi-year monitoring of flowering and fruiting of *Iris pumila* L. can shed light on various evolutionary issues such as mechanisms of flower color polymorphism maintenance as well as provide information on population structure and dynamics that is important for this species *in situ* conservation. In the monitoring process flowering is recorded each year in 35 experimental plots (more than 4000 m^2 in total) on two-day basis during flowering period (early spring) in population occupying undisturbed natural habitat in the Natural Protected Reserve of Deliblato Sands. That enables determination of spatial position, flowering date, flowering span, flower color, and pollination success for more than thirteen thousand individual flowering ramets per year. Preliminary findings indicate that in the second year of monitoring flowering started almost a month earlier and had almost two times greater flowering span compared to the first year. Number of flowering individual ramets was lower, but fruit to flower ratio was by the order of magnitude higher in the second year of the study.

Keywords: *Iris pumila*, natural populations, flowering seasons

This study is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173025).

Light induces variation in size and shape of *Iris pumila* flower parts in two natural habitats

OP6-2

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We employed the technique of geometric morphometrics to study variation of geometric size and shape of *Iris pumila* flower parts (standard, fall and style) from plants growing in two natural habitats (exposed and shaded) in a protected natural reserve of the Deliblato Sands. We applied analysis of variance (ANOVA) for centroid size (CS) and multivariate analysis of variance (MANOVA) with all shape variables (Procrustes coordinates) as dependent variables, and with habitat as fixed and clone as a random factor. Landmarks and semilandmarks of standards, falls and styles were positioned in MakeFan6 and TpsDig program. The CVA (Canonical discriminant analysis) was used to visualize the differences of shape between the habitats with contrasting ambient light conditions. The heterogeneity of light conditions affected the flower shape. The mean val-

ues of centroid size (as a measure of geometric size) of *I. pumila* standards and falls were lower in open habitat than in shaded environment. The results of ANOVA revealed a statistically significant effect of habitat for standards, fall and style ($p = 0.0009$, $p = 0.0223$ and $p = 0.0358$**,** respectively,) and between-clone variability in size of all flower parts was also observed (all $p < 0.0001$). According to the results of MANOVA analysis significant differences in shape between habitats and clones (all $p < 0.0001$) were observed. Considering that the light intensity is significantly different in open and shaded habitats, we can only assume that this is the key factor that influences the size and shape variation of *I. pumila* floral parts.

Keywords: shape of flower parts, light conditions, geometric morphometrics, *Iris pumila*

This study is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173025).

How do *Iris pumila* plants respond to photo-oxidative stress in the wild: the variation of leaf functional traits?

OP6-3

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Adaptive plastic responses to variable light conditions include all aspects of plant phenotype – morphology, physiology and biochemistry. To elucidate how ambient light intensity modulates the phenotypic expression of leaf functional traits in *Iris pumila*, twenty clonal genotypes inhabiting an exposed and a shaded site at the Deliblato Sands were selected and reciprocally transplanted between the two sites. Fifteen years later, one fully developed youngest leaf was harvested from each of these transplants during the summer, and analyzed for malondialdehyde (MDA) content (an environmental stress indicator), leaf morphophysiological traits (specific leaf area, SLA; leaf dry matter content, LDMC and succulence) as well as the total concentrations of leaf pigments (chlorophylls, Chl; carotenoids, Car; and anthocyanins, Anth). The MDA content was found to be about 20% greater in sun exposed leaves than in those developed under the vegetation canopy, indicating a stronger abiotic stress prevailing at the open compared to the shaded habitat. The leaf morphophysiological traits varied significantly between the alternative radiations environments. While LDMC and succulence increased with irradiance, SLA decreased. A higher succulence and LDMC, accompanied with a lower SLA in the exposed leaves compared to their shaded counterparts reflected greater, functionally advantageous, water conservation efficiency at open habitats. The total Anth content was elevated in leaves growing in full sunlight compared to those from the vegetation shade, acting as a light attenuator against the photo-oxidative damage of chloroplasts. The Car : Chl ratio was also increased in the sun-exposed leaves relative to the shaded ones, as an adaptive plastic response of thermal energy dissipation to stressful photo-oxidative conditions commonly occurring in natural habitats of *Iris pumila*.

Keywords: adaptive phenotypic plasticity, functional leaf traits, *Iris pumila*, photo-oxidative stress

POSTER PRESENTATIONS

Anatomical and physiological changes in *Iris pumila* leaves in unpolluted and polluted environment

PP6-1

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Plants are capable of sensing various types of stress and plant stomata, as well as photosynthetic pigments may play an important role in response to altered environmental conditions in order to maintain optimal photosynthetic efficiency, carbon uptake and water metabolism. In this survey, we estimated the stomatal density and photosynthetic pigments concentration of *Iris pumila* leaves as an indicator of antropogenically induced stress (traffic pollution). Leaf samples were taken from one unpolluted site – Deliblato sands (natural protected area), and one polluted site (along the main road Belgrade-Novi Sad), where replicas of the clones selected in the unpolluted site were previously transplanted. We measured anatomical (Stomatal density) and physiological traits (Chlorophyll a (Chla), Chlorophyll b (Chlb), Carotenoid (C) and Total chlorophyll (ChlT) concentration, the ratio of Chlorophyll a to Chlorophyll b, and the ratio of Chlorophyll a to Carotenoids). Estimated parameters differed significantly between studied sites. We detected statistically significant increase in stomatal density in polluted sites. Chla, Chlb and C concentrations, as well as ChlT and Chla/C showed significantly lower values in a polluted environment. Cha/b ratio showed significantly higher values in plants originating from the polluted habitat. We can conclude that there was a decrease in photosynthetic pigments in polluted habitat, as well as change of Chla/C ratio in favor of carotenoids that serve as photo protective pigments. All these conclusions confirm that stomatal density and concentration of relevant pigments can serve as a stress indicator in this protected species.

Keywords: anthropogenic pollution, stomatal density, photosynthetic pigments, *Iris pumila*

This study is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173025).

Morphological integration of vegetative traits in *Lamium purpureum* originating from two contrasting light habitats (Open and Shade), in two natural populations and grown in two densities (High and Low)

PP6-2

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Plants as sessile organisms have to continuously adjust their growth, development and reproduction to variable surroundings, especially in relation to light as an essential abiotic factor. Plants growing in crowded conditions inevitably compete for light, namely, for an ability to photosynthesize. In this study, we addressed correlation structures between traits essential for the response to changes in light quality and light intensi-

ty, in *Lamium purpureum* plants originating from two habitats (Open and Shade), in two natural populations (Vršac mountain and Zuce) and grown in two densities (High and Low). Every single plant was planted in a single pot and this arrangement allowed competition only for light and not for the other resources. We measured eight traits after 8 weeks of growth: height, first and second leaf length, first and second leaf width and number of leaves, number of internodes and number of shoots per plant. We aimed to test if there are statistically significant phenotypic correlations between traits (morphological integration) and if they depend on the population, habitat and density treatment. Correlation structures differed between habitats and treatments in both populations. Plants in Vrsac/Shade habitat and Zuce/Open habitat, showed the lowest level of between trait integration in the High light treatment. Between-population differences in correlation structures were more numerous in the High light treatment for both habitats.

Keywords: morphological integration, conspecific density, *Lamium purpureum*

This study is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173025).

Spatial and temporal environmental differences for pendunculate oak (*Quercus robur* L) pollen traits

PP6-3

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The physiological characteristics of pollen, germination percentage and germination energy, depend on the plant species, the genetic variability and also on environmental conditions. In this study we analyzed the differences of oak pollen germination percentage and germination energy between locality and years of collecting pollen, in optimal nutrient medium of 15% sucrose. The pollen samples were collected in 2011, from four locations (Košutnjak, Novi Beograd, Ada Ciganlija and Bojčinska šuma). The results of analysis of variance confirmed intra-individual difference within locality ($p < 0.0001$) for the length of the pollen tube. The germination percentage (54.0-60.1%) and energy (132.7-160.9 μm) were not significantly different between localities ($p > 0.05$). The spatial variability, of the percentage of germination and germination energy, has been monitored for six years, on one tree at the Banovo brdo location. The results of one way analysis of variance, confirmed a statistically significant difference between the years ($p < 0.0001$) for both traits. The lowest percentage of germination was recorded in 2005, 28.2% and maximum 89.7% in 2011. The pollen taken in 2004 had minimum germination energy of 81.8 μm, while the maximum value of 196.1 μm was recorded in 2006. The relationship of temperature and precipitation in the period immediately prior to flowering significantly affected the physiological properties of pollen. The smaller percentage of germination in 2005 was affected by lower temperatures and higher precipitation compared to 2011 as a dry year. Spatial and temporal environmental differences had statistically significant impact on the variability of physiological traits of pollen.

Keywords: pollen germination percentage, pollen germination energy, *Quercus robur* L.

This study is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173025).

The intra-individual variability in germination and germination energy of oak (*Quercus robur* L.) pollen grains, tested on nutrient media with different percentage of sucrose

PP6-4

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The optimal substrate for the germination of pollen depends primarily on the plant species and then the composition of the nutrient medium. The intra-individual variability in the percentage of germination and germination energy (pollen tube length) was estimated at six nutrient media with different concentrations of sucrose (0, 5, 10, 15, 20, and 25%). The pollen was collected in late April and early May from eight trees in Ada Ciganlija. Trees were statistically significantly different in the percentage of germination and germination energy, according to the results of analysis of variance ($p < 0.0001$). The effect of the nutrient media was also statistically significant ($p < 0.0001$). The inter-individual difference (between the trees) depended on the concentration of sucrose in nutrient media. The maximum value of the germination percentage (%) was recorded in medium with 20% sucrose for the trees 1, 3, 4, 5, 6 (47.55, 25.13, 44.73, 11.09 and 37.66, respectively). The substrate with 15% sucrose was optimal for germination energy, the value's pollen tube length (μm) for the trees 1, 2, 3, 5 and 6 were 113.5, 118.08, 78.72, 51.66 and 121.52, respectively. The biochemical processes for a the germination and growth of pollen tubes are different, so that the surface with 15% and 20% sucrose was more optimal for germination percentage, while the surface with 15% of sucrose in the medium was more optimal for germination energy. All of the above facts infer that pollen viability is a complex physiological process which, in addition to genotype effect, is also affected by nutrient media.

Keywords: pollen germination, pollen germination energy, pedunculate oak, nutrient medium.

This study is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173025).

The correlation patterns between *I. pumila* flower traits and between their developmental stability in two natural habitats with contrasting light conditions

PP6-5

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Morphological variability and developmental stability of flower traits were examined in two natural habitats situated in the protected Deliblato Sands Special Natural Reserve. Pearson's correlation coefficients were calculated among flower morphological traits and among developmental stability of *I. pumila* flower traits in natural habitats with contrasting light intensity (full daylight and vegetation canopy). The most phenotypic correlations among the flower parts were found to be moderate or low in magnitude except for the strong positive correlation between the fall and standard length in both habitats (full daylight $r = 0.88$ and vegeta-

tion canopy r = 0.92). The correlations between the developmental stability of flower traits were low and statistically insignificant in both habitats. The congruence of the correlation matrix of *I. pumila* flower morphological traits (full daylight and vegetation canopy habitats) was strong and statistically significant (r = 0.83, p < 0.05). The correlation matrices of developmental stability of flower traits between the two habitats were not statistically similar. The correlation matrix of morphological traits and correlation matrices of developmental stability of the same characteristic differed between habitats. In the open habitats, these matrices were different while in shaded habitats were similar to each other (r = 0.56, p < 0.05), according to the results of Mantel test. The floral morphological variation in *I. pumila* appeared to be tightly integrated regardless of ambient light conditions. The strong correlation between the fall and standard in both habitats with alternative light intensity indicates close connection in their developmental process.

Keywords: flower trait correlations, developmental stability, *Iris pumila*

This study is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173025).

The pedunculate oak (*Quercus robur* L.) leaf shape variation between five natural populations in northern Serbia

PP6-6

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The technique for multivariate shape analysis – geometric morphometrics was applied with the aim to evaluate an interpopulation variability of pedunculate oak (*Quercus robur* L.) leaf shape. A total of 500 leaves were collected in five natural populations in northern Serbia (Sombor, Subotica, Vršac, Bojčinska šuma and Ada Ciganlija). The eleven homologous landmarks (LM) were digitized on images of leaves using the tpsDig software. The Procrustes coordinates were computed using Morpho J software. Inter- and intrapopulation variation of oak leaf shape was tested by MANOVA using Procrustes coordinates data set. A principal component analysis (PCA) was carried out to quantify the shape variation associated with each shape dimension. The canonical variate analysis (CVA) was used to visualize the observed interpopulation differences in the leaf shape. The first two axes explained 85.34% of the variance in the data set (first = 60.22%, second = 25.12%). According to the first axis, there were intrapopulation differences. The leaves from Bojčinska šuma and Sombor were narrower and had longer leaf petiole in comparison to the leaves from Ada Ciganlija, Subotica and Vrsac populations. Second axis revealed differences in leaf shape between populations Vrsac and Subotica. Population Vrsac leaves were narrower with longer leaf petiole, while leaves from population Subotica were wider with shorter leaf petiole. According to the MANOVA analyses, the differences in the leaf shape between populations and between trees were significant (p < 0.0001), while differences among leaves from an individual tree were not significant (p > 0.05).

Keywords: leaf shape, geometric morphometrics, *Quercus robur*

This study is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173025).

Study of genetic principles of pea pod dehiscence

PP6-7

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The origin of the agriculture was one of the key points in human history, and a central part of this was the evolution of new plant forms, domesticated crops. The transformation of wild plants into crop plants can be viewed as an accelerated evolution, the result of human and natural selection. Domestication triggered changes representing adaptations to cultivation and human harvesting, accompanied by genetic changes. Common set of traits have been recorded for unrelated crops, named domestication syndrome. These include loss of germination inhibition and increase of seed size, linked to a successful early growth of planted seeds as well as loss of pod dehiscence. In this work several pairs of wild versus domesticated forms were selected, together with primitive forms (landraces) representing transitory steps. These pairs as well as mapping populations of crosses were morphometrically, histologically and physiologically characterized and assessed for pod dehiscence phenotypes. The main objective of the study is to identify genetic and structural of pea pod dehiscence by comparative analysis of wild and domesticated pea genotypes. Nighty nine F9 RILs of wild *Pisum elatius* VIR320 x cultivated pea WL1238 and 136 F5 RILs of JI64 *P. elatius* and JI92 landrace (primitive domesticate) were used for genetic mapping. Based on previously published position of Dehiscence pod (Dpo1) loci, we re-mapped respective region. In addition to these genes used for "anchoring" of the locus, several candidate genes were chosen based on the published data.

Keywords: pea, domestication, pod dehiscence

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Herbivore load in a montane population of a dioecious forest forb: Is gender important?

PP6-8

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The presence of sex-specific herbivory in natural populations of plants is a question of growing scientific interest. Herbivorous insects are considered an important factor in evolution of plant mating systems. Dominant hypothesis is that individuals with a primarily male function are more prone to herbivory, i.e. herbivore load is expected to be biased towards pollen-bearing morphs. Gender-dimorphic species of genus *Mercurialis* are informative model systems in addressing numerous questions in plant evolutionary ecology, including the interactions with herbivores. In this study, the model species was dioecious forb *Mercurialis perennis* L. (Euphorbiaceae), a typical member of undisturbed understory communities. Our aim was to explore the pattern of herbivore damage in a large natural population of *M. perennis* (Metodje, Mt Kopaonik). A total of 566 leaves were sampled and each individual leaf was scanned and scored. We estimated the herbivore load by scoring damage to leaf area on an ordinal scale (1 to 5 scale; 1- no damage, 5 – severe damage). Data were analyzed

using contingency tables and G-test. Herbivore load was further examined in relation to plant gender, and a set of vegetative and reproductive traits (height, number of flowers, above-ground vegetative mass and mass of leaves). Our results did not confirm the pattern of male-biased herbivory in natural conditions, expected on basis of previous hypothesis. Plant gender did not emerge as an important factor to explain the observed variation in herbivore loads; the obtained results point to a more complex relationships between growth, reproduction and defense against herbivores.

Keywords: sex-specific herbivory, herbivore load, *Mercurialis perennis*, dioecy

Exploring the leaf shape variation under anthropogenic disturbance in dioecious forest forb (*Mercurialis perennis*): a geometric morphometric approach

PP6-9

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Nowadays plants are exposed to increasing levels of diverse human activities that impose new challenges and constraints on growth and development. Anthropogenic disturbance has a dramatic impact on overall plant physiognomy, and specifically on leaf morphology. Urban and suburban forest recreational sites have attracted considerable research interest recently. To explore the persisting recreational impact on differences in leaf shape and whole plant physiognomy, we conducted a field study, using dioecious forb *Mercurialis perennis* L. (Euphorbiaceae), a typical member of undisturbed understory communities, as a model system. We selected adjacent sites in a suburban forest, experiencing contrasting disturbance regimes, under otherwise concordant conditions. Patterns of leaf shape variation and possible sex-specific pattern in response to disturbance were analyzed using geometric morphometric approach. Organ-level shape data was integrated with data on plant height, leaf and internode number to explore the response at the whole plant level. The results show that plants growing under heavy disturbance regime had greater number of shorter and wider leaves on shorter stems. After removing the effect of size on shape, leaf shape pattern associated with disturbance was revealed. MANOVA for shape variables showed highly significant effect of site, but nonsignificant effect of sex. These shape changes are not attributable solely to differences in size and growth inhibition and are indicative of a stress-induced morphogenic response. This survey is the first one to use geometric morphometric method to depict influence of anthropogenic disturbance on leaf shape variation in a natural population.

Keywords: anthropogenic disturbance, leaf shape, geometric morphometrics, sexual dimorphism

This study is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173025).

The variation pattern of antioxidative enzymes in wild populations of *Iris pumila* inhabiting contrasting light environments: a reciprocal transplant experiment

PP6-10

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High solar irradiation is abiotic factor that can cause oxidative stress in plants, especially when co-occurs with drought and elevated temperatures. To investigate the adaptive responses of major antioxidative enzymes, superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) to high-light stress in *Iris pumila*, a rhizomatous perennial plant, we conducted a reciprocal transplant experiments within its natural habitats in the Deliblato Sands (Serbia). The leaf tissue of 20 reciprocally transplanted genotypes from two natural populations inhabiting an open and a shaded habitat were used for the immunoblot analyses of SOD, APX and CAT. In the open habitat, Cu/ZnSOD expressed two isozymes, whereas in the shaded one three Cu/ZnSOD isozymes were observed. The total amount of Cu/ZnSOD was lower in sun-exposed genotypes compared to their shaded counterparts. Conversely, the amount of MnSOD appeared to be greater in genotypes exposed to full sunlight than in those growing under the vegetation shade. The enzyme APX expressed three isozymes in all genotypes from both populations, but the total APX content was greater in leaves developing in full sun-light than in those from the vegetation shade. A small increase in the amount of CAT polypeptide was observed in genotypes growing in the shaded habitat compared to their counterparts growing in the open, in both populations. The results suggest that the adaptive response of *I. pumila* genotypes to high-light stress encompasses a concerted change in both the expression and the content of major antioxidative enzymes regardless of their population origin.

Keywords: high-light stress, antioxidative enzymes, reciprocal transplant experiment, *Iris pumila*

Flowering and reproductive performance of *Iris variegata* genotypes in different light conditions

PP6-11

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Plants grown under canopy shade show a range of responses to the change in light quantity as well as in red/far-red ratio of the ambient light, i.e. light quality. These responses, known as the shade-avoidance syndrome, or near neighbor detection response, are characterized, among others, by changes in flowering phenology and reproductive output. The individual's flowering pattern is defined by the timing or temporal distribution of flowering (start of flowering, average flowering period), the duration of flowering, as well as the number of flowers produced. We analyzed the genetic variability and phenotypic plasticity of flowering pattern and reproductive success in 97 genotypes of *Iris variegata* originating from contrasting light habitats in Deliblato Sands and expressed under different experimental light conditions. Rhizome segments were taken from each of these clones and transplanted in the experimental garden near the Institute for Biological Re-

search in Belgrade. Significant differences between genotypes were found for two traits (start of flowering, average flowering time). Genotypes originating from open and understory habitats significantly differed in three traits (number of flowers, number of capsules/number of flowers, seed mass/capsule). Significant effect of light treatment was found for three traits (number of capsules/number of flowers, seed mass/capsule, average seed mass). Statistically significant correlations between explored traits were generally similar but also to some extent habitat- and treatment-specific. Comparing these results with research carried out on congeneric species we noted that there were similar responses for some traits, but also significant differences in some components of flowering and fruiting success.

Keywords: genetic variability, *Iris variegata*, light environment, flowering phenology, reproductive output

This study is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173025).

VII

Mechanisms Underlaying Health Compounds Biosynthesis in Fruits (COST ACTION FA1106)

INVITED TALKS

Genetic improvement of fruits and vegetables for health

IT7-1

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Horticultural crops are rich sources of potent phytochemicals, which may play a role in preventing or ameliorating various chronic human diseases such as cancer, coronary vascular disease, Alzheimer's disease and diabetes. Numerous studies were dedicated to understanding the factors controlling the content of biologically active phytochemicals such as antioxidants, vitamins, etc. in fresh fruit and vegetables. The state-steady levels of these compounds during ripening may be attributed to both genetic and environmental factors, such as cultivar, cultural practices, environmental cues, fruit manipulation and postharvest environment. Recently special attention has been given to exploitation of natural biodiversity as an integrated part of modern marker assisted breeding to improve the nutritional quality and especially the content of bioactive compounds. This presentation will attempt to review the recent work on arginine and vitamin C and emphasis will be given to manipulation of their biosynthetic pathways to produce elite varieties enriched in these health promoting substances.

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New insight into the control mechanisms underlying fleshy fruit development and ripening

IT7-2

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Fleshy fruit development is characterized by a series of developmental transitions leading to major metabolic reorientation and structural changes. These developmental shifts are coordinated by a complex network of interacting signalling pathways. The co-ordinated changes in the levels of several plant hormones during the transition steps suggest their dynamic involvement in these processes. Though it is widely accepted that interplay between hormones is instrumental to the coordinated unfolding of most plant developmental processes, yet the key integrating molecular players triggering the developmental transitions remain poorly characterized. Using the tomato as a model species for fleshy fruit development, the molecular events underlying the transition from flower to fruit and from unripe to ripe fruit were investigated via combined genome-wide transcriptomic profiling and reverse genetics approaches. The data highlight the role of auxin and ethylene-dependent transcriptional control as part of the mechanisms by which the fruit developmental program switches into a ripening process. In particular, new light is shed on the role of *Auxin Response Factor* (*ARF*) genes in mediating interactions between ethylene and auxin signalling and set these transcriptional regulators at the cross-roads of both hormones. The down-regulation of some *ARF* genes resulted in

ripening phenotypes uncovering new roles for ARFs during fleshy fruit development. For instance, *Sl-ARF4* is shown to control sugar metabolism in tomato fruit and to regulate ripening-related quality traits and postharvest behaviour. On the other hand, down-regulation of Sl-ARF2 leads to severe ripening inhibition associated with altered expression of key ethylene biosynthesis and signaling genes, and major regulators of the ripening process, like *RIN*, *CNR*, *NOR*, *TAGL1*. The data bring unprecedented evidence supporting the role of auxin in the regulation of fruit ripening and define Sl-ARF2 as a new component of the control mechanism underlying the ripening process of climacteric fruit.

The role of transcription factors in regulation of tomato fruit ripening and quality

IT7-3

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MADS box genes comprise a family of transcription factors involved in numerous aspects of eukaryotic development. They are especially well characterized in plants regarding their roles in floral development and extending to fruit development, ripening and ultimately fruit quality. In tomato, a spontaneous, single locus deletion mutation within two adjacent MADS box genes, *LeMADS-RIN* and *LeMADS-MC* locus known as *ripening-inhibitor* (*rin*), abolished the function of both genes. The mutant presents a non-ripening fruit phenotype caused by mutation of the *LeMADS-RIN* gene and indeterminant inflorescence development and enlarged sepals resulting from the defect in the *LeMADSMC gene*. *LeMADS-MC* belongs to the *APETALA1* MADS-box family clade while *LeMADS-RIN* belongs to the *SEPALLATA* group.
We deployed a gene-specific RNAi approach to repress and 35S-ectopic overexpression of other MADS box family members to explore their possible roles in fruit maturation and ripening. The primary focus was on genes that were expressed in tomato carpels including *TAGL1*, *TAGL11* and *MBP3*. These tomato genes represent the orthologs of the Arabidopsis *SHP1* and *SHP2* and *STK* genes involved in specification of carpel and ovules in Arabidopsis flowers.

Metabolic engineering of bioactive compounds in tomato fruit allows for comparative assessments of their dietary health benefits

IT7-4

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Phenylpropanoids are derived from phenylalanine and comprise an important class of plant secondary metabolites that include specialized bioactives with medicinal properties and important phytonutrients, that promote human health. A number of transcription factors have been used to upregulate specific branches of phenylpropanoid metabolism, but by far the most effective has been the fruit-specific expression of *AtMYB12*

in tomato, which resulted in an astonishing 10% of fruit dry weight accumulating as flavonoids and hydroxycinnamates. AtMYB12 not only increases the demand of flavonoid biosynthesis, but also increases the supply of carbon from primary metabolism, and the supply of energy and reducing power, by upregulating glycolysis, the TCA cycle, the oxidative pentose phosphate pathway, fuelling the shikimate and phenylalanine biosynthetic pathways to supply more aromatic amino acids for secondary metabolism. AtMYB12 directly activates at least some genes encoding enzymes of primary metabolism. The enhanced supply of precursors, energy and reducing power achieved by AtMYB12 expression can be harnessed to engineer high levels of novel metabolites in tomato fruit, offering an effective production system for high value polyphenols, and foods fortified in health-promoting phytonutrients. Such model foods can be used to assess, quantitatively, the health promoting bioactivities of different dietary phytonutrients, though comparative nutrition.

Tomato fruit carotenoid biosynthesis: regulation and evolutionary aspects

IT7-5

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Cultivated (*S. lycopersicum*) is part of the lycopersicon section, comprising 13 species. In the fruits stay green thourghout ripening, while in a few species (*S. cheesmaniae*, *S. galapagense*, *S. pimpinellifolium* and *S. lycopersicum*) they show a change in color from green to yellow/orange or red. In order to understand the molecular basis of these differences in ripe fruit color, we conducted combined metabolic and transcriptional profiling of two green-fruited (*S. neorickii* and *S. arcanum*) one yellow-fruited (*S. cheesmaniae*) and two red-fruited (*S. pimpinellifolium* and *S. lycopersicum*) species. The pigment composition of leaves and fruits at different stages of development was analyzed by High Pressure Liquid Chromatography and online Photodiode Array detection (HPLC-PDA) and the expression of pigment biosynthesis genes was analyzed through RNA-Seq. The results suggest that a complex remodeling of multiple structural and regulatory genes involved in carotenoid and chlorophyll biosynthesis underlies the differences in color between green- and red-fruited species.

Suppression of a tomato prolyl 4 hydroxylase results in multiple alterations on fruit development, ripening and health components

IT7-6

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Proline hydroxylation is a major post-translation modification of hydroxyproline-rich glycoproteins (HRGPs) that is catalyzed by prolyl 4-hydroxylases (P4Hs). Their involvement in plant growth and development has been recently investigated in Arabidopsis, tobacco and carnation while little is known about their role in tomato. The tomato genome comprises 10 putative P4Hs with most of them being expressed during fruit development. Preliminary experiments to partially suppress their expression using Virus Induced Gene Silencing resulted in alterations on cell division and expansion of tomato leaves. Therefore, transgenic tomato plants expressing an RNAi construct were produced in order to suppress a tomato P4H highly expressed during fruit development and ripening. Several independent lines down-regulating the target P4H were identified and

nine of them were further characterized. The expression of the target P4H was completely suppressed during fruit ripening and the total hydroxyproline content in fruits was lower in most of the lines. All of the lines exhibited a reduction in fruit diameter while the number of viable seeds was significantly reduced. Moreover, a delay was observed in pedicel abscission which was associated with expression of key abscission progression genes. Most importantly alterations in the composition of tomato fruits were observed. Collectively, these results indicate that the target P4H plays a significant role in tomato fruit development and abscission.

VIII

Abiotic and Biotic Stress and Ecophysiology

INVITED TALKS

Strigolactones. Key players in the adaptation of plants to the abiotic environment

IT8-1

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The strigolactones are signaling molecules in plants with multiple roles. They are a new class of plant hormones that regulate plant development and are a rhizosphere signal inducing the germination of root parasitic plant species of the Orobanchaceae, and stimulating plant root colonization by symbiotic arbuscular mycorrhizal (AM) fungi. The biosynthesis of strigolactones is strongly stimulated by low phosphate conditions. On the one hand this results in the activation of AM fungi to help the plant to exploit a larger soil volume for phosphate. On the other hand, as a result, plant architecture is adapted to deal with these conditions (a.o. reduced shoot branching and altered root architecture). A possible role for strigolactones in the response of plants to other stresses such as drought, possibly through an interaction with ABA, is also slowly being uncovered. The multiple roles of the strigolactones with positive as well as negative effects have resulted in a large structural diversity with by now over 20 different strigolactones identified across the plant kingdom and many more the structure of which still needs to be elucidated. To further unravel the importance of this structural diversity we are further elucidating the biosynthetic pathway of strigolactones. The role of strigolactones in the adaptation of plants to abiotic stresses, the regulation of their biosynthesis and the current knowledge of their biosynthetic pathway and the biological importance of the structural diversity will be discussed.

Keywords: strigolactones, phosphate, drought, AM fungi, parasitic plants

H_2S and NO signalling in plants

IT8-2

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Besides already well known actions in animal organisms and described effects on stomata closing in plants, nitric oxide (NO) has an influence on seed dormancy, flower development, root gravitropism, gene expression, plant defence reactions in pathogen attack, photomorphogenesis, Zn-excess tolerance, antioxidative protection from UV-B radiation etc. Similar issues are now being addressed with regards to hydrogen sulphide (H_2S). A body of literature exists which shows that H_2S can affect the growth of plants, but more recent work has been published that suggests that H_2S is a more fundamental molecule which is produced by plants and used to control different plant functions. Known as a signalling molecule in both animal and human tissues, H_2S participates in signalling crosstalk in plants during stress, helping them to overcome unfa-

vourable growth conditions through an increase of stress enzymes' activity, preventing membrane destruction and enhancing accumulation of osmo-protective metabolites such as proline. It is probably the interaction of H_2S with metabolism of other reactive species which accounts for many of the effects seen. Furthermore, we showed that H_2S mediates stomatal opening using *A. thaliana* L. and *Capsicum annuum* L. plants as model systems. Using a specific NO fluorescence probe it was shown that H_2S reduced NO accumulation to a large extent, which may account for the effects of H_2S seen.
Further research should reveal if the interactions of H_2S and NO signalling pathways may regulate some other metabolic responses in plants as well, with possible influence of H_2S on plant productivity and quality.

Keywords: H_2S, NO, signalling

Essential oils elicit defense genes in potato: Can volatiles released from damaged plants prime defense in their undamaged neighbors?

IT8-3

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During infestation by herbivores or pathogens some plants emit large amounts of volatiles into the atmosphere as a part of a defense system. These compounds can directly repel microbes and animals or attract the natural predators of attacking herbivores. However, there is a lot of evidence that plant volatiles can mediate plant to plant communication providing information on herbivores in neighboring plants. In this study, the effects of volatile compounds of essential oils (EOs) isolated from *Nepeta rtanjensis* Diklić and Milojević and *Tanacetum vulgare* L. on defense mechanisms in potato were investigated *in vitro*. Expression of defense related genes encoding for lipoxygenase (*LOX*), serine type proteinase inhibitor (*Pin2*), phenylalanine ammonia lyase (*PAL1*) and pathogenesis-related endo-1,3-β-D-glucanase (*PR-2*) and thaumatin-like protein (*PR-5*), were analyzed by qRT-PCR in potato plantlets after 2, 4, 6, 8, 10, 12, 24, 48 and 72 h of continuous exposure to EOs. The abundance of dominant volatile compounds of both EOs in potato surrounding atmosphere was monitored by Proton Transfer Reaction Mass Spectrometry over the entire exposure period. Additionally, the activity of antioxidant enzymes, peroxidases, catalases and superoxide dismutases, were measured in order to determine the level of stress related response in exposed potato plantlets. Results of the gene expression analyses revealed that EOs induced the alternations in activity of all analyzed genes, while the status of antioxidant enzymes showed slightly elevated activity, both suggesting that EOs from *N. rtanjensis* and *T. vulgare* are capable to prime defense, but not to induce severe stress in potato under laboratory conditions.

Keywords: priming defense, potato, essential oils, gene expression, antioxidant enzymes

This study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Projects OI173015 and III41011.

Alyssum markgrafii as a model organism to study metal hyperaccumulation

IT8-4

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Plants from the genus *Alyssum* are well known hyperaccumulators capable to accumulate up to 3% of dried mass. *Alyssum markgraffii* O.E. Shulz is a Balkan endemit nickel hyperaccumulator able to accumulate up to 12 mg g^{-1} of nickel in its tissues. To investigate molecular mechanisms underlying metal hyperaccumulation and associated hypertolerance, shoot cultures of *A. markgraffii* were subjected to high nickel concentration (1-10 mM $NiCl_2$).Toxic effects of excess nickel were observed through morphological parameters together with increased reactive oxygen species (ROS) production and lipid peroxidation. Nickel exposure decreased chlorophyll and carotenoid concentration. Addition of sodium benzoate, potent ROS scavenger, showed concentration-dependent disturbing effect on nickel hyperaccumulation, lowering the content of accumulated nickel in *A. markgraffii* shoots. It is evident that nickel treatment with 1 mM $NiCl_2$ increased the amount of potential nickel transporter – nicotianamine. Using Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM-EDS), we showed that nickel was sequestrated outside of trichomes in *A. markgraffii*. In addition, nickel treatment did not affect the expression of metal transport protein (MTP) and heavy metal ATPase 3 (HMA3), potential nickel transporters. Further investigation related to the expression of potential nickel transporter (YSL group), as well as the investigation of nickel localization on the cellular level, using fluorescent dye and transmission electron microscopy, should provide more information about mechanism relying on nickel hyperaccumulation. Our work on metal hyperaccumulation provides insights not only into how a complex physiological trait evolved, but also into how plant metal homeostasis networks can be effectively modified for phytoremediation, phytomining and bio-fortification.

Keywords: *Alyssum*, nickel, hyperaccumulator, hypertolerance

This work was supported through grants No. 173005 and 173015 by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

SELECTED TALKS

The influence of *Trichoderma* spp. treatment on water regime, ABA content and gene expression in leaves and roots of tomato in drought conditions

OP8-1

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The aim of this work was to examine the influence of *Trichoderma* spp. treatment on water regime, ABA content and gene expression in leaves and roots of tomato plants in drought conditions. The experiment has started on tomato plants which were transplanted in the phase of established six leaves and then grown under controlled conditions. Seven days after transplantation water suspension of *Trichoderma* (T) isolate was added to half of the plants. At the stage of 9-10 leaves, half of the T treated and T untreated plants were not watered, until the soil water content decreased from 35% to 10% vol. Consequently relative water content (RWC) in the leaves of both T treated/untreated leaves decreased by about 25%. However, T treatment affected the response of plants so that RWC was higher in both control and drought conditions for 13%. Higher RWC of control T treated plants was in correlation with higher stomatal conductance (Gs), however, under drought conditions better water status is achieved by stomatal closure. The content of ABA tended to increase in leaves of plants subjected to drought, particularly in the case of T treatment. ABA content was higher in roots of T treated plants under optimal water supply while in drought there was no significant effect of T treatment. The beneficial effect of T treatment on water preservation in droughted plants provided by lower Gs is connected with increased ABA content in leaves. The results are discussed in connection with expression of several genes involved in SA and JA response.

Keywords: soil fungi, water stress, plant-microbe interaction

Effect of dynamic changes of vegetative compatibility types in *Cryphonectria parasitica* populations on biological control of chestnut blight in Croatia

OP8-2

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Chestnut blight, caused by ascomycete fungus *Cryphonectria parasitica*, is a dangerous disease responsible for the decline of chestnut forests. Fortunately, biological control of this disease is enabled by naturally occurring dsRNA virus, Cryphonectria hypovirus 1 (CHV1) that reduces virulence and reproductive capacity

of the fungus, consequently causing healing of cankers and recovery of infected trees. Transmission of CHV1 occurs horizontally via hyphal anastomosis and vertically into conidia, but not into ascospores. However, horizontal transmission of CHV1 is limited by the vegetative (in)compatibility system of the fungal host. Research conducted over a period of ten years revealed qualitative and quantitative changes of the vegetative compatibility (vc) types of *C. parasitica* populations in Croatia. Formally dominant vc types are becoming less frequent over time, some vc types are no longer present, and same novel vc types emerged in the studied populations. This could be the result of sexual reproduction and/or migrations from adjacent areas and it could, at least partially, account for decrease in percentage of hypovirulent (virus-infected) fungal isolates detected in these populations. Due to these changes of *C. parasitica* populations in Croatia, naturally present virus-induced hypovirulence may not be sufficient for the protection of the chestnut forests and human-mediated biocontrol using carefully selected CHV1 isolates might become necessary.

Keywords: chestnut blight, *Cryphonectria parasitica*, vc type diversity, biological control

Effect of *in vitro* induced water deficit on lipid peroxidation intensity and antioxidant capacity of sugar beet

OP8-3

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Accompanied by oxidative stress as a signal to activate defense mechanisms, various abiotic factors induce peroxidation of membrane lipids. The aim of this research was to analyse changes in lipid peroxidation (LP) intensity and antioxidant capacity of selected sugar beet genotypes during micropropagation under the conditions of water deficit. Performed research represents a part of a more comprehensive study, the purpose of which is understanding the difference in acclimation to drought as the major abiotic factor limiting sugar beet production in Serbia. The best solution for overcoming this obstacle in successful sugar beet growing is breeding for drought tolerance. Tested sugar beet genotypes were grown and multiplied *in vitro* on standard nutrient medium. Axillary shoots were placed on the micropropagation media with 3% and 5% PEG for 28 days, which caused physiological drought stress. All genotypes could be divided into three groups according to the level of LP, however only two of eight genotypes had significantly higher LP intensity during drought conditions when compared to their controls (10.1-29.1%), as well as significantly reduced antioxidant activity (22.2-59.9%). A slight decrease in LP intensity and enhanced antioxidant capacity (up to 92.9%) in comparison to control were recorded in treatments of the rest of investigated genotypes. As for correlation between tested biochemical parameters and growth conditions, all genotypes differed in their response to PEG concentration. Obtained results showed that LP intensity and antioxidant capacity could be used as biochemical parameters in assessing drought tolerance of individual genotypes in further selection of sugar beet.

Keywords: antioxidant activity, biochemical markers, micropropagation, sugar beet

High PAR and UV-B radiation-induced differential responses in green and white leaf sectors of *Pelargonium zonale* in relation to sugar, antioxidative and phenolic metabolism

OP8-4

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In this study we investigated the specific effects of high photosynthetically active radiation (PAR) and ecologically relevant UV-B radiation (0.90 W m^{-2}) on antioxidative, phenolic and sugar metabolism in variegated *Pelargonium zonale* plants. The green-white leaf variegation in these plants presents a suitable model system for examining "source-sink" interactions within the same leaf. High PAR (1350 µmol m^{-2} s^{-1}) and UV-B radiation induced tissue specific responses in variegated *P. zonale* leaves. While UV-B radiation had a pronounced effect on phenolic content in the white tissue, high PAR intensity stimulated accumulation of phenylpropanoids and flavonoids with preferential antioxidative *vs.* UV-screening function in green tissue. High PAR stimulated the increase of antioxidative metabolism in both leaf sections. However, the greater enhancement of ascorbate peroxidase and catalase activities and ascorbate content under HL+UV-B than HL only in green sectors, indicated that UV-B radiation and high PAR synergistically stimulated antioxidative defense. These results indicate that green tissue can be considered as high light acclimated, provided with an efficient defense against potential oxidative pressure under high PAR, along with significant protective role of UV-B radiation. Efficient sugar transport from green to white tissue was stimulated by both UV-B radiation and high PAR intensity. By stimulation of starch and sucrose breakdown and carbon allocation in the form of soluble sugars from "source" (green) tissue to "sink" (white) tissue, UV-B radiation stimulates a compensatory mechanism for phenylpropanoid and flavonoid biosynthesis in white tissue, due to the lack of photosynthesis.

Keywords: high light intensity, flavonoids, sugar distribution, UV-B radiation, variegated *Pelargonium zonale*

POSTER PRESENTATIONS

Impaired electron transport in thylakoid membranes of young spruce needles exposed to high light stress

PP8-1

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Photoinhibitory implications in photosynthetic apparatus of two developmental stages of Norway spruce (*Picea abies* L. Karst.) needles, current-year (CY, three months old) and previous-year (PY, one year older), were investigated. Chlorophyll *a* fluorescence of young (CY) and mature (PY) needles, collected early in the morning, was measured after acclimatization to the room temperature and after the stress treatment (HL, ~850 μmol m^{-2} s^{-1}; 29 ± 1 °C; 1 h) conducted in growth chamber.The maximum quantum yield of photosystem II (PSII), Fv/Fm, measured before stress application showed fully functional PSII in both CY and PY needles. Increased performance index on absorption basis (PI_{ABS}) in PY revealed their higher overall photosynthetic efficiency. Light stress (HL) slightly decreased Fv/Fm in both CY and PY needles, but remained high above limited value for PSII functionality (Fv/Fm>0.75). Specific fluxes per active reaction center (RC) including absorption (ABS/RC) and trapping of excitation energy (TR_0/RC), electron transport (ET_0/RC) and dissipation of excessive absorbed energy (DI_0/RC) were also analyzed. CY needles had decreased PI_{ABS} and ET_0/RC after stress treatment. Other fluxes per RC showed significantly higher values in CY, when compared with PY needles. In conclusion, we can highlight better acclimation response of mature needles under photoinhibitory light. In spite of the same primary photochemistry, young needles were strongly affected by light stress which was related to inhibited electron transport in thylakoid membranes.

Keywords: *Picea abies,* high light stress, photoinhibition, chlorophyll a fluorescence, photosynthesis

Cadmium phytoextraction using willow clones (*Salix* spp.) – effect of citric acid as chelating agent

PP8-2

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The uptake and accumulation of different heavy metals in plants depend on soil properties and plants ability for phytoremediation. Willows are promising candidates for Cd phytoextraction. The application of chelating agents has shown positive effects on increasing the solubility of heavy metals in soil and enhancing phytoextraction. In this study, the citric acid (CA) was used as chelating agent, due to its properties being a natural low molecular weight organic acid. The aim of this work was to investigate the effects of increased Cd con-

centration in the soil on the plant tolerance, accumulation and translocation of Cd in three different willow clones (*Salix viminalis*, *S. matsudana* and *S. alba*), as well as their potential for phytoextraction. Cuttings were grown in the greenhouse using the soil culture method. Treatments consisted of Cd (control, 3 and 6 ppm), applied separately and in combination with citric acid (20 mM kg^{-1} of dry soil). Total amounts of Cd in various plant parts and soil were determined using an Atomic Absorption Spectrometer. Generally, willows were resistant to applied concentrations of Cd, and didn't show any visible phytotoxic symptoms. Morpho-physiological parameters of clones were differently affected by Cd and CA treatments. Results have shown statistically different impact of CA on proline content in leaves. The addition of citric acid has increased transport of the accumulated Cd from the roots to the aerial part in *S. viminalis* and *S. alba* in comparison with the same treatments without CA. Bioaccumulation factor of all treatments was higher than 1, reliably suggesting good potential of selected willow clones for phytoextraction.

Keywords: phytoremediation, cadmium accumulation, willow clones

Mangiferin and total phenol content in two *Iris* species during the vegetative season in genotypes originating from contrasting habitats

PP8-3

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Polyphenols (phenolic acids, flavonoids, xanthones) are a large and chemically diverse class of secondary metabolites which occur widely in plants. *Iris* species are known to be rich in isoflavonoids, flavonoids and C-glucoxanthones. Mangiferin, naturally occurring C-glucoxanthone, was the first xanthone to be investigated pharmacologically and has been found to exhibit various pharmacological and biological effects. *Iris* pumila and *Iris* variegata are native to the area stretching through central and southeastern Europe. Genotypes of these two congeneric species inhabited locations with contrasting light conditions (exposed grassy and forest habitats) in Deliblato Sands, protected sandy area 40 km NE from Belgrade, Serbia. Above- (leaves) and below-ground (rhizomes) plant organs were taken for secondary metabolite analysis in the beginning and at the end of the vegetative season (spring and autumn). The purpose of This research was to screen two *Iris* species for their mangiferin and total phenolic content using HPLC and Folin-Ciocalteu method. In addition, we wanted to establish if clones of each species growing in contrasting habitats differed in the content of these compounds. Mangiferin amount in leaves was higher in spring then in autumn in both *Iris* species. The same pattern was observed in rhizomes of *I.variegata*. Total phenol content in spring was higher in leaves comparing to rhizomes in genotypes inhabiting both localities. Contrary, in autumn, higher total phenolics were observed in rhizomes of genotypes originating from both habitats in both *Iris* species.

Keywords: *Iris*, mangiferin, total phenolic content, contrasting light habitats, vegetative season

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, research grant 173015 and grant 173025.

Allelopathic effect of aqueous extracts of *Urtica dioica* L. on germination and growth of some cereals

PP8-4

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Allelopathy refers to beneficial or harmful effects of one plant on another plant, from the release of chemicals from plant parts by leaching, root exudation, residue decomposition and other processes in both natural and agricultural systems. Commonly cited effects of allelopathy include reduced seed germination and seedling growth. Nettle (*Urtica dioica* L.) is a species of wild edible plants, which grows on neglected places like weeds, and is encountered along the edges of fields planted with agricultural crops. The allelopathic potential of *U. dioica* aqueous extracts on seed germination, seedling growth and fresh weight of wheat, barley and oat was studied. The experimental design was completely randomized with six treatments (extract concentration of 0%, 5%, 10%, 20%, 40% and 80%) with thirty seeds. Obtained results showed that the effect of aqueous extract of nettle on tested parameters was dependent on the concentration and type of the plant. Different concentrations of extracts stimulated germination of wheat (88.3-93.3%), but inhibited it in barley (56.6-73.3%). Germination in oat was stimulated at lower and inhibited at higher concentrations of extracts. Root elongation was stimulated at concentration of 5%, while higher concentrations had inhibitory effect in all cereals (by 40.2-84.3% less than control). Aqueous extract of *U. dioica* had adverse effect on oat seedling growth (even 63.8% less than control). Contrary, seedling growth was increased at lower concentrations in wheat and barley, and inhibited at concentrations of 40% and 80%. The greatest inhibitory effect on the fresh weight was recorded at concentration of 80% in all investigated cereals.

Keywords: wheat, barley, oat, bioassay

Effects of salt and water stress on wheat root development

PP8-5

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The main objective of this paper was to monitor the development of the root system of wheat cultivars under different levels of water and salt stress. The HAS-RSDS root phenotyping platform is a semi-automatic diagnostic system for continuous monitoring with the rhizocolumn system during the developmental stages. The experiment was conducted with fourteen wheat cultivars (*Triticum aestivum* L.) from Serbia (5), Austria (4) and Azerbaijan (5) which were chosen based on different levels of salt and drought tolerance shown in field trials. The experiment was carried out in a greenhouse under four different watering/salt conditions: 1. well watered (60% field capacity) without salt (NaCl) added (control 1); 2. water limited (20% field capacity) and no salt (NaCl) added (control 2); 3. well watered (60% field capacity) and saline conditions (0.2% NaCl) and 4. water limited (20% field capacity) and saline conditions (0.2% NaCl). Digital images of root architecture were

collected from different side angles, from the bottom view through inside the soil using boroscope technology. Under water limited and salt stress conditions, the varieties *Gallio* and *Capo* showed the best root density at side of rhizocoloumn at flowering stage, whereas the half of the varieties poorly tolerated salt and water stresses. The obtained results from root phenotyping together with field investigations could help the breeders in the selection and crossing programs to achieve good level of drought/salt tolerance in wheat genotypes.

Keywords: abiotic stress, root density, *Triticum aestivum* L.

This research was funded by the Program of Transnational Access to European Plant Phenotyping Network (grant agreement no. 284443) and by the Ministry of Education, Science and Technological Development of Serbia (TR31066).

The effect of lead stress on *Paulownia elongata* biomass production in hydroponics

PP8-6

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Paulownia elongata S.Y.Hu is a fast-growing woody species tolerant to urban pollution, suitable for aforestation of degraded and mining areas and use in intense traffic areas. So far it was shown to be tolerant to increased concentrations of lead in urban atmospheric dust, but its tolerance to increased accessibility of lead in the root zone was not determined. The aim of the present experiments was to determine the effect of lead stress in conditions of adequate nutrients availability as well as under P and Fe deficiency. Shoots of *Paulownia elongata* were used for the establishment of *in vitro* cultures. In multiplication phase MS medium was used with 6 mg L^{-1} N^6benzyladenine (BA) and 0.5 mg L^{-1} indole-3-butyric acid (IBA). MS medium with 0.8 mg L^{-1} IBA and 0.8 mg L^{-1} 1-naphthaleneacetic acid (NAA) was used for rooting. Rooted cuttings were further grown hydroponically in modified Hoagland solution. In the pre-treatment plants were exposed to the phosphorus and iron deficiency and then treated with lead, adding $Pb(NO_3)_2$ at concentration of: 20, 50, 100 and 250 µM. Minimum inhibition of growth was observed in the plants which were provided with all nutrients in the course of treatment. When plants were exposed to a lack of phosphorus, inhibition of growth by lead was most pronounced. At the concentration of 250 µM shoot mass was about three times lower than in the control, while the root mass was two times lower. Pronounced chlorosis and a significant reduction in the concentration of chlorophyll *a* was observed in plants treated with lead after iron deficiency pre-treatment, while there was no significant reduction in dry weight of shoots and roots after lead stress. The results may have potential significance for improving biomass production of this species on soils contaminated with lead.

Keywords: lead, stress, *Paulownia elongata,* hydroponics

The oxidative stress in *Ambrosia artemisiifolia* L. shoots grown *in vitro* induced by *Nepeta rtanjensis* and *N. cataria* essential oils

PP8-7

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The possibility of using essential oil (EO) of *Nepeta rtanjensis* and *N. cataria* as bioherbicides against *Ambrosia artemisiifolia* L., an important weed and allergen species, was explored. *In vitro* culture experimental system of *A. artemisiifolia* has been established and ragweed shoots were exposed to the atmosphere enriched with volatile compounds (VOCs) emitted from EOs. *N. rtanjensis* EO was characterized by high amounts of 4aα,7α,7aβ-nepetalactone (*trans,cis*-nepetalactone), while *N. cataria* EO possessed high amounts of 4aα,7α,7aα-nepetalactone (*cis,trans*-nepetalactone). Reduction in *A. artemisiifolia* shoots growth and their discoloration, in the presence of *N. rtanjensis* and *N. cataria* EOs (2% and 4%, final nepetalactone concentrations) was observed after 2 weeks of exposure. An antioxidative defense system of *A. artemisiifolia* was activated by increasing peroxidases activity or by inactivating catalases and superoxide dismutases activity. Overall, stronger inhibitory effect on shoot growth, CAT activity, and stimulating POX activity was observed for *N. cataria* EO. *N. rtanjensis* EO was more efficient in inhibiting rooting and root growth, and in suppressing SOD activity. Therefore, EOs of *N. rtanjensis* and *N. cataria* might be recommended as potential bioherbicides against highly allergenic and weed species *A. artemisiifolia*. Further exploration of EOs' mode of action, confirmation of phytotoxicity in field conditions, and feasibility assessment for the commercial production and application in agricultural practice, is the course of our further work.

Keywords: *Ambrosia artemisiifolia*, nepetalactone, antioxidative enzymes, *Nepeta rtanjensis, Nepeta cataria*

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173024 and III41011).

Physiological responses to drought conditions in transformed *AtCKX1* and *AtCKX2* potato (*Solanum tuberosum* L.) plants

PP8-8

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Cytokinins (CKs) have an important role in plant responses to abiotic stresses, such as drought. It is known that during the stress, an alteration of CK transport from the roots occurs and influences gene expression in the shoots and, thus, elicits appropriate responses to the stress. Moreover, possible enhancement of CKX ac-

tivity during abiotic stress, probably mediated *via* abscisic acid (ABA), has been reported recently. In this study we have investigated effects of CKs on response to drought in transformed potato plants, bearing *AtCKX1* or *AtCKX2* genes under the control of CaMV 35S promoter. As previously shown, selected transformed potato lines grown *in vitro* were characterized by slightly elevated (*AtCKX1* line) or manifold increased (two *AtCKX2* lines) CKX activity, resulting in differently reduced bioactive CK contents and changed ABA levels. Shoots of non-transformed and transformed lines from *in vitro* culture were rooted, and plants were transferred to commercial compost, acclimated and grown under controlled conditions. Stress was imposed by withholding water (SWC reduced from 35% to 6%). Water potential and stomatal conductance of mature, fully expanded leaves, as well as shoot fresh weight were determined. Differences in measured parameters between *AtCKX2* lines, on the one side, and *AtCKX1* and non-transformed lines, on the other side, were observed. Obtained results indicate that response to drought in potato plants probably depends on CKX activity, CK homeostasis and their effects on ABA levels.

Keywords: drought, AtCKX, cytokinins, potato

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Phytoplasmoses: from devastating disease to latent infection (balanced parasitism)

PP8-9

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Phytoplasmas, members of the provisional taxon '*Candidatus* Phytoplasma', form a monophyletic group within the class Mollicutes. They are obligate pathogens of plants and insects, their vectors, which transmit phytoplasmas in a persistent manner. Phytoplasmas are associated with more than 1,000 plant diseases, which affect many economically important vegetable and fruit crops, ornamental plants, timber and shade trees. Disease severity varies from mild or even latent to severe, economically important or devastating. The most typical symptoms of phytoplasma infection are proliferation of axillary buds (witches' broom formation), virescence and phyllody (development of green and/or leaf-like structures instead of floral organs) which may lead to flower sterility. Besides these symptoms, many other less specific ones have been associated with phytoplasma infection, resulting from biotic stress caused to infected plants. However, some phytoplasma infections are desirable, mainly in ornamental horticulture. Although four phytoplasma effectors have been functionally characterized so far, how such extreme malformation of plants is induced by phytoplasma remains largely unclear. In some cases the same or undistinguishable phytoplasmas have been associated with diverse symptoms in different plant hosts, while distinct phytoplasmas have been associated with similar or the same symptoms in a single species. Cases of latent infection of host plants to phytoplasma infection are not rare and are particularly interesting from a biological point of view. They raise the question of which factors are able to induce such a relationship and whether they can potentially be used to reduce phytoplasma impact on infected plants.

Keywords: symptom, effectors, tolerance

Pb, Cd and Zn stress influence seed germination and seedling growth of invasive woody plants

PP8-10

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The effect of heavy metals cadmium, lead and zinc on seed germination and early seedling growth of invasive tree species *Ailanthus altissima* (Mill.) Swingle, *Acer negundo* L. and *Ulmus pumila* L. was investigated. Seeds were germinated in 0 μM, 20 μM, 50 μM and 90 μM Pb nitrate, Cd nitrate or Zn sulfate. Seedlings were hydroponically grown in controlled conditions. Toxic metal stress imposed on these woody plants had no significant reduction in seed germination parameters. Germination capacity of seeds treated with the highest concentration of Cd and Pb nitrate was higher in *A. altissima* (88.66% and 94.67%, respectively) compared to *A. negundo*. Pb and Cd stress did not influence significantly *A. altissima* shoot and root growth. It was also found that the optimal Fe supply in Pb-stressed *A. altissima* plants increased photosynthetic efficiency and chlorophyll *b* content. These seedlings had 4-7 fold higher biomass production so that *A. altissima* plants could be recommended for phytoremediation. In Zn-stressed conditions these seedlings were tolerant to elevated concentrations in early developmental stage, but later, the strongest concentrations inhibited root growth and leaf development. It was found that Cd reduced *U. pumila* seedlings shoot length, but not significantly root length, while Pb did not influence shoot length and stimulated root length. As seed germination and seedlings early development were possible in the presence of high concentrations of Pb, Cd and Zn, investigated invasive species could be considered facultative tolerant to these toxic metals.

Keywords: toxic metals, stress, *Ailanthus altissima, Acer negundo, Ulmus pumila*

Arsenic-induced oxidative stress and antioxidant response of *Festuca rubra* L. growing on fly ash deposits

PP8-11

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Arsenic (As) is a highly toxic metalloid present in fly ash (FA) generated from coal combustion in thermal power plants and has been recognized as an environmental and human health hazard. Therefore, arsenic phytoremediation of fly ash requires suitable plant species that are tolerant to high levels of arsenic in the aboveground tissue. This study examined the metabolic adaptation of *Festuca rubra* L. under arsenic stress. Field studies were carried out in May 2009 on passive FA lagoons of thermoelectric power plant 'Nikola Tesla – A' in Obrenovac (L3 – 3-year-old lagoons; L2 – 11-year-old lagoons) and in Botanical Garden 'Jevremovac' in Belgrade (CS – control site). Concentrations of As in leaves of *F. rubra* at L3 and L2 (5.08 and 4.80 μg g^{-1}, respectively) were elevated in comparison to CS (3.43 μg g^{-1}). Arsenic accumulation caused oxidative stress in *F. rubra* leaves at both FA sites, as indicated by significant increases in MDA levels ($p<0.05$; $p<0.001$). High concentrations of As reduced the efficiency of PSII photochemistry (Fv/Fm), chlorophylls and carotenoids content ($p<0.001$). However, with increase in the concentration of As, the content of anthocyanins, phenolics and ascor-

bic acid as well as DPPH radical scavenging activity in leaves of *F. rubra* increased at both FA sites ($p<0.001$). Results of our study indicate that in spite of high oxidative stress and reduced photosynthetic efficiency, this grass species is characterized by significant activation of the antioxidant protection under arsenic stress. Therefore, *F. rubra* possesses high adaptive potential to grow and survive hostile conditions on fly ash deposits.

Keywords: arsenic, *Festuca rubra,* fly ash, oxidative stress, adaptations

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The allelopathic activity of leaf and fruit leachates of introduced invasive *Amorpha fruticosa* L.

PP8-12

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Allelopathic activity can be one of the key mechanisms that allow the spread of invasive species into new habitats and dominance in the community. *Amorpha fruticosa* is a highly invasive species in semi-aquatic ecosystems and one of the most invasive species in Serbia. The aim of this study was to determine whether this species has allelopathic activity, through research on the effect of leaches from the leaves and fruits on seedling growth of lettuce (*Lactuca sativa* L.). Because allelopathic activity varies under the influence of environmental factors, the plant material was collected from several habitat types: 1) indigo bush (*A. fruticosa*) riverine scrub community; 2) artificial broadleaved deciduous forestry plantation of *Populus canadensis;* 3) sedge and reedbeds communities, on the banks of standing water. Effects of leachates from dry leaves and fruits (with dormant seeds) of test species (*A. fruticosa*) were assayed on agar (Sandwich Method). Radicle growth inhibition of indicator species (*L. sativa*) served as a measure of the inhibitory capacity of *A. fruticosa*. Allelopathic activity varied depending on the type of plant organs tested and habitats from which the sample was taken. Leaf leachates of *A. fruticosa* showed high allelopathic activity (radicle growth inhibition: 42-82%), while leachates from fruits showed lower allelopathic inhibitory effects (radical growth inhibition: 20-57%). Highest allelopathic activity was obtained from leaf material collected from sedge and reedbeds communities, on the banks of standing water. Water stress, caused by large amounts of water in the land, may be one of the key factors that influence higher production of allelochemicals in *A. fruticosa.*

Keywords: allelopathy, *Amorpha fruticosa*, invasive, leachates

Effect of meteorological factors on heading time and plant height in wheat

PP8-13

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Change of meteorological factors throughout years causes different reactions of wheat genotypes in terms of trait expression. Effects of environmental factors (sum of active temperatures, total precipitation and insolation) on heading time and plant height were analysed in twenty-seven wheat genotypes of different geographical origin from 2009-2013. The selected genotypes were classified into three groups according to the heading time (early, medium early and late), and the height (low, medium or high). Canonical correlation analysis was used to determine the interdependence between environmental factors and wheat phenotypic traits. Within all three groups for plant height, Eigen value of the first canonical factor explained 86.9-98% of the variability, indicating one predominant factor in the expression of this trait. Within the groups for earliness, Eigen value of the first canonical factor explained 64.9-76.3% of the variability, indicating more predominant factors in the expression of this trait. The coefficient of Canonical correlations (r) for plant height (0.50-0.64) showed a significant interdependence of all three groups of genotypes with all environmental factors used as dependent variables. Unlike height, in genotypes differing in earliness r differed significantly as an indicator of interdependence with the dependent variable. Specifically, in the early genotypes highly significant value was observed (r=0.81), in the medium early significant (r=0.46), while in the late genotypes the value was not significant (r=0.23). Since both analysed traits are determined by reduced height genes (Rht) and photoperiod genes (Ppd), this study accurately indicates their expression in different environmental conditions.

Keywords: wheat, heading time, plant height, meteorological factors

Allelopathic effects of three moss species on seed germination of *Lactuca sativa*

PP8-14

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Although bryophytes represent the second most diverse group of land plants, little is known about their allelopathic interactions with other plants. The fact that despite their small body some of them can form large colonies, excluding other plants, indicates that allelopathy may be an important strategy in their competition for resources. To screen for allelopathic substances, we tested the aqueous extracts of the mosses *Abietinella abietina* (Hedw.) Fleisch., *Leucodon sciuroides* (Hedw.) Schwägr. and *Homalothecium sericeum* (Hedw.) Schimp. against seed germination of *Lactuca sativa* L. All moss species were collected in Pešter plateau, in the springtime 2014. Thirty seeds were put on double-layered filter paper in each Petry dish, supplied with 4 mL of moss extract of different concentrations and incubated for three days. Four replicates were made for each concentration (3% and 30%) and for the control (0%). At 3% concentration, statistically insignificant increase in germination rate was observed for *A. abietina* and *H. sericeum* extracts compared to that of the control group. In contrast, all aqueous extracts of 30% concentration inhibited seed germination, but only *H. seri-*

ceum extract significantly reduced germination rate of lettuce seeds (P<0.05). These observations imply that *H. sericeum* may possess allelopathic potential, but more detailed analyses are needed to confirm this and to identify the substances responsible for these effects.

Keywords: bryophytes, extracts, biotic relationship

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Biochemical changes in *Hypnum cupressiforme* during *in situ* exposure to atmospheric pollution

PP8-15

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The technique of moss bags is widely used for atmospheric pollution biomonitoring. The moss *Hypnum cupressiforme* is ubiquitous and thus widely used for such surveys. In this study we have tested biochemical properties of *H. cupressiforme* exposed at three different positions within Botanical Garden Belgrade, and at pre-defined light and humidity conditions as control (16h light/8h dark, 18 °C). The parameters such as pigments (chlorophyll- a, -b, carotenoids), total phenolic and antioxidative capacities. The exposure time was 60 days during the summer 2014. The sample analyses were performed each 15 days. The results showed a strong incongruence in plant status in moss bags that were exposed under real conditions and the bags kept under controlled conditions. In the moss exposed in bags within the study sites, decrease of the pigment content with the exposure time was clearly evident, in contrast to the moss which was under controlled conditions where the plants were grown under close to optimal conditions. The phenolic content was significantly increased, up to four times, in the exposed moss bag in comparison with plants exposed in atmospheric conditions from 15 to 60 days. In the control group, the phenolic content was up to seven times lower compared to the exposed moss. The antioxidative capacity was lowest in the control group. The results clearly showed that the exposed plants were stressed, and that capacity for using them as such in active biomonitoring, decreases with time exposure and the plant losing viability.

Keywords: moss, pollution, biomonitoring, stress

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Allelopathic potential of an emulsion of *Nepeta rtanjensis* Diklić et Milojević essential oil on common chickweed [*Stellaria media* (L.) Vill.] seed germination

PP8-16

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The effect of an emulsion of essential oil of *Nepeta rtanjensis* Diklić et Milojević on seed germination and seedling growth of common chickweed [*Stellaria media* (L.) Vill.] was examined. In the first experiment, the dynamic and total germination of seeds were evaluated after applying the essential oil emulsion at six concentrations, ranging from 0.003-0.1%. That segment of the experiment was carried out in Petri dishes under constant temperature of 20 ± 1 °C. Seeds were germinated in the dark over the initial 48 h and then illuminated during 10-min counting sessions at intervals of 1-3 days. In another experiment, the dynamic and total germination of *S. media* seeds were evaluated in soils treated with five concentrations (0.0625-1%) of the emulsion of *N. rtanjensis* essential oil. Pots containing 100 g of soil treated with 3 ml of emulsion were used to germinate 50 seeds in each at 26 ± 1 °C/21 ± 1 °C temperature and under 14h/10h light/dark photoperiod. After emergence, the seedlings were sprayed with emulsions that had the same concentrations of essential oil as those used for spraying seeds. Seeds in Petri dishes did not germinate at oil concentrations exceeding 0.00625%, and germination was at a very low percentage at that concentration. The essential oil had affected the dynamic but not total germination of seeds in pots. Concentrations of the essential oil had effect on the seedling parameters measured (height, number of nodes, fresh and dry weight, and number of dead plants).

Keywords: allelopathy, *Nepeta rtanjensis,* essential oil, *Stellaria media*

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Elm yellows and Dutch elm disease in Croatia

PP8-17

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Elm yellows (EY) is a disease caused by phytoplasmas, non-helical, wall-less, unculturable bacteria of the class *Mollicutes* and provisional genus '*Candidatus* (*Ca.*) Phytoplasma' **.** '*Ca.* Phytoplasma ulmi' (16SrV-A subgroup) is predominantly infecting elms in Europe, although other phytoplasmas were occasionally detected as well. Dutch elm disease (DED) is caused by ascomycete of the genus *Ophiostoma*. First DED pandem-

ic was caused by less aggressive species, *O. ulmi*, and the second, ongoing pandemic is caused by more aggressive species, *O. novo-ulmi*. In Croatia, DED has spread in the early 20th century and decline of elms observed since then is mainly associated with this disease, while phytoplasmas causing EY have not been studied. However, DED was mainly detected based on external and internal disease symptoms without detailed investigation of the DED pathogen. In this study, samples of *Ulmus laevis*, *U. minor* and *U. glabra* from across Croatia were analyzed. Phytoplasmas were detected by 16S rRNA gene amplification and sequencing in 64 out of 139 samples. Three phytoplasmas were identified: '*Ca*. Phytoplasma ulmi' (16SrV-A subgroup), '*Ca*. Phytoplasma asteris' (16SrI group) and '*Ca*. Phytoplasma solani' (16SrXII-A subgroup), but the infection was most frequently caused by '*Ca*. Phytoplasma ulmi'. Dutch elm disease was confirmed by isolation of the fungus from 55 out of 139 trees and results of fungal growth rate and morphology test indicated that infections were exclusively caused by *O. novo-ulmi*. Furthermore, for the first time it was proven that phytoplasmas and *O. novo-ulmi* can cause mixed infection in natural elm populations.

Keywords: elms, phytoplasma, phytopathogenic fungi, pathogen detection, mixed infection

Local and systemic oxidative stress response of cucumber plants to potassium naphthenate treatement

PP8-18

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Naphthenic acids represent a complex mixture of aliphatic and alicyclic carboxylic acids found in crude oils and oil sands bitumen. They are toxic components in refinery wastewaters and in oil sands extraction waters. Because of their structure similar to plant hormones of the auxin and gibberellin families, they were studied as stimulators of plant growth. This study investigated the changes in the activities of several antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPOX), as well as the extent of lipid peroxidation (LP) and electrolyte assimilation of cucumber (*Cucumis sativus* L.). Plants were treated with potassium naphthenate (K-naph) at different concentrations (0.1-400 μM). It was proved that the antioxidant system of cucumber was sensitive to the application of K-naph since the parameters of its antioxidant status changed already at a very low concentration of this agent. The difference was observed between local and systemic response, assessed in roots and leaves, respectively. The highest concentrations of K-naph (100, 200, 400 μM) caused electrolyte "leakage" and the highest oxidative stress. Roots of the plants at these concentrations showed significantly higher antioxidant enzymes activities, but LP was elevated in leaves. Low naphthenate concentrations (0.1, 1, 10 μM) decreased antioxidant enzymes activities, both locally and systemically. These results indicate that naphthenic acids could act as stimulators or inhibitors depending on the concentration.

Keywords: potassium-naphthenate, cucumber, oxidative stress, response

Activity of nitrogen assimilation enzymes in soybean seedlings infected with hemibiotrophic fungi

PP8-19

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The purpose of this research was to compare how soybean seedlings (*Glycine max* L., cultivar Bečejka) cope with different nutrition acquisition strategies of hemibiotrophic fungi: *Rhizoctonia solani* Kühn and *Sclerotinia sclerotiorum* (Lib.) de Bary. Severe changes at morphological and histological level after inoculation with both fungi were accompanied by significant changes in nitrogen assimilation enzymes activities in leaves and roots of 21-day-old soybean plants. Infected seedlings had decreased nitrate reductase (NR) (2-fold the amount of control, on average) and glutamate synthase (GS) activity (40-60%), except in leaves infected with *S. sclerotiorum*. Glutamate dehydrogenase (GDH) activity increased 46-75% after the pathogen infection, being highest during *R. solani* infection. High GDH values in infected organs (0.26-0.47 μmol NADH mg^{-1} protein) point to enhanced nitrogen remobilization process from infected tissue, possibly to restrict available nutrients to pathogens, among other things beneficial to plant. Differences in GS and GDH activities in the same organs depending on pathogen infection showed that plants cope differently with these fungi at this stage of development, or that time of switching from bio- to necrotrophic lifestyle differs between investigated pathogens. Due to adaptable lifestyle of hemibiotrophic fungi, mechanistic details that allow pathogen to control host metabolic pathways are unknown, and for this reason the understanding of plant nutrient acquisition could be of great importance in the development of novel disease control strategies.

Keywords: biotic stress, hemibiotrophic fungi, nitrogen metabolism

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Effect of nitrogen nutrition on water use efficiency of wheat cultivars under well-watered and drought conditions

PP8-20

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Optimal nitrogen (N) nutrition has been shown to alleviate the negative effects of drought stress (DS) on plants. In this study, the effects of different ratios of N nutrition on the water use efficiency (WUE) of ten wheat cultivars were investigated under DS and non-DS conditions. The experiment was conducted in a greenhouse with four growth conditions:

1. Well watered (60% field capacity) in the presence of sufficient N (60 mg N kg^{-1} soil)
2. Well watered (60% field capacity) in the presence of low N (3-4 mg N kg^{-1} soil)
3. Water limited (20% field capacity) in the presence of sufficient N (60 mg N kg^{-1} soil)
4. Water limited (20% field capacity) in the presence of low N (3-4 mg N kg^{-1} soil).

Water use profiles of individual plants were recorded during the whole cultivation period from which the efficiency of water usage, as well as the effect of N availability on water utilization was determined. WUE was significantly decreased by N limitation in well watered conditions, as well as in drought stressed plants. This shows that under N-limitation wheat plants have decreased capacity to use soil water. The WUE at the level of seed production was not affected by N limitation under drought stress in two wheat cultivars (NS Avangarda and Siete Cerros). These cultivars could be used as potential parents for development of new wheat cultivars with enhanced production under drought and N-limited conditions.

Keywords: wheat, nitrogen nutrition, water use efficiency, drought

Black locust and white poplar ecophysiological adaptations to pollution stress at the fly ash deposits of the 'Nikola Tesla – A' thermoelectric plant (Obrenovac, Serbia)

PP8-21

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The ecophysiological traits of two woody species, *Robinia pseudoacacia* L. (planted) and *Populus alba* L. (naturally colonized), were assessed in terms of trace element (As, B) accumulation, photosynthetic efficiency (Fv/Fm), total chlorophyll (Chla+b) and carotenoid (Tot Carot) content, and MDA levels in populations growing at the 'Nikola Tesla – A' thermoelectric power plant's fly ash ponds, weathered for 3 (L1) and 11 years (L2), compared to their natural habitat. Research showed that the trace element content in leaves of both species at the ash deposits was higher compared to plants from the reference site ($p<0.001$). Despite decreasing as ash aged, the B content in leaves of both species at both ponds was at toxic levels for plants. As ash age increased, so did As concentrations in both species, with levels in white poplar leaves at L2 and black locust leaves at L1 and L2 being in the toxic range. In such conditions, white poplar exhibited stable photosynthetic efficiency at both ponds due to the stable photosynthetic pigment content and the functional integrity of cell membranes. At L2, symptoms of oxidative stress in black locust manifested in the form of reduced Fv/Fm ($p<0.001$), elevated levels of lipid peroxidation ($p<0.05$), and lower levels of chlorophyll and total carotenoids ($p<0.001$) compared to plants at the reference site. The results show that white poplar exhibited higher adaptive potential at L1 and L2, while black locust had reduced adaptive potential to the stressful conditions on the weathered ash at L2 of the 'TENT-A' ash deposit site.

Keywords: fly ash, *Robinia pseudoacacia* L., *Populus alba* L., pollutants, adaptations

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Physiological and biochemical characterization of maize genotypes from drought tolerant mini-core collection under osmotic stress

PP8-22

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Drought is one of the most important environmental stresses and the major limitation to plant growth, development and yield. Osmotic stress caused by drought may lead to oxidative stress, due to enhanced generation of reactive oxygen species (ROS) in plants. In order to regulate and remove the excess of harmful ROS, plant cells possess a complex antioxidant system, consisting of low-molecular mass antioxidants and antioxidant enzymes. This experiment was conducted on 41 genotypes from Maize Research Institute Zemun Polje gene bank drought tolerant mini-core collection of maize (15 introduced inbred lines, 13 local and 13 introduced landraces), by studying parameters of growth, free proline content and activities of soluble root peroxidases (POD) at early seedling stage under optimal and water stress conditions imposed by polyethylene glycol (PEG). In all the genotypes, PEG-induced osmotic stress resulted in growth reduction (i.e. length, fresh and dry weight) of both roots and shoots, accompanied by proline content increase, particularly exhibited in inbred lines. The highest variability among the genotypes was observed in respect to peroxidases activity. Depending on the germplasm type (landrace or inbred line), changes in POD activity varied from approximately 50% stimulation to 10% reduction in landraces, and from 25% stimulation to 35% reduction in maize inbred lines, respectively. Application of Principal Component Analysis revealed almost similar distribution of the genotypes in both experimental environments. The results obtained indicated seedlings characteristics as relevant early indicators of drought tolerance.

Keywords: genetic resources, seedlings, water deficit, *Zea mays* L.

Fourier transform-infrared studies on the chemical characteristics of leaf surface of variegated *Pelargonium zonale* in drought conditions

PP8-23

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Fourier transform-infrared (FTIR) spectroscopy was used as a direct and non-destructive method to analyze changes in chemical composition of white and green leaf tissue induced by drought. Additionally, leaf stomatal conductance and temperature were measured by infrared camera and porometer. In drought and control conditions in white parts of leaves Gs decreased compared to green. FTIR analysis showed characteristic bands in the region from 3000 to 1600 cm^{-1} originating from OH, H_2O and CH_2. Different compounds such as wax esters, aromatic compounds, proteins, polysaccharides and phenolics were identified in epidermal cells of both white and green leaf parts. Spectra obtained from green and white parts of control plants were similar, while plants from drought differed in the following bands: 1732 cm^{-1} (wax esters) and 1716 cm^{-1} (ketones). In addition, the CC stretching vibration seen as band at 1558 cm^{-1} and 1519 cm^{-1}, characteristic for phenolic compounds, increased under drought conditions. The band at 1542 cm^{-1} that occurred in all spectra of control plants was most probably due to the presence of proteins (amide-II band). In the spectral re-

gion 1500-1300 cm^{-1} different bands could be observed: at 1471 cm^{-1} and 1319 cm^{-1} for CH_2. The broad band at 1105 cm^{-1} can be assigned to the COC stretching vibration of esters. The band at 1066 cm^{-1} presented polysaccharide region and it stayed almost unchanged significantly in drought conditions. In conclusion, intensity and position of bands described imply a significant change in metabolism of epidermal cells in drought conditions in the white and green parts of leaves.

Keywords: drought, white and green part of leaf, chemical composition

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Influence of salicylic acid on morphological and physiological characteristics of *Impatiens walleriana* L. under water stress induced by polyethylene glycol

PP8-24

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Impatiens walleriana is a highly popular ornamental plant worldwide. The major problem in *I. walleriana* production, transport and sale display is related to tendency to wilt quickly when drought-stressed thus becoming undesirable to customers. Salicylic acid (SA) is now considered as an endogenous growth regulator that influences many plant physiological processes including regulation of plant defense, and plays a significant role in plant protection against various abiotic stresses. To evaluate the role of exogenously applied SA against the effect of drought simulated by polyethylene glycol (PEG 8000), growth and physiological parameters of *I. walleriana* were analyzed. *In vitro* developed shoots of *I. walleriana* were grown on MS media containing different levels of PEG (0, 1, 2 and 3%) and treated with SA at varying concentrations (0, 1, 2 and 3 mM). Increasing PEG concentrations progressively reduced plant growth parameters (plant height, number of leaves, fresh weight and proliferation rate) and decreased relative water content, chlorophyll and total pigments in *I. walleriana* leaves. In contrast, SA treatments under simulated water deficit conditions enhanced *I. walleriana* growth performance. Additionally, a decline in the photosynthetic pigment contents and the level of water loss in leaves of drought stressed plants were significantly reduced with SA application. Taken together, these results show that SA improves performance of *I. walleriana* under stress conditions, suggesting that exogenous application of SA can be used to increase tolerance of *Impatiens* plants against water deficit.

Keywords: *Impatiens walleriana*, polyethylene glycol, salicylic acid, abiotic stress

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Anatomical response of Mg-treated sugar beet plants to Cd stress

PP8-25

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In order to analyze the effect of magnesium nutrition on anatomical leaf structure in Cd-treated plants, the pairs of completely developed sugar beet leaves were sampled. Plants were subjected to different Mg treatments, in semi-controlled conditions. After 44 days, one half of plants of each Mg treatment were exposed to $1x10^{-6}$ M Cd. Plants were harvested seven days after the application of Cd. Anatomical analysis was done on leaves sampled from the section of the petiole and lamina between the third and fourth lateral vein and the main vein section. Leaf cross-sections were obtained using a Leica CM 1850 cryostat at a cutting interval of 25 µm. In addition to leaf morphological analysis, anatomical parameters of lamina, leaf main veins and petiole were measured. All observations and measurements were performed using an Image Analyzing System Motic Images Plus. The influence of Cd on lamina and petiole was presented as the relative change value of Cd-treated plants, compared to Cd-untreated plants. Increasing the concentration of Mg led to almost linear decrease of Cd concentration in leaves and roots. Influence of Cd on anatomical characteristics of sugar beet leaves was dependent on Mg nutrition. Various Mg treatments induced significant changes of anatomical characteristics only in the sugar beet lamina. Differences between the analyzed groups were most significant for area of the leaf main vein and the percentage of phloem cross-section area.

Keywords: leaf anatomy, cadmium stress, nutrient management

Comparison of the impact of low concentrations of yttrium on maize and sunflower

PP8-26

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Rare earth element yttrium (Y), which has found a widespread use in modern industry, is actually not so rare, since its concentration in the Earth's crust (31 ppm) is higher than that of e.g. cobalt (29 ppm) and its effects on crops were not extensively studied so far. Therefore, the aim of this experiment was to examine the effect of low concentrations of Y on growth and metabolism of young maize and sunflower plants. Plants were grown under semi-controlled conditions of a greenhouse, in water cultures, in the presence of 0 (control), 10^{-5}, 10^{-4} or 10^{-3} M Y, in the form of $Y(NO)_3{\cdot}5H_2O$. The presence of Y generally reduced plant growth. At concentration of 10^{-3} M, Y severely impaired sunflower but not maize growth. Sunflower root and stem dry weight significantly decreased at 10^{-3} M Y (50% and 83% of control, respectively), and leaf dry weight already at 10^{-4} M Y. In maize, leaf dry weight declined significantly in the presence of 10^{-3} M Y, whereas the reduction of shoot dry weight was significant already at 10^{-5} M Y and of root at 10^{-4} M Y. Total leaf area of sunflower significantly declined in the presence of 10^{-4} M Y, and of maize already at 10^{-5} M Y. Reduced productivity of photosynthesis in both species, and consequently reduced plant dry weight, can be explained by significant reduction of leaf ar-

ea but also by reduction in concentrations of photosynthetic pigments in the presence of 10^{-3} and 10^{-4} M Y. At applied concentrations Y did not show any stimulating effect on growth of young maize and sunflower plants.

Keywords: yttrium, *Helianthus annuus, Zea mays*

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Specificity of aquatic macrophytes *Phragmites communis* Trin, *Salvinia natans* L. and *Utricularia vulgaris* L. in the accumulation of manganese and iron in the Bardača area (Republic of Srpska)

PP8-27

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The paper deals with Fe and Mn content analysis in water, sediment and tissue of *Phragmites communis* Trin, *Utricularia vulgaris* L. and *Salvinia natans* L. on two localities in the area of Bardača (Necik and Sinjak – active fish ponds) in May–October the period. During the season, Fe and Mn concentration in the water was in the range of maximum permitted values (0.05-0.240 mg L^{-1} for Fe and 0.037-0.086 mg L^{-1} for Mn), while manganese value in the sediment during October was higher than regulated (618.33 mg kg^{-1}). Fe and Mn concentrations in the tissue of studied macrophytes varied in relation to species, locality and period of sampling. The highest Fe concentration was recorded during June for *Salvinia natans* (1.646 mg kg^{-1}) on both examined localities, while the highest determined Mn value was during May in *Utricularia vulgaris* (620 mg kg^{-1}). In terms of Fe content, the accumulation sequence in studied macrophyte species on both researched pools was declining in the following sequence: *Salvinia natans* > *Utricularia vulgaris* > *Phragmites communis* – rhizome > *Phragmites communis* – above-ground part. For Mn, bioaccumulation sequence was somewhat different: *Utricularia vulgaris* > *Salvinia natans* > *Phragmites communis* – above-ground part > *Phragmites communis* – rhizome. Results obtained in the paper indicate a different capacity for Fe and Mn accumulation between the studied species, which may be of great importance at selection of potential species in phytoremediation technique.

Keywords: heavy metals, phytoremediation, *Phragmites communis* Trin, *Utricularia vulgaris* L., *Salvinia natans* L.

Changes in polyphenols content in soybean and *Datura stramonium* after treatment with the herbicides and Delfan Plus®

PP8-28

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The most common herbicide combination for weed prevention in soybean is Pulsar 40 and Harmony 75WG, but when applied during hot days these herbicides may induce oxidative stress in plants. Delfan Plus®

is a biostimulator with high amino acid content which can be used in combination with herbicides. Manufacturer advertises it as a supplement for faster protein synthesis and thus, overcoming stress conditions caused by high and low temperatures, drought, herbicides etc. In order to investigate the ability of Delfan Plus® to mitigate the effect of herbicides on soybean plants (and its weed species *Datura stramonium* L.), we analyzed the accumulation of polyphenolic compounds (total polyphenols, tannins, flavonoids and proanthocyanidins), as well as phytotoxicity and grain yield. In addition, we determined the antioxidant capacity of plant extracts in DPPH and NBT-tests. Soybean cultivar Sava was treated with Pulsar 40 + Harmony 75WG (1 L ha^{-1} + 8 g ha^{-1} and 2 L ha^{-1} + 16 g ha^{-1}, respectively), with or without Delfan Plus® (1 L ha^{-1} and 2 L ha^{-1}, respectively). Biochemical parameters analyzed showed that Delfan plus® did not decrease the level of stress in plants. Most parameters increased their values 7 days after the treatment, compared to day 1, which was expected. Specimens of *D. stramonium* were especially stressed after herbicide treatment. Although soybean yield was somewhat higher after the treatment with normal dose of herbicides and Delfan plus® (3.28 t ha^{-1}), there were no statistical differences between the treatments. Hence, the question of the application of this biostimulator in agronomic practice may be raised.

Keywords: abiotic stress, herbicides, soybean, biostimulators, polyphenols

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Tomato fruit growth under regulated deficit irrigation

PP8-29

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Under current climate conditions of drought and scarce water supply, the challenge for agricultural production is to increase irrigation water use efficiency and sustained crop yield. Regulated deficit irrigation (DI) is a new deficit irrigation strategy which may decrease demand for agricultural use of water for many crops, including tomato. The aim of this study was to investigate the effects of DI on tomato fruit growth, the activity of cell wall-associated peroxidase and ABA content in the pericarp of tomato cultivar Ailsa Craig. The experiment was done in controlled conditions and plants under DI received 50% of water given to fully irrigated (FI) plants. ABA content in fruit pericarp was measured by ELISA test and cell wall-associated peroxidase activity by a guaiacol test. Fruit growth rate was significantly higher in FI plants than DI and the final diameter of tomato fruits was higher in FI than in DI fruits. ABA content in tomato pericarp showed significant difference in the initial stages of fruit development (15 and 20 dpa) in DI treated plants compared to FI. During tomato fruit development ABA content declined until the end of cell growth phase without significant differences between DI and FI plants. The significant increase in the activity of the enzyme cell wall-associated peroxidase in DI tomato fruit pericarp coincided with the end of cell growth and the beginning of the ripening process. These results pointed out that this enzyme may control tomato fruit maturation and may have influence on fruit growth rate.

Keywords: regulated deficit irrigation, tomato fruit growth, ABA, cell wall-associated peroxidase

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Galactan content and localization as a measure of compression wood severity in *Picea omorika* (Pančić) Purkyně

PP8-30

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Response to leaning, as a part of the gravitropic response of the tree, in conifers is a formation of compression wood (CW). Changes in morphology and anatomy that characterize CW are reduced tracheid length, increased cell wall thickness, reduced lumen diameter, rounder cell cross-sectional profile, and the presence of intercellular spaces. Concerning its chemical composition, CW is highly lignified and consequently contains less cellulose, increased amounts of galactan, and lower amounts of mannan and xylan. *Picea omorika* (Pančić) Purkyně is a slow growing Balkan endemic coniferous species, considered to be one of the most adaptable spruces. Our investigation is aimed at understanding the reaction wood response in a slow growing conifer species under conditions of severe and long-term bending stress, which would correspond to the impact of winter snow loads or snow falls on juvenile conifers in their natural habitats. Galactan content was increased in stem samples of bent *P. omorika* trees compared to the control. It decreased from the stem base to the top, in line with the decrease in calculated bending moment, i.e. with the decrease in compression wood severity. Additionally, immunostaining using the LM5 antibody showed galactan localization almost exclusively to the compression wood. Expected accompanying decrease in arabinan, glucan, xylan and mannan contents, was also registered.

Keywords: bending stress, compression wood, galactan

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Effect of high irradiation on chlorophyll *a* fluorescence in young and mature fig leaves

PP8-31

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Combination of high irradiation and elevated temperature most often causes inactivation of oxygen evolving centre (OEC) of photosystem II (PSII). In this work we aimed to investigate influence of high irradiation on PSII photochemistry in young (YL) and mature leaves (ML) of common fig (*Ficus carica* L.) exposed to elevated temperature. Both leaf types were detached from the tree, acclimated at room temperature in dark for 12h and then exposed to 35 ± 1 °C combined with low (LI, ~50 µmol m_{-2} s_{-1}) or high irradiation (HI, ~800 µmol m^2 s^1) for 4h. To evaluate PSII photochemistry, normalizations and subtractions of polyphasic fluorescence transients (OJIP) were used. While YL revealed negative L-band after HI treatment, indicating high energetic connectivity of PSII, positive L-band shown in ML suggested light induced antenna dissociation and lower PSII stability. Inflection in Kband in

YL after exposure to HI treatment suggested slight destabilization of OEC activity, while positive K-band detected in ML reflected complete inactivation of OEC. Although both leaf types showed significant reduction of maximum quantum yield of PSII (F_v/F_m) and performance index (PI_{ABS}), stronger decline was observed in ML. In conclusion, exposure of ML to HI resulted in impaired electron donation due to inactivated OEC accompanied with severe decline of PSII efficiency and overall performance. Combination of HI and increased temperature in YL increased thermostability of PSII, associated with the resistance of OEC and enhanced energy connectivity between PSII units that was also reflected by a lower decrease in F_v/F_m and PI_{ABS} compared to ML.

Keywords: chlorophyll *a* fluorescence, OJIP, *Ficus carica* L., PSII thermostability

The effects of different fertilizers on cherry tomato (*Lycopersicon esculentum* Mill. 'Sakura F1') leaf proline and phenolics contents and antioxidative capacity under drought stress

PP8-32

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Excessive use of inorganic fertilizers has led to many environmental and agricultural drawbacks, and the use of eco-friendly fertilizers for organic agriculture is expanding. The aim of this study was to examine the impact of three bio-fertilizers suitable for organic farming (Bio-algeen S-92, Slavol and Ergonfill) on some physiological parameters of cherry tomato seedlings (*Lycopersicon esculentum* Mill. 'Sakura F1') and their effect on plants subjected to drought stress. The following physiological parameters were examined: proline and total phenolics content, and antioxidant capacity in the extract of the leaves of cherry tomato seedlings. Proline content was determined by spectrophotometric method using ninhydrin reagent, phenol content by spectrophotometric method using Folin-Ciocalteu reagent, and total antioxidant capacity by FRAP method. Proline and total phenolics contents in all tested variants were significantly higher in leaves of cherry tomato seedlings exposed to drought stress. Effect of applied fertilizer when plants were not subjected to stress was not significant on either of the parameters analyzed. Fertilizers application induced significant increase in phenolics content and antioxidative capacity in stressed plants, the effect being greatest with Bio-algeen S-92. Fertilizers also affected the dynamics of proline accumulation in stressed plants. The research results showed that the proper application of all used fertilizers can within the limits of genetic predisposition of cherry tomato, significantly increase phenolic content and total antioxidant capacity of the plant, which is an important precondition for better adaptation of plants to drought stress.

Keywords: tomato, drought stress, bio-fertilizers, proline, phenolics

The effect of long-term salt stress on different genotypes of the moss *Atrichum undulatum*

PP8-33

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The ancestor of bryophytes belongs to the first terrestrial plants. Thus, many of them exhibit a high degree of abiotic stress tolerance, which enables them to survive in the harsh environment. Different genotypes of the moss *Atrichum undulatum* were chosen and axenically grown in controlled *in vitro* conditions. The five genotypes were chosen from ecologically various sites across Europe. Clear genetic incongruence and dissimilarities were confirmed among accessions. The shoots were exposed to long-term (28 d) salt stress by addition of NaCl (0-500 mM) to the basal medium. Survival, indices of multiplication, secondary protonemal diameters, and biochemical parameters such as chlorophyll and carotenoid contents or chlorophyll a/b ratio clearly showed the differences among genotypes exposed to long term salt stress. These parameters proved Italian (highmountain) genotype to be the most resistant to long-term salt stress. Although the multiplication and emergence of new shoots decreased strongly with the increase of salt concentration in all genotypes, Hungarian and Italian genotypes at lower concentration of added salt developed the best (according to the numbers of new shoots appearing). Lower concentrations of salt in the media were also long-standing conditions for German and Italian genotypes. Biochemical parameters were not congruent with developmental ones. German genotype did not express significant variation in total, a, b and ratio of chlorophylls and carotenoid contents. The results obtained clearly showed that stress caused reaction is not equal and the same in all the representatives of species, and thus can be highlighted as genotype specific.

Keywords: bryophyte, abiotic stress, tolerance

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173024 and OI173030).

The effect of salt stress on yellow wort (*Blackstonia perfoliata*) seed germination

PP8-34

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Blackstonia perfoliata (L.) Huds. belongs to the family Gentianaceae. Due to a high content of biologically active secondary metabolites (gentiopicrin, swertiamarin and sweroside) this species has been described as a possible substitute for *Centaurii Herba* – a bitter remedy for treatment of digestive system disorders. Yellow wort is widespread in European and African regions of the Mediterranean and in Western Europe, mainly on calcareous grasslands habitats and fixed sand dunes. Therefore, the environmental conditions such as salinity and osmotic stress are very important for the growth and development of this species. The germination re-

covery from salt stress conditions of three populations of *B. perfoliata* collected from different natural habitats was examined. Germination rate, germination velocity and rate of germination recovery were tested. Results showed that germination characteristics depended on NaCl pre-treatment and were population-specific. Increase in salt concentration inhibited seed germination and germination recovery rate. Additionally, seeds of *B. perfoliata* population collected from sandy locality of Vojvodina showed different germination pattern in comparison with seeds collected from saline habitat; salt treatment improved their germination rate, but also attenuated germination velocity response.

Keywords: salinity, stress, *Blackstonia perfoliata*, germination

This study was supported by Grant No. OI173024, Ministry of Education, Science and Technological Development of the Republic of Serbia.

Volatile compounds of three *Nepeta* species inhibit seed germination, reduce seedling growth and induce oxidative stress in garden cress (*Lepidium sativum* L.)

PP8-35

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Phytotoxic effects of volatile organic compounds (VOCs) from *Nepeta* species on agricultural and weed species have been previously demonstrated, and are usually attributed to iridoid monoterpenes nepetalactones. Here we studied the effect of VOCs of three endemic *Nepeta* species, differing in their qualitative nepetalactone content, on seed germination and seedling growth of garden cress (*Lepidium sativum* L.). Garden cress was exposed to the atmosphere enriched with VOCs released from the leaf surface of three *Nepeta* species. Identification of VOCs was performed by headspace GC-MS, while the concentrations of nepetalactone in the atmosphere of culture vessels were detected by PTR-MS. Volatiles released from *N. rtanjensis* and *N. sibirica* shoots reduced seed germination and inhibited seedling growth of garden cress. Phytotoxic effect of *N. rtanjensis* and *N. sibirica* originates from the dominant bioactive compound of these species – nepetalactone. Stereochemistry of nepetalactone considerably determines its phytotoxic potential, *trans,cis*- isomer is more active than the *cis,trans* nepetalactone. Phytotoxic effect of nepetalactone is reflected through its effect on biochemical processes that are the consequence of plants disturbed antioxidative system: the inhibition of activities and changed profiles of peroxidases, catalase, Fe- and Cu/Zn-superoxide dismutase isoforms were observed.

Keywords: phytotoxicity, peroxidase, catalase, Fe-superoxide dismutase, CuZn-superoxide dismutase

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Molecular characterization of *Fusarium* spp. isolated from maize and cereals

PP8-36

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Fusarium spp. is one of the most damaging cereal pathogens that provokes serious economic losses worldwide. Also, this pathogen produces mycotoxins which pose severe danger to animal and human health. Thus, it is very important to control and eliminate risks caused by this fungus. For making improvement in overcoming these issues, accurate separation of *Fusarium* species is needed. Besides identification based on morphological and pathogenic characters, novel molecular genetic methods are available nowadays. The aim of this research was characterization of fifty *Fusarium* spp. isolates from maize and cereals and possible detection of recently discovered species *F. gerlachii* and *F. vorosi*. Characterization was done by DNA sequence-based analysis using two specific primer pairs (ITS/ITS4, ef1/ef2). Specific genome fragments were sequenced and analyzed. Sequences were compared to the data from GeneBank, NCBI (National Center for Biotechnology Information). Genetic similarities between sequences were determined using software MEGA, version 6.06. All tested isolates appeared to represent *F. graminearum sensu stricto* species. Molecular detection, sequencing and phylogenetic analysis provide more accurate classification of fungi species, identification of unknown isolates, establishment of relationships between species and determination of toxogenic profiles.

Keywords: cereals, DNA sequencing, *Fusarium* spp., maize, molecular characterization

Application of Tempo EPR spin probe for *in vivo* detection of salt-induced oxidative stress in *Centaurium erythraea* Rafn

PP8-37

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Electron paramagnetic resonance spectroscopy (EPR) was applied for *in vivo* detection of oxidative stress induced by high ionic strengths in *Centaurium erythraea* Rafn (*Gentianaceae*), a herbaceous perennial often found on saline soils. Shoots of *C. erythraea* were cultured *in vitro* for 4 weeks on solid 1/2 MS medium, or 1/2 MS medium supplemented with 200 mM NaCl to induce salt stress and thus stimulate the production of reactive oxygen species (ROS), which is a common response of plant cells subjected to various types of biotic or abiotic stresses. The reduction of a stable cell-permeable aminoxyl radical spin probe, Tempo, was measured to assess the oxidative status of the control and salt-treated samples. In both types of samples, the reduction of Tempo showed zero-order kinetics. After one hour, the control reduced only 9% of the initial amount of the spin probe, whereas the sample grown with 200 mM NaCl reduced it by 20%. This may indicate that the plants grown in the presence of 200 mM NaCl produced higher amounts of ROS which are able to reduce the aminoxyl radical. The obtained results indicate that EPR can be used as a method for *in vivo* evaluation of the redox state of plants under stress conditions, and could also be useful in determining the salt-stress tolerance of plants. Further studies, including different salt concentrations, and plant species, have to be conducted to verify these findings.

Keywords: *Centaurium erythraea*, EPR, Tempo, oxidative stress, salt stress

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Prospective protein markers for heat tolerance screening in potato, *Solanum tuberosum* L.

PP8-38

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Potato, *Solanum tuberosum* L. ssp. *tuberosum*, is the fourth most important food crop in the world and major vegetable crop in Serbia. Most of the commercially important cultivars of potato are well adapted to cool climates, whilst adversely affected by high temperatures. In order to develop a procedure for efficient screening of potato cultivars/genotypes regarding heat tolerance, we were investigating expression and accumulation of heat stress-related HSP18, HSP21, HSP101 and eEF1A proteins by immunobloting in six potato cultivars. Potato was grown in the irrigated field in Zemun Polje (randomized complete-block experimental design) and leaf samples for protein analyses were collected after high temperature incidents in summers 2011 and 2012. Besides, relevant agronomic yield parameters were determined each year. Years 2011 and 2012 were extremely hot; summer 2012 was the warmest since records began in Serbia. Positive, linear correlation has been determined between yield per plot and accumulation of HSP18, HSP101 or eEF1A under heat stress (HS) in examined potato cultivars. Negative correlation has been determined between height of primary shoots, as well as above-ground biomass, and accumulation of HSP18, HSP101 or eEF1A. Explicitly, potato genotypes/cultivars which accumulated higher amounts of HSP18, HSP101 and eEF1A under HS in the field, also had shorter primary shoots, lower above-ground biomass and higher tuber yield. HSP21 abundance under HS, however, did not correlate with any of the measured agronomic parameters. Our results indicate that among investigated proteins, HSP18, HSP101 and eEF1A might be considered as prospective protein markers for selection of high-productive potato genotypes under HS.

Keywords: potato, heat tolerance, HSP, eEF1A

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Effects of salicylic acid on the expression of superoxide dismutase in potato, *Solanum tuberosum* L., under heat stress *in vitro*

PP8-39

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Importance of salicylic acid (SA) in the enhancement of heat tolerance has been, so far, recognized in several plant species. SA-inducible thermotolerance mechanisms may involve induction of certain heat shock proteins and modulation of antioxidant enzymes activity, such as catalase, ascorbate peroxidase and superoxide dismutase (SOD). We were investigating effects of SA pretreatments on the expression of SOD in several potato cultivars/genotypes grown *in vitro* after short-term (6 h) exposure to 35 °C or 45 °C. Results of our study indicate that SA can modulate expression of all three major types of SOD, FeSOD, MnSOD and Cu/ZnSOD, in leaves of potato microplants under heat stress. The effect of SA was especially prominent considering Cu/Zn-SOD, the most abundant type of SOD present in potato microplants that determines total SOD activity in the cell. Interestingly, pretreatment with 10^{-5} M SA stimulated Cu/ZnSOD accumulation in leaves of microplants maintained at standard growth temperature (20 °C). Stimulatory effect of 10^{-5} M SA was accentuated at 35 °C, while slight increase in abundance of Cu/ZnSOD has been observed at 45 °C in most potato genotypes. Other SA pretreatments (10^{-6} M and 10^{-4} M SA) did not cause substantial increase in Cu/ZnSOD levels at 35 °C or 45 °C, as well as standard growth temperature. Our findings indicate that the effect of SA on Cu/ZnSOD accumulation in potato depends on both temperature treatment and SA concentration. To our knowledge, this is the first report on SA effects on SOD expression in potato under heat stress.

Keywords: salicylic acid, potato, SOD, heat stress

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (TR31049).

Heat-induced accumulation of protein synthesis elongation factor 1A implies an important role in heat tolerance in potato

PP8-40

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Protein synthesis elongation factor 1A (eEF1A) is a cytosolic protein that plays a central role in the elongation phase of translation. Some of the non-canonical eEF1A activities, such as chaperone activity in preventing protein aggregation, might be important in developing plant abiotic stress tolerance. In order to in-

vestigate the possible role that eEF1A plays in heat tolerance, we compared the protein profiles of eEF1A under heat stress (HS) and optimal temperature conditions in six potato cultivars/genotypes grown in the field. Results of 1D-PAGE-immunoblot analysis revealed significant accumulation of eEF1A in all examined potato cultivars under HS, as well as significant genotypic differences in the level of the accumulated protein. Protein samples of three cultivars, differing in HS-induced eEF1A accumulation, were further analyzed. 2D-PAGE immunoblots revealed approximately 9-11 eEF1A protein spots with isoelectric points (pI) 5.8-8.8. Two protein isoforms/polypeptides (pI 7.9, 8.8) showed significant increases in abundance under HS in all genotypes, while one additional isoform/polypeptide (pI 8.2) was strongly induced only in eEF1A-high-accumulating cultivar. Certain increase in the volume of other eEF1A protein spots (pI 5.8-7.5) was detected only in moderate- and high-accumulating cultivars. In order to determine eEF1A gene copy number in potato, Southern analysis was conducted. The results revealed existence of eEF1A multigene family which, depending on the genotype, probably comprises 6-9 gene copies. Taken together, the results of our study indicate existence of multiple eEF1A isoforms in potato, some of which are strongly HS-inducible. Heat-induced accumulation of eEF1A implies an important role of this protein in potato heat tolerance.

Keywords: potato, eEF1A, heat stress, heat tolerance

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Effects of drought on morphological and physiological parameters of tomato leaf

PP8-41

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It is well-known that drought stress is characterized by reduction of the soil water content, diminished leaf water potential and turgor loss, stomatal closure and decreased rate of cell enlargement and growth. The magnitude of these changes and their consequences for plant metabolism, growth and development depend on the degree of stress and plant resistance to drought. The aim of presented results was to investigate the effects of drought on leaf characteristics (length, area and specific leaf area) and stomatal conductance in two different tomato cherry cultivars (LA1420 and Plovdiv). The experiment was conducted in glasshouse conditions. Plants were exposed to drought by withholding irrigation of substrate in the pots (soil water content *ca*. 25%), while in control conditions plants received full substrate water holding capacity (soil water content *ca.* 75%). Leaf length was measured by a ruler, their DW after drying procedure, while the leaf area was measured by area meter. Specific leaf area (SLA) was calculated on the base of the relationship between leaf area and leaf DW. Stomatal conductance was measured by porometer. Obtained results showed genotypic differences between tomato cultivars in the sensitivity of investigated parameters to drought. Higher stomatal sensitivity to drought and consequently greater reduction of stomatal conductance and transpiration in cultivar LA1420 comparing to Plovdiv, contributed to the to ability of LA1420 plants to retain a relatively high level of leaf hydration necessary to sustain leaf area development in drought conditions.

Keywords: drought, tomato, leaf growth, stomata

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Detection of RNOS in poplar tissue culture under PEG-induced drought stress

PP8-42

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In the present climate change context, adverse effects of water deficit on plant productivity became increasingly important. A better understanding of the physiological and biochemical mechanisms conferring increased tolerance to drought is important in part of breeding strategies. Drought increases accumulation of reactive oxygen species (ROS) and causes oxidative stress in plants. In vivo staining methods are a reliable way to visualize and localize ROS and reactive nitrogen species (RNS) in plant cells and tissues. In vitro experiment was carried out in order to evaluate the resistance of poplar M1 clone on drought stress. The stress was imposed on poplar tissue culture by polyethylene glycol (PEG) 6000 applied at different concentrations (100 and 200 mOsm PEG) during 6 days. After PEG treatment, roots, leaves and stems of poplar were analysed by fluorescence microscopy. For detecting total intracellular ROS, dichlorodihydrofluorescein-diacetat (H_2DCFDA) was used, for NO radical, 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetat (DAF-FM) and for $ONOO^-$ 3′-(p-aminophenyl) fluorescein. The increase of all RNOS (ROS, NO i $ONOO^-$) in leaves due to the increase of PEG concentration was established. Drought stress provoked oxidative and nitrosative stress in poplar leaves. In roots and stems, opposite tendency was observed regarding ROS content, while NO and $ONOO^-$ were not changed significantly. It can be assumed that roots and stems are more tolerant to drought stress but also hypoxic conditions around the root could be possible explanation for a decreased content of ROS.

Keywords: drought, PEG, poplar, RNOS, tissue culture

Leaf- and stem-wetting events and water translocation during early rehydration of resurrection *Ramonda serbica* plants

PP8-43

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The rapid and highly efficient water absorption and its retranslocation within resurrection plant tissues upon rewatering, both leaves and the stem are crucial in early plant rehydration. The recovery of physiological activities in poikilohydric flowering species *Ramonda serbica* is largely determined by the efficacy of water absorption and its flow through desiccated plant organs. The fact that this chasmophytic plant species regain from the physiological inactivity characteristic for dehydrated state within 48-72 hours and indicate that the water uptake and its transport rely on more than its absorption by roots and xylem acropetal transport as well as the existence of the specific path of absorbed water within plant tissues. The leaf and stem surface, leaf trichomes and marcescent leaves participate in water uptake. We found that the water running down the hairy

surface of the leaf is channeled towards the rosette centre and then "funneled" downward along the contracted stem. Water deposited onto the leaf surface was very efficiently evenly distributed over the entire leaf surface by capillary forces. It penetrated into the leaf via stomata and cuticular cracks. The lower epidermis played a role in water absorption; the water accumulated in lacunar space beneath epidermal cells in space in-between neighboring vessels and played a role in rehydration of mesophyll cells. The specific net-like organization of the stem vascular tissue promotes the efficient redistribution of water transported in either xylem or phloem between the roots, stem and leaves, during both dehydration and especially during early rehydration.

Keywords: resurrection, rehydration, water transport, capillary forces

Anatomy and crop productivity – relevance of anatomical studies in crop responses to drought

PP8-44

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Increased drought stress is one of the most important tasks for agricultural research. Since plants react to their environment by adjusting both physiological functions and structure, the yield of crop plants in drought conditions is therefore a combination of anatomical, physiological and biochemical processes which depend on the environment as well as on genetic features. Anatomical aspects, as an integral part of plant growth and its resistance to drought, depend on division, elongation and differentiation of the cells, and investigation in crop anatomy is focused on adaptations at the level of cells, tissues, and organs, necessary for obtaining appropriate levels of water, nutrients and light and should be able to describe the role of each feature in helping the plant to maintain the yield. The size of the fruit is usually the key factor that determines the yield and depends on water and assimilate transport. Since vascular tissue plays a crucial role in long-distance water transport, and also determines the movement of chemical signals from shoot to fruit, methods that enable measuring the size of the vessels and estimating hydraulic conductance could be useful tool in evaluation of plant resistance to drought. In this paper we will provide an overview of applications of various anatomical techniques and methods useful for studying hydraulic network in plants.

Keywords: fruit, vascular tissue, transport

AtCKX2 transgenic potato plants exhibit altered tolerance to salt stress *in vitro*

PP8-45

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Cytokinin oxidase/dehydrogenase (CKX, EC 1.4.3.18/1.5.99.12) is the key enzyme involved in the catabolism of plant hormones cytokinins. Potato (*Solanum tuberosum* L. cv. Désirée) plants transformed with the

AtCKX2 gene from *Arabidopsis thaliana* were tested for salt stress tolerance *in vitro*. Three transgenic, cytokinin-deficient lines were grown for 20 days on MS media containing 0, 50, 100, 150 or 200 mM NaCl. Tolerance to salt stress was evaluated by measuring plant height, rooting frequency, the sensitivity index, fresh weight, relative water content and chlorophyll content – and comparing these parameters to those measured in non-transformed control plants grown on the same media. The results suggest that cytokinin-deficient lines are more sensitive to higher levels of salinity (150 and 200 mM NaCl) than the non-transformed control plants. Furthermore, the transgenic line *AtCKX2*-51 exhibited the greatest sensitivity to higher concentrations of salt. However, the situation is different at moderate salt concentrations (50 and 100 mM NaCl), where transgenic *AtCKX2* plants exhibit greater tolerance than the control plants. While greater sensitivity of *AtCKX2*-transgenic plants to high concentrations of salt might be accounted for by enhanced triggering of senescence in these plants, the possibility that cytokinin deficiency might confer greater tolerance to moderate levels of salinity suggests that the relationship between the altered cytokinin homeostasis and salt stress tolerance in potato could be much more complex.

Keywords: chlorophyll, cytokinin deficiency, potato, relative water content, salt stress

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Effects of salicylic acid on heat tolerance in potato, *Solanum tuberosum* L., microplants

PP8-46

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Salicylic acid (SA) is considered as an endogenous growth regulator and signaling molecule involved in defense responses of plants to the presence of pathogens and abiotic stress. The effects of SA on potato heat tolerance were investigated *in vitro*, in a two-stage protocol which included SA pretreatments and treatments of two unrelated cultivars/genotypes and long-term exposure to 35 °C. Pretreatments with 10^{-6}-10^{-5} M SA caused a significant increase in the number of microplants developed from nodal explants in cv. 'Marabel', while in cv. 'Liseta' SA protective effect was more prominent during treatments with 10^{-6}-10^{-4} M SA. In more responsive cultivar 'Liseta', SA also stimulated shoot elongation and root initiation under long-term heat stress. Treatments with relatively high SA concentrations reduced root elongation in both examined cultivars at either 35 °C or standard growth temperature. To determine whether SA affects the expression of heat-shock proteins (HSP) in potato, accumulation of cytosolic HSP17.6 and chloroplastic HSP21 were investigated in SA-pretreated or SA-treated shoots of two cultivars after exposure to 35 °C for 6h. Although SA stimulated accumulation of HSPs in both cultivars, cv. 'Liseta' accumulated larger amounts of HSP17.6 and HSP21 during heat stress at all applied SA concentrations. Results of our study demonstrate that SA may promote heat tolerance in potato. In addition, genotype differences in SA sensitivity regarding heat tolerance-enhancement were confirmed.

Keywords: salicylic acid, potato, heat tolerance, HSP

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (TR31049 and OI 173015).

Cell wall peroxidase activity in tomato skin fruits under limited irrigation

PP8-47

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Tomato fruit size depends on the rate and duration of fruit growth. Biochemical investigations of plant cell growth mechanism showed the increase in peroxidase activity or its different isoforms during maturation or cessation of tomato fruit growth. The effects of regulated deficit irrigation (RDI) on tomato fruit growth and cell wall peroxidase activity in tomato exocarp were investigated in growth chamber conditions in two tomato hybrids (Sunpak F1 and Astona F1). The RDI treatment was 50% of water given to fully irrigated (FI) plants. RDI significantly reduced fruit diameter in both hybrids. The activity of peroxidase was significantly higher in RDI treated plants compared to FI plants. In the fruits of RDI treated plants peroxidase activity began to increase in the phase when fruit growth started to decline with the peak of enzyme activity of 6.1 and 9.4 HRPEU g^{-1} FW (Sunpak F1 and Astona F1) reached in the phase of mature green fruits when fruit growth rate was minimal. These data potentially identified that tomato exocarp cell wall peroxidase in RDI treated plants may have a role in restricting fruit growth rate.

Keywords: peroxidase activity, tomato fruit growth, exocarp, regulated deficit irrigation

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Fingerprinting antioxidant activity in *Alyssum markgrafii* shoot culture during treatment with nickel

PP8-48

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In order to fingerprint antioxidant response induced by excess nickel, shoot cultures of rare Balkan hyperaccumulating species *Alyssum markgrafii* O.E. Shulz were subjected to nickel concentrations of 0.5 and 1 mM. A new approach in studying 'summary parameters', characteristic for whole classes of antioxidative metabolites and key enzymes, was introduced. This method was based on five assays which yield information about the general antioxidant status and specific aspects of antioxidative system rather than the exact data for single antioxidant species. In our study we conducted three luminometric assays: luminol converting peroxidase assay (LUPO), the total antioxidant capacity (TAC) of low molecular weight antioxidants (LMWA) and superoxide scavenging activity (SOSA) of high molecular weight compounds including superoxide dismutases. We also applied well known assays for catalase (CAT) and glutathione reductase (GR) activity. Additionally we quantified other morphological and physiological parameters. Toxic effects of nickel were observed through impeded growth, reduction in biomass, lowered chlorophyll and total phenolic content. Fingerprint of the antioxidant response at 0.5 mM nickel treatment showed increased total antioxidant capacity, LUPO and GR activity, while SOSA and CAT activities were lower than in control plants. At 1 mM nickel TAC

and GR showed no statistically significant activities compared to control plants, while SOSA, CAT and LUPO activities were lower than in control plants. Subsequent analysis of total non-protein thiol content of LMWA revealed that this class of compounds possessed the strongest impact on overall antioxidant activity, at least in the case of 0.5 mM nickel treatment.

Keywords: *Alyssum markgrafii,* hyperaccumulator, antioxidant response

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (OI173005 and OI173015).

Effects of different nitrogen and phosphorus concentrations on antioxidative response of *Lemna gibba* plants to silver toxicity

PP8-49

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Silver is non-essential heavy metal for plants known to cause oxidative stress. Several factors, such as the presence of nutrients, can affect the ability of plants to adequately respond to abiotic stress. The objective of This study was to investigate the effect of different nitrogen (N) and phosphorus (P) concentrations on antioxidative response of plants to ionic colloidal silver (Ag). To provide insight, Lemna gibba plants were exposed to three N and P levels (100%, 50% and 25%) for seven days and then to nominal 100 μg L^{-1} and 1000 μg L^{-1} Ag for 48h. Plants grown at lower N and P concentrations had significantly higher initial enzyme activities such as ascorbate peroxidase (APX), guaiacol peroxidase (GPOX) and superoxide dismutase (SOD) as well as elevated concentrations of ascorbic acid and total phenols. Increased antioxidative enzymes activities indicate oxidative stress-tolerance. Silver caused oxidative injury to *L. gibba* cells, evident from decreased protein and chlorophyll concentrations and increased concentrations of hydrogen peroxide (H_2O_2) and products of lipid peroxidation (TBARS). However, the production of H_2O_2 and TBARS as well as the degradation of proteins and pigments caused by Ag treatment was less pronounced in plants grown at lower N and P concentrations when compared to full strength medium. This could be explained by higher degree of protection from oxidative stress caused by Ag in plants grown at lower N and P supply. These results indicate that N and P supply, by modifying antioxidant activity in plants, can affect their tolerance to various kinds of stresses.

Keywords: *Lemna gibba*, silver, nutrients, oxidative stress, antioxidative response

Influence of UV radiation on the content of secondary metabolites in tomato grown in different environmental conditions

PP8-50

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In this study we investigated the effects of ambient ultraviolet (UV, 280- 400 nm) radiation on the composition of secondary metabolites of high nutritional value (pigments and flavonoids) in *Lycopersicon esculen-*

tum (tomato) fruits. Tomato plants were grown during summer in the open field and two types of polytunnels, PT1 (UV-A 1.45 mV cm^{-2}, UV-B 3.84 µV cm^{-2}, PAR 750 µmol m^{-2} s^{-1}) and PT2 (UV-A 0.37 mV cm^{-2}, UV-B 0.03 µV cm^{-2}, PAR 760 µmol m^{-2} s^{-1}) and fruits were taken in august. The contents of lycopene, β-carotene and flavonoids (quercetin and kaempferol) were determined by HPLC in the tomato exocarp and pulp. Regardless of UV radiation exposure, higher amounts of lycopene, flavonoids and β-carotene were measured in exocarp compared to the pulp. Accumulation of phenolics, in both exocarp and pulp was the highest in fruits collected in the field. Similarly, the concentration of epidermal flavonoids was the highest in the leaves of plants from the open field. These results support the protective functions of flavonoids as UV-screeners and antioxidants from high PAR. Moreover, the content of lycopene was the highest in exocarp and β-carotene in the pulp of fruits exposed to full ambiental UV radiation doses, compared to fruits collected in the polytunells. The results implicate that the controlled exposure to solar UV radiation during crop growth may be used as a stimulator of biosynthesis of compounds with high antioxidative capacity thus improving the fruit quality and nutritional value.

Keywords: UV radiation, *Lycopersicon esculentum*, pigments, flavonoids

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (III43010).

Volatile compounds of golden fern (*Asplenium ceterach* L.) detected during the process of rehydration

PP8-51

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Volatile organic compounds (VOC) emitted by fronds of desiccation tolerant fern *Asplenium ceterach* L. during the rehydration process were investigated. Head-space GC-MS analysis was used in order to determine the volatile compounds in dry and rehydrated sample of golden fern. It was found that the VOC profile of golden fern is based mainly on isomeric heptadienals (over 25%) and decadienals (over 20%), other linear aldehydes, alcohols and related compounds. Aerial parts of fresh and dry fronds do not contain monoterpene-, sesquiterpene- and diterpene-type hydrocarbons or corresponding terpenoids. In order to determine the composition of the VOC during the process of rehydration, we have applied proton-transfer reaction mass spectrometry (PTR-MS). PTR-MS is a sensitive technique that allows real-time detection of VOCs emitted from plants. We have used dry plants to establish a base line and then distilled water was added to induce hydration process of the golden fern plant. Masses in the range from m/z 21 to m/z 300 were measured with dwell time of 200 ms and for the time period of 24 hours. The experiment was repeated for five plants. PTR-MS measurements revealed that the amounts of compounds, determined by head-space GC-MS, generally exhibit a significant increase after addition of water. After about 20 hours the values measured by PTR-MS for these masses are reduced to a new base line that corresponds to the rehydrated plant.

Keywords: golden fern, rehydration, PTR-MS, headspace GC-MS

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (III41011 and ON173024).

Antioxidant enzymes activity and physiological parameters of black locust families exposed to heavy metal stress

PP8-52

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Exposure of plants to redox inactive (Cd, Zn, Ni, Al, etc.) heavy metals results in oxidative stress through indirect mechanisms such as interaction with the antioxidant defense system, disruption of the electron transport chain, or induction of lipid peroxidation, therefore disrupting the cellular homeostasis in plants. In the present work, plants of four black locust families (54, 56, 115, 135) were submitted to excessive concentrations of cadmium, nickel and lead in hydroponic solution. Oxidative stress was assessed examining the activities of antioxidant enzymes such as catalase (CAT), cytosolic ascorbate-peroxidase (APx) and guaiacol-peroxidase (GPx) which are involved in scavenging of hydrogen-peroxide free radical. Thiobarbituric acid (TBA) assay was used to assess lipid peroxidation. Physiological parameters included analysis of proline content, photosynthetic pigments content, and nitrate-reductase activity in leaves and roots of tested families. All measurements were made spectrophotometrically. The increase of lipid peroxidation was evident in leaves in almost all applied treatments. Family 54 was the most susceptible to lipid peroxidation in leaf tissue. Activities of both GPx and APx were increased in the roots of tested families in comparison to activity in leaves, while CAT activity was strongly affected by applied HM treatments, in comparison to control. Accumulation of free proline varied among investigated families and applied treatments. In general, the lowest content of proline was found in the family 115 at all applied treatments. This result may indicate that some other mechanisms are also involved in tolerance under heavy metals stress.

Keywords: oxidative stress, black locust, heavy metals

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ANALYSIS
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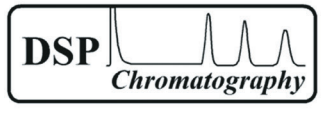
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