BOOK OF ABSTRACTS

3rd International Conference on Plant Biology

(22nd SPPS Meeting)

9-12 JUNE 2018
BELGRADE

Serbian Plant Physiology Society

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

Faculty of Biology, University of Belgrade

3rd International Conference on Plant Biology

(22nd SPPS Meeting)

9-12 June 2018, Belgrade

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PROGRAMME

Saturday 9th June

09:00-14:00	*Registration*	
14:00-14:30	*Opening Ceremony*	

Section 2 • Plant Stress Physiology

Chairs: Sonja Veljović-Jovanović & Ivana Maksimović

14:30-15:00	(Plenary lecture) **Hrvoje Fulgosi**	Sifting the elements of FNR-TROL bifurcation
15:00-15:30	(Plenary lecture) **Autar Mattoo**	Tomato (*Solanum lycopersicum*) lipoxygenase (LOX) gene family: Delineating gene members associated with growth, development and abiotic stresses
15:30-15:50	(Invited talk) **Tamara Rakić**	Two-year study of ecophysiological parameters of *Miscanthus* × *giganteus* grown on tailing pond at the mine "Rudnik" (Serbia)
15:50-16:10	(Invited talk) **Vladimir Crnojević**	Data science in biosystems
16:10- 16:40	*Coffee break*	
16:40-17:00	(Invited talk) **Ingeborg Lang**	Tolerance to heavy metals – some examples in bryophyte species
17:00-17:15	(Selected talk) **Predrag Bosnić**	Silicon mediates sodium (Na+) transport in maize under moderate NaCl stress
17:15-17:30	(Selected talk) **Milan Borišev**	Dynamics of Cd accumulation and metabolic adaptation of *Salix alba* grown hydroponically
17:30- 17:45	(Selected talk) **Slavica Dmitrović**	Nepetalactone-rich essential oil mitigates BASTA-induced ammonium toxicity in *Arabidopsis thaliana* L. by maintaining glutamine synthetase activity
17:45-18:00	*Group Photo*	
18:00-19:00	*Poster session: Plant Stress Physiology (Section 2)*	
19:00-21:00	*Welcoming cocktail (Rectorate of the University of Belgrade)*	

Sunday 10th June

09:00-14:00 *Registration*

Section 1 • Plant Growth, Development, Metabolism and Nutrition

Chairs: Snežana Zdravković-Korać & Miroslav Nikolić

09:30-10:00	(Plenary lecture) **Guido Grossmann**	Cellular growth regulation in roots - how to adapt in a complex environment
10:00-10:20	(Invited talk) **Ondrej Novák**	Tissue- and cell-specific analysis of phytohormones
10:20-10:40	(Invited talk) **Ksenija Radotić**	Plant cell walls – mechanical and chemical modifications underpin growth and stress response
10:40-11:00	(Invited talk) **Herman Heilmeier**	Bioavailability of elements for effective phytoremediation and phytomining: the role of rhizosphere processes
11:00- 11:30	*Coffee break*	
11:30-11:50	(Invited talk) **Václav Motyka**	Comprehensive phytohormone profiling during Norway spruce (*Picea abies*) somatic embryogenesis
11:50-12:05	(Selected talk) **Danijela Paunović**	Are receptor tyrosine kinases chimeric AGP's?
12:05-12:20	(Selected talk) **Jelena Pavlović**	Silicon increases iron use efficiency in cucumber- a strategy 1 model plant
12:20-12:35	(Selected talk) **Katarina Ćuković**	Characterization of *Arabidopsis GLN1;5* knockout mutant
12:35- 14:00	*Lunch break*	

Sunday 10th June

Section 4 • Phytochemistry

Chairs: Vuk Maksimović & Vladimir Mihailović

14:00-14:30	(Plenary lecture) **Alain Tissier**	Engineering plant diterpenoid pathways in yeast: increasing yield and expanding product diversity
14:30-14:50	(Invited talk) **Roque Bru Martinez**	Metabolic engineering and elicitation strategies to produce stilbenoids in plant cell cultures
14:50-16:10	(Invited talk) **Sokol Abazi**	New fatty acids discovered for the first time in *Vitex agnus-castus*
16:10-16:30	(Invited talk) **Peđa Janaćković**	Do plant volatiles reflect taxonomy?
16:30- 17:00	*Coffee break*	
17:00-17:20	(Invited talk) **Angelos Kanellis**	The *Cistus creticus* terpene synthase gene family
17:20-17:40	(Invited talk) **Marina Soković**	Terpenes and terpenoids: linking bioactivity, opportunities and challenges
17:40-18:00	(Invited talk) **Jules Beekwilder**	Plant terpenes and bioplastics
18:00-18:15	(Selected talk) **Jelena Dragišić Maksimović**	Enzymatic behavior of edible berries – "Beroxidases"
18:15-18:30	(Selected talk) **Elma Vuko**	Inhibition of satellite RNA associated cucumber mosaic virus infection by essential oil of *Micromeria croatica* (Pers.) Schott
18:30-18:45	(Selected talk) **Dorisa Çela**	Structure elucidation of a new alkaloid and other 11 known compounds isolated from *Gymnospermium* species
18:45-19:45	***Poster sessions: Plant Growth, Development, Metabolism and Nutrition; Phytochemistry (Sections 1 and 4)***	

Monday 11th June

Section 5 • Applications in Agriculture, Pharmacy and Food Industry

Chairs: Jasmina Glamočlija & Slavica Ninković

09:00-9:30	(Plenary lecture) **Mondger Bouzayen**	New factors controlling fruit development: epigenetic modifications associated with the fruit set transition in tomato
09:30-10:00	(Plenary Lecture) **Andrew Allan**	New breeding technologies for fruit trees
10:00-10:20	(Invited talk) **Slađana Žilić**	Food and pharmacy application of anthocyanins originating from colored grains
10:20-10:40	(Invited talk) **Eligio Malusa**	Microbial-based inputs: opportunities and challenges for sustainable and resilient agricultural productions
10:40-11:10	*Coffee break*	
11:10-11:30	(Invited talk) **Dragana Miladinović**	Old problems, new tools - Integrated approach to oil crop breeding
11:30-11:45	(Selected talk) **Brankica Tanović**	Prospects of cabbage leaf debris use in the control of *Fusarium* wilt of pepper
11:45-12:00	(Selected talk) **Nina Devrnja**	Effects of tansy essential oil on fitness and digestion process of gypsy moth larvae
12:00-12:15	(Selected talk) **Zora Dajić-Stevanović**	Advantages and limitations of phytogenic feed additives
12:15-14:00	*Lunch break*	

Monday 11th June

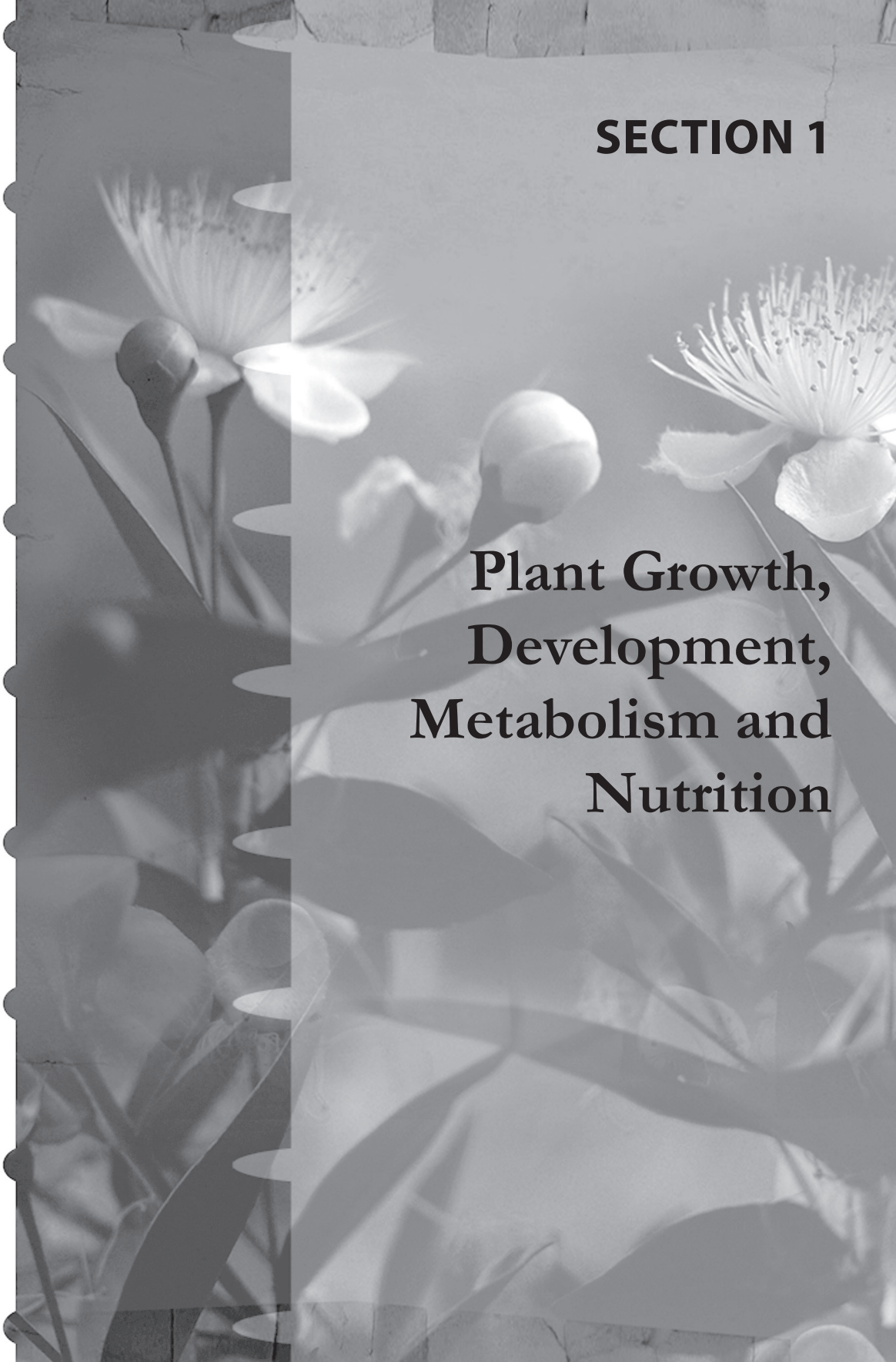
Section 3 • Biodiversity, Conservation and Evolution of Plants

Chairs: Jelena Aleksić & Aleksej Tarasjev

14:00-14:30	(Plenary lecture) **Hendrik Poorter**	Meta-Phenomics: Converting data into knowledge
14:30-15:00	(Plenary lecture) **Antonio Granell Richart**	The biodiversity present in European tomato, phenotypes galore and a first insight in the underlying genetics
15:00-15:20	(Invited talk) **Zlatko Šatović**	Origin and genetic diversity of Croatian common bean landraces
15:20-15:50	*Coffee break*	
15:50-16:10	(Invited talk) **Aneta Sabovljević**	Conservation physiology of bryophytes
16:10-16:30	(Invited talk) **Nataša Barišić Klisarić**	Biomonitoring: Plants' (in) perspective
16:30-16:50	(Selected talk) **Sanja Budečević**	Morphological diversity of functionally distinctive floral organs in *Iris pumila*: Does the flower color matter?
16:50-17:05	(Selected talk) **Žaklina Marjanović**	First data on arbuscular mycorrhizal communities from selected climatic borderline forest ecosystems of the Balkan Peninsula
17:05-17:20	(Selected talk) **Tijana Banjanac**	Verification of interspecies hybridization within the genus *Centaurium* Hill using *EST-SSR* molecular markers
17:20-18:20	*Poster sessions: Applications in Agriculture, Pharmacy and Food Industry; Biodiversity and Conservation, Evolutionary Plant Biology (Sections 5 and 3)*	
18:20-18:30	*Closing Ceremony*	
18:30-19:00	*SPPS General Assembly Meeting*	
21:00-01:00	*Gala dinner: Restaurant "Vizantija"*	

Tuesday 12th June

10:00-16:00	*Excursion: Special Nature Reserve "Carska bara"*



SECTION 1

Plant Growth, Development, Metabolism and Nutrition

PLENARY LECTURE

Cellular growth regulation in roots - how to adapt in a complex environment

PL1

Guido Grossmann

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In nature, plant roots grow in highly heterogeneous conditions, which require both systemic and local response mechanisms to modulate cellular growth and adapt organ morphology. How environmental cues are integrated into growth regulation and how systemic communication and cell-autonomous responses are interlinked in this process, remain challenging questions in plant biology. To facilitate the investigation of root-environment interactions, we combine the use of microfluidic lab-on-a-chip platforms with live-cell imaging of roots. Over the past years, we developed a series of microfluidic devices for on-chip root growth under precisely controlled conditions. Here, we present the dual-flow-RootChip, a microfluidic organ-on-a-chip platform for asymmetric perfusion of Arabidopsis roots to investigate root growth, development, and signaling under simulated environmental heterogeneity. Applications range from monitoring physiology and signaling (e.g. calcium sign.) under asymmetric conditions, tracing molecular uptake and selective drug treatments to local infection or colonization by fungi or bacteria.

Using the dual-flow-RootChip, we revealed cell-autonomous regulation of root hair growth upon differential availability of inorganic phosphate (Pi). Independently of the overall Pi status of the root, we found that hair cells can triple their growth rate within minutes, when extracellular Pi levels rise, pointing to a direct modulation of the tip growth machinery and resulting in hair growth that followed the nutrient gradient.

To understand the molecular mechanisms of growth regulation in root hairs, we further dissected the tip growth machinery and unveiled a phased assembly process during the early stages of hair emergence. The transition between different phases depends on timed regulation of Rho of plants (ROP) GTPases. We identified a novel candidate that acts upstream of ROPs, pre-forms a polar domain at the plasma membrane, and is necessary and sufficient to recruit ROPs in order to trigger the initiation of tip growth.

INVITED TALKS

Tissue- And Cell-Specific Analysis of Phytohormones

IT1-1

Vladimír Skalický[1], Ioanna Antoniadi[2], Aleš Pěnčík[1], Martin Kubeš[3], Jan Vrána[4], Jan Šimura[1,2], Miroslav Strnad[1], Karin Ljung[2], Ondrej Novák[1]
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Phytohormones play crucial roles in the control of various physiological processes. Whilst metabolism provides the building blocks for plant growth and development, the phytohormonal groups are essential to control the rate of growth of individual plant parts and to integrate the activities of these parts. High-resolution measurements of phytohormones are therefore necessary for physiological studies of their mode of action.

We focus on efficient cell and organelle isolation, combining different approaches such as density gradient ultracentrifugation or fluorescence-assisted cell/organelle sorting (FACS/FAOS) with novel approaches, a simple one-step purification protocol based on in-tip micro Solid-Phase Extraction and a class-specific miniaturized immunoaffinity chromatography method. We have also developed several fast chromatographic separations and highly sensitive tandem mass spectrometry (LC-MS/MS) methods for simultaneous profiling of the phytohormone metabolites. New analytical tools will provide comprehensive insights into plant hormone regulatory networks, such as detailed distribution of plant hormones in specific tissues, cells and organelles. This will allow us to obtain e.g. nuclear fractions of high-purity and intactness in an adequate yield suitable for future analytical assessments. Our preliminary data point out that auxin and CK profiles in plant cell are quite complex and do not include just expected active molecules, but also other key representatives that cover phytohormone biosynthesis, conjugation and degradation. By employing these novel methods, we will be able to gain a much better understanding of how genetic and experimental manipulations affect plant hormone levels, which will foster a more complete understanding of how these hormones act.

Keywords: plant hormone, metabolite profiling, resolution, sensitivity.

Plant cell walls – mechanical and chemical modifications underpin growth and stress response

IT1-2

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Cell wall mechanical properties play a key role in the plant growth and in physical support/protection from environmental stress. At the cellular level, cell wall nanomechanics and its growth-related dynamics are not completely resolved. Atomic force microscopy (AFM) stiffness tomography enables us to monitor stiffness distribution in the cell wall layers and its evolution during different growth phases of living suspension-cultured plant cells. At the beginning and at the end of cell growth, the average stiffness of the cell wall is low and the wall is mechanically homogenous. In the exponential growth phase, the average wall stiffness increases, with increasing heterogeneity and the polysaccharide/lignin ratio obtained by Fourier-transform infrared (FTIR) spectroscopy. In trees, mechanical stimuli induce compression/tension forces leading to the formation of reaction wood in the tree stems. Severe and long-term bending stress in juvenile spruce results in a severe compression and dramatic change in biomass distribution. The evidence is provided that cell wall structure and composition is a precise indicator of compression severity. Fluorescence-detected linear dichroism (FDLD) microscopy reveals a decrease in cellulose fibril order in compression wood compared with normal wood. Cell wall mechanical features, based on structural modifications, underpin plant response to long-term UV radiation stress. In the needles of the juvenile spruce exposed to elevated UV-B or UV-C radiation in the environment, cellulose crystallinity that is important for mechanical properties, increases in order control> UV-C treatment>UV-B treatment. The FTIR spectra indicated radiation-induced changes in xylan, cellulose and lignin quantity and structure.

Keywords: cell walls, mechanical stimuli, UV radiation, chemical modifications

This work was supported by the Grant No. 173017 from the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Bioavailability of elements for effective phytoremediation and phytomining: the role of rhizosphere processes

IT1-3

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The success of phytoremediation and phytomining depends heavily on the bioavailability of target elements in the soil, which, among others, is a function of pH and redox potential. In order to desorb the target elements from the soil matrix, the natural capacity of plants to increase availability of elements in their root environment can be used. Here we report on rhizosphere mech-

anisms of various plant species to increase bioavailability of germanium (Ge), rare earth elements (REEs) and also toxic elements for phytomining and phytoremediation.

Several species of forbs and grasses were grown on various substrates, either without or with addition of organic acids, in mono- and mixed cultures both in the greenhouse and in the field. The concentration of metal(loid)s was analyzed in the dried plant material via ICP-MS following micro-wave digestion with concentrated HNO_3 and HF. Germanium and REEs were also determined in different soil fractions after sequential extraction.

The addition of carboxylates dramatically increased the mobility of Ge in soils and Ge contents in the plant material. The accumulation of Ge in aboveground plant material was by a factor of 10 higher in grasses than in forbs. In contrast, forbs accumulated higher concentrations of REEs than grasses. For those plants with a high capacity for lowering pH and releasing carboxylates from roots (e.g. genus *Lupinus*), which is a common strategy of plants to mobilize poorly available nutrients such as Fe, Mn and P in the rhizosphere, we could demonstrate their ability to mobilize Ge and REEs.

Keywords: bioavailability, germanium, rare earth elements, phytoextraction, rhizosphere

Comprehensive phytohormone profiling during Norway spruce (*Picea abies*) somatic embryogenesis

IT1-4

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Conifer somatic embryogenesis (SE) is controlled by a number of different chemical factors. Among them, phytohormones with a complex network of their metabolic and signaling pathways play an irreplaceable role. Concentrations of endogenous phytohormones including auxins, cytokinins (CKs), abscisic acid (ABA), jasmonates and salicylic acid (SA) were monitored over the course of SE in Norway spruce (*Picea abies*). We utilised HPLC-ESI-MS/MS to provide currently the most comprehensive overview of phytohormone levels in somatic *P. abies* embryos. The results revealed that the concentrations of particular phytohormone classes varied substantially between proliferation, maturation, desiccation and germination. Endogenous ABA showed a maximum concentration at the maturation stage, which reflected the presence of exogenous ABA in the medium and showed its efficient perception by the embryos as a prerequisite for their maturation. Auxins, both indole and non-indole, also had concentration maxima at the maturation stage, suggesting a role in embryo polarisation. Endogenous jasmonates were detected in conifer somatic embryos for the first time, and reached maxima at germination. According to our knowledge, we have presented evidence for the involvement of the non-indole auxin phenylacetic acid, *cis*-zeatin- and dihydrozeatin-type CKs and SA in SE for the first time. The presented concentration changes of various classes of phytohormones during SE suggest close correlations between endogenous phytohormone profiles and particular developmental stages of somatic embryos in conifers.

Keywords: somatic embryogenesis, phytohormone, Norway spruce, *Picea abies*

This work has been funded by the Czech Science Foundation (16-14649S).

SELECTED TALKS

Are receptor tyrosine kinases chimeric AGP's?

ST1-1

Danijela Paunović, Milica Bogdanović, Milana Trifunović Momčilov, Slađana Todorović, Ana Simonović, Angelina Subotić, Milan Dragićević
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Arabinogalactan proteins (AGPs) are cell wall proteoglycans with important roles during plant growth and development. They comprise one of the most complex families of macromolecules found in plants, which is in part due to the incredible diversity of the glycans decorating the protein backbone, as well as the heterogeneity of the protein backbones. While this diversity is certainly responsible for the wide array of physiological functions associated with AGPs, it hampers efforts for homology-based identification. We have developed a new method for filtering AGP sequences that exploits one of their key features, the presence of hydroxyprolines, which represent glycosylation sites. This method was used to filter potential AGPs from *Centaurium erythraea* RNA-seq data. Most of the filtered sequences had no identifiable domains, while the most frequent identified domains were the Protein kinase and Protein tyrosine kinase domains identified in the same sequences, followed by well-known AGP associates, Leucine rich repeats, Probable lipid transfer, Plastocyanin-like and Fasciclin. It is noteworthy that the Protein (tyrosine) kinase domain has thus far eluded experimental evidence for linkage with AGPs in any plant species, probably due to its transmembrane nature. The implicated sequences were examined in depth and compared to homologs from Arabidopsis.

Keywords: arabinogalactan proteins, bioinformatics, hydroxyproline prediction, finding-AGP

This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (TR31019, OI173024)

Silicon increases iron use efficiency in cucumber – a strategy 1 model plant

ST1-2

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Silicon (Si) and iron (Fe) are respectively the second and the fourth most abundant minerals in the earth's crust. While the essentiality of Fe has been discovered in the middle of the 19th century, Si is still not fully accepted as an essential element for higher plants. Due to poor Fe availability for higher plants, especially in alkaline and calcareous soils, Fe deficiency represents a major limiting factor for crop production worldwide, affecting both crop yield and quality, with a strong negative impact on human health.

Here we investigated the key physiological, biochemical and molecular parameters involved in the processes of root acquisition and tissue utilization of Fe by cucumber (*Cucumis sativus* L.), as both Strategy 1 model and Si-accumulating species.

Silicon nutrition increased the accumulation of apoplastic Fe and Fe-mobilizing compounds in roots, as well as upregulated the expression of genes (*AHA1, FRO2, IRT1*) encoding the main components of the reduction-based Fe uptake machinery (Pavlovic et al., 2013). In leaves, Si affected relative Fe distribution by enhancing Fe remobilization from old leaves via increased NA accumulation and expression of the *YSL1*, which stimulated Fe chelation and its retranslocation to younger leaves (Pavlovic et al., 2016). This for the first time demonstrated a new beneficial role of Si, *i.e.* in increasing nutrient acquisition, transport and utilization by crops.

Keywords: cucumber (*Cucumis sativus* L.), iron deficiency, leaf retranslocation, root acquisition, silicon.

References:

Pavlovic J., Samardzic J., Kostic L., Laursen K.H., Natic M., Timotijevic G., Schjoerring J.K., Nikolic M. (2016): Ann. Bot. 118, 271-280.

Pavlovic J., Samardzic J., Maksimović V., Timotijevic G., Stevic N., Laursen K.H., Hansen T.H., Husted S., Schjoerring J.K., Liang Y., Nikolic M. (2013): New Phytol. 198, 1096-1107.

Characterization of *Arabidopsis GLN1;5* knockout mutant

ST1-3

Katarina Ćuković[1], Milan Dragićević[2], Snežana Zdravković-Korać[2], Ana Simonović[2], Milica Bogdanović[2], Slađana Todorović[2]

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Glutamine synthetase is a key enzyme of plant nitrogen metabolism that assimilates ammonia into glutamine. The *Arabidopsis* genome encodes one chloroplastic (*GLN2*) and five cytosolic isoforms, *GLN1;1* through *GLN1;5*, with different expression patterns, kinetic properties, regulation and functions. Physiological roles of different isoforms have been elucidated mainly by studying knockout mutants. However, the role of *GLN1;5*, which is expressed in dry seeds, remained unknown. To elucidate the *GLN1;5* function, we have studied a *GLN1;5* knockout line (GLN1;5KO), homozygous for T-DNA insertion within the *GLN1;5*. The *GLN1;5* deficiency results in a phenotype with slightly delayed bolting and fewer siliques. The dry weight of GLN1;5KO seeds is 73.3% of WT seed weight, with seed length 90.9% of WT seeds. Finally, only 18.33% mutant seeds germinated in water within 10 days, in comparison to 34.67% of WT seeds. KNO_3 strongly stimulated germination of both GLN1;5KO and WT seeds, while germination in increasing NH_4Cl concentrations potentiates the differences between the two genotypes. It can be concluded that GLN1;5 activity supports silique development and grain filling and that it has a role in ammonium reassimilation within the seed, as well as assimilation and/or detoxification of ammonium from the environment.

Keywords: Arabidopsis, glutamine synthetase, knockout mutant, phenotype, germination

This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 173024)

POSTER PRESENTATIONS

Modifications in parenchyma cell wall structure related to stem twining in monocotyledonous liana *Dioscorea balcanica* Košanin

PP1-1

Aleksandra Lj. Mitrović[1], Jasna Simonović Radosavljević[1], Jelena Bogdanović Pristov[1], Gabor Steinbach[2], Dušica Janošević[3], Marina Vuković[1], Győző Garab[4], Ksenija Radotić[1] (mita@imsi.rs)

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Anatomical adaptation of liana plants includes structural changes in cell walls of different tissues: fibers, vessel elements and tracheids. The contribution of parenchyma cells to stem twining is mostly unknown. Plants control the orientation and alignment of cellulose fibrils during the deposition in the cell walls with high precision, creating required anisotropy of the cell wall. Our aim was to determine possible changes in cellulose fibrils orientation and structural order in stem parenchyma cell walls related to stem twinning in liana plants.

We applied different microscopy techniques: light microscopy, scanning electron microscopy and differential polarization laser scanning microscopy (DP-LSM) for fluorescence detected linear dichroism imaging (FDLD), on stem cross sections of straight and twisted internodes of monocotyledonous liana *Dioscorea balcanica*. Histochemical analysis showed no difference in parenchyma cell wall structure between straight and twisted internodes. Also, no difference in "cellulose fiber order" in parenchyma cell walls related to stem twining was found by FDLD microscopy. However, SEM micrographs suggested the difference in cellulose microfibril orientation in secondary cell walls of parenchyma cells related to stem twining.

Our results indicate that adaptations to stem twining in liana plants involve modifications in cellulose microfibril orientation in parenchyma cell walls. Although the orientation of cellulose microfibrils dictates, among other properties, cell shape, living stem parenchyma cells in *D. balcanica* retain their shape regardless of stem twining, which is possibly enabled by retaining "cellulose fibril order".

Keywords: cellulose, cell wall, parenchyma, *Dioscorea balcanica* Košanin.

This work was supported by Grant Nos. 173017 and 173015 of the Ministry of Education, Science and Technological development of the Republic of Serbia.

Higuchi's fractal dimension in plant histology

PP1-2

Aleksandra Lj. Mitrović[1], Dušica Janošević[2], Snežana Budimir[3], Ksenija Radotić[1], Lloyd Donaldson[4], Slađana Z. Spasić[1,5]

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Fractals are self-similar patterns, from exactly the same to nearly the same, and hence they are common in Nature. Fractal analysis of digital images or signals already has the application in the assessment of properties of tumors, viral infections, blood vessels, neurons, EEG signals, heart rate variability, etc. In different fields of science, especially in botany, application of different fractal methods is necessary to obtain a complete picture of structures or processes.

We suggest a new method for 2D Higuchi's fractal dimension estimation for use in plant analytical microscopy. To present its performance, we used two different sets of digital microscopic images: light microscopy micrographs collected during *Tacitus bellus* direct shoot organogenesis from leaf explants *in vitro*, and confocal laser scanning microscopy images of stem cross sections of juvenile *Picea omorika* trees exposed to static bending stress. Estimated Higuchi's fractal dimension of presented sets of micrographs enables quantification, separation and alignment of subsequent morphogenic stages of shoot organogenesis on the time scale, i.e. quantitative gradation of structural changes of wood cell properties on a compression severity scale, respectively.

Suggested fractal analysis method, combined with statistical analysis, could be used for quantification of structure complexity that characterizes cells and tissues during different growth and developmental processes or stress related structural changes in plants, as well as for the evaluation of the synchronization of those processes. It allows fast computational analysis of micrographs and is independent of the type of microscopy used.

Keywords: 2D Higuchi fractal analysis, shoot organogenesis, compression wood.

This work was supported by Grant Nos. 173017, 173045, 173015 from the Ministry of Education, Science and Technological development of the Republic of Serbia.

Transformation of tomato cultivar Moneymaker with *Agrobacterium tumefaciens*

PP1-3

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Tomato (*Solanum lycopersicum*) is not only one of the most important vegetable crops, but a widely used model for genetic improvement of dicotyledonous crops. Even though the first report on tomato transformation using *Agrobacterium tumefaciens* has been published over 30 years ago, numerous protocols published since then demonstrate that tomato transformation is neither standardized nor routine. Tomato cultivars and genotypes greatly differ in their susceptibility to transformation and response to growth regulator and antibiotics combinations used during regeneration and selection. Hereby we report a successful protocol for "Moneymaker" transformation with *A. tumefaciens* GV3101:pSM90 bearing pAGT174 with Kanamycin resistance cassette. Whole larger leaves (from ~10 cm high plantlets), rather than leaf segments were selected for inoculation, as the explant size significantly affected regeneration rate. Combination of zeatin (1 mg L^{-1}) and IAA (0.1 mg L^{-1}) was used for preculture and throughout the selection process, with addition of Timentin (200 mg L^{-1}) and increasing concentrations of Kanamycin (from 20 to 50 mg L 1) during the selection and regeneration. The regeneration on Kan occurred almost exclusively in transformed explants and proceeded via organogenesis with the callusing stage. Using Timentin instead of commonly used Cefotaxime to kill the bacteria proved beneficial for Moneymaker regeneration. In this system the regeneration frequency was 7%. Plantlets that survived Kan selection were PCR-tested, and 89.6% of them were transformed. The protocol is convenient and robust in terms of very low false-positive rate (10.3%).

Keywords: Moneymaker, transformation, Timentin, Zeatin

This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 173024)

Distribution of some arabinogalactan protein epitopes during somatic embryogenesis and organogenesis on leaf explants of centaury (*Centaurium erythraea* Rafn)

PP1-4

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Arabinogalactan proteins (AGPs) are a family of ubiquitous hydroxyproline-rich glycoproteins present in plasma membranes, cell walls and secretions of plants. Since AGPs are highly glycosylated, more than 90% of their total molecular mass comes from carbohydrate moieties consisting of various arabinogalactosyl chains (AG sugar chains), which are thought to be important for the

diverse functions of AGPs. AGPs are implicated in many aspects of plant growth and development, including cell differentiation, organogenesis and somatic embryogenesis (SE). The localization of AG sugar chains in plant tissues can be visualised using monoclonal antibodies (mAbs) that can detect different AGP epitopes. The aim of this study was to investigate changes in the localization of AGPs during induction of indirect SE and shoot organogenesis on leaf explants of centaury. Immunofluorescence labelling of leaf sections was performed with a set of mAbs (MAC207, JIM4, JIM8, JIM13, JIM15, LM2 and LM14). The results revealed that AGPs recognized by all mAbs tested were expressed in numerous meristematic cells from which somatic embryos develop. The AGP epitope recognised by the JIM4 antibody showed stronger intensity of immunofluorescence in the cell walls of protodermal cells of globular somatic embryos, whilst MAC207 and JIM13 epitopes were detected in cotyledonary somatic embryos. Strong immunofluorescence of the AGPs epitopes recognized with JIM4 and MAC207 mAbs were observed in the cells of apical meristem and leaf primordia of adventitious buds. These results suggest that AGPs play an important developmental role during formation of somatic embryos and adventitious buds from leaf explants of centaury.

Keywords: somatic embryo, shoot organogenesis, JIM4, JIM13, MAC207

This work was funded by the Serbian Ministry of Education, Science and Technological Development, Grant No. TR 31019

Glandular trichomes on the leaves and flowers of *in vitro* cultured *Micromeria thymifolia* (Scop.) Fritsch: morphology, distribution and histochemistry

PP1-5

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The genus *Micromeria* Bentham (Lamiaceae) comprises more than 70 aromatic species distributed throughout the temperate belt. Micromeria species produce considerable quantities of the essential oils, the biosynthesis, accumulation and secretion of which are generally restricted to glandular trichomes. *Micromeria thymifolia* (Scop.) Fritsch is a Balkan endemic species, traditionally used as a condiment and medicinal plant in the Mediterranean area. In view of the potential pharmacological and commercial value of this species, the glandular trichomes of *in vitro* cultured *M. thymifolia* on both vegetative and reproductive organs were examined, in relation to their morphology, distribution and histochemistry of the main secretion compounds, using light and scanning electron microscopy. Leaf indumentum of *in vitro* grown *M. thymifolia* comprised

three morphological types of glandular trichomes. Peltate trichomes were present on both leaf sides, particularly concentrated at the periphery of the leaf lamina. Type I capitate trichomes, with pear-shaped secretory head cell, were positioned at an angle to the leaf surface. Type II capitate trichomes were upright and had single-celled head with a subcuticular space. Type I capitate trichomes were more abundant than the type II capitate trichomes, which displayed similar density on both leaf sides. The calyx exhibited the same types of trichomes that occur on the vegetative organs. Both peltate and type II capitate trichomes present on the calyx had well-developed subcuticular space. A thick papillate indumentum present on the corolla appeared to bear only uniseriate non-glandular trichomes. Histochemical analysis revealed the complex nature of the material secreted by all types of *M. thymifolia* glandular trichomes.

Keywords: glandular trichomes, histochemistry, *in vitro* culture, *Micromeria thymifolia*, morphology

This research was financially supported by The Ministry of Education, Science and Technological Development of Serbia, Grant Nos. 173015, 173030, 43007.

Somatic embryogenesis in hairy roots of *Gentiana utriculosa* L.

PP1-6

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The aim of this study is establishment of hairy root and shoot cultures of *Gentiana utriculosa* L. by *Agrobacterium rhizogenes* strain A4M70GUS as well as development of superior genotypes with improved secondary metabolites production. Transformation efficacy was 10.66% and 5 stable hairy root clones were established whose transformation was confirmed by PCR analysis and Southern blotting. They differed in phenotype characteristics and growth parameters. Thus clones cl 4, cl 9 and cl 12 elongated and branched while clones cl 1 and cl 13 produced callus. Hairy roots biomass increased 1.5-2-fold on both agar solidified and liquid media. Spontaneous somatic embryo regeneration occurred either directly on roots, or indirectly from callus formed on the hairy roots. The highest number of somatic embryos (27 embryos per 400 mg FW) was obtained in cl 9, grown in MS 1/2 liquid medium. Further development of somatic embryos on hormone-free medium was poor. Most embryos became vitrified and only a small number developed into normal plantlets, up to 22.5% in cl 9. Inclusion of cytokinins (0.1 mg L^{-1} kin or 0.2 mg L^{-1} BA) alone or with GA3 0.1 mg L^{-1} did not improve SE development. Addition of activated charcoal into the germination media significantly diminished vitrification and necrosis and stimulated branching during somatic embryo germination into plantlets. Excised somatic embryo-derived shoots were successfully maintained and multiplied on MS media containing kin at 0.1 mg L^{-1} and activated charcoal at 1 g L^{-1}.

Keywords: hairy roots clones, spontaneous somatic embryogenesis

This work was supported by the Serbian Ministry of Education, Science and Technological Development Grant No. 173015.

Comparison of antioxidative metabolism in the green and yellow leaf tissues of *Liriodendron tulipifera* 'Aureomarginatum'

PP1-7

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As opposed to common *Lirodendron tulipifera* that has completely green leaves, variety 'Aureomarginatum' has two different kinds of tissue: yellow which is positioned on the outside of leaves and green which is centered. For this research the specified tissues were separated and the following parameters of antioxidative metabolism in them were examined: activities of superoxide dismutase (SOD, EC 1.15.1.1) and peroxidase (POD, EC 1.11.1.1) as well as phenol content. The completely green leaves of common *L. tulipifera* have also been analysed and the obtained results did not indicate any significant metabolic difference between them and green parts of the leaves in 'Aureomarginatum' variety. It has been noticed that yellow leaf parts have different SOD and POD isoenzyme profiles as well as their higher activity.

Keywords: *Liriodendron tulipifera*, green and yellow tissue, antioxidative metabolism

Diurnal rhythmicity of the phototropic bending parameters in sunflower seedling hypocotyls

PP1-8

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Sunflower is a species traditionally used in studies of plant tropic responses. Unlike Arabidopsis, sunflower seedlings prefer high light conditions, demonstrating fast daily elongation with short elongation arrests at dawn. Phototropic (PT) bending magnitude and other key parameters comprising the PT bending capacity (lag phase duration, time available for bending and bending rate) were the main goal of our research. They showed strong rhythmicity in all diurnal photoperiods, absent only in seedlings grown in conditions of continuous light. PT bending capacity, which was low at dawn, increased during the day reaching highest values in the afternoon some 10h after the dawn and then low values during the night. Two additional pivotal points located some 1-2h after light transitions (dawn and dusk) were visible in all parameters except in the changes of PT bending magnitudes. Lag phase was inverted to other parameters as its duration significantly decreased at dawn, had a minimum in the afternoon and then strongly increased 2h after the dusk reaching a night maximum 4h later. Prolonging the length of the last day induced decrease in lag phase duration irrespectively of the presence or absence of light after the dawn. This response can therefore be considered as the ability of plantlets to anticipate the approach of daytime.

Keywords: phototropic bending, lag phase duration, diurnal rhythmicity

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Involvement of endogenous phytohormones in the phototropic bending capacity of potato

PP1-9

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Phototropic bending is a complex plant response that serves to adjust the spatial position of plant body for optimal light utilization. Ability of *in vitro* cultured potato shoots to perform light-driven movements follows a diurnal rhythmicity with changes imposed mainly by light transitions. Here we monitored changes in the levels of major endogenous phytohormones and their connection with phototropic bending capacity in light-to-darkness transitions at the end of the day. The study compared events in the 16/8h (long day, LD) photoperiod with growth in continuous light (CL). The strongest effect and an inverse relation were observed in the levels of auxins, indole-3-acetic acid (IAA) and its major primary catabolite, 2-oxindole-3-acetic acid (oxIAA). IAA contents were suppressed both in darkness and CL, being replaced with high levels of oxIAA. Other phytohormone groups like gibberellins, abscisic acid, salicylic acid and jasmonates also showed concentration changes triggered by transition from light to darkness and growth in CL. A wide spectrum of cytokinin compounds was also screened showing rhythmicity both in LD and CL conditions and a general shift of daily level maximum to subjective night in CL conditions. Changes in IAA levels alone are sufficient to explain the observed variability of phototropic bending capacity of potato shoots.

Keywords: Endogenous phytohormones, phototropic bending, potato, diurnal rhythmicity

This work was supported by the Serbian Ministry of Education, Science and Technological Development (Grant No. 173015), Czech Science Foundation (16-14649S), and Swiss National Science Foundation, SCOPES (Grant No. 15222).

Effects of nitrogen form and concentration on the development of *Ocimum basilicum* L. var. *basilicum* L.

PP1-10

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Nitrogen is an essential element of plant cells at structural, genetic and metabolic levels and is involved in many processes of plant metabolism. The aim of this study was to examine the influence of different forms and concentrations of nitrogen on the content of photosynthetic pigments, protein content and activity of antioxidant enzymes (A-POX, G-POX and P-POX) in sweet basil *Ocimum basilicum* L. var. *basilicum* L. (cultivar Genovese). Plants were maintained *in vitro* under two forms of nitrogen - KNO_3 alone (6.18 mM, 12.36 mM and 24.7 mM) or in combination with NH_4NO_3 (4.7 mM KNO_3 with 5.15 mM NH_4NO_3; 9.4 mM KNO_3 with 10.3 mM NH_4NO_3; 18.8 mM KNO_3 with 20.6 mM NH_4NO_3), applied at low, medium and high concentrations. It was found that after one month of growth under these conditions, the concentration of photosynthetic pigments was higher under nitrate treatments, and the highest concentrations were recorded under highest

nitrogen content. Differences in the total soluble protein content varied significantly under nitrate treatments, depending on the concentration. Activity of antioxidant enzymes varied regarding the nitrogen forms as well as concentrations. Significant differences were noticed in A-POX activity, and the highest activity was under low KNO_3 content. The highest activity of G-POX was under high content of KNO_3 and NH_4NO_3. Opposite of G-POX, P-POX was induced under low content of KNO_3 and NH_4NO_3. It may be concluded that the form and concentration of nitrogen have a significant effect on the processes of primary metabolism in *Ocimum basilicum* L. var. *basilicum* L. (cultivar Genovese).

Keywords: *Ocimum basilicum*, nitrogen, pigments, antioxidant enzymes

This investigation was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. III41010).

Effects of 6-benzyladenine on the tuberization process in two *AtCKX*-transformed potato lines under strong tuber-inducing conditions *in vitro*

PP1-11

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Transformed potato plants, bearing genes for cytokinin oxidase/dehydrogenase (CKX), the key enzyme in cytokinin (CK) catabolism, represent a valuable model-system for investigations of CK role in the fine tuning of tuberization. CKs are known to affect tuber initiation *in vitro*, while their effects on other processes, such as tuber enlargement and dormancy break, are less clear. In this study we have investigated dynamics of tuber formation (observed every 5 days during 30-day long experimental period) and parameters of tuber enlargement (tuber diameter and mass measured at the end of experiment) under strong tuber-inducing conditions *in vitro* (continuous darkness and high sucrose concentration [8% w/v] in the medium) in two transformed potato lines, bearing *AtCKX1* or *AtCKX2* genes. As previously shown, selected transformed potato lines grown *in vitro* were characterized by slightly elevated (line *AtCKX1*-36a) or manifoldly increased (line *AtCKX2*-51) CKX activity, resulted in differently reduced bioactive CK contents. 6-benzyladenine (BA), being neither substrate nor inhibitor of CKX, was added to the medium (at the concentration of 1 μM) in order to investigate whether it could substitute for the lack of endogenous CKs and their effect on tuberization in *AtCKX* potato lines. Tuber initiation on single-node stem cuttings showed different dynamics in two investigated *AtCKX* lines, although in both lines it was completed earlier than in non-transformed control. BA at 1 μM enhanced and accelerated tuber formation in control and *AtCKX1*-36a. In both *AtCKX* lines, tuber diameter and mass were decreased, which could be restored to control values by addition of BA. BA also shortened tuber dormancy.

Keywords: tuberization, *AtCKX*, cytokinins, potato

This work has been funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 173015) and the Czech Science Foundation (16-14649S).

The effect of sucrose on the density and distribution of leaf glandular trichomes of *in vitro*-grown *Inula britannica* L.

PP1-12

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Inula britannica (family Asteraceae) belongs to a group of aromatic plants used in traditional medicine, horticulture, chemical, food and cosmetic industry. As with many aromatic plant species, adaxial and abaxial surfaces of *I. britannica* leaves are covered with glandular and non-glandular trichomes. Trichomes, hair-like structures, which originate from the epidermal cells, are genetically determined, but external factors also have important influence on their development. The glandular trichomes contain chemical compounds with various functions, including defense. Changes in number/density of trichomes and in composition/concentrations of their compounds may have important consequences for plant adaptation to different external factors. *In vitro* cultures may be an alternative choice to manipulate and provide a better control of abiotic factors that may affect the type, size, number and density of leaf trichomes as well as synthesis of secondary metabolites. Sucrose used in growth medium is required by plant cells as energy and carbon source, and can also act as an osmotic agent that may cause osmotic stress and modifications in growth and development.

The aim of present study was to analyze the effect of different sucrose concentration in the growth medium on the density and distribution of leaf glandular trichomes of *in vitro* grown *I. britannica*. It was observed that all applied concentrations of sucrose have effect on density and distribution of glandular trichomes. The highest density of glandular trichomes was noticed on the abaxial side of the leaf of plants cultivated at the sucrose concentration of 0.3 M.

Keywords: *Inula britannica*, aromatic plants, micropropagation, glandular trichomes, osmotic stress

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia Grant Nos. 173015 and 173024.

Morphogenesis of *Artemisia alba* Turra as a key for targeting secondary metabolism *in vitro*

PP1-13

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Artemisia alba Turra is a fragrant shrub, whose essential oil and non-volatile constituents have been utilized in traditional medicine and explored scientifically. The effects of morphogenic changes on several biochemical parameters of the plant have been studied in tissue culture conditions. Auxin and cytokinin treatments resulted in the development of two main morphotypes. While

PGR-free control and IBA treated plants were characterized with the development of both aerial and root tissue, the combined IBA and BA treatments resulted in root inhibition and callusogenesis at the explant base. The root-suppressed morphotype expressed inhibition of some antioxidant enzymes, as compared with normally rooted plants. Root suppression was also characterized by a drop in the content of salicylic acid, as well as abscisic acid and its catabolites. This effect was accompanied by elevation of the levels of jasmonic acid and its conjugate jasmonic acid-isoleucine, as well as of the polyphenolics content, thus leading to a preservation of lipid peroxidation levels, as compared with non-treated control. In addition to polyphenolics, the terpenoid profile of *in vitro* cultivated plants was also affected, as rooting was related to domination of monoterpenoids, and root inhibition – to domination of sesquiterpenoids in the oils from aerial parts. The obtained results imply that morphogenesis plays an important role in the interplay between enzymatic and non-enzymatic defense of *A. alba in vitro* and seems to be an important factor for targeting the biosynthesis of secondary metabolites in this species.

Keywords: *Artemisia alba* Turra tissue culture, *in vitro* morphotypes, essential oil profile, polyphenolics production, antioxidant defense

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Selection of stable reference genes in *Centaurium erythraea* Rafn during *in vitro* somatic embryogenesis and mechanical wounding

PP1-14

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Centaurium erythraea Rafn (Gentianaceae) is a medicinal plant, rich in secondary metabolites, mainly secoiridoid glucosides and xanthones, known for numerous beneficial effects on human health. In addition, *C. erythraea* possesses remarkable developmental plasticity and is easily cultured *in vitro*, so it represents a suitable model system for studying developmental biology. One of the most notable illustrations of plant cell totipotency is the capability of certain somatic plant cells to initiate embryogenic development through somatic embryogenesis (SE). It has been proposed that mechanical wounding during *in vitro* manipulations of plant material can promote cell differentiation and somatic embryo development. Our recent research is based on elucidating the gene expression profiles of centaury tissues in response to wounding and in different SE stages using quantitative real-time PCR. Considering the fact that these processes exhibit a dynamic genetic activity, the selection of stable reference genes is paramount in order to obtain unbiased conclusions. Hereby we report selection of stable reference genes in *C. erythraea* for studying gene expression during somatic embryogenesis and wounding. Thirteen frequently used reference genes were selected and their expression stability was assessed in different developmental stages, including globular and cotyledonary stages of embryos, as well as wounded tissues. Specific sets of primers were designed relying on previously obtained next-generation RNA sequencing data. The results were interpreted using two algorithmic approaches - geNorm and NormFinder - and the most stable reference genes from a set of tested candidate genes were determined.

Keywords: reference genes, gene expression, somatic embryogenesis, wounding

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Fructan accumulation in the internodes and photosynthetic performance during development of two barley cultivars

PP1-15

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Fructans represent the major temporary stem carbon storage compounds which accumulate in the stem and leaves. In barley (*Hordeum vulgare* L.) stem, accumulated fructans can be easily mobilized in the grain after flowering, which significantly affects the overall yield. The aim of this study was to reveal the dynamics of remobilization of the fructan reserves in the three lower internodes with transcription levels of the genes involved in their synthesis and degradation as well as the efficiency of photosystem II during development in two spring barley cultivars Astor and Jaran. Cultivar Jaran with better photosynthetic efficiency, as opposed to cultivar Astor, had lower fructan content. These results can be explained by coordinated expression pattern of the sucrose: sucrose 1-fructosyltransferase (*1-SST*), sucrose: fructan 6-fructosyltransferase (*6-SFT*) involved in the synthesis and the expression of fructan-1-exohydrolase (*1-FEH*) gene involved in fructan remobilization leading to better sink strength and generally lower fructan content in the stem of cultivar Jaran. Fructan synthesis and remobilization are under a strong influence of genotype and enzyme activities were directed towards synchronized synthesis of fructans and their mobilization to the grain. The obtained results will contribute to the selection and breeding processes of spring barley cultivars.

Keywords: fructans, internode, *1-SST*, *6-SFT*, *FEH*

Enhanced gibberellin catabolism promotes somatic embryo induction from spinach apical root sections

PP1-16

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Gibberellins (GA) are essential for induction of somatic embryogenesis from spinach apical root sections. To elucidate the role of GA in this process, expression of genes encoding enzymes that catalyze the final step of GA_{20} oxidation to bioactive GA, GA3-oxidase (GA3-ox), and GA degradation, GA2-oxidase (GA2-ox), was assessed. In spinach, there is only one GA3-ox and three GA2-ox (GA2ox 1, GA2 ox2, GA2 ox3). Expression of these genes was tested in the explants cultivated on noninductive medium (NM), supplemented with 20 μM NAA, inductive medium (IM), containing 20 μM NAA + 5 μM GA_3, or plant growth regulator (PGR)-free medium during a 28 d induction period. Root-tips isolated from seedlings and immediately frozen for RNA isolation were used as a control. In the explants cultivated on PGR-free medium, expression of GA3-ox increased gradually up to 10-fold and was constantly higher than in control. GA2-ox1, with an increase of up to 60-

fold, was the most highly expressed GA2-ox, while the expression of GA2-ox2 and GA2-ox3 only slightly increased compared to the control. In the explants cultivated on NM, expression of GA3-ox decreased slightly until the 7th day of cultivation and then increased up to 2-fold until the end of the experiment, while the expression of GA2-ox1 and GA2-ox2 was only slightly higher than in control. However, explants cultivated on IM showed the constant and significant decrease of GA3-ox (down to 7-fold) and increase of GA2-ox2 (up to 20-fold) expression. Here, we propose that continuous decrease in GA3-ox and increase in GA2-ox2 expression were favorable conditions for somatic embryo induction.

Keywords: gene expression, gibberellins, somatic embryogenesis, spinach

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Bud regeneration from root-tips of *Allium atropurpureum* Waldst. & Kit.

PP1-17

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A. atropurpureum is a natural rarity of Serbia, growing only in the area of Vojvodina. The vulnerability of its habitat by fragmentation and frequent and uncontrolled chemical treatment of surrounding agrarian areas, affects its status and the impoverishment of natural populations. Therefore, establishing a protocol for efficient *de novo* regeneration of this species for *ex situ* conservation was the aim of the present study. For callus induction, the apical root sections of axenic seedlings were cultivated on medium supplemented with 5 μM 2,4 D + 5 μM BA for 8 weeks. The obtained calli were friable, pale beige, without regeneration capacity. However, within these calli, a compact yellowish callus formed, and this type of callus had the capacity for bud formation when cultivated on media containing 0, 1, 5 or 10 μM TDZ, Kin or BA for 8 weeks. Calli cultivated on medium supplemented with 10 μM Kin exhibited the highest bud forming capacity, with the lowest level of hyperhydricity and albinism. Shoot bunches were further hardened on plant growth regulator-free medium for 8 weeks, and then single plants were detached and subcultivated on media with 0, 1, 5 or 10 μM GA_3 and grown at 12 °C, for bulblet induction. All plants formed bulblets, but bulblets cultivated on GA_3-containing media multiplied by setting up to 10 secondary bulblets. Obtained bulblets are suitable propagules, as they easily develop into plants. This protocol is convenient for clonal propagation of this endangered plant species, as well as for other applications in its research and improvement.

Keywords: *Allium atropurpureum*, buds, bulblets, roots, tissue culture

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Effect of N^6-benzyladenine (BA) on biomass production and morphological characteristics of *Hypericum barbatum* Jacq. shoot cultures

PP1-18

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This is the first report of the effect of cytokinin N^6-benzyladenine (BA) on biomass production and morphological characteristics of *Hypericum barbatum* Jacq. shoot cultures. Shoot cultures of *H. barbatum* were established from epicotyls isolated from *in vitro* grown seedlings and cultivated on MS/B_5 medium supplemented with BA (0.1, 0.2, 0.5 and 1.0 mg L^{-1}). Control shoots were cultured on MS/B_5 hormone-free medium. One-month-old shoot cultures were evaluated for the production of fresh weight (FW) and dry weight (DW), multiplication index (MI), index of compactness (IC) and number of dark glands per leaf (NDG). Present results demonstrated that application of 0.5 mg L^{-1} BA markedly enhanced the accumulation of shoot FW (16-fold) and DW (7-fold) compared to control cultures. Similarly, shoots grown on medium with 0.5 mg L^{-1} BA displayed significantly increased MI values than those cultured on 0.1 and 0.2 mg L^{-1} BA (about 2.2-fold), as well as on 1.0 mg L^{-1} BA (1.6-fold). The IC values of BA-supplemented shoots were remarkably enhanced (from 1.5 to 2.7-fold) in comparison to control shoots and this evidence was also visually confirmed by the plant shape. It was found that medium supplemented with BA slightly increased NDG values (1.4-fold) of multiplied shoots compared to control shoots. Present results indicated that BA is an effective cytokinin for rapid multiplication and biomass accumulation of *H. barbatum in vitro* shoots. This protocol for multiplication of *H. barbatum* shoots provides a solid base for the establishment of various *in vitro* cultures that can be explored for the production of biologically active secondary metabolites.

Keywords: Biomass production, *Hypericum barbatum* Jacq., Multiplication, N^6-Benzyladenine, Shoot cultures.

Effects of nitrogen concentration and interspecific competition on vegetative growth and resources allocation of invasive *Symphyotrichum lanceolatum* (Willd.) G.L. Nesom complex and native *Achillea millefolium* L.

PP1-19

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Symphyotrichum lanceolatum (Willd.) G.L. Nesom complex is nonindigenous perennial plant that invades wet habitats and has negative consequences for floristic diversity. *S. lanceolatum* complex is treated as a single species consisting of ssp. *hesperius* and ssp. *lanceolatus*. The aim of this research is to investigate the effect of suboptimal nitrate concentration and competition on

vegetative growth and resource allocation of species *S. lanceolatum* complex and *Achillea millefolium* in hydroponic culture. Three plant cultures were grown in two nutrient concentration treatments, low N concentration (6.4 mg L^{-1}) and control (14.0 mg L^{-1}). Culture types encompass monoculture of invasive *S. lanceolatum* and native *A. millefolium* and mixture between those two species in a ratio 1:1. The treatment lasted 3 weeks and the plants were 6 weeks old when the contents of N, P and K in plant biomass were determined. In order to determine the allocation of biomass and resources, different mass relations and fractions were examined as well as specific and relative leaf area. The results showed that at the beginning of its development *S. lanceolatum* allocates biomass to the roots, as well as rhizome, and thus creates the potential for greater resource acquisition. This growth strategy differs from *A. millefolium* which allocated the most part of its biomass into aboveground parts. Content of nutrients in the biomass showed that *S. lanceolatum* is more competitive in nutrient acquisition. It is anticipated that this kind of growth strategy of *S. lanceolatum* contributes to the rapid spread of this species.

Keywords: invasive plants, *Symphyotrichum lanceolatum*, competition, vegetative growth, resources allocation

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Stimulative effect of silicon on the growth of alfalfa (*Medicago sativa* L.)

PP1-20

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Silicon (Si) is considered as a useful element for plants, since it plays an important role in their growth and development. It can also be involved in the metabolism of higher plants exposed to abiotic and biotic stress. In this study, the effect of Si on the growth and physiological processes of alfalfa was examined. Plants were grown in the semi-controlled conditions in greenhouse in nutrient solution (½ Hoagland) supplemented with 2 mL L^{-1} of Siliplant (dose recommended by the producer), which contains 7% of Si. Plants were exposed to Si treatment from the beginning of their growth. The experiment was set in five replications, with eight plants per pot, which were grown for one month and then analyzed. Due to silicon treatment, plant biomass has changed, and higher differences were found in stem fresh and dry weight (67% and 70%, respectively), compared to roots and leaves. The number of leaves per plant increased more than 25% and leaf area by 58.6% in the presence of Si. Free proline concentration was 87% higher than in the roots and as much as 1.6 times higher in the shoots grown in the presence of Si, with respect to the control. Intensity of transpiration and concentration of photosynthetic pigments were not significantly different in plants exposed to Si. Since Si showed positive effects on alfalfa growth and no apparent negative side effect at applied concentration, it may be used as a potential growth enhancer.

Keywords: silicon, *Medicago sativa*, transpiration, proline, photosynthetic pigments

Shoot regeneration from root segments in *AtCKX* transformed centaury lines grown at graded NaCl concentrations *in vitro*

PP1-21

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Common centaury (*Centaurium erythraea* Rafn) is a medicinal plant that can inhabit saline soils. Previous *in vitro* studies revealed centaury's tolerance to salinity concerning shoot regeneration and development. Centaury is known to spontaneously regenerate shoots from root segments *in vitro* on hormone-free medium, probably due to endogenous levels of cytokinins (CKs) and indolyl-3-acetic acid and their balance in roots. In this study we have investigated *in vitro* shoot regeneration from root segments, i.e. frequency of shoot regeneration, the average number of re-generated shoots per explant and shoot biomass production in three transformed centaury lines, bearing *AtCKX1* or *AtCKX2* genes. Graded concentrations of NaCl (0–200 mM) were added to the nutrition medium during 8-week cultivation period. As previously shown, selected transformed centaury lines grown *in vitro* were characterized by differently reduced bioactive CK contents, as a consequence of different expression of transgenes. The main goal was to elucidate if changed CK homeostasis interferes with salt-tolerance and affects shoot regeneration in centaury. The select-ed *AtCKX* centaury lines significantly differed from each other in terms of capacity for spontaneous shoot regeneration and biomass production *in vitro*. NaCl lowered shoot regeneration in all of the investigated lines, although they differed in their sensitivity to salt. Both *AtCKX2* lines showed increased sensitivity to NaCl with the highest sensitivity found in one with the highest potential for spontaneous regeneration (*AtCKX2*-26). Concentration of 50 mM NaCl enhanced biomass produc-tion only in control and line *AtCKX1*-29. Our results indicate connection between CK homeostasis, regeneration capacity and tolerance to salinity.

Keywords: centaury, regeneration *in vitro*, *AtCKX*, salinity, cytokinin

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Secondary somatic embryogenesis in *Centaurium erythraea*

PP1-22

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Centaurium erythraea Rafn is a widespread medicinal plant from the Gentianaceae family. Grown *in vitro*, centaury displays enviable developmental plasticity, often being capable of regenerating the whole plant from root or shoot tissues through pathways of organogenesis and somatic embryogenesis. Somatic embryogenesis (SE) is especially interesting for mass plant propagation, production of virus-free material, synthetic seeds and cryopreservation, and is known to produce less somaclonal variation than organogenesis. In *Centaurium*, both pathways have been reported to occur both spontaneously and induced by plant growth regulators (2,4-D and CPPU). It has been noted that subculturing of embryogenic callus for multiplication over extended period of time results in the reduction of embryogenic potential. One way to overcome this problem is initiation of secondary embryogenesis from primary cotyledonary embryos. Here we report successful secondary embryogenesis in *C. erythraea* induced both directly and indirectly from cotyledonary somatic embryos. Several combinations of different 2,4-D and CPPU concentrations were tested for efficiency to initiate secondary embryogenesis in the dark. After four weeks, several parameters were recorded: presence of callus and new cotyledonary embryos, number of cotyledonary embryos per explant and whether new embryos were formed directly or indirectly. Secondary embryos were capable of producing tertiary somatic embryos, effectively entering cyclic SE. Secondary embryos, as well as primary, were able to germinate into plantlets by transferring them on hormone-free medium.

Keywords: *Centaurium erythraea*, somatic embryogenesis, 2,4-D, CPPU

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Arabidopsis EML histone readers are essential for seed development

PP1-23

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Histone post-translational modifications (PTMs) and their recognition by histone readers, play a major role in regulating gene expression. Despite extensive studies, there is a lack of systematic knowledge of histone readers in plants. *EMSY-like (EML)* gene family was identified in *Arabidopsis thaliana* (Arabidopsis) based on homology with maize *RIF1*. RIF1 and EML proteins share a unique protein domain organization, characterized by the ENT and AGENET domains, found only in proteins with chromatin-associated functions. We demonstrated that EML1 and EML3 bind histone H3 lysine 36 (H3K36), a PTM usually associated with active gene expression and that they are localized in different compartments during seed development. To functionally characterize Arabidopsis EML proteins, we generated *eml1-2*, and *eml3-4* homozygous single mutant, as well as *eml1-2 eml3-4* double mutant stocks. Consistent with their localization pattern, mutants in *EML1* or *EML3* showed delayed embryo development, while in the *eml1-2 eml3-4* we observed a more significant delay compared with its WT. In addition, combined loss of function of *EML1* and *EML3* displayed early seed abortion and also caused ovule abortion, suggestive of a synergistic effect. Therefore, our preliminary results suggest that *EML* genes, specific histone mark readers in seeds, have an essential role in seed development.

Keywords: *Arabidopsis thaliana*, post-translational modifications, histone reader, seed development

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The influence of light quality on growth parameters of pepper (*Capsicum annuum* L.) plantlets

PP1-24

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The quality and quantity of light are important factors in the production of quality vegetable plantlets. Effective portion of the spectrum for photosynthesis is in the red and blue range; however, in the production of vegetable plantlets additional LED lighting that emits light just within the range of these wavelengths is often applied. The aim of this study was to determine the effect of red, blue and combinations of red and blue LED lights as well as common fluorescent light, on morphological traits, SPAD index and the content of chlorophyll, carotenoids and phenols in the leaves of pepper (*Capsicum annuum* L.), in two terms of analysis during seedlings growth. The

highest content of chlorophyll, carotenoids, SPAD index and the largest leaf area were established in the treatment red + blue LED illumination. The lowest values of leaf width and length were found in plants grown under red LED light, while the lowest plantlet height was obtained in plants grown under fluorescent lights. From these results we conclude that additional LED lighting with the combination of blue and red parts of the spectrum can stimulate growth and development of pepper seedlings.

Further research is required in order to find the most favourable ratio of before mentioned wavelengths of the spectrum in particular stage of growth and development, as well as genotype variability, because numerous studies confirm different responses to light quality among varieties of the same plant species.

Keywords: LED lights, pepper, pigments, phenols, SPAD index

Leaf glandular trichomes and secreted material of *in vitro*-grown tansy (*Tanacetum vulgare* L.): micromorphology, structure, histochemistry and essential oil analysis

PP1-25

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The morphology and structure of tansy glandular trichomes were characterized for the first time using scanning electron microscopy and light microscopy, and their secretion was analyzed by histochemical staining. Leaf indumentum of tansy was covered with non-glandular and glandular trichomes of biseriate type, distributed on both adaxial and abaxial surface. Both types of trichomes were also distributed along the stem, but with lower density. Light microscopy showed that biseriate glandular trichomes consisted of two broad basal cells embedded in the epidermis, a short single-celled stalk, and a large round multicellular head. The histochemical analysis of the glandular secretion, performed through several staining methods, revealed the complex nature of the secreted products, including lipids, terpenes, alkaloids and polysaccharides.

GC/MS phytochemical analysis of volatile compounds secreted by glandular trichomes, identified 42 compounds, representing >97% of the total volatiles in EO, obtained by hydrodistillation in a Clevenger-type apparatus. The monoterpene fraction was dominant, with the prevalence of oxygenated monoterpenes (38.5%), while the majority of sesquiterpene fraction referred to oxygenated sesquiterpenes (22.6%). Two dominant compounds, which determined the essential oil chemotype, were *trans*-thujone (22.7%) and neryl-isovalerate (20.6%).

The essential oil protects the aerial parts of the plant against herbivores and pathogens and the biological activity of the secreted products indicates their potential as pesticides or pharmaceuticals.

Keywords: *Tanacetum vulgare* L., *in vitro*, glandular trichomes, essential oil

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Seasonal dynamics of the rhizosphere phosphorus and citrate exudation by grapevine roots in a low P soil: a field experiment

PP1-26

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Low phosphorus (P) availability is a common constraint for crop productivity in both acid and calcareous soils due to formation of insoluble P complexes. As far as we are aware, grapevine is not considered a P-efficient species, whereas P deficiency is rarely reported under the field conditions.

We performed a field experiment under extremely low P conditions (Olsen P < 2 mg kg^{-1}) using own-designed rhizotron (80 cm depth), installed in 8-y-old vineyard with cultivar Chardonnay, grafted on 5BB rootstock. The device enables easy access to the new intact roots while preventing root drying. We collected the exudates from root tips every 2 h (during daytime) at the different growth stages (i.e. shoots 10 cm, flowering, berries pea-size and veraison). At the same time, both rhizosphere and bulk soil samples were collected and subjected to the sequential P analyses. The results of HPLC analyses of the root exudates showed that citrate can be considered as the main P-mobilizing compound, with clear seasonal and diurnal patterns: the highest anion exudation rate was recorded at flowering (lower pick) and veraison (higher pick), and at each growth phase in the morning (10 a.m.) and in the afternoon (4 p.m.), with a rapid depression at 1-3 p.m. Consequently, the relative share of soluble P fractions (H_2O- and $NaHCO_3$-extractable) in the rhizosphere was very high (20% of the total P fractions), leading to the leaf P concentration of 0.2% DW which is above the critical P-deficient level (0.15% DW) for grapevine leaves at flowering.

Keywords: citrate, grapevine, phosphorus, rhizosphere, root exudates.

Seasonal variations of photosynthetic efficiency in invasive species *Amorpha fruticosa* L.

PP1-27

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Indigobush (*Amorpha fruticosa* L.) is widely distributed invasive species native to North America. It was deliberately introduced to Europe primarily to prevent soil erosion, but recently it is often planted for decoration, honey production and biomass energy production. Uncontrolled expansion of Indigobush represents a significant threat to native species due to its rapid growth and reproduction as well as its allelopathic activity that negatively affects nearby vegetation. The aim

of this study was to analyze and compare changes in photosynthetic performance of Indigobush and accompanying woody species (*Quercus robur* L. and *Alnus glutinosa* (L.) Gaertn.). Therefore, photosynthetic performance was analyzed by measuring direct chlorophyll fluorescence twice during the vegetation season, in May and July. Indigobush seedlings grow in the Pokupsko basin forest complex near Karlovac (Croatia) in stands of varying size and age. For this investigation, three locations were chosen that were about the same size and age. Our results showed that performance index (PI_{abs}) in Indigobush increased significantly in July at all three locations compared to both accompanying species. Moreover, more enhanced electron transport per reaction center (ET_0/RC), the efficiency that an electron moves further than primary electron acceptor Q_A– (ET_0/TR_0), maximum quantum yield of primary photochemistry (TR_0/ABS) as well as quantum yield of electron transport (ET_0/ABS) followed by an increase in structure-function index (SFI_{abs}) in Indigobush was observed in July. Our results suggested that Indigobush was able to utilize absorbed light energy more efficiently than accompanying native species.

Keywords: Indigobush, invasive alien species, performance index, structure-function index

Mucilaginous exudate from the apical root sections of *Allium ascalonicum* provokes autoinhibition of proliferation and subsequent bud regeneration

PP1-28

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Mucilage secreted from *in vitro*-cultured organs has been frequently reported in numerous *Allium* species. Despite its adverse effect on cell proliferation and subsequent *de novo* regeneration, this phenomenon has never been thoroughly studied. In *Allium ascalonicum* (shallot), only the uppermost part of the root tip responds to a callus induction treatment by forming callus with bud-forming capacity. The very same site of the root secretes mucilage during the course of its cultivation on callus induction medium (CIM). To address this issue, mucilaginous exudate (10 µL), collected from previously cultured root-tips of shallot, was applied directly to the root-tips isolated from *in vitro*-cultured shallot plants. Untreated root-tips were used as a control. All explants were cultivated on CIM containing 5 µM 2,4 D + 5 µM BA for 10 weeks, and then on regeneration induction medium supplemented with 5 µM BA for 4 weeks. A remarkable difference in cell proliferation and subsequent bud regeneration between treated and control root-tips were observed. Calli formed from treated root-tips were 3-fold lighter (53.7 mg vs. 169.9 mg) and regenerated buds with lower frequency (14.64% vs. 89.2%) and smaller mean bud number per explant (0.1 vs. 15.4), and these buds were shorter (0.65 mm vs. 16.4 mm) compared to the control. Root-tips isolated from older roots secreted the mucilage at the higher frequency, formed smaller calli and regenerated fewer buds than those isolated from younger roots. Inhibition of cell proliferation indicates the potential of this exudate in control of cell proliferation in the treatment of some diseases.

Keywords: *Allium ascalonicum*, bud regeneration, mucilaginous exudate, roots, shallot

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Potassium iodide promotes bud regeneration from the apical root sections of shallot plants

PP1-29

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The apical root sections of *Allium ascalonicum* plants respond to a callus inducing treatment by forming callus from the root apical meristem. It has been shown in *Arabidopsis thaliana* that balance between superoxide and hydrogen peroxide (H_2O_2) in the root tip determines root meristem size by controlling the transition from cell proliferation to differentiation. Hence, the increased size of root meristem may affect callus formation and subsequent bud regeneration from the root-tips. To test this hypothesis, *in vitro*-grown shallot plants were pretreated with aqueous solutions of H_2O_2 (100, 500 or 1000 μM) or potassium iodide (KI, 1 or 10 mM) for 24h. Plants incubated in water were used as a control. The apical root sections (1 cm) isolated from these plants were cultivated on callus induction medium, supplemented with 5 μM 2,4-D + 5 μM BA, for 8 weeks and then on regeneration induction medium, containing 5 μM BA, for 4 weeks. Obtained results indicate the significant impact of KI on *de novo* bud regeneration. Explants treated with 1 mM KI regenerated buds with significantly higher frequency (83.6% vs. 25.2%) and the mean bud number (6.19 vs. 0.21) compared to the control. The frequencies of bud regeneration and the mean bud numbers obtained for H2O2-treated explants were significantly lower compared to KI-treated explants (43.5% 52.0% and 1.0-2.3, respectively). Assuming that H_2O_2 decreases size of the root meristem, as was shown in *Arabidopsis*, the results obtained in this work suggest that KI, a H_2O_2 scavenger, increased bud-forming capacity presumably by increasing the size of root meristem.

Keywords: *Allium ascalonicum*, bud regeneration, hydrogen peroxide, potassium iodide, shallot

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Combination of high sucrose concentration with exogenuous cytokinins affects endogenous phytohormone profiles of kohlrabi seedling explants during *de novo* organogenesis

PP1-30

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Fluctuations in local phytohormone levels are considered to be important for initiation of developmental events in the process of plant organogenesis. Simultaneously, sugars as signaling molecules affect plant metabolism and growth, potentially interacting with hormonal regulation. Thus, the aim of the presented study was to see whether there were any changes in cytokinin (CK) homeostasis in 4 different stages (T1-T4) during *de novo* shoot organogenesis of kohlrabi (*Brassica oleracea* var. *gongylodes* cv. Vienna Purple) seedlings under the influence of exogenous CKs, *trans-*

zeatin (*trans*Z) and thidiazuron (TDZ), combined with high sucrose concentration. Explants grown on CK-free medium with 3% sucrose were used as control. Generally, analysis of variance showed statistically significant impact of CK treatment and sucrose concentration, as well as their interaction in all investigated stages, including seedling development before calli formation (T1 and T2), calli formation (T3) and when *de novo* shoots occurred (T4). The obtained CK profiles exposed remarkable increase in total CK levels in samples treated with *trans*Z, particularly when 9% sucrose was used in nutrition medium, reaching up to 50,000 pmol g^{-1} FW. This trend was observed for all CK physiological and structural groups. On the other hand, application of a synthetic urea-type CK, TDZ, contributed to little or no increase in the endogenous CK levels regardless of the sucrose concentration. Our results demonstrated that phytohormones metabolism may be triggered by sucrose signaling in kohlrabi shoot organogenesis.

Keywords: kohlrabi, seedling, sucrose, cytokinin, *de novo* shoot organogenesis

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Cytokinin *N*-glucosides: occurrence, metabolism and biological activities in plants

PP1-31

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Cytokinins (CKs) are essential hormones for plant growth and development. One of the mechanisms controlling homeostasis of CKs in plants is their glucosylation at the *N7*- and *N9*-positions of the purine ring, generally regarded as irreversible and down-regulating CK pathway. The products of this metabolic pathway, CK-*N7*- and *N9*-glucosides, are known to have widespread distribution in higher plant species, representing even the major CK metabolic forms in most of them. In our comprehensive screening, however, only very low levels or a total absence of CK-*N7*- and *N9*-glucosides were found in fungal kingdom and non-vascular plants such as algae and mosses. These data together with predominance of *cis*-zeatin-type CKs in these species indicate a close interconnection between CK-*N*-glucosyltransferase pathway and formation of *cis*-zeatins in the evolutionary context.

The levels and quantitative ratios of CK-*N7*- and *N9*-glucosides differed during ontogenesis in selected plant species. While CK-*N9*-glucosides exhibited mild antisenescent activities expressed by chlorophyll retention in detached oat leaf segments in the dark, CK-*N7*-glucosides were essentially inactive. This may be due to stronger metabolic conversions demonstrated in the same experimental system for *trans*-zeatin-*N9*-glucoside leading to the formation of some storage forms of bioactive CKs such as *trans*- and *cis*-zeatin-O-glucosides compared to its *N7*-glucoside.

Our data substantially question the tenacious notion of biological inactivity and irreversibility of CK-*N*-glucosides, namely those glycosylated at *N9*-position, and will be discussed with respect to arguing against the general image of CK-*N*-glucosides as inactive and irreversible compounds.

Keywords: cytokinin, *N*-glucoside, glucosyltransferase, zeatin, phytohormone

This work has been funded by the Czech Science Foundation (16-14649S).

Effects of temperature stimuli on seed germination of common ragweed (*Ambrosia artemisiifolia* L.)

PP1-32

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The activity of temperature stimuli on seed germination requires additional research focus in the context of changing climatic conditions in Serbia and globally. The present study examined the effects of duration and intensity of single and multiple exposures of stratified and non-stratified common ragweed seeds to temperature stimuli. In the first step, seeds were incubated at eight constant temperatures to determine their three cardinal germination temperatures: basic temperature (T_b), optimal temperature (T_o) and maximum temperature (T_m). The data were then used to calculate the thermal time of seed germination. Seeds from the same original sample were then exposed to single or multiple temperature stimuli over different periods of time at three temperatures: 23 °C, 28 °C, or 33 °C. Seeds exposed to a single temperature stimulus at each of the tested temperatures were found to have thermal times that increased with treatment duration. Over the same duration periods, thermal time values decreased with increasing temperature. Common ragweed seeds that were exposed to repeated temperature stimuli had lower thermal time values when the difference was greater between germination temperature and stimulus temperature. Exposure of ragweed seeds to either single or multiple temperature stimuli reduces thermal time which is required for germination, compared to untreated seeds, i.e. such exposure stimulates germination.

Keywords: *Ambrosia artemisiifolia*, thermal time, single temperature stimulus, multiple temperature stimuli

The present study was part of Grant Nos. TR 31043 and TR 31037 funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Phosphorus solubilizing bacteria isolated form ascocarp of *Tuber magnatum Pico* (*Pseudomonas sp.* (TMG021) influence root morphology and phosphate metabolism of potential truffle host (*Populus alba* L.)

PP1-33

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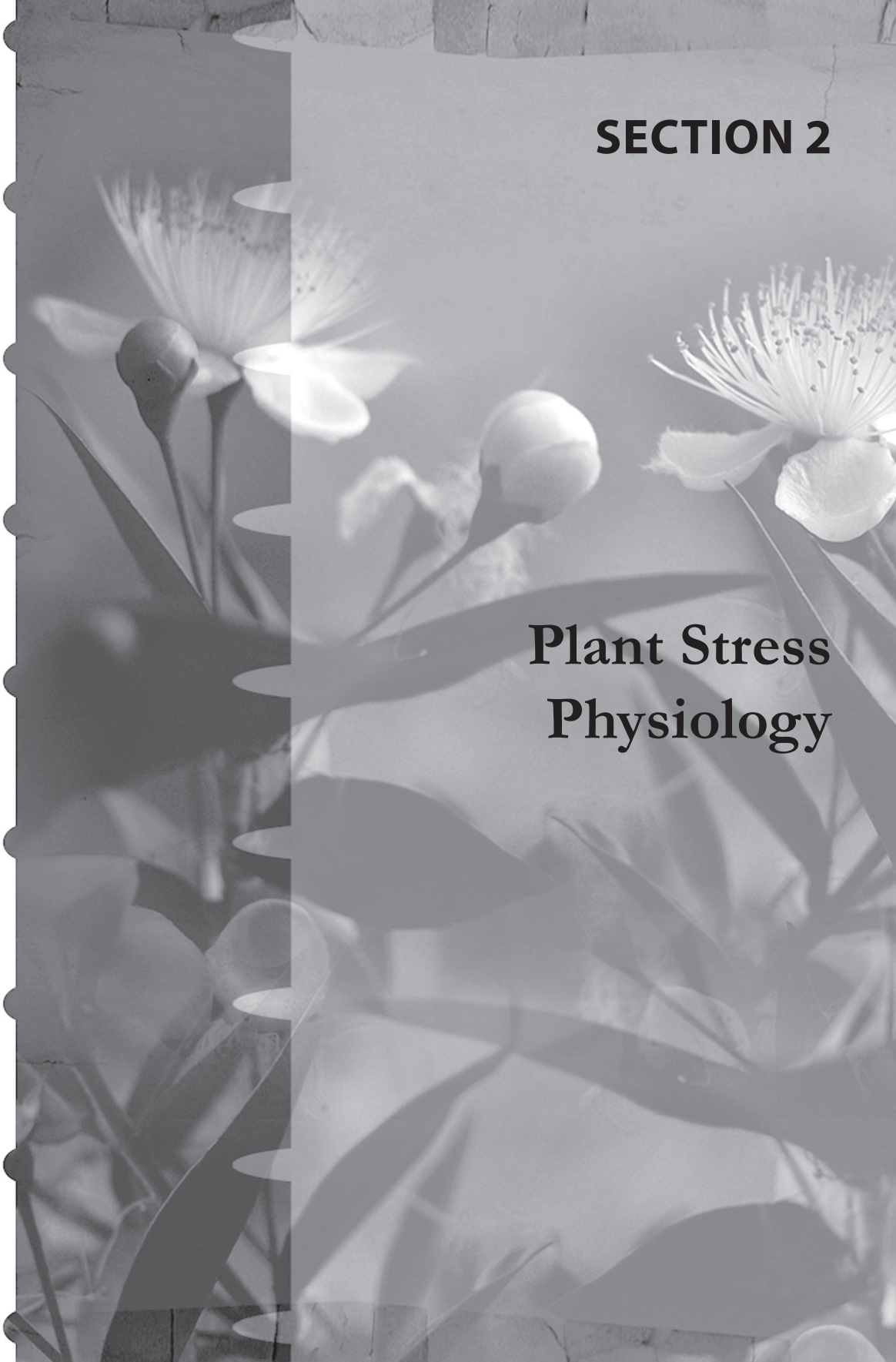
The most prized ectomycorrhizal (ECM) fungi *Tuber magnatum* Pico, the white truffle of Piedmont, lives in nutrient rich neutral to weakly alkaline soils. The factors that could explain the formation of this ectomycorrhizal type are varying soil water content and regularly low available phosphorus (P). In such soils, P is usually saturated as water insoluble $Ca(PO_4)_3$ or organically bound insoluble phosphates like phytate. As the ascocarps have been proved to host extremely rich microbiome, the question arised whether the soil phosphorus solubilisation (PS) in this tripartite symbiosis is performed by the mycelia themselves or by bacterial helpers. In the first part of our investigation we have screened isolated bacterial strains for $Ca(PO_4)_3$ solubilisation or for phytase activity and detected certain number of very effective strains. Among those, strain named *Pseudomonas sp.* TMG021 expressed both. This strain was used for further experiments set up for screening the influence on plant part of ECM, *Populus alba* L. Micropropagated plants were inoculated with the liquid culture of bacteria on the variety of culturing media (woody plant media (WPM); WPM with exchanged source of P ($KHPO_4$ was exchanged with $Ca(PO_4)_3$ or phytate). Noninoculated plants grown on WPM were used as a control. Phosphorus metabolism was recorded by ^{31}P NMR measurements of total P, phosphates and sugar phosphates in roots and stems. The results imply that presence of bacteria on plant roots change root morphology as well as phosphorus metabolism of investigated plants.

This work was supported by Grant No. 173017 financed by The Ministry of Education, Science and Technological Development of the Republic of Serbia.



SECTION 2

Plant Stress Physiology

PLENARY LECTURES

Sifting the elements of FNR-TROL bifurcation

PL2-1

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The last step of photosynthetic light-to-chemical energy conversion is the electron transfer from reduced ferredoxin (Fd) to NADP+. This reaction is catalysed by the flavoenzyme FNR (ferredoxin:NADP+ oxidoreductase), which utilizes two reduced Fds to produce one molecule of NADPH. In order to prevent Fd from promiscuous electron partitioning, the electron transfer reaction Fd>FNR>NADPH has to proceed rapidly and preferentially at the vicinity of photosystem I. FNR dimer is tethered to vascular plant photosynthetic membranes via an integral membrane protein TROL (thylakoid rhodanase-like protein). Soluble protein Tic62 also participates in FNR sequestration and activity control. FNR-TROL interaction prioritizes linear electron transfer (LET). When FNR complex is released from the TROL, highly efficient electron sink is activated. This pathway is faster than methyl viologen-mediated ROS propagation and represents so far undescribed mechanism of photosynthesis regulation. Apart from thylakoid membranes, TROL is targeted to the inner chloroplast envelope. Apparently, regulation involving stromal processing peptidase is crucial for this sorting. Finally, FNR-TROL bifurcation is embedded into redox responses of the entire plant and may even represent the source element linking photosynthesis with other plant defense and stress responses.

Keywords: photosynthesis, ROS, anthocyanins, protein import and sorting, EPR, PAM

Tomato (*Solanum lycopersicum*) Lipoxygenase (LOX) Gene Family: Delineating Gene Members Associated with Growth, Development and Abiotic stresses

PL2-2

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Lipoxygenases (LOXs) (EC 1.13.11.12) catalyze the oxygenation of fatty acids to their hydroperoxy derivatives critical to the biosynthesis of hormone jasmonic acid/methyl-jasmonate. To date little information is available about the LOX gene family in tomato, and less so about which members impact growth or development or respond to different abiotic stresses. We present complete identification of LOX gene family in tomato and characterization of members of this family of genes associated with growth, development and five different abiotic stresses (cold, drought, heat, salinity and wounding). Genome wide analysis of tomato LOXs identified 19 LOX genes annotated as lipoxygenases in Solanaceae Genome Consortium (SGN) Database. Their phylogenic

analysis revealed closeness of distinct members with known rice and Arabidopsis LOX gene members. Transcriptome expression analysis by q-PCR identified transcripts that accumulate during fruit development and those that accumulate during early fruit ripening transition and prolonged ripening. Exposure-time dependent kinetics in response to each abiotic stress (cold, drought, heat, salinity and wounding) employed was quite revealing and separated LOX gene members that are either induced, suppressed or non-responsive to a defined abiotic stress. Possibly, these data signify the importance of responsive LOX gene family members in mitigating specific plant stress. The new information unearthed here about LOX gene family in tomato will be advantageous for future genetic manipulation to understand key biological processes associated with each of them.

Keywords: lipoxygenase, gene families, tomato

INVITED TALKS

Two-year study of ecophysiological parameters of *Miscanthus* × *giganteus* grown on tailing pond at the mine "Rudnik" (Serbia)

IT2-1

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Flotation tailings pond is heavily loaded by Pb, Cu, Zn, Fe, Mn and Cd, and represents a great threat for the environment. The main objective of this study was to evaluate the ecophysiological response to heavy metal stress of *Miscanthus* × *giganteus* plants grown on the mining flotation tailings. All plants survived the two-year experiment. The concentrations of Cu, Zn and Cd in the roots and rhizomes of *M.* × *giganteus* increased several times in the second year compared to the first one indicating their accumulation in plant over time. Despite high heavy metal concentrations in the underground and aboveground plant parts, *M.* × *giganteus* can be considered as excluder suitable for phytostabilization of mine tailings ponds. The excessive amounts of heavy metals in leaves led to almost two-fold increase in membrane lipid peroxidation although total antioxidant capacity was not changed. Significant reduction in chlorophyll a content and low photochemical capacity for efficient utilization of strong light intensities were noted in all heavy-metal stressed plants. The lowered overall photosynthetic capacity and heavy metal stress resulted in markedly lower plant stature and biomass yield. Only older leaves showed visible signs of metal toxicity, chlorosis and premature leaf senescence. However, the parameters of chlorophyll a fluorescence (Fv/Fm, ΦPSII, qP and ETR) were not severely affected by heavy metal accumulation in leaves, suggesting the tolerance of PSII photochemistry. Our results show that *M.* × *giganteus* is able to grow under heavy metal stress and is efficient in phytoremediation of the flotation tailings ponds.

Keywords: chlorophyll *a* fluorescence; flotation tailings pond; photosynthesis; phytoremediation; tolerance

Tolerance to heavy metals – some examples in bryophyte species

IT2-2

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Contamination of the environment with heavy metals is manifold and often manmade. The metals are present as aerial dispersion being washed out by rain or they occur in the soil e.g. on mining sites. Contaminated environments are usually characterized by a barren, metal spiked substrate, high irradiation and drought. Bryophytes represent a group of early land plants and are pioneers to settle even in such inhospitable habitats. They can tolerate high levels of metals as they mainly depend on uptake of nutrients from the air wet deposition. The reasons for this astonishing tolerance remain largely unknown but could be key factors to understand biotic stress tolerance in plants.

Here, we focus on four different bryophyte species (*Hypnum imponens*, *Mielichhoferia elongata*, *Physcomitrella patens* and *Pohlia drummondii*). Some of these species occur on metal spoil heaps, some are from uncontaminated sites. In laboratory experiments, the specimens were introduced to controlled amounts of copper, zinc or cadmium (provided as $-Cl_2$, $-SO_4$, or -acetate) by addition to the substrate or by application as simulated rain. The bryophyte samples were analysed for growth parameters, stress physiology markers and cellular localisations of metal deposition. The inclusion of the model species *Physcomitrella patens* allows for complementary analyses of abiotic stress tolerance at the proteomics level.

Certain bryophyte species still keep an evolutionary conserved tolerance to metal contamination. Our *in vitro* experiments on a dozen different species show that the overall tolerance to zinc is higher than to copper or cadmium.

Keywords: bryophyte, metal tolerance, cellular localisation

SELECTED TALKS

Silicon mediates sodium (Na^+) transport in maize under moderate NaCl stress

ST2-1

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Silicon (Si) is known to alleviate NaCl stress in various plant species; however, the mechanisms by which Si regulates sodium (Na^+) transport are poorly understood. Here we investigated the expression of key Na^+ transporter genes in maize (*Zea mays* L.) plants exposed to moderate NaCl

stress (40 mM), paralleled by measurements of Na concentration in various tissues, compartments and organelles. Silicon supplied plants accumulated less Na in both root apex and cortex, but allocated more Na^+ to the shoot via the xylem and also enhanced its return to the root via the phloem. This was accompanied by increased expression of *ZmSOS1* and its kinase *ZmSOS2* in the root apex and root cortex facilitating Na^+ exclusion, and in the root stele for Na^+ loading into the xylem. Silicon differentially regulated the expression of Na^+ transporter gene *ZmHKT1;1* in different tissues and consequently decreased Na^+ unloading from the xylem in root and increased both xylem unloading and phloem loading of Na^+ in leaves. Furthermore, Si upregulated the transcript level of tonoplast *ZmNHX5* for Na^+ loading into the vacuole, thus decreasing Na^+ accumulation in the chloroplasts. In response to moderate salinity stress in maize, Si shifts the typical glycophyte behavior of this species towards that of a halophyte.

Keywords: maize, salt stress, silicon, sodium transporters, vacuole

Dynamic of Cd accumulation and metabolic adaptation of *Salix alba* grown hydroponically

ST2-2

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Toxic levels of heavy metals in the environment could be efficiently reduced by plants with specific metabolic properties, able to uptake, accumulate and tolerate heavy metals in their roots and shoots. Willow species are excellent tool for phytoextraction or phytomining technologies applied for in situ remediation of polluted sites. In this research, two *Salix alba* genotypes were grown hydroponically for 106 days. Cadmium treatment was applied in two concentrations (3 and 10 mg kg^{-1}) during last 14 days of the experiment. Photosynthetic performance, activities of antioxidant enzymes, content of compatible osmolytes and cadmium accumulation were used as evaluation criteria, every day during Cd exposure. Cd accumulation gradually increased, reaching a saturation plateau after 9 days. Plants showed fast time-dependent acclimatization response. Antioxidant enzyme activity was increased and lipid peroxidation reduced 4 to 12 hours after exposure to Cd. Compatible osmolyte proline had no significant role in adaptation to heavy metal stress. Concentrations of Cd accumulated in leaves and stems were higher than 100 mg kg^{-1} (up to 281.4 and 546.3 mg kg^{-1}, respectively), thus defining analyzed genotypes as hyperaccumulators of Cd. Rapid growth, high biomass yield, and good adaptability in correlation with high genetic potential of Cd accumulation, determines analyzed willow genotypes as a valuable resource for phytoremediation applications, such as phytoextraction and phytomining. High accumulation of Cd determined in roots (up to 874 mg kg^{-1}) also indicates high potential for phytostabilisation procedures.

Keywords: phytoremediation, phytoextraction, phytomining, heavy metals, willows

Nepetalactone-rich essential oil mitigates BASTA-induced ammonium toxicity in *Arabidopsis thaliana* L. by maintaining glutamine synthetase activity

ST2-3

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Phosphinothricin, active ingredient of the commercial herbicide BASTA, acts as an inhibitor of glutamine synthetase (GS), a key enzyme in ammonium assimilation, which leads to the elevation of ammonium levels in plants and further to various physiological alternations, ammonium toxicity and lethality. *Nepeta rtanjensis* Diklić & Milojević essential oil (*Nr*EO), rich in iridoid monoterpenoids nepetalactones, has been previously highlighted as a potential bioherbicide inducing oxidative stress in model plants. Interestingly, simultaneous foliar application of *Nr*EO and BASTA, two agents showing differential mode of herbicidal action, suspends BASTA-induced toxicity in *Arabidopsis thaliana* plants by maintaining sub-toxic and/or sub-lethal ammonium concentration in tissues. *Nr*EO effectively reduces BASTA efficacy, and the degree of antagonism between these agents escalates at increasing BASTA and *Nr*EO levels. Regulation of GS activity, as influenced by BASTA, *Nr*EO, and their joint action, partially occurs at transcriptional, posttranscriptional, and/or posttranslational levels, and is organ-specific. Furthermore, BASTA and *Nr*EO interaction mitigates the effects of these agents, applied independently, on chlorophyll, soluble sugars and organic acids metabolism. Results suggest the existence of complex regulatory mechanisms determining antagonistic BASTA and *Nr*EO interaction, and highlight the possible applications of the BASTA/*Nr*EO mixture in agricultural practice. Furthermore, the possibility of using *Nr*EO as a bioherbicide in BASTA-treated crop fields to mitigate the effect of BASTA residues in contaminated soils, is suggested here as an environment-friendly approach for weed control.

Keywords: BASTA, phosphinothricin, nepetalactone, antagonism, glutamine synthetase, ammonium toxicity

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant Nos. OI173024, OI173015, OI173040 and III41011).

POSTER PRESENTATIONS

Effects of elevated Zn on photosynthesis in *Miscanthus × giganteus* plants

PP2-1

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Miscanthus × giganteus plants were subjected to elevated Zn concentrations in soil during the three-month period of their growth. High concentrations of Zn in soil led to an increase in its concentrations in the roots, rhizomes, stems and leaves. However, according to low bioconcentration, bioaccumulation and translocation factors, *M. × giganteus* represents Zn excluder plant species. Excess zinc induced visible leaf damages in the form of leaf chlorosis and necrosis present only in lower leaves. High amounts of Zn significantly decreased the chlorophyll *b* content, stomatal conductance, transpiration rate, intercellular CO2 concentrations, photosynthetic rate, and parameters of chlorophyll *a* fluorescence (F_m, F_v, F_v/F_m). Nevertheless, there was no severe reduction in the maximal quantum yield of PSII photochemistry. Obtained results indicate high tolerance of *M. × giganteus* to elevated Zn concetrations, and its ability to grow on Zn-contaminated soils.

Keywords: chlorophyll *a* fluorescence; pigments; photosynthesis; phytoremediation; Zn tolerance

Chlorophyll *a* fluorescence, leaf pigment content and antioxidant protection of *Anacamptis morio* (Orchidaceae) grown on carbonate, ultramafic and siliceous soils

PP2-2

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The aim of this study was to determine the photosynthetic and antioxidant response of the orchid *Anacamptis morio* to chemical properties of carbonate, ultramafic and siliceous (chert) soils. Acidic soil solution and the lowest amount of organic carbon were characteristic of siliceous soils, whereas toxic amounts of Co, Cr and Ni were measured in ultramafic soils followed by large

amount of Mg and small content of Ca. Furthermore, the highest content of Co, Cr, Ni and Mg were detected in roots and leaves of plants grown on ultramafic soils. The maximal efficiency of PSII photochemistry (Fv/Fm), the actual PSII efficiency (ΦPSII) and electron transport rate (ETR) significantly differed among plants from different geological substrates, but remained at high levels. The photochemical fluorescence quenching (qP) was the highest in *A. morio* grown on the ultramafic soils, whereas the non-photochemical fluorescence quenching (NPQ) was the highest in plants from the carbonate soils. Plants from ultramafic soils had the lowest amounts of chlorophylls and carotenoids, and the highest amount of anthocyanins in leaves. The total antioxidant capacity (DPPH) and the content of malondialdehyde (MDA) were low in plants from the ultramafic and siliceous soils, suggesting that depletion of antioxidants decreased oxidative stress. Positive correlations were found between anthocyanins, free and bound phenolics and qP, NPQ, ETR, Fv/Fm and ΦPSII in plants from carbonate, ultramafic and siliceous soils. In summary, our results show that *A. morio* grown on different geological substrates possesses high photosynthetic potential due to strong antioxidant activity.

Keywords: *Anacamptis morio*, PSII photochemistry, anthocyanins, phenolics, antioxidant activity

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant Nos. 173030 and 173018).

Influence of moderate water stress on the fruit antioxidant composition of two tomato cherry genotypes

PP2-3

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Tomatoes are characterized by high fruit antioxidant capacity, which is often correlated with the concentrations of ascorbic acid and carotenoids. Also, antioxidant capacity is highly genotype specific and it can be affected by different abiotic stresses, including drought. Taking into account the increased importance of cherry tomato, the aim of the presenting study was to investigate their healthy promoting traits under drought stress.

Two cherry tomato genotypes of the MAGIC TOM population were selected - Cervil and Criollo. The plants were exposed to moderate drought stress in a greenhouse conditions and at the stage of the 2nd flower truss anthesis until fruit harvesting (red-ripe stage) and soil humidity was maintained around 25% of maximum water retention capacity. Control plants were optimally irrigated until the end of the experiment (70% of maximum water retention capacity). Carotenoids were analysed by HPLC. Vitamin C was determined by microplate assay, while antioxidant capacity was determined by Trolox equivalent assay.

Results showed that drought stress had a negative effect on fresh weight in both genotypes, but more expressed in Crillo than in Cervil fruits. The analysis of carotenoids revealed that the effect of drought on β-carotene, lycopene and phytoen accumulation was also genotype-specific

and more pronounced in Crillo than Cervil fruits. Total antioxidant capacity and vitamin C content increased in both genotypes, but more expressed in Cervil fruits. Although drought effect on the antioxidant capacity, vitamin C and carotenoid content was genotype-specific, these results indicated that moderate drought has potential to improve fruit healthy promoting traits of cherry genotypes.

Keywords: antioxidant system, moderate drought stress, cherry tomato

This study was supported by the EU Commission (FP7 project AREA) and the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 31005).

Heavy metals effect on morphogenesis of *Atrichum undulatum* in controlled conditions

PP2-4

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In vitro culture of the acrocarpous moss species *Atrichum undulatum* from the moderate environment of forest soils was established with the aim to study effects of heavy metals on its morphogenesis. Well-developed gametophores were used to study either 5-week or 5-day long heavy metal stress. Heavy metals, namely cadmium, zinc and copper were added to solid medium as non-harmful acetate salts in concentrations of 200 and 700 μM. The survival rate, index of multiplication, secondary protonema development, content of photosynthetic pigments, total phenols and antioxidative capacity were measured after heavy metal stress. Survival rates on medium containing copper, zinc and lead were very high in all treatments, while for cadmium in higher concentrations, survival rate was up to 52% in long exposure and 100% in short exposure experiments. As expected, index of multiplication significantly decreased for all heavy metals and salts applied. The pattern of secondary protonemal development could not be determined and related to treatment or metal. DPPH test showed antioxidant activity to be rather intensive during copper application. A decrease in the concentration/content of pigments (total chlorophyll and carotenoids) and total phenolics was documented with the increase of heavy metal salt concentration. The differences in morphogenesis of the moss *A. undulatum* exposed to heavy metals are documented and zinc can be regarded as less toxic compared to copper and cadmium.

Keywords: bryophyte, toxic metals, development, eco-physiology

This work was supported by the Ministry of Education, Science and Technological development of the Republic of Serbia (Grant Nos. 173024 and 173030).

Correlations between ABA content and physiological parameters up to 48 h after *Trichoderma*-Tomato interaction and drought

PP2-5

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At the stage of 4 developed leaves, tomato plantlets (Solanum lycopersicum Mill. cv. Ailsa Craig) were subjected to four treatments: optimal water supply (75% soil water capacity), cessation of watering and cessation of watering with addition of the *T. brevicompactum* SZMC 22661 spore suspension (8 x 10^6 CFU) to the root zone. Measurements of the soil water content (SWC; *Theta* probe), content of chlorophyll and epidermal flavonols (Dualex Scientific sensor), stomatal conductance (Gs; porometer), leaf water potential (ψH2O; pressure probe) and abscisic acid content (ABA; ELISA test) were performed 2, 6, 24 and 48 hours from the onset of treatments.

Plants responded to *Trichoderma* treatment by significant decrease in Gs already 2 h after application. This was coupled with decreased ψH2O and increased ABA content in both leaves and roots. In the same time point, plants that were not watered had high Gs but ABA content has doubled, both in roots and leaves. Plants without *Trichoderma* treatment and those that were treated have experienced drought 24 h and 48 h after the cessation of watering, respectively. Higher Gs of droughted plants that were in contact with *Trichoderma* was in accordance with higher ψH_2O and lower ABA content, both in roots and leaves in comparison to plants without *Trichoderma* treatment.

Keywords: *Trichoderma brevicompactum*, stomatal conductance

Fresh chives in pots on the Belgrade's balconies and terraces How do the urban environmental factors affect their quality?

PP2-6

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Chive (*Allium schoenoprasum* L.) is a small bulbous perennial which is used as a spice plant and as a culinary herb. This plant contains compounds with potent antioxidant activity and has beneficial effect on human health. Chive can be easily grown without special care. Accordingly, it would be the best that people grow spice and healthy plants on their own balconies and terraces to have fresh raw plant like chive every day. The objective of this study was to determine the impact of urban environmental factors on the quality of chives planted at 5 different locations in Belgrade. The

parameters of quality were phenolics profile and heavy metals content, while exposure to stress was determined by measuring the activity of antioxidant enzymes, peroxidases (POX) and superoxide dismutases (SOD) in fresh plants. The phenolics profile of plant aerial parts was characterized by LC-MS/MS. Twelve phenolic compounds were quantified, among which kaempferol 3-*O*-glucoside and quercetin 3-*O*-glucoside were dominant. Positive correlation between POX/SOD activity and concentrations of the forementioned compounds was observed. The concentrations of Cd, Cr, Cu, Pb and Zn in the leaves of investigated chives were measured using ICP-OES technique. The levels of Cd in edible parts of chive at all investigated locations were higher than permissible limit. Mean Cr concentration in leaves of chive was high only in central urban zone. The process of urbanization has been expanded with the negative consequences on plants, which can be seen from increased POX activity and high levels of heavy metals in leaves of chives.

Keywords: *Allium schoenoprasum* L., urban ecosystem, plant phenolics, antioxidant enzyme, heavy metals

This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant Nos. ON173015; ON172058 and ON172019).

The level of malondialdehyde (MDA), H2O2 and catalase activity in *Hedera helix* leaves at low environmental temperatures

PP2-7

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The leaves of *Hedera helix* are metabolically active throughout the year and are exposed to significant temperature changes. The aim of our work was to investigate several parameters of oxidative stress in *H. helix* leaves in the period of the lowest annual temperatures since oxidative stress is one of the main consequences of plant exposure to low temperatures. We examined the level of malondialdehyde (MDA) and H_2O_2, and catalase activity in the leaves of five *H. helix* plants during the period November 2017- February 2018 at Banja Brdo (Banja Luka). The average monthly temperatures in the research period were: 6.9 °C (November), 4.6 °C (December), 4.8 °C (January) and 0.7 °C (February), while the temperatures at the moment of sampling were 3.7 °C (November), 3.8 °C (December), 6.8 °C (January) and 0.2 °C (February). The results showed a significant increase in catalase activity and H_2O_2 concentrations at lower temperatures. MDA concentration was maintained at a constant level during the study. We noticed that the changes in the studied oxidative stress parameters, in addition to changes in the environment, depend on the biochemical-physiological status of each individual plant. The paper presents results for *H. helix* growth periods with the lowest environmental temperatures, but the main goal is to observe changes in these parameters in comparison with temperature changes during the whole year and different vegetative periods.

Keywords: *Hedera helix*, catalase, low temperature, H_2O_2, MDA

Allelopathic effect of apple hairy root exudates on the germination and seedlings growth of *Arabidopsis thaliana in vitro*

PP2-8

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In the previous study, phenolic acids and polyphenolic flavonoids were identified as putative allelochemicals in apple cv. Golden Delicious hairy roots growth medium. In order to examine their allelopathic potential, the effect of hairy root exudates on the germination and seedling growth of the test species *Arabidopsis thaliana* was elucidated. *Arabidopsis* seeds were cultivated in Erlenmeyer flasks containing 25 mL of liquid medium in which apple hairy roots were cultivated for 4 weeks. The effect of apple hairy root growth medium on *Arabidopsis* was evaluated after 5 and 10 days of seed cultivation. Apple hairy root exudates had no significant effect on *Arabidopsis* germination rate, while growth characteristics of seedlings were significantly affected both after 5 and 10 days of treatment. The roots of treated seedlings were significantly shorter (35.1%), with lower number (74% decrement) of lateral roots compared to control after10 days of treatment. The growth and morphology of *Arabidopsis* shoots and leaves have been affected too. Thus, the shoots of treated seedlings were almost twice shorter compared to control after 5 days of treatment. At the 10th day, this distinction became more pronounced, when two fully expanded leaves were visible in treated seedlings, while control had completely developed rosettes with six leaves. In addition, leaves of treated seedlings were smaller and pale in colour compared to control. qPCR expression analysis of genes involved in regulation of the cell cycle showed reduction of transcript levels in roots of treated seedlings, with the highest expression decrease detected for *CDKA1* and *CYCB2;4* cyclin.

Keywords: allelopathy, allelochemicals, cell cycle genes, phenolic acids, polyphenolic flavonoids

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. OI173015).

Transgenic hairy root *in vitro* culture system of apple as a tool for allelopathic studies

PP2-9

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Since the investigation of plant allelopathic interactions in the rhizosphere is difficult to perform because of its high complexity, microbial and environmental factors interactions, the generation of autonomous root culture could be appropriate system for the studies of allelopathic

potential of apple (*Malus* x *domestica* Borkh.). Thus, we have focused our efforts on the generation of transformed hairy-roots in apple cv. Golden Delicious using the 15834 strain of *Agrobacterium rhizogenes*. The agrobacteria inoculation by dipping the basal cut off end of the stem of *in vitro* grown apple shoots into bacterial suspension proved to be the best option for transformation, providing efficiency of 39.3%. Molecular characterization of hairy roots by RT-PCR analyses confirmed their transgenic nature. *In vitro* conditions for apple hairy root growth and maintenance were optimized using different mineral solutions and auxins. The liquid half-strength MS medium with the addition of IBA at 0.025 mg L^{-1} was chosen for the initial stimulation of growth. For their prolonged cultivation and maintenance, the alternation of IBA-containing and IBA-free media is recommendable. Fast-growing lines continuously maintained in IBA-free medium were used for further research. Potential allelochemicals including phenolic acids (chlorogenic, caffeic, syringic, p-coumaric and ferulic), glycosilated flavonoids (phloridzin, rutin, quercitrin, isoquercitrin, kaempferol-3-glucoside) and flavonoid aglycons (floretin, quercetin and naringenin) have been detected by UHPLC/DAD/(+/-)HESI-MS/MS analyses in the growth medium, in which these transformed roots were maintained for 4 weeks, as well as in the root tissue itself. The obtained hairy-root cultures offer a complementary system for studying and validating the allelopathic potential of apple roots.

Keywords: *Agrobacterium rhizogenes*, allelopathy, allelochemicals, growth conditions, hairy roots.

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Does ABA pretreatment affect ecophysiological status of bryo-halophyte *Entosthodon hungaricus* during salt-stress?

PP2-10

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In vitro culture of a peculiar moss halophyte *Entosthodon hungaricus*, which can be found exclusively on salty grasslands, was established with the aim to study the effect of exogenous ABA treatment of the moss gametophores prior to their growth in NaCl enriched environment. Axenic *in vitro* growth environment was established to avoid synergistic or antagonistic effects of other varying factors and to achieve fully controlled conditions. ABA is known to influence the activation of certain molecular mechanisms and expression of adequate ABA-responsive genes in different vascular plants under dehydration and salt stress.

E. hungaricus gametophores were grown for 72 hours on medium containing ABA (50 μM), and afterwards transferred to different levels of NaCl (0-500mM) for 21 days. The survival rate, index of multiplication, content of photosynthetic pigments, total phenols and antioxidative capacity were recorded. The results obtained clearly showed that exogenous ABA resulted in higher survival rate in all treatments. However, ABA had no influence on new gametophore production, as expected. However, in higher salt concentrations, secondary protonema appeared in ABA pretreated plants

and not in control, non-treated plants. In contrast to control plants where the pigment content decreased, ABA treated plants transferred to NaCl enriched medium maintained the chlorophyll and carotenoid content rather constant. In plants grown on the highest salt concentration, both high DPPH scavenging activity and total phenols content were detected. Exogenously added ABA significantly affected all parameters measured in the moss halophyte *E. hungaricus* during salt stress and further investigations are in progress.

Keywords: ABA, mosses, stress tolerance

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Ecophyisiology of the moss *Hypnum cupressiforme* under controlled heavy metal stress conditions

PP2-11

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The pleurocarp moss *Hypnum cupressiforme* is widely used to study the deposition of heavy metals from the atmosphere. However, there are no studies on this species under controlled conditions i.e. excluding synergistic and/or antagonistic effects of other environmental parameters. To achieve this type of controlled conditions, we have established *in vitro* culture of this species for optimized growth and used gametophore tips to study the effects of zinc and cadmium. Long (5 weeks) and short (5 days) exposures to zinc and cadmium on the ecophysiological status of the moss *H. cupressiforme* were examined. The survival rate, index of multiplication, secondary protonemal development and content of photosynthetic pigments were measured. Survival rate after both short and long exposure to zinc acetate (200 and 700 μM) was 100%, while in cadmium-acetate treatment survival rate was 50% at 700 μM already during short exposure. Long exposure to cadmium was even more harmful at both concentrations applied. In general, the index of multiplication was low after short and long exposures, and it significantly decreased in cadmium treatments. Zinc appears less harmful than cadmium in the same type of experiments. The total chlorophyll content was significantly different in zinc and cadmium treatments, highly decreasing in cadmium treatments. Carotenoids even slightly increased at the higher zinc concentration, while they linearly decreased with higher cadmium concentration and time of exposure. In summary, zinc is well tolerated by the model moss *H. cupressiforme* as shown in this environmental simulation study. Cadmium is more harmful for *H. cupressiforme*, both in short and long exposure.

Keywords: bryophyte, zinc, cadmium, development

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant nos. 173024 and 173030)

Induction of potato (*Solanum tuberosum* L.) defense genes by French marigold (*Tagetes patula* L.) essential oil

PP2-12

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When attacked, plants activate defense mechanisms including volatile emission. Besides having a pest repellent effect, volatiles can induce defense response in surrounding unharmed plants. French marigold (T*agetes patula* L.) essential oil (EO) was isolated from the above-ground parts of plants collected from nature by hydrodistillation using Clevenger apparatus. GC/MS analysis revealed the presence of 42 compounds, of which 97% (32 compounds) have been identified. The monoterpene fraction was dominant (76.9%) with terpinolene (32.4%) and limonene (14.7%) as the most abundant compounds. In order to investigate the potential of marigold EO to induce the defense of potato, we analyzed 3 genes related to the induced resistance (IR): pathogenesis related *PR-2* and *PR-5* genes, and proteinase inhibitor *Pin2*. The potato plants were exposed to EO volatiles during 4, 8 and 12 h. Two leaves of EO-treated or untreated control plants were collected, and plants were then additionally injured by mechanically wounding leaves across the leaf surface. After 24 h of wounding, another two leaves from each plant were collected and expression of the IR related genes was analyzed by qPCR method. EO induced altered expression of PR genes, while *Pin2* wasn't affected by the treatment. Mechanical wounding increased the expression of all genes after 4 and 8 h of EO treatment, with up to 4-fold change in *PR-2*. However, in plants exposed to EO for 12 h before wounding, the level of transcription in *PR-2* and *Pin2* was similar to that detected in untreated controls. These findings support the efforts for environmentally-safe pest control in crops by using natural compounds.

Keywords: *Solanum tuberosum* L., *Tagetes patula* L., essential oil, induction of defense

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Chilling tolerance in maize: morphological and physiological changes in maize inbreds under low temperature conditions

PP2-13

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Cold stress has become a serious problem in maize seed production, due to the on-going climatic changes. Severe summer droughts call for earlier sowing when temperatures are sub-optimal for seed germination and early plant development. Also, different susceptibility of hybrid parents prevents synchronization in male and female flowering and results in the absence of fertilization. A pilot experiment on three maize inbreds with different sensitivity was conducted with

the aim of elucidation of plant response mechanisms to cold stress conditions. The experiment was conducted outside in pots under low and optimal temperature conditions. At the third leaf stage, a set of morphological and physiological traits was analyzed. Agronomic traits and yield of the plantelets transferred to the field were evaluated, too. Number of plants, shoot fresh weight (SFW), flavonoid, anthocyanin and chlorophyll content showed significant differences among the analyzed inbreds under the cold stress treatment. Only SFW of all analyzed shot/root traits was significantly different between the most tolerant and the most sensitive inbred. The most tolerant inbred had significantly higher content of pigments and lower chlorophyll content. Fluctuating temperatures during the cold treatment had the most important impact on plant response to the stress. In this way, the genuine response of the analyzed genotypes to low temperatures occurring in fields in the period of early sowing was analyzed. More detailed research including greater number of physiological parameters together with molecular characterization of these genotypes will be done in order to better understand mechanisms underlying tolerance to low temperatures in maize.

Keywords: maize, cold stress, physiological traits, morphological traits

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The effects of drought stress on tomato plants grown under different light regimes

PP2-14

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The aim of this study was to investigate the effects of drought stress on particular physiological parameters, molecular mechanisms and their interactions on tomato plants grown under two different light regimes - PAR 300 µmol m^{-2} s^{-1} (LL) and PAR 850 µmol m^{-2} s^{-1} (HL), respectively. Wild type (WT) (*Lycopersicum esculentum* Mill. cv. Ailsa Craig) and ABA deficient mutant *flacca* were used in this experiment. Plants were grown in the chamber with controlled temperature, light, humidity and photoperiod. The experiment was started when plants were in the phase of four leaves. Soil water content, stomatal conductance (Gs), concentration of epidermal flavonoids and water potential (ΨI) were measured in fully irrigated (FI) and in drought exposed plants (D). Biochemical parameters (ABA, ascorbate, proline and carbohydrate content) were also determined in FI and D phase.

ABA content was increased in drought in both plant types at LL and HL, which was accompanied by decrease of Gs in WT and increase in *flacca*. Expression of genes involved in ABA signaling, such as *NCED1*, *PPC2* and *ABI1* were in line with the obtained ABA changes. High light increased ascorbate content in WT and *flacca*. Ascorbate was further increased by drought in HL. Proline (Pro) content in drought was significantly increased in both plant types irrespective of the light intensity. However, carbohydrate content varied depending on light and water status.

Results imply the importance of light intensity on drought tolerance.

Keywords: tomato, drought, PAR

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Interaction effects of Fe nutritional status and Cd stress on PSII activity and photosynthetic pigments content in plants of *Ailanthus altissima* (Mill.) Swingle

PP2-15

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Cadmium induced phytotoxicity is based on inhibition of photosynthetic electron transport but it also negatively affects the water balance, total chlorophyll and carotenoids, oxidative phosphorylation in mitochondria, inhibits stomata opening, H^+/K^+ ion exchange and ATPase membrane activity. It significantly affects several enzymes, most often decreasing their activity etc. In most cases Cd uptake and translocation are driven by competition to Ca, Fe, Zn for nutrient transporters and channels. The iron nutritional stress can increase Cd uptake and translocation, due to induced transport mechanisms by Fe deficiency, and involvement of Cd in plant photochemistry. In this experiment we tested if there was an influence of Fe status on Cd induced changes of some photosynthetic parameters. Two-week Cd supply at the concentration of 50 μM in hydroponic culture reduced total chlorophyll and carotenoids content ($p<0.05$) in young leaves of *Ailanthus altissima*, in all treatments. Besides this, in iron deficient plants Cd significantly inhibited the efficiency of PSII (Fv/Fm), half-rise time for maximum fluorescence (t1/2) and chlorophyll a/b ratio, while the same parameters in iron sufficient, Cd treated plants were at the control level. Pronounced effect of Cd on the measured parameters in *Ailanthus altissima* plants exposed to iron deficiency indicated higher sensitivity of PSII to Cd toxicity in iron deficiency conditions.

Keywords: Cd phytotoxicity, Fe nutritional status, *Ailanthus altissima*, PSII activity, photosynthetic pigments

Salinity effect on germination of parasitic (*Cuscuta campestris*) and non-parasitic (*Ipomoea purpurea*) members of Convolvulaceae family

PP2-16

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Parasitic plants and soil salinization represent growing problems for agriculture, causing substantial yield losses of crop plants worldwide. *Cuscuta* spp., members of the family Convolvulaceae are stem holoparasites, acquiring all their nutrients from the host plants and are among the most widely distributed and aggressive agricultural pests. Although their seeds contain minimum amounts of storage compounds and their photosynthetic activity is insufficient to support meta-

bolic needs, they are able to sustain growth for several days and up to 20 cm until they successfully attach to a host plant. The aim of the present study was to explore the effect of various salinity levels on the germination properties, hydrolytic and antioxidant enzymes and growth in host absence of *Cuscuta campestris* in comparison to its non-parasitic relative, *Ipomoea purpurea*. Unlike the non-parasitic plant, which was not significantly affected up to 100 mM NaCl, germination percentage of *Cuscuta* seeds was overall lower and further decreased by salinity. Growth ability before attachment to host was also impeded. While both α-amylases and proteases were involved in *Ipomoea* germination and affected by salinity, in *Cuscuta protease* activity was hardly detectable. The parasite proved also to be less effective in L-proline accumulation and antioxidant response. *Cuscuta* was shown to be much more sensitive to salinity at germination and early growth stage, although previous findings proved it was successful in host attachment and parasitization at similar NaCl concentrations.

Keywords: germination, parasitic plant, salinity

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Non-invasive mapping of redox status in the aflatoxin-stressed maize and wheat seeds by 2D electron paramagnetic resonance imaging

PP2-17

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Cereal seeds are susceptible to infection by the opportunistic pathogen *Aspergillus flavus* that consequently leads to the contamination with aflatoxins. In this work, EPR imaging was applied for mapping redox state of the maize and wheat seeds contaminated with aflatoxin, through detection and localization of paramagnetic spin probes *in vivo*. The sample recordings were made in the L-frequency domain (1.1 GHz). The 3-Carbamoyl-2,2,5,5-tetramethyl-1-pyrrolidinyl-N-oxyl (3-Carbamoyl-PROXYL) spin probe was used for the estimation of changes in the redox state of the aflatoxin-stressed seeds. The EPR-active probes were reduced by cellular reducing agents forming EPR inactive species (hydroxylamines). The reduction rate, which among other factors depends on the redox status of the cell, provides information about the redox environment in the region of interest. A higher and more localized 3-Carbamoyl-PROXYL spin-active probe signal has been found in uncontaminated seeds than in aflatoxin-stressed seeds. Our results imply that aflatoxin contamination leads to the change in the seeds' redox status which provides useful information about its impact on seed metabolism.

Keywords: aflatoxins, seeds, redox status, electron paramagnetic resonance imaging

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Physiological responses of basil cultivars to prolonged nutritional stress

PP2-18

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Large-scale losses in plant productivity may occur when plants are exposed to nutrient stress, which is particularly important for worldwide cultivated crops. In the present study, three basil genotypes (green leaved - *Ocimum basilicum* L. var. *basilicum*, purple leaved - *Ocimum basilicum* L. var. *purpurascens* and lemon basil - *O.* x *citriodorum* Vis.) were tested in terms of the ability of the seedlings to withstand the prolonged nutrient stress. From germination until the first month of growth, basil seedlings were grown *in vitro* in media with low, medium and high content of nutrients. It was demonstrated that seedlings from investigated basil cultivars were capable to overcome stress and to maintain homeostasis through maintenance or increase of SOD, CAT, A-POX, G-POX and P-POX. The activities of antioxidant enzymes varied among genotypes as well as seedling parts and among tested enzymes, A-POX, G-POX and P-POX seem to have crucial role in defense from prolonged stress. According to obtained results, among tested genotypes, lemon basil was less, and purple basil was more susceptible to nutrient stress. The changes in nonspecific antioxidant biomarkers could be highly valuable for studying the mechanism underlying stress tolerance in basil genotypes and could facilitate the development of tolerant basil cultivars. Appropriate mineral nutrition for diverse genetic material of basil cultivars is essential to minimize the damage caused by nutrient stress.

Keywords: basil, stress, antioxidant enzymes, nutrients, genotypes

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Role of specialized metabolites in *Brassica oleracea* var. *acephala* abiotic stress response

PP2-19

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Cruciferous vegetables belong to the family Brassicaceae (Cruciferae), with many genera, species, and cultivars and with a great cultivation potential under various climatic conditions. One of the oldest vegetable from Brassicaceae family, originating in the eastern Mediterranean is non-headed kale (*Brassica oleracea* var. *acephala*). Plants from this group possess good tolerance to unpleasant environmental conditions and are tolerant to the extreme temperature fluctuations caused by climate change. In the recent years, with the growing interest in healthy food,

varieties from this group have gained worldwide popularity due to low calorie value and high content of health-beneficial compounds including vitamins and non-vitamin compounds such as carotenoids, glucosinolates and their derivatives, and polyphenols. Due to their specific biological functions, compounds that are associated with health benefits in humans often play critical roles in plant growth and survival in the changing environment. The aim of the present study was to investigate the role of specialized metabolites in *B. oleracea* var. *acephala* stress adaptation capability. Agar-grown plants were exposed to abiotic stresses (salt stress and low temperature stress) and compared with the control. Then we determined morphological changes, stress markers and the levels of main groups of specialized metabolites.

Keywords: *Brassica oleracea* var. *acephala*, abiotic stress, specialized metabolites

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Drought resistance is an excellent aspect of the autochthonous pear cultivars in regard to the production of one-year-old seedlings

PP2-20

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The study was conducted in three successive years to examine the possibility of producing in nursery one-year old seedlings of autochthonous pear cultivars grafted on quince Ba-29 and wild pear seedlings (*Pyrus communis* L) during the first year after bud grafting. The aim of this paper is to determine morphological characteristics, compatibility of autochthonous pear seedling cultivars (Sijerak, Kaličanka, Medunak, Kačmorka, Buzdovanka and Mirisavac) with the quince BA-29 and wild pear seedlings and water attaining capability of the leaves in one-year old seedlings of autochthonous pear cultivars.

Production of seedling material of autochthonous cultivars with vegetative rootstocks 'Quince Ba-29' will be enormous contribution to even partial preservation of fruit genofond that is the unity product of our ecological environment and autochthonous biocenosis.

The results of this research show that the plant height and the stem diameter of one-year old seedlings are characteristics of autochthonous pear cultivars, from which rapid growth and uniformity of scions depend.

The leaves of cultivar "Kaličanka" had the highest water attaining capability. The lowest level of the stated capability was recorded for the leaves of cultivar "Kačmorka".

The results of this research also showed that the water attaining capability of the leaves in one-year old seedlings of autochthonous pear cultivars as an indicator of their resistance to drought was genetic characteristic of the cultivars.

Keywords: autochthonous cultivars, morphological characteristics, one-year old seedlings, resistance to drought, water attaining capability

Thermodynamic properties of isothermal dehydration process of two maize hybrids under the influence of 24-epibrassinolide

PP2-21

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In this paper, the influence of different concentrations of 24-epibrassinolide (24-EBL) on thermodynamic quantities during dehydration process of two maize hybrids (ZP434 and ZP704) was analyzed. Analyses were conducted on control samples (not treated with 24-EBL) and samples treated with 24-EBL. It was established that seedling parts (radicle, plumule and rest of the seedling (RoS)) related to ZP704 hybrid, in comparison with those related to ZP434 hybrid, show higher tolerance (in terms of energy) to impact of 24-EBL during dehydration process. Based on performed glass transition temperature analysis of control samples for both hybrids, it was concluded that the formation of tight glass matrices increases the glass transition temperature of a glassy matrix made of carbohydrates and reinforced H-bonding network. Obtained results showed that this phenomenon is more favored in the case of ZP434 control sample. Also, based on enthalpy-entropy compensation theory, it has been found that water desorption mechanism in tested hybrids is controlled by entropy, where the results demonstrated that the molecular re-arrangements are at high levels. Based on BET (Brunauer-Emmett-Teller) and modified BET approaches, it has been found that adaptation of ZP704 hybrid treated with 24-EBL is more acceptable than in the case of ZP434 hybrid.

Keywords: 24-epibrassinolide, maize hybrids, dehydration, thermodynamic properties, sugar-protein matrix

Effect of low concentrations of NaCl on accumulation and distribution of Na, K, Ca and their ratios in different plant species

PP2-22

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Uneven distribution of precipitations during vegetative season, periods of drought, inappropriate quality of irrigation waters and particularities of soil composition are the main factors leading to salinization of agricultural soils. The area of salt-affected soils is increasing worldwide, leading to yield losses and deterioration of plant-derived food and feed.

To assess the extent to which the steady presence of sodium chloride in relatively low concentrations (0.1-1.2 g L^{-1}) affects plants, an experiment was set in semi-controlled conditions of a greenhouse. Safflower (*Carthamus tinctorius* L., Asteraceae), coriander (*Coriandrum sativum* L., Apiaceae) and oilseed rape (*Brassica napus* L., Brassicaceae) were grown in water cultures, on half-strength Hoagland's nutrient solution, to which NaCl was added 2 weeks after planting. Plant growth, ash content, accumulation and distribution of Na in relation to K and Ca were analyzed 3 weeks following the beginning of the treatment.

The percentage of ash significantly increased in roots of safflower, leaves, stems and roots of coriander and leaves and stems of oilseed rape, whereas the percentage of dry matter significantly declined in coriander and oilseed rape. Concentration of Na increased in all plants and organs, to different extents. Ratios of concentrations Na/K, Na/Ca and (Na+K)/Ca increased in all species; K/Ca significantly increased in roots of safflower and coriander and declined in leaves of coriander. Even though dry weight of plants did not significantly change in the presence of NaCl at applied concentrations, significant changes in their composition were evident.

Keywords: salt stress, elemental composition, safflower, coriander, oilseed rape

Total antioxidant activity in wheat and pea seedlings treated with uncoated and polysaccharide coated CeO_2 nanoparticles

PP2-23

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CeO_2 nanoparticles ($nCeO_2$) are popular because of the unique redox property-transition between oxidation states (Ce^{3+} and Ce^{4+}). Their tendency for agglomeration has led many researchers to coat $nCeO_2$ with different polymers, but little is known about the impact of coated nanoparticles on plant metabolism. Increased $nCeO_2$ application enhances risk for the environment due to their accumulation in soil, air and water. Metal toxicity causes abiotic stress and leads to overproduction of reactive oxygen species (ROS), damaging important biological molecules in plants.

In this research, we performed a three-week treatment of seedlings of two agricultural plants in hydroponics with 200 mg L^{-1} of uncoated and glucose-, levan- and pullulan coated $nCeO_2$ (G-CeO_2, L-CeO_2 and P-CeO_2). Our aim was to study the effect of nanoparticle coating on Ce uptake, and on changes in total antioxidant activity (TAA), the indicator of oxidative stress in monocotyledonous and dicotyledonous crop species. Concentration of Ce in shoots of treated seedlings was determined using ICP-OES.

The uptake of Ce differed in the treated plant species. Coating of $nCeO_2$ increased Ce uptake in pea, but decreased in wheat. However, Ce content was 20-fold higher in wheat compared to pea plants, regardless of the nanoparticle coating. Extremely high Ce content measured in wheat coincided with the decrease in TAA. On the other hand, low Ce content measured in pea coincided with no changes in TAA.

Presented results suggest the difference in $nCeO_2$ uptake and its physiological effects between monocotyledonous and dicotyledonous plant species, but further research is necessary.

Keywords: coating, CeO2, nanoparticle, plant, stress

Wounding alters gene expression of secoiridoid glucosides metabolic pathway in leaves of common centaury

PP2-24

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Common centaury (*Centaurium erythraea* Rafn) is rich in secoiridoid glucosides (SG's): sweroside, swertiamarin and gentiopicrin. These glycosides are believed to be a part of dual defense system in which the SG's are bio-activated by hydrolytic enzymes. Upon tissue disruption, SG's are released from storage compartments and hydrolyzed via β-glucosidase to yield unstable and highly reactive aglycones. This two-component system provides plants with an immediate chemical defense against herbivore-induced wounding of leaves. Plants react to mechanical damage by activating a set of genes, the products of which are involved in defensive functions. Current study was conducted in order to determine how wounding affects the expression of SG metabolic pathway genes. Gene expression patterns of five SG biosynthetic pathway-related genes (*CeGPPS*, *CeGES*, *Ce8HG0*, *CeIS*, and *Ce7DLGT*) and of β-glucosidase (*CeBglu*) were examined in a time-dependent manner to determine the molecular mechanisms underlying wounding-induced changes in SG metabolism. Gene expression results were correlated with SG profiles in centaury shoots. The relative expression of *CeGES*, *Ce8HGO* and *Ce7DLGT* showed an increasing trend, reaching maximum at 24h/48h after wounding. Interestingly, two enzymes of the pathway with opposite functions, Ce7DLGT catalyzing the glycosylation reaction, and CeBglu having role in deglycosylation, showed opposite gene expression profiles. In conclusion, severe changes in gene expression profiles in response to wounding might lead to the reprogramming of SG metabolism in centaury leaves, and thus alter its defense strategies against herbivores.

Keywords: *Centaurium erythraea*, gene expression, mechanical injury, secoiridoid glucosides, UHPLC-MS/MS analysis.

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The investigation of sugar beet responses to drought at the Institute of Field and Vegetable Crops, Novi Sad

PP2-25

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Drought is the prime abiotic factor that limits sugar beet (*Beta vulgaris* L.) production in Serbia and other regions where the crop is not usually irrigated. As increased irrigation is not an economically viable solution, the most effective one is development of varieties adapted for successful growth in drought-prone environments. Within the framework of ongoing projects, in Institute of Field and Vegetable Crops Novi Sad (IFVCNS) research was performed with the aim to select drought tolerant sugar beet genotypes, improve production under water deficit conditions, and clarify the physiological processes of drought tolerance in sugar beet. Genotypic diversity for

drought-related tolerance indices were assessed in the field trials and the strength of association between them and crop performance was measured. As the drought tolerance is a complex trait, very difficult to evaluate in the field, the study was also conducted through the greenhouse experiments and *in vitro* screening. The plant material was studied for morphological and physiological parameters of water regime and the expression of genes that are known to respond to osmotic stress. There are reasons to believe that fullerenol ability to form hydrogen bonds with water molecules makes this nanoparticle a potential intracellular water depot, which can be used if osmotic stress occurs. In collaboration with colleagues from the Faculty of Science, Novi Sad we have started to analyze the influence of fullerenol on sugar beet plants exposed to drought stress. Results indicate that application of fullerenol can modify intracellular water metabolism and enable adaptation of plants to drought stress.

Keywords: drought, sugar beet, Institute of Field and Vegetable Crops

High temperature-related changes in gas-exchange parameters in oak (*Quercus* spp.) populations

PP2-26

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One of the first evidence of climate changes is a rising temperature that significantly affects plant physiology. The elevation of temperature is one of the leading problem at the global scale, thus selection of plants with adaptive response to high temperatures is necessary for reforestation process. The key aim of this research was to understand physiological mechanisms involved in high temperature acclimatization. The impact of high temperature (HT) stress in short term exposure was analyzed in two oak species: Pedunculate oak (*Quercus robur* L.) and Turkey oak (*Quercus cerris* L.) originating from four populations in different forest localities in Serbia. Eight-month old seedlings were grown under semi-controlled conditions in glasshouse and were exposed to the acute HT stress (temperature range: 42-47 °C) for two and five days. Plant response to HT comprised of different traits, depending on the exposure time, intensity of the heat waves and particularly on the selected population. Elevated temperature led to disturbance of photosynthetic gas exchange parameters, water status, concentration of proline and chlorophyll content. Significant declines of photosynthetic and transpiration rates, water use efficiency and stomatal conductance were observed in all tested populations. In addition, proline content was significantly increased in comparison to control treatment, which might be attributed to the strategies of plants to cope up with heat stress by accumulation of compatible osmolyte. Taking into account all analyzed parameters, it can be concluded that among investigated oak species, population of *Q. cerris* has the greatest adaptive potential with fast acclimatization response to high temperatures.

Keywords: oak population, heat stress, photosynthesis, proline accumulation

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Effect of silicon on growth and biochemical traits of *Coriandrum sativum* L. in saline conditions

PP2-27

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Saline stress in plants is reported to be one of the most important abiotic stresses. In most cases, salinity problems are linked to an excess of NaCl in the irrigation water, but other salts like Na2SO4 are also often present. Recent studies show that Si may have a prominent role in plant defense against salt stress. In the present study the effect of Si addition to Na_2SO_4-containing Hoagland's nutrient solution, on growth and biochemical traits of *Coriandrum sativum* L. was examined. Si in the form of fertilizer (Siliplant- NNPP Nest-M, Moscow Russia), was added to half-strength Hoagland's nutrient solution to which 25 mM Na_2SO_4 was added, in semi-controlled conditions. Due to the complex composition of fertilizer, control treatment that contains only nutrient solution and Si was set up. Si addition to 25 mM Na_2SO_4-containing solution adversely affected plant growth by declining fresh weight of root (54%) and stem (66%). Plants cultivated in the presence of Si and 25 mM salt (in growth solution) transpired 20% less than plants grown in the absence of Si but in the presence of salt. The increase of 10% of vitamin C content in leaf tissue was recorded, while free proline content in stem of coriander was increased by actually 30% in the presence of Si. Addition of Si to Hoagland's nutrient solution caused early flowering of plants relative to other treatments. Water flow analyses, as well as free proline and vitamin C concentration proved Si addition to be efficient in alleviating the adverse effect of Na_2SO_4.

Keywords: fresh weight, proline, salinity, vitamin C, Si

Accumulation of heavy metals by wetland plant species *Phragmites australis* growing on mine tailings ponds

PP2-28

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Many macrophyte species act as biological filters by accumulating heavy metals present in aquatic ecosystems and may be used to reduce element leakage from mine tailings by phytostabilization. Mine tailings ponds are heavily loaded with Pb, Cu, Zn, Fe and Mn (Pb-Zn mine Rudnik, central Serbia) and with Cu (Cu mine Bor, eastern Serbia). The aim of this study was to find out the level of accumulation of metals in roots and shoots of naturally grown wetland species *Phragmites australis* on the edges of mine tailings ponds. Therefore, the concentrations of Pb, Cu, Zn, Fe and Mn were analyzed in sediments and in different plant organs. The obtained results showed that the roots contained significantly higher metal concentrations in comparison with rhizomes and aboveground plant organs. This indicates that the establishment of emergent macrophyte *P. australis* on the edges and shallow soils of mine tailings ponds can be a safe method to stabilize the metals and decrease their concentration in water that is finally released into the environment.

Keywords: common reed, macrophytes, phytoremediation, phytostabilization, tolerance

Long-term high temperature effect on field-grown pumpkins (*Cucurbita maxima* Duchesne and *Cucurbita moschata* Duchesne)

PP2-29

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Temperature stress during reproductive phase of life is among environmental constrains with the most adverse effect on pumpkins, at least in the fields of South and East Europe. The growing season (May-September) of 2017 was characterized by 56 days of high (>30 °C) and 21 days of extremely high (>35 °C) maximum daily temperatures, differing from the temperate season of 2016 (33 and 0 days), and from the 1981-2010 averages of 32.4 and 3.3 days, respectively. This was an opportunity to analyze the effects of the stress on pumpkin quality and agronomic traits. The investigation was undertaken on field-grown *Cucurbita maxima* and *Cucurbita moschata* collections, each comprising 41 genetically divergent accessions. All appropriate agro-technical procedures, including irrigation, were applied in both seasons of the experiment (2016, 2017); therefore high temperature was the only stress to which the plants were exposed. The most affected by the stress was carotenoid content, which was 64.1% (*C. maxima*) and 75.4% (*C. moschata*) lower comparing to the values noted in the temperate season. Concerning other quality traits, protein content was 44.6% reduced in *C. moschata*, and slightly (6.1%) increased in *C. maxima*. Dry weight and total soluble solids contents were moderately increased in both species; and sugar content in *C. moschata* only. Refractive index and pH were unaffected by the stress. Concerning agronomic traits, temperature stress decreased the fruit weight (23.1, 17.3%) and therefore the yield of both *C. maxima* (27.0%) and *C. moschata* (16.1%), exhibiting no significant effect on number of fruits per plant.

Keywords: *Cucurbita maxima*, *Cucurbita moschata*, field conditions, high temperature stress

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Impact of nitrogen form on some stress parameters in Ni-treated kohlrabi (*Brassica oleracea* var. *gongylodes*)

PP2-30

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Brassica oleracea is characterized by high S concentrations. High tolerance of this plant towards heavy metals is very much based on non-enzymatic metal-binding and ROS scavenging properties of S-compounds. In the present experiment, kohlrabi (*Brassica oleracea* var. *gongylodes*) seedlings were transferred, after germination, on nutrient solution (Hoagland 1:4), which was modified to contain either 2.5 mM NO_3-N or 1.25 mM NO_3-N + 1.25 mM NH_4-N. One half of each treatment was exposed to Ni (100 µM). After 7 days of cultivation under controlled conditions, leaf fresh mass, pigment concentrations, total S and non-protein thiol (NPT) concentrations in leaves were determined. Also, the activities of glutathione reductase (GR E.C. 1.6.4.2) and ascorbate peroxidase

(APX E.C. 1.11.1.11) in leaves were measured. Ni increased S content only in NO_3-stems. Pigment and NPT concentrations were decreased by Ni only in NO_3-leaves, while in NO_3/NH_4-leaves pigment concentrations were higher under Ni stress. In Ni-treated seedlings, GR and APX activities were decreased only in NO_3-leaves. In NO_3-/NH_4-leaves APX was unchanged, while GR was significantly increased. The results show that partial substitution of NO_3-N form by NH_4-N produces favorable effects under Ni stress, by protecting non-enzymatic part of antioxidative system and even stimulating GSH production.

Keywords: ammonium, kohlrabi, Ni, nitrate, stress parameters

The work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. OI 173030).

Joint action of BASTA and *Nepeta rtanjensis* essential oil suppresses the effects of individual agents on *Arabidopsis thaliana* antioxidant system

PP2-31

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The present study describes the interactive effects of BASTA and *Nepeta rtanjensis* essential oil (*Nr*EO), two agents showing differential mode of herbicidal action, on *Arabidopsis thaliana* antioxidant system. BASTA is known to act through inhibiting glutamine synthetase activity and inducing ammonium accumulation and toxicity, while *Nr*EO and its major component nepetalactone induce oxidative stress in target plants. *A. thaliana* plants were exposed to BASTA through *in vitro* culture medium, while *Nr*EO volatiles were supplied through the atmosphere of culture vessels. When applied independently for 10 days, BASTA (at 5 and 10 mg L^{-1}) and *Nr*EO (2 and 4%), altered the activity and abundance of catalase (CAT) and peroxidase (POX) in 4 week old *A. thaliana* plants. The decrease in CAT activity in *A. thaliana* shoots and roots induced by *Nr*EO treatment was followed by a decrease in CAT abundance. On the other hand, BASTA induced significant increase in both CAT activity and abundance in roots. Similar trend, which was more pronounced in roots, was observed for POX activity. Superoxide dismutase (SOD) activity in *Arabidopsis* shoots and roots were not significantly affected by BASTA or *Nr*EO treatments, although decrease and increase in Mn-SOD abundance were recorded following BASTA and NrEO application, respectively. Simultaneous application of *Nr*EO and BASTA mitigated the effects of the two agents applied independently. This was especially evident for CAT and POX activity. The results indicate the existence of complex control mechanisms underlying BASTA interaction with *Nr*EO, which involve the activation or inactivation of antioxidant defense system enzymes, and their regulation at the transcriptional level.

Keywords: *Arabidopsis*, antioxidant enzymes, BASTA, essential oil, *Nepeta rtanjensis*

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Antioxidative response of certain water macrophytes to seasonal environmental changes

PP2-32

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The changes of antioxidative metabolism in aquatic macrophytes (*Phragmites communis* Trin., *Utricularia vulgaris* L. and *Salvinia natans* L.) were investigated, with the aim to examine plants response to different environmental changes during growing season (May-October). The research included physical-chemical properties of the water and the activity of leaf antioxidative enzymes: peroxidase (POD, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11) catalase (CAT, EC 1.11.1.6) and polyphenol oxidase (PPO, EC 1.14.18.1). The obtained results showed that all examined species had increased POD, CAT and PPO activities during maturing, while APX activity was decreased. The investigation of antioxidative metabolism of water macrophytes in the natural environment can be used for bioindication of water quality in the habitat.

Keywords: antioxidative metabolism, water macrophytes, environment

Effects of juglone on growth and enzyme activities in lettuce and cucumber seedlings

PP2-33

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Juglone (JG) is a naturally occurring naphtoquinone, which can be found in plants of the Juglandaceae family. JG has shown strong allelopathic effects, including toxicity and growth inhibition, on different plant species. The degree of JG effects varies depending on the plant species. In this study, effects of JG on seedling growth and activities of the antioxidative enzymes catalase (CAT) and pyrogallol peroxidase (POD) were monitored in lettuce (*Lactuca sativa*) and cucumber (*Cucumis sativus*). Fluorescence of intact seedlings was also monitored during the course of the experiment. Seeds were germinated on filter paper wetted with either distilled water or JG solution (20 μM and 200 μM). Seedling length and enzyme activities were measured every other day during 7 days. Seedlings of both species showed significant growth inhibition under JG treatment, especially at later stages, and the effect was more pronounced in lettuce. Catalase activity gradually decreased with aging in lettuce, while it increased in cucumber, but JG did not affect CAT in either species. In cucumber, POD activity increased with JG concentration as well as with the duration of the treatment, while in lettuce no significant changes were observed. Eight POD isoforms were detected by native PAGE in cucumber, two of them specific for JG induced stress, while in lettuce only two POD isoforms were detected, and none of them was affected by JG.

Keywords: juglone, allelopathy, peroxidase, catalase, fluorescence

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Nickel accumulation and tolerance of *Noccaea praecox* (Brassicaceae)

PP2-34

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The aim of our study was to test the tolerance and accumulation potential of *Noccaea praecox*, a facultative serpentinophyte, to Ni. The seeds were collected from an ultramafic site on Mt. Zlatibor, and cultivated for 90 days in a peat based substrate amended with nickel (0, 250, 500, 1000 mg kg^{-1}) under controlled temperature and light conditions. Gas exchange parameters (net photosynthetic rate, transpiration rate, stomatal conductance and intracellular CO_2 concentration) and concentration of Ni in plant tissues were determined, as well as concentrations of photosynthetic pigments and anthocyanins. A dose-dependent increase in Ni concentrations in the roots and shoots of treated plants was recorded. Concentrations of Ni in the shoots exceeded hyperaccumulation threshold in all treatments, with the highest concentration of 14257 mg kg^{-1} DW. No statistically significant difference in the biomass of roots and shoots of treated plants was recorded in comparison to the control. Concentrations of Chl a, Chl b and carotenoids did not show a statistically significant dose-dependent response to elevated Ni concentrations in the shoots, whereas concentrations of anthocyanins increased with increasing Ni concentrations. Localization of Ni in the adaxial and abaxial epidermis of *N. praecox* leaves was performed by SEM-EDS, and the highest concentrations were found in the elongated epidermal cells, whereas concentrations in the guard cells were several-fold lower. Our results indicate high Ni bioaccumulation potential of the studied *N. praecox* accession without adverse effects to analyzed gas exchange parameters.

Keywords: nickel, hyperaccumulation, *Noccaea praecox*, ultramafics

Grafting influence on the potassium concentration in tomato fruits under salt stress conditions

PP2-35

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Salinity is one of the most important ecological problems that affects irrigated agriculture in the world. Increased soil salinity inhibits plant growth through osmotic and ionic stress, but can also decrease availability of certain macro- and micro-nutrients. The effect of grafting technique is considered as environmentally friendly tool for overcoming soil salinity problem. Two commercial

tomato cultivars were used to determine whether grafting could prevent decrease of K concentration under salt stress conditions. The cultivars Buran F1 and Berberana F1 were grafted onto rootstock "Maxifort" and grown under three levels of elevated soil salinity (S1- EC 3.80 dS m-1, S2- 6.95 dS m-1 and S3- 9.12 dS m-1). Salt stress at the third salinity level (EC 9.12 dS m-1) induced the highest alteration of K concentration of both grafted and non-grafted plants (about 11%) in comparison to the control. The possibility of grafting tomato plants to improve influx of potassium in salt stress conditions is discussed.

Keywords: tomato, grafting, potassium

Response of antioxidative enzymes to drought and salicylic acid application in *Impatiens walleriana* grown *ex vitro*

PP2-36

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Impatiens walleriana is one of the most popular Impatiens species characterized by fleshy, succulent leaves and a variety of flower colours. The major problem in production and sale of this ornamental species is related to its tendency to quickly wilt when drought stressed. Since one of the most devastating consequences of drought is the onset of oxidative stress, we have studied the responses of antioxidative enzymes to drought and applied salicylic acid (SA) as a potential stress-alleviating regulator. The *I. walleriana* seeds were germinated in a growth chamber under controlled conditions. Plants (8 weeks old) were divided into four groups: (1) regularly watered plants (W), (2) regularly watered plants with single application of 2 mM SA (WS), (3) plants exposed to drought (D), and (4) drought-exposed plants with single application of 2 mM SA (DS). The activities of antioxidative enzymes including superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (POX) were assessed in all four groups. Our results show that drought and SA (WS, D, and DS) increase activity of Cu/ZnSOD and MnSOD in comparison to control (W). Total CAT activity was increased in D, but not in WS and DS groups; however additional CAT isoforms were observed in these groups. POX activity was generally high in D and especially in DS group. It can be concluded that SA improves drought tolerance in *I. walleriana* grown ex vitro probably by modulating the activity of antioxidative enzymes and can be used as a drought-ameliorating agent.

Keywords: drought, salicylic acid, antioxidative enzymes

This research was sponsored by Ministry of education, Science and Technological Development of the Republic of Serbia (Grant Nos. TR31019 and TR31049).

Expression of dehydrins in *Impatiens walleriana* exposed to drought

PP2-37

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Impatiens walleriana is an important ornamental plant with a tendency to quickly wilt when exposed to dehydration, which considerably affects its commercial value. However, *Impatiens* also has great potential to rehydrate upon watering. Effective protection of this valuable ornamental from water stress requires understanding the events associated with dehydration and rehydration at the molecular level. Dehydrins (DHNs) are highly hydrophilic proteins whose expression in many plant species has been correlated with several types of abiotic stresses, including drought, salinity, and cold. Dehydrins comprise Group II of late embryogenesis abundant (LEA) proteins, known to be associated with plant responses to water deficit. DHNs lack stable tridimensional structures, so they are considered intrinsically disordered proteins. They are characterized by three conserved sequence motifs marked as a K (Lys-rich), Y (Tyr-rich) and S (Ser-rich). To study the expression of Dhn genes in response to drought, we have sequenced *I. walleriana* transcriptome and identified *IwDhn* transcripts from RNA-seq data using HMMER (hmmscan, v3.1b2) and Pfam 31 database. Quantitative RT-PCR revealed that two *IwDhn* genes are extremely upregulated in drought, while one has constitutive expression. It can be speculated that fast rehydration after drought stress is at least in part result of the protective role of dehydrins.

Keywords: drought, dehydrins, gene expression

This research was sponsored by Ministry of education, Science and Technological Development of the Republic of Serbia (Grant No. TR31019).

Preliminary Observation of the Effects of Different Concentrations of Copper on Germination of Seeds in Maize Hybrid ZP434

PP2-38

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Copper as an essential element for plants, besides useful, has also negative effects at high concentrations. This problem was investigated by effects of various concentrations (10^{-4}, 10^{-5} and 10^{-6} M) of copper (as a $CuSO_4$) on germination and thermodynamic changes at maize hybrid (ZP434) seedlings. Fresh mass of the 25 parts of the maize seedlings was measured (plumule, radicle, rest of seedlings), and their mass after drying at 60, 105 and 130 °C. Mass changes correspond to different water fractions (bulk water, cytosolic water and chemically bond water) in the seedling

parts, and based on this mass determination the changes of enthalpy, entropy, and free energy were calculated. Content of copper in plant parts were determined according to standard AAS methods. Results of the changes of absolute and relative mass of the seedling parts indicate that the mass of plumule and the radicle decrease in the higher doses of copper, while the mass of the rest of seedling grows. This indicates that an increase in the copper dose acts inhibitory on the redistribution of the mass between the rest of the seedling, plumule and radicle, indicating inhibition of the growth. Changes in thermodynamic parameters shows decrease of value of Gibss free energy in the rest of seedling at increased copper doses. This finding coincides with the reduction in the transfer of mass from the rest of seeds into the plumule and the radicle, and consequently, the slowing of growth of corn seedlings at higher copper doses.

Keywords: Copper stress, maize seedlings, heavy metal, thermodynamic parameters

Allelopathic potential of *Amorpha fruticosa* L. rhizospheric fly ash

PP2-39

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The allelopathic potential of a particular plant species is determined through the content of biologically active compounds (allelochemicals) in soils, originating from the plants. The aim of this study was to determine the allelopathic potential of fly ash collected under the dominant plant species *Amorpha fruticosa* L., whose invasive populations inhabit fly ash deposits at the "Nikola Tesla - A" thermoelectric power plant in Obrenovac. Results in this study show a higher content of ferulic acid and 3,5 DHBA in rhizospheric fly ash of *A. fruticosa* than in control fly ash (without vegetation cover). Content of rutin was significantly higher in rhizospheric fly ash of *A. fruticosa* compared to control fly ash, whereas the difference in the content of quercetin was not significant. Phenolic acids and flavonoids content in control and rhizospheric fly ash of *A. fruticosa* show the same decreasing order: quercetin > 3,5 DHBA > ferulic acid > rutin. The inhibition of radicle elongation of indicator species *Trifolium pretense* L. was significantly higher in biotests with rhizospheric fly ash of *A. fruticosa* versus control fly ash. Furthermore, positive correlations were found between radicle growth inhibition and the content of ferulic acid, 3,5 DHBA and rutin from rhizospheric fly ash. In summary, results in this study show that invasive plant species *A. fruticosa* increases the content of phenolic acids and flavonoids in the sterile substrate such as fly ash. These phenolic compounds have the properties of allelochemicals which can lead to inhibition of growth of some herbaceous species in the plant community.

Keywords: invasive species, soil phenolics, allelochemicals, radicle growth inhibition

This work was supported by the Ministry of Education, Science and Technological Development (Grant Nos. III41010, OI173018 and TR31070.

Allelopathic potential of *Robinia pseudoacacia* L. invasive populations growing on limestone quarry deposit

PP2-40

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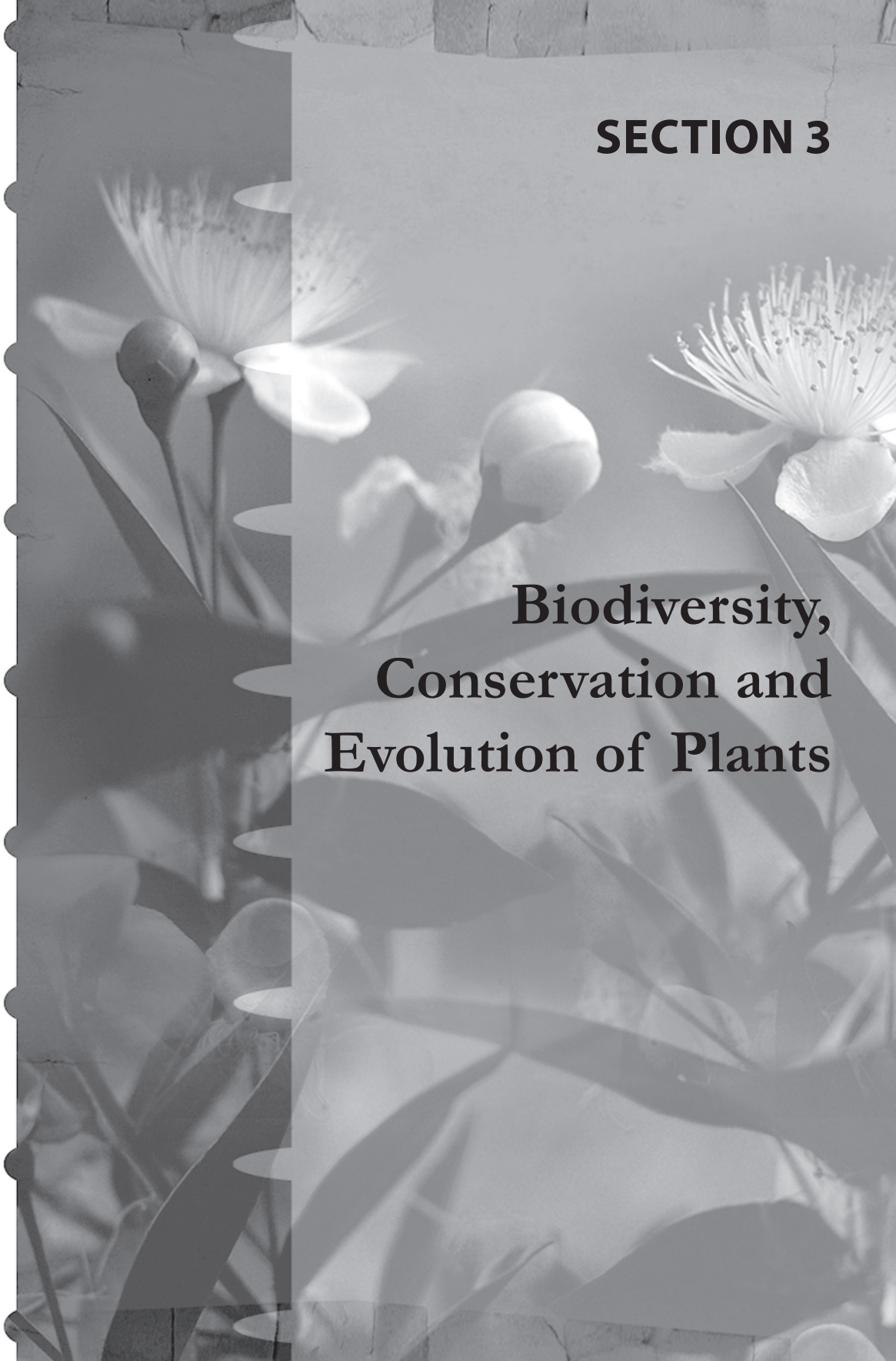
Black locoust (*Robinia pseudoacacia* L.) represents an invasive species in Europe that spreads rapidly in different early succession habitats. It tolerates diverse range of soil conditions, but prefers good aeration of soil and avoids moist and compacted soils. The main objective of this study was to determine the seasonal differences in the allelopathic activity of the invasive *R. pseudoacacia* populations from the limestone quarry Krš (Veliki Šenj, Kragujevac, Serbia), examining the effects of leaf and litter leachates on the seedling growth of indicator species *Trifolium pratense* L. As an allelopathic bioassay, a sandwich method with agarose substrate was used. Inhibition of radicle growth of *T. pratense* in relation to control decreased in the following order: leaf (autumn) > leaf (spring) > leaf litter. The content of the total phenolic acids and flavonoids decreases in the identical following order: leaf (autumn) > leaf (spring) > leaf litter. Positive correlations were found between radicle growth inhibition and the content of phenolic acids ($p < 0.001$) and flavonoids ($p < 0.01$). The allelopathic activity and the content of total phenolic acids and flavonoids depend on the season. The highest content of total phenolic acids and flavonoids, and allelopathic activity was identified in the leaves collected in the autumn season, most likely due to the accumulation of allelochemicals in leaves during the vegetation season. In contrast, the weakest allelopathic activity, as well as the smallest content of phenolic acids and flavonoids, was found in leaf litter, most likely due to the process of leaching, evaporation and degradation of potential allelochemicals.

Keywords: invasive species, allelochemicals, leachates, radicle growth inhibition

This work was supported by the Ministry of Education, Science and Technological Development (Grant Nos. III41010 and TR31070).



SECTION 3

Biodiversity, Conservation and Evolution of Plants

PLENARY LECTURES

Meta-Phenomics: Converting data into knowledge

PL3-1

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A tremendous but non-systematic effort has been made during the past 90 years to characterize the response of a wide array of plant species to their environment. This has happened at various levels, such as biochemistry, physiology, anatomy as well as at the level of whole-plant carbon budget and growth. As a result, a large number of phenotypic data are publicly available. Unfortunately, this information is not unified in a quantitative and structured way which allows for a comparative analysis. My aim is to fill this important gap by building a large database containing the responses of circa 70 growth-related variables for a range of plant species, and for 12 different environmental factors (light quantity, light quality, UV-B, CO2, O3, nutrients, drought, water-logging, submergence, temperature, salinity, soil compaction). In this talk I will show how ecophysiological data can be used to construct dose-response curves. The data will be subsequently used in a range of contrasts that test whether specific subgroups of species (ecological, phylogenetical, functional) behave in a distinct way to light and CO2.

This approach - that I refer to as 'meta-phenomics' - not only can serve as a benchmark for future and comprehensive phenotyping efforts, but it will also represent a very valuable tool per se in understanding the integrated response of plants to their environment.

Keywords: a-biotic environment, dose-response curves, meta-analysis

Reference: dose-response curves can be found at www.metaphenomics.org.

The Biodiversity present in European tomato, phenotypes galore and a first insight in the underlying genetics

PL3-2

The TRADITOM partners as in traditom.eu (presented by A. Granell)
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Europe is considered a secondary diversification site for tomato since its introduction in XVII century Europe by Spanish Conquistadores resulted in an explosion of variability. In the frame of the EU-funded project TRADITOM we have started to characterize the genotypic and phenotypic variability present in more than 1700 traditional varieties available on farms and in public repositories throughout Europe. A total of 400 Gb of genotype information have been produced and approximately 2x the whole TRADITOM collection has been cultivated (over 15k plants), with different subpopulations growing in a total of 12 locations, spreading over 5 different countries. For each of the 1700 varieties, a total of 19 qualitative and 117 quantitative traits have been measured and a total of >100 Gb of images, scans of the plants at the fruit level have been recorded. Two core collections have been cultivated in 3 different locations, and agronomic characteristics, flavor

and nutritional composition analyzed. TRADITOM collection genotyping has revealed that despite the reduced variability, it is still possible to map geographical and phenotypic characteristics to the tomato genome. Extensive phenotyping revealed ample variability in traits related to plant architecture and fruit characteristics: fruit size, shape, Brix and also in fruit ripening time. A subset of the European traditional variety set showed extended shelf life and the ability to grow and set fruit under limited water supply, which is of high interest. I will present some of this variability present in the TRADITOM collection and our progress in understanding the basis of such variability.

Keywords: tomato, biodiversity, TRADITOM

INVITED TALKS

Origin and Genetic Diversity of Croatian Common Bean Landraces

IT3-1

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Most of the common bean (*Phaseolus vulagris* L.) production in Croatia is based on local landraces, grown by small-scale farmers in low input production systems. The landraces are commonly known by their traditional names based primarily on seed coat pattern and colour. In order to assess the genetic diversity of Croatian common bean landraces, 174 accessions were analysed using a DNA marker for phaseolin type, microsatellite markers and a DArTseq method of SNP genotyping. The information on phaseolin type and the results of the distance- and model-based analysis of population structure coupled with the morphological characterization of the accessions enabled the thorough classification of the accessions, that was, for the most part, congruent. Out of 174 accessions, 135 were assigned to 14 newly formed morpho-genetic groups originating from three distinct domestication events: (1) Mesoamerican, (2) Andean - indeterminate type, and (3) Andean - determinate type. The rest of accessions could represent putative hybrids among morpho-genetic groups including the hybrids among the gene pools. This study would be a first step into a genome-wide association mapping using a panel of Croatian common bean landraces. In conjunction with a detailed data on bioactive nutrient content, a whole genome association mapping analysis will be carried out to identify quantitative trait loci (QTLs) related to bioactive nutrient content.

Keywords: *Phaseolus vulgaris* L., landraces, genetic diversity, DArTseq, gene pools

Conservation physiology of bryophytes

IT3-2

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The new scientific discipline of conservation physiology has arisen recently (2006), with the aim to incorporate physiological knowledge into ecological models, and improve predictions of organism responses to environmental change and human activities and provide tools to support management decisions in conservation science. Since 2006 most investigations in this field were dedicated to the higher animals, and less than 10% could be considered in flowering plants. There was almost no research in invertebrates, spore-bearing organisms, and flagellate plants (including bryophytes). However, the Belgrade Bryophyte Biology Group (BBGB) is working on these problems and here we present the results on the European bryophyte species of conservation interest. The phenomena like apospory, endophyte, biotic and abiotic factor effects, breaking spore dormancy, reintroduction, endoreduplication, recent hybridization, alloploidy and endopolyploidy in target bryophyte species will be discussed linking the physiological responses and conservation issues. All these phenomena were documented, induced, or reduced in the laboratory and field tests in different target species. Thus, this concept of conservation physiology emphasizes the basis, importance, and ecological relevance of physiological diversity at a variety of scales, and gives insights in the latest approach to conservation of different biological entities in rapidly changing environments. The importance of multidisciplinary approach to bryophyte conservation issues will be documented by concrete case studies.

Keywords: conservation, *ex situ*, *in vitro*, mosses

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Biomonitoring: Plants' (in) perspective

IT3-3

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Anthropogenic pollution has been an ever-present threat, even in natural areas protected under state legislations. Timely protection and prevention through continuous biomonitoring is therefore a necessity.

Communities, organisms or their parts should detect the quality of the environment reliably enough and at an early stage in order to be referred to as bioindicators, biomonitors, biomarkers or biosensors. Such model organisms include animals and plants, with invertebrates, lichens and mosses as the most frequent. Nevertheless, the use of higher plants in terrestrial ecosystems may be even more feasible.

Plants as sessile organisms are considerably influenced by different environmental factors, as well as by pollution. Reaction to different pollution types in higher plants could depend on whether it was monocotyledon or dicotyledon, annual or perennial, woody or herbaceous. Changes in the environment could reflect in altered physiology, anatomy, morphology or development. In order to determine the 'normal state' of a given monitoring model system, we have to determine specificities of every single reaction. Developmental instability, measured by estimates of fluctuating and radial asymmetry, is an exception, because an optimal level *i.e.* perfect symmetry is predefined.

In our research we are testing biomonitoring potential of different vegetative and reproductive traits, as well as of developmental instability measures in invasive woody species with broad distribution - *Robinia pseudoacacia*, in widely planted ornamental *Iris germanica* and in natural populations of Dwarf Bearded Iris - *Iris pumila* that inhabits protected natural areas.

Keywords: anthropogenic pollution, biomonitoring, developmental instability

SELECTED TALKS

Morphological diversity of functionally distinctive floral organs in *Iris pumila*: Does the flower color matter?

ST3-1

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Flowers are complex phenotypic structures composed of four functionally distinct organs arranged in concentric floral whorls: sepals in the first, petals in the second, and two inner whorls consisting of reproductive structures: stamens in the third whorl and tricarpellary gynoecium, in the centre. Color, size and shape of these floral organs are considered to be important signals that can attract the pollinators, advertising the plants' floral reward (commonly nectar and pollen). Here we use the methods of geometric morphometrics to compare size and shape variation of three functionally different floral organs: falls, standards and style arms, among five distinct color morphs (blue, violet, dark blue, dark violet and yellow). Pairwise comparisons of the centroid size means showed that all floral organs differed significantly among the analysed color phenotypes. The mean shape of falls, standards and style arms appeared to be flower color-specific as well, but most noticeably between yellow - on one side, and blue/violet morphs - on the other. Procrustes ANOVAs revealed the significant presence of directional and fluctuating asymmetry for falls and standards among all five color morphs, with the exception of style arms. The observed morphological diversification of floral organs among distinctive *I. pumila* color phenotypes could be the adaptive outcome of the interactions with their pollinators.

Keywords: shape, flower color, morphological diversity, *Iris pumila*, floral organs

First data on arbuscular mycorrhizal communities from selected climatic borderline forest ecosystems of the Balkan Peninsula

ST3-2

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Mycorrhiza is an obligatory symbiosis between fungi and 99% of terrestrial plant species, majority of which form the so called arbuscular mycorrhiza (AM). However, one of the European regions with the highest plant diversity rates, the Balkan Peninsula, has never been explored for diversity of AM fungi (AMF). AM has previously been considered dominant type of mycorrhiza in tropics and communities formed by herbaceous plants, while northern temporal and boreal forest ecosystems have been explored for AMF diversity only recently. Unexpectedly, these forests, thought to be dominated by ectomycorrhiza, appeared to host rich AMF communities as well. In South European regions such topics have rarely been addressed.

Through two independent experiments in selected regions of the Balkan Peninsula (Slovenia and Serbia), we aimed to explore the gradients of natural grass field-transition zone-forest AMF communities, in order to determine diversity rates and composition differences caused by vegetation, climatic and soil parameters. For approaching AMF communities, we have applied comparative 18S DNA NGS based analyses of sampled roots and spore morphology based analyses from soil samples taken from the rhizosphere. Investigated ecosystems were located in calcareous regions, where the soils were in weakly acidic-neutral-weakly alkaline gradient, depending on the level of influence of Mediterranean climate and altitude. Vegetation differences were significant, but the AMF diversity was surprisingly high in Pinus and Tilia forests dominated by typically ECM trees. We discuss obtained results comparing the investigated variables in different climate zones of the Balkan Peninsula.

This research has been financed by Swiss National Scientific Foundation through SCOPES initiative project IZ73Z0_152740. ZM is also financed by Grant No. III43010 financed by Ministry of Education, Science and Technological Development

Verification of interspecies hybridization within the genus *Centaurium* Hill using *EST-SSR* molecular markers

ST3-3

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Clarification of phylogenetic relations within the genus *Centaurium* Hill is very difficult due to poor discrimination of species by traditional morphological characteristics. Moreover, polyploid species complexes and interspecies hybridization are recognized as very important evolutionary mechanisms responsible for the speciation within this genus.

Investigation of interspecies hybridization within the genus *Centaurium* using nine *EST-SSR* loci, mined from *C. erythraea* transcriptome, involved a total of 140 individuals belonging to three populations of *C. erythraea*, four populations of *C. littorale* ssp. *compressum* and two populations of a taxon classified as *C. pannonicum*, all originating from the area of Subotica-Horgoš sands (Vojvodina, Serbia). Ploidy level of the plants was estimated by flow cytometry analysis, which revealed hexaploid nature of the individuals belonging to the hybridogenic taxon *C. pannonicum*, while parent taxa *C. erythraea* and *C. littorale* ssp. *compressum* were detected as tetraploids. Validation of microsatellite markers was performed by Lab-on-a-Chip technology using Agilent 2100 Bioanalyzer. *PCoA* analysis and hierarchical cluster analysis (*UPGMA*), performed on the basis of the *Bruvo* genetic distances between the individuals, clearly separated three groups of individuals corresponding to the three taxa: *C. erythraea*, *C. littorale* ssp. *compressum* and *C. pannonicum*. Selected *EST-SSR* markers, together with previously developed high-resolution metabolic classifiers, supported the hybrid nature of the *C. pannonicum* individuals, which clustered between the two parental taxa.

Developed *EST-SSR* markers can be transferred onto other types of ubiquitous hybrids within the genus *Centaurium* and thus may generally contribute to the elucidation of taxonomical and phylogenetic positioning of the taxa within the genus.

Keywords: *Centaurium*, *EST-SSR,* interspecies hybridization, polyploidy

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. 173024

POSTER PRESENTATIONS

Flower color polymorphism in *Iris pumila* - Why we definitely need *in situ* reflectance spectroscopy in population analysis and evolutionary studies of this model system

PP3-1

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Dwarf bearded iris - *Iris pumila* L. is a perennial clonal plant that exhibits huge flower colour polymorphism with white, yellow and various shades of purple and violet flowers. Maintenance of such polymorphism is very interesting evolutionary question in its own right. Moreover, flower colour is also used in determining individual clones, their size and population diversity - all very important aspects of further population and evolutionary studies on this model system. However, visual identification or use of digitalized images, while being a necessary first step, is not sufficient for this task. They are often subjective, dependent on light conditions, and covering only part of the spectrum - one that is visible to humans. In published papers that utilized flower colour in *I. pumila*, number of analyzed different colour variants ranged from nine to only three colour classes. Chemical analysis of different colour variants is a very useful tool and has also been applied, but it is hardly applicable for most population level analyses. We therefore consider portable reflectance spectrometer as the best available tool for those multiple tasks, and present preliminary results of its application *in situ* with wavelengths chosen to match the visible spectrum of *I. pumila* main pollinators.

Keywords: flower colour polymorphism, spectroscopy, reflectance, *Iris pumila*

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. OI 173025).

Characterization of ns rapeseed germplasm collection based on the content of fatty acids and tocopherols

PP3-2

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Rapeseed (*Brassica napus* L.) is the most common source of vegetable oils in Europe. One of the main breeding objectives is to create rapeseed genotypes with highly appreciated nutritional characteristics. The aim of this study was to investigate fatty acid and tocopherol constituents (α-, β-, γ-tocopherols) in a collection of 49 NS rapeseed genotypes and to identify genotypes with desired content of fatty acids and tocopherols using multivariate statistical methods: principal

component, cluster and two-way cluster analysis. Principal Component Analysis revealed 5 PC components with Eigen value >1, which explained 78.70% of the total variability. The PC analysis identified oil quality traits that contributed most to the variation of analyzed genotypes and can be used for facilitating the selection of desirable characteristics in rapeseed breeding. Both cluster analysis and two-way cluster analysis helped the identification of genotypes with similar fatty acid and tocopherol composition. Two main groups could be identified on the dendrogram, the first having two genotypes and the second comprising 44 genotypes. Three genotypes did not belong to any group. The extent of variation within the breeding material was clearly illustrated and the genotypes from one side, and analyzed oil quality traits from the other side, were effectively classified on the heatmap. The obtained results confirmed that classification and characterization of rapeseed germplasm and the selection of superior genotypes for commercialization or as parents in future hybridization program can be effectively performed by using multivariate analysis.

Keywords: rapeseed, fatty acids, tocopherols, multivariate analysis

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Do pollinators prefer bigger floral organs? A case study on *Iris pumila* L.

PP3-3

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Angiosperm flowers serve as an advertisement for pollinators and are, therefore, believed to be shaped by pollinator-mediated selection. The large flower size, which is one of the distinct characteristics of the genus *Iris*, might have evolved under the strong selective pressures imposed by pollinators, either because larger flowers indicate more rewards or because the pollinators can detect them from a greater distance. To test the role of visual floral signals in attracting pollinators and, consequently, pollination efficiency, we compared the phenotypic expressions of flower height and centroid size of petaloid floral organs: falls, standards and style arms, as well as anthocyanin absorption between naturally pollinated and non-pollinated flowers of *I. pumila* plants grown in a common-garden experiment. Our results indicated that *I. pumila* pollinators generally preferred taller flowers with greater organ sizes compared to the alternative ones. However, the direction of pollinator-mediated selection appeared to be strongly flower organ-specific: positive on fall, negative on style arm and neutral on standard size. The observed results are in agreement with the functions that each of these floral structures has in the pollination process: standards are a long-distance reward signals, falls are landing platform for pollinating insects, while style arms, as upper parts of the pollination tunnels, promote pollen deposition. We failed to corroborate the existence of pollinator-mediated selection on anthocyanin absorption. This suggested that some other aspects of flower colour might be more attractive to insect pollinators, or that different biotic or abiotic factors could account for the maintenance of flower colour polymorphism in *I. pumila*.

Keywords: floral organ size, anthocyanins, pollinator attraction, *Iris pumila*

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. 173007.

Flooding influence on developmental instability and specific leaf area of Black poplar *Populus nigra* L. in Special Nature Reserve Gornje Podunavlje

PP3-4

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The development and management of water resources by humans has altered natural flow regimes and flooding patterns in the river systems, reducing frequency, extent and duration of many European floodplains. All these affect habitats and the sustainability of the high biodiversity observed along river systems. Riparian tree species are heavily dependent on the floods both to provide new sites for their natural regeneration from seeds and to recharge water table levels in the rooting zone. Riparian species, as *Populus* and *Salix*, may tolerate weeks and even months of flooding; where *Populus nigra* L. can tolerate flooding up to 60 days. Within this research, we evaluated *P. nigra* L. developmental instability and leaf morphological trait variability in flood vs. non flood zones along Danube basin within Special Nature Reserve Gornje Podunavlje. The leaf samples (800 leaves) were taken from localities on both sides of embankment, four localities in non-flooding area and four in direct flooding area. The specific leaf area with petiole is leaf-morphology trait, while fluctuating asymmetry index is an indicator of leaf developmental instability. All traits showed significant difference within different localities nested in habitats and intraindividual variability was observed too. The main values of fluctuating asymmetry indices were slightly higher in flooding zones, which indicates the leaf developmental instability. Based on the data presented, *P. nigra* L. is flood tolerant. In restoration programs of recovery areas with long periods of flooding, this riparian tree species can be used.

Keywords: developmental instability, leaf morphology, flooding, *Populus nigra* L., Special Nature Reserve Gornje Podunavlje

This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. OI 173025).

Developmental instability and herbivore damage in a dioecious forest forb from contrasting light habitats

PP3-5

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The relationship between fluctuating asymmetry (FA) as an indicator of developmental instability and herbivore damage is a topic of growing research interest, the question becoming more complex in dioecious species and contrasting environments. Here, we explored the patterns of herbivore damage and developmental instability in natural populations of dioecious understory forb *Mercurialis perennis* located at Mt. Kopaonik, 1700 m asl. Males and females from two contrasting light habitats, open (sun-exposed field) and shaded (spruce forest) were examined with respect to: fluctuating asymmetry (FA) as the measure of developmental instability, herbivore damage (percentage of leaf area consumed) and leaf size traits, taking into account possible sexual dimorphism. Leaf measurements were used to calculate univariate and multivariate FA indices. In addition to developmental instability between and within habitats, intraindividual leaf asymmetry patterns were explored as well. Our results showed that herbivore damage was significantly higher in open habitat, as well as one out of four univariate FA indices and the multivariate index. Both foliar damage and FA were higher in a more stressful habitat; within habitats, they were not positively correlated. Significant intraindividual variation in FA was not found. Intersexual differences were observed for herbivore damage and leaf size traits, including total leaf area. Herbivore damage was significantly male-biased in open habitat. In shade, sex-biased herbivory was not found, probably because overall level of damage was very low. The analysis of intersexual differences in developmental stability measured by leaf asymmetry provided no evidence that female plants were more sensitive to environmental stress.

Keywords: developmental instability, fluctuating asymmetry, folivory, sex-biased herbivory

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Norway maple superior specimens in urban and natural habitats

PP3-6

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Detecting superior specimens is of crucial importance in meeting the challenges of urbanization and global warming. Norway maple's superior specimens are the basis for the protection of the species and the biodiversity. Analyzing morphological and phenotypic characteristics of Norway maple in urban and natural habitats, 76 superior specimens were detected.

On average, the samples in natural habitat Rudnik 3 are the tallest, while the shortest are samples from urban population in Belgrade. Trees in Belgrade have the smallest breast diameter, while the biggest ones are detected in Rudnik 2 population. The tree of the biggest biometric characteristics is in natural habitat Rudnik 2 and the one with the smallest biometric characteristics is in urban population in Belgrade. Individuals superior in size are in populations Rudnik 2 and Rudnik 3, while the trees of the best vitality are in Rudnik 1. Trees in urban population are of a good vitality and decorative values and close to Rudnik 1 population in their morphological and phenotypic characteristics. Therefore, good adaptability of Norway maple to challenging environmental conditions in urban areas is confirmed. Selected genotypes are the good basis for collecting seeds and production of planting materials as well as for further research.

Phenotypic variations are transmitted further in sexual and asexual ways, and deviations in Norway maple morphology in different environments allow survival of the species in different habitats. According to the results of the study, Norway maple is promising for various uses and favorable for survival under changed environmental conditions.

Keywords: Norway maple, superior specimens, variability, phenotype, climate change

Molecular tools for the study of genetic diversity in Faba bean

PP3-7

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Faba bean (*Vicia faba* L.) is an important food and feed legume used mainly in eastern and northern Africa and western Asia but having a sound prospect to become one of the eight priority grain legumes for reducing poverty, hunger and malnutrition in mankind. Renewed interest in faba bean breeding and cultivation has led to the enrichment of *ex situ* seed collections worldwide and to the intensification of efforts on their phenotypic and genotypic characterization. Nuclear microsatellites are still markers of choice for genotyping at various scales. However, selection of reliable and informative nuclear microsatellites may be a tedious task. At present, more than 600 microsatellite loci for genotyping in faba bean are available. We report our efforts on assessing performance of both genomic and genic nuclear microsatellites for genotyping in faba bean.

Keywords: faba bean, legumes, nuclear microsatellites

Variation in the amount of Hsp70 among different floral organs in three alternative color phenotypes of *Iris pumila*

PP3-8

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The 70kD heat shock proteins (Hsp70s) are highly conserved molecular chaperones that play essential roles in numerous cellular processes, including the folding of newly synthesized and stress-denatured proteins, the translocation of proteins across membranes, as well as the degra-

dation of aggregated proteins. Here we examined the variation in the abundance of Hsp70s in different floral organs of the three distinctive color morphs in *Iris pumila*. Flowers of twenty seven genotypes raised in a common-garden and belonging to three colour classes: yellow, dark blue and dark violet, were collected (9 per each class) for an immunoblot analysis. The content of Hsp70 was quantified in four floral organs: falls, standards, stamens and style arms. The immunoblot analysis revealed that the expression of Hsp70s was floral organ-specific. While only one Hsp70 isoform was expressed in the standards and falls, the stamens and style arms expressed two isoforms. The relative abundance of Hsp70 varied among differed flower organs as well. The lowest value was recorded in the standards and the highest in the stamens, whereas its abundance in the falls and style arms appeared to be intermediate. Such variation pattern was detected exclusively in the dark violet colour morphs. In the yellow flowers, however, the amount of Hsp70 tended to be lower compared to the dark blue or dark violet colour morphs. This study provides original evidence for organ-specific Hsp70 expression in flowers of *I. pumila* plants, likely due to the differential metabolic activities occurring in the cells of these functionally distinctive floral structures.

Keywords: Hsp70, floral organs, *Iris pumila*, flower colour

Genetic diversity of *Nepeta rtanjensis* based on RAPD data

PP3-9

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Nepeta rtanjensis Diklić et Milojević (*Lamiaceae*) is an endemic, herbaceous plant, found only in a very small area of Mount Rtanj in Southeast Serbia. The whole species is represented by several hundred specimens distributed within three known populations: Greda, Mirčin del and Javor. Like many species of the genus *Nepeta*, *N. rtanjensis* is also famous for its secondary metabolite production (mainly nepetalactones and phenolics) and it is well known that these compounds show antibacterial, antifungal, cytotoxic, phytotoxic and antioxidant activities. Because of its limited distribution and the status of critically endangered species, it is important to find as much as possible information about the population genetic structure of this species. Therefore, we performed randomly amplified polymorphic DNA (RAPD) analysis to get an insight into the genetic diversity and variation of this species within and between the populations. DNA material used in this process was isolated from leaves collected from plants in their natural habitats. To our knowledge, this is the first employment of RAPD markers in the assessment of the *N. rtanjenis* genetic structure. Genetic variability between populations was not detected, but only small variations between individual plants from the same populations were recorded. These preliminary data give us a good base for further studies towards elucidating the genetic structure of this rare species using more reliable *EST-SSR* markers, designed based on *N. rtanjensis* transcriptome.

Keywords: *Nepeta rtanjensis*, DNA, RAPD, population genetics

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

Anatomical and micromorphological investigations of *Artemisia absinthium* L. (*Asteraceae*) from Serbia

PP3-10

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In this work anatomical and micromorphological investigations of vegetative organs of *Artemisia absinthium* L. (*Asteraceae*), wild-growing in Serbia, were conducted. The aim of this study was to examine the general anatomy and micromorphology, as well as to find possible valid taxonomic characters. Microscopic slides were prepared following the standard histological procedures. Triarch type of the vascular bundle is present in primary root structure, whereas typical secondary growth occurs in older roots. Also, large secretory ducts, with a brownish content, are present in the cortex. The stem is polygonal in shape and characterized by collateral vascular bundles. Clearly visible endodermis layer is noticed. The largest parenchyma cells occur in the pith. Also, small secretory ducts occur in the cortex and in the pith of the stem. Petiole has ellipsoidal shape, with similar anatomy to the stem. Concerning leaf anatomy, the isolateral palisade structure is observed. On the surface of all aerial vegetative organs, numerous morphologically variable T-shaped nonglandular, as well as very prominent, large glandular trichomes, with brownish content, were found. All of the data may be considered as possible taxonomic characters which could help in species identification and infrageneric taxonomy of the genus *Artemisia*. Thus, these findings are of importance for future anatomical, micromorphological and phytochemical investigations of this and related species.

Keywords: *Artemisia absinthium*, *Asteraceae*, anatomy, micromorphology

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Cryopreservation of *Viola cornuta* shoot tips using vitrification procedure

PP3-11

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Cryopreservation represents a suitable method for long term storage of different plant genetic resources. The aim of this study was to develop protocol for cryopreservation of *Viola cornuta* shoot tips using one step freezing method with chemical dehydration of tissue with modified Plant Vitrification Solutions (PVS2 or PVS3). Shoot tips (1-2 mm) of two-week cold acclimated shoots were cultured on ½MS medium with 0.3 M sucrose for one day before treatment with loading solution (2 M glycerol, 0.4 M sucrose) for 30 min. Osmotic dehydration with PVS2 solution (30%

glycerol, 15% ethylene glycol and 15% DMSO in liquid ½MS medium with 0.4 M sucrose) were tested at 0 °C or 24 °C. Osmotic dehydration with PVS3 (50% sucrose, 50% glycerol in liquid ½MS medium) were tested at 24 °C for 45 min. After the treatment the explants were directly immersed in liquid nitrogen (LN) for at least one day. Re-warming was performed at 42 °C in water bath for 2 min. After re-warming, the PVS solutions were replaced with unloading solution containing 1.2 M sucrose for 20 min. Re-warmed shoot tips were cultured on ½MS medium with 0.1 mg L^{-1} BAP. We observed that PVS2 solution is cytotoxic for *V. cornuta* shoot tips and cannot be used for cryopreservation. However, cryopreservation with PVS3 solution was successful, where 71.9-100% shoot tips survived treatment before immersion to LN and 31-40% survived after re-warming from LN. Regrowth of cryopreserved shoot tips with new well-formed leaves was obtained after four weeks of culture.

Keywords: horned pancy, Plant Vitrification Solution, PVS2, PVS3

This study was supported by the Grant No. TR31019 from the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Towards *ex situ* conservation of rare and endangered moss *Tayloria splachnoides*: biotechnical approach

PP3-12

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Tayloria splachnoides is an uncommon moss species rarely found in high mountains of central Europe and in Scandinavia. It is red-listed in many European countries: Finland, Norway, Sweden, Austria, Czech Republic, Poland, Slovakia, Switzerland, Bulgaria, Romania and Slovenia. *In vitro* culture of an accession from Slovakian High Tatra Mountains was established with the aim to study massive micropropagation of this widely threatened species. The effect of plant growth regulators, different media types, and sugar content were tested to obtain well developed gametophores. Index of multiplication and secondary protonema diameter were measured. According to the results achieved, it can be emphasized that the best media type for *T. splachnoides* micropropagation was sugar- and plant regulators-free Murashige and Skoog medium, at 18 °C, and 16/8 light/dark condition. Considering protonema diameter, KNOP medium enriched with sucrose (7.5-15 mg L-1) was the most appropriate. In contrast, BCD enriched with sucrose had the opposite effect, i.e. decreasing the secondary protonema diameter. KNOP medium enriched with cytokinin BAP (0.1 μM) combined with auxin IBA (0.1 μM) clearly induced the largest secondary protonema diameter. Gametophore appeared only on KNOP medium supplemented with plant growth regulators, but no clear pattern can be inferred, which implicates no clear agents in bud induction on secondary protonema. Further investigations are urgently needed and in progress.

Keywords: conservation, rare, moss, development, propagation

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Metal content in aerial parts of the species *Teucrium montanum* L. sampled from habitats with serpentine and calcareous substrate

PP3-13

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The purpose of this comparative analysis is determination of the total quantity of metals (Mg, Ca, K, Ni, Fe, Mn, Zn, Cu, Cr and Pb) in soil samples and aboveground plant parts of plant *Teucrium montanum* L. (*Lamiaceae*) sampled from 23 different localities on serpentine and calcareous habitats on the territory of Serbia. Metal contents were determined by means of Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). The preparation of the samples was done by wet digestion. The obtained results showed that the quantities of certain metals (Mg, Fe, Ni and Mn) in the soil from the serpentine habitats were greater in comparison with other metals (Ca, Zn and K) that were more frequently found in the soil from the calcareous habitats. The results demonstrated that the analysed plant samples from the serpentine habitats contained higher quantity of Fe, Ni and Cr as opposed to the plant samples from the calcareous habitats that contained greater quantity of Ca and Zn. The results of the conducted research showed that the examined plant species accumulates analysed metals in different quantities, which mostly depends on the type of substrate from which the species is sampled.

Keywords: metal content, *Teucrium montanum*, calcareous substrate, serpentine substrate

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Flavonoid and carotenoid content in floral organs of *Iris pumila*

PP3-14

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Flavonoids and carotenoids are plant pigments that play a variety of functional roles in higher plants. Beside their impacts on flower color, they are involved in plant growth, development and protection against a wide range of environmental stressors. They act either as light attenuators and free radical scavengers (e.g. anthocyanins) or participate in non-photochemical quenching (carotenoids). To quantify the accumulation of flavonoids and carotenoids in different floral organs, fifty *Iris pumila* genotypes grown in a common-garden and belonging to different color classes (yellow, blue, violet, dark blue and dark violet) were assessed. One flower per each genotype was harvested and analyzed spectrophotometrically for the content of three flavonoids (anthocyanins, flavones, flavonols) and total carotenoids in different floral organs: falls, standards, stamens and style arms. While the quantity of flavonoids was found to be floral organ- and color morph-specific, the amount of carotenoids varied only among individual floral organs. The variation pattern of the analyzed compounds displayed identical trend - the lowest amounts were recorded in the sta-

men and style arm, and the highest in the standard and fall. The content of carotenoids appeared to be the highest in floral organs of the yellow color morphs, whereas in the blue, violet, dark blue and dark violet flower phenotypes its abundance was a half of that recorded for the yellow one. Conversely, the anthocyanin content was rather high in the floral organs of dark violet and dark blue color morphs, but extremely low in those of the yellow one. Our results clearly suggest that a greater accumulation of both plant pigments in sterile rather than in fertile flower organs could be causally related to their specific function in the process of sexual reproduction, where the former serve to attract plant pollinators, while the latter promote an efficient pollen deposition.

Keywords: anthocyanins, carotenoids, *Iris pumila*

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Characterization of storage seed reserves of *Chenopodium quinoa* using Raman spectroscopy

PP3-15

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Quinoa (*Chenopodium quinoa* Willd.) is a crop whose seeds are storage of proteins, mineral nutrients, lipids and carbohydrate reserves, which are localized in cotyledons and perisperm. The aim of this study was to localize carbohydrates and proteins in quinoa seed and find differences in chemical composition between two genotypes (Puno and Titicaca) using Raman spectroscopy and chemometric tools (PCA). The experiment was carried out during the 2016 growing season in rainfed conditions in the north of Serbia (area of Subotica). The experiment was set up in a split-split plot system, with four replications on the soil type chernozem. Raman spectroscopy analysis was performed using a XploRA Raman spectrometer at 785 nm on longitudinal quinoa seed sections. Raman spectra were recorded in two seed regions: cotyledon and perisperm. Spectra were recorded in the range from 200 to 1750 cm^{-1}. Analysis of the scores of the principal components revealed a division into two different groups, classified according to genotype differentiation observed in both seed compartments (cotyledons and perisperm). The analysis of the loading pointed out the region of the spectrum that contributes to genotype separation, e.g. the band at 472 cm^{-1} regarding the glucosidic ring vibration and is probably related to the amylopectin content in perisperm region. According to the loading plot corresponding to cotyledons part, the highest loadings were observed in spectral range from 1100 to 1650 cm^{-1}, including the most important bands originating from Amide I, II and probably protein with globoid crystals of phytin, which were responsible for genotype separation.

Keywords: quinoa, Raman spectroscopy, starch, Amide I and II, phytin.

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The longevity of annual wild *Helianthus* seeds in short- to medium-term storage conditions

PP3-16

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Wild species are important in sunflower breeding as a source of genetic variability, as the required characters are not always available in the cultivated sunflower gene pool. The use of wild species is complex due to interspecific cross incompatibility, while regeneration of accessions can be difficult because of low self-fertility. Viability of seeds over extended periods is thus important as it directly influences the frequency of regenerations and seed availability in active collections. Viability of 7 annual wild sunflower species stored up to 14 years at 4 ± 2 °C and 55 ± 5% relative humidity is reported.

Initially the germination slightly increased, which was most visible in *H. niveus*, and after 3 to 5 years started to decrease, but the curve parameters were species characteristic. After 8 years, the evaluated species had on average 20% lower germination. *H. argophyllus*, *H. petiolaris* and *H. praecox* retained highest germination after 8 years: 70, 65 and 60% respectively. The largest decrease was found for *H. neglectus* and *H. annuus* where germination decreased to 50%. Lowest starting germination was found for *H. debilis* (62%) and *H. niveus* (55%), but unlike *H. niveus* where germination decreased similar to other species, *H. debilis* was the most stable in retaining viability together with *H. argophyllus*. The presented results contribute to better understanding of seed longevity in storage and are important when determining the monitoring frequency and the need for regenerations.

Keywords: *Helianthus*, seeds, longevity, conservation, genebank

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Impact of traffic-borne dispersal of invasive *Asclepias syriaca* L. in Special Nature Reserve Deliblato sands

PP3-17

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The effectiveness of roadside corridors for the migration of invasive plant species has often been linked to altered site conditions along roads due to increased disturbance, thus reducing the competitive strength of the native roadside flora. The invasion histories of some roadside plant species indicate that, in addition to site-related changes, traffic also promotes invasiveness.

Asclepias syriaca (common milkweed) is a fast-growing competitor with effective clonal spreading and seed dispersal ability. A single common milkweed plant can produce a few thousand seeds with long, white flossy hairs that can easily be carried by wind. *A. syriaca* is common along the borders of the protected area - Deliblato sands, and is spreading into this area, mostly along the roads. Impact of traffic and accompanying human activities as dispersal vectors on seed deposition of *A. syriaca* in Deliblato sands has been investigated and our results demonstrate that, besides altered site conditions and abundance along particular Special Nature Reserve border, traffic-borne dispersal is an important cause of the higher incidence of *A. syriaca* on roadside verges. There was significantly higher number of *A. syriaca* plants along roads with heavier traffic. Also, higher frequency of road use results in higher incidence of new establishment of *A. syriaca*.

Transport and human activities around roads can also explain the occurrence of isolated founder populations and discontinuous distributional patterns of common milkweed in this area.

Keywords: invasive species, *Asclepias syriaca*, traffic-borne dispersal, Deliblato sands

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Comparative study of plant morphology and leaf anatomy of near threatened terrestrial species, *Orchis morio* L.

PP3-18

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A terrestrial orchid, *Orchis morio* L. is one of the near threatened species in Croatia. Although it inhabits different habitats at a different altitude, a variety of anthropogenic influences, including habitat degradation and transformation, succession caused by the abandonment of traditional management activities (e.g. grazing, mowing) as well as expected climate changes, have been recognized as the most serious threats for its survival. To succeed in a variable environment, *O. morio* develops specific adaptive responses. We examined 16 morphological and 10 anatomical parameters of *O. morio* plants growing on two different habitats at a different altitude (steppe-like grassland and hilly grassland area) in Northeast Croatia. The results showed that most of the analysed morphological parameters (e.g. plant height, the total number of leaves, the width of the steam below the inflorescence, inflorescence length, total number of flowers), were similar in both study sites while leaf size and anatomy were significantly different. At the hilly grassland area, orchid leaves have significantly larger upper and lower epidermal cells, thicker cuticles and mesophyll layers. In conclusion, changes in morphology and leaf anatomy of *O. morio* depend on the local environmental conditions, but further investigations are necessary to improve our knowledge about its adaptation strategies and to provide an important base for the effective conservation of this threatened species.

Keywords: *Orchidaceae*, ecological adaptation, elevation gradient, Croatia.

Cryopreservation of apple

PP3-19

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In this work we employed two vitrification-based techniques (vitrification and droplet vitrification) to cryopreserve *in vitro* grown shoot tips of apple 'Gala Must' (*Malus* × *domestica* Borkh.). After preculture, shoot tips were osmoprotected at room temperature in a solution containing 2 M glycerol and 0.4 M sucrose for 20 min, and then dehydrated in the following plant vitrification solutions: PVS2 (13.7% sucrose, 30% glycerol, 15% ethylene glycol, 15% DMSO), PVS A3 (22.5% sucrose, 37.5% glycerol, 15% ethylene glycol and 15% DMSO) for 30, 40 and 50 min at 0 °C and PVS3 (50% glycerol and 50% sucrose) for 40, 50 and 60 min at room temperature. Explants dehydrated with PVS2 and PVS A3 were cryopreserved by vitrification while those dehydrated with PVS A3 and PVS3 were cryopreserved using droplet vitrification. In vitrification protocol, regrowth of the cryopreserved shoot tips dehydrated with PVS2 ranged between 20–40%. Dehydration with PVS A3 resulted in considerably higher regrowth rates (15–75%) using the same protocol. The highest values of regrowth were achieved with the longest treatment duration (50 min) for both vitrification solutions. As for droplet vitrification, regrowth of cryopreserved explants dehydrated with PVS A3 varied between 45–70%, and between 45–50% for those dehydrated with PVS3. The highest regrowth values, 70% and 50%, were achieved after 40-min PVS A3 treatment and 50-min PVS3 treatment, respectively. These results prove the feasibility of the PVS A3-based vitrification for the long-term storage of this genotype.

Keywords: *Malus* × *domestica* Borkh., vitrification, droplet vitrification, liquid nitrogen

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. TR-31064).

Seasonal variability in leaf chemistry of *Iris variegata* L. genotypes growing in contrasting light conditions

PP3-20

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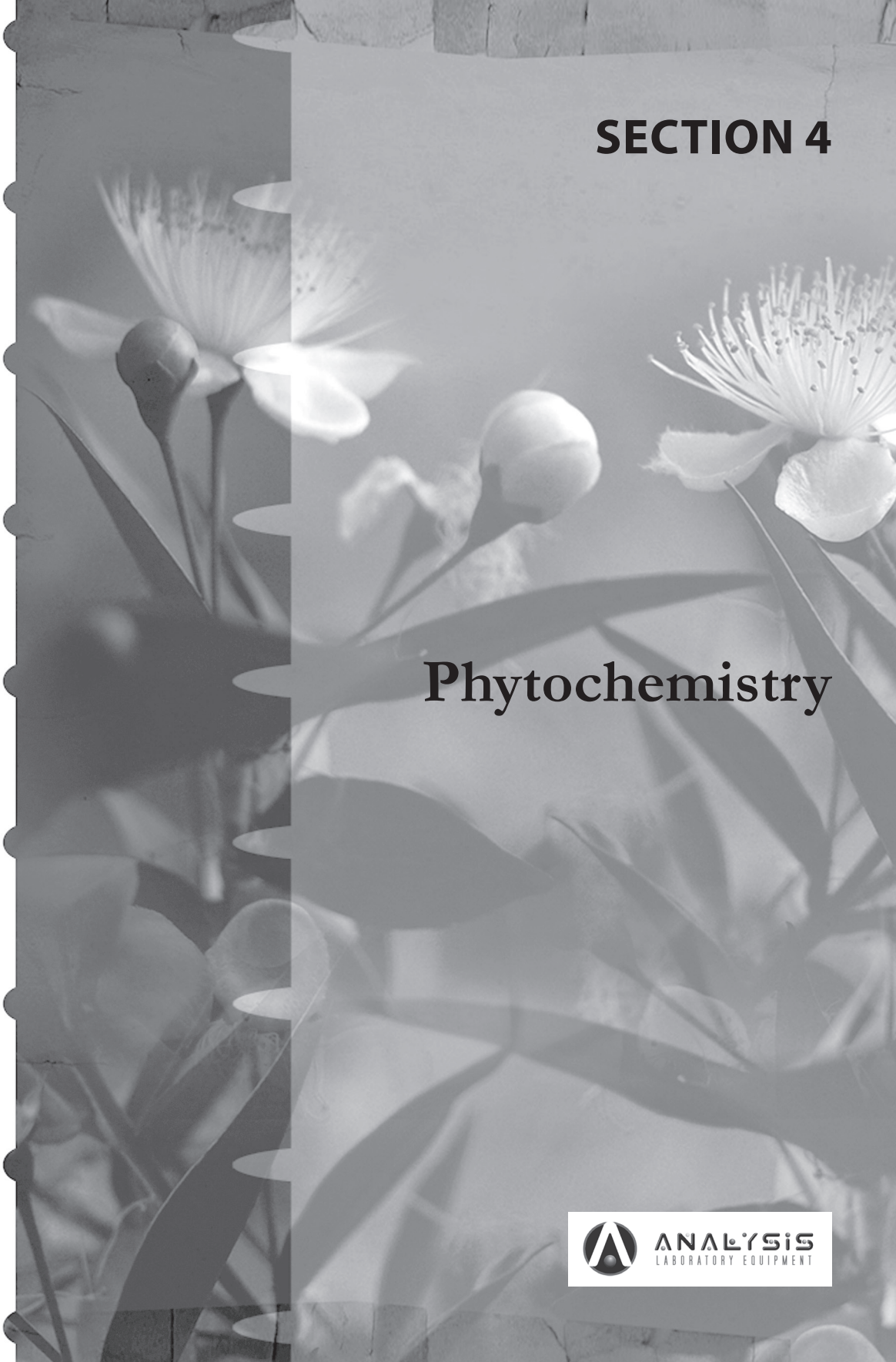
During their evolution plants developed a set of mechanisms to adapt to the varying environmental conditions. Light is one of the most dynamic components of the terrestrial environment that affects plant physiology and development. Optimization of light harvesting for photoautotrophic growth inevitably induces specific adjustments in all aspects of plant phenotype: morphology, anatomy, phytochemical composition, flowering phenology etc. As sessile organisms capable of perceiving quantitative and qualitative features of light surroundings, plants need to be particularly plastic in their response to different light environments. For this study genotypes of

Iris variegata that occupy different light habitats in Deliblato sands were selected: a) open habitats where they were exposed to full sunlight and b) woodland understories with lower light intensity and changed light quality (vegetative shade). Specimens of those *I. variegata* genotypes were grown under two experimental light treatments: 1.) high intensity and higher red-far red light ratio and 2.) low intensity and lower red-far red light ratio. Leaves were collected during spring, summer and autumn of one experimental year, dried in silica gel and extracted with methanol. Samples were subjected to UHPLC/qqqMS profiling of phenolics (phenolic acids, flavonoids, and xanthones), and subsequently to chemometric analysis. Results showed that dissimilar light conditions in applied treatments markedly affected *I. variegata* phenolics composition. Repeated measures model ANOVA revealed a significant effect of seasons for all analyzed phenolics. Concentrations of half of the analyzed chemical compounds were significantly different under two light treatments. The correlations between traits were in almost all cases significant and positive.

Keywords: secondary metabolites, light treatments, *Iris variegata*, Deliblato sands

This study is supported by the Ministry of Education, Science and Technological Development, Grant Nos.OI173025, OI173024 and OI173015.



SECTION 4

Phytochemistry

ANALYSIS
LABORATORY EQUIPMENT

PLENARY LECTURE

Engineering plant diterpenoid pathways in yeast: increasing yield and expanding product diversity

PL4-1

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We are using the yeast *Saccharomyces cerevisiae* as a platform for engineering the biosynthesis of plant diterpenoids. For this, we developed a set of yeast expression vectors for hierarchical modular cloning based on Golden Gate cloning which we call MoClo-Yeast (Scheler et al., 2016). A set of parts was established that are compatible with MoClo-Yeast, including yeast native promoters, synthetic promoters (inducible or constitutive), yeast native terminators and synthetic terminators. With MoClo-Yeast up to 6 transcription units can be assembled in just two cloning steps and over 15 in three cloning steps. MoClo-yeast is particularly well suited for combinatorial cloning. We have employed this for example to optimize the production of *cis-abienol*, a tobacco diterpenoid which can be used as a precursor for the synthesis of Ambrox. The highest titer in shake-flasks was over 1 g L^{-1} *cis-abienol*, boding well for further optimization in improved culture conditions. Carnosic acid is a phenolic diterpenoid present in species of the *Lamiaceae*, including rosemary (*Rosmarinus officinalis*) and several sage species (*Salvia sp.*). Carnosic acid and its derivative carnosol are potent antioxidants which are used in the food and cosmetic industry as natural preservatives. With MoClo-Yeast, we were able to elucidate and reconstitute the biosynthesis of carnosic acid which comprises two diterpene synthases (diTPS) and two cytochrome P450 oxygenases (CYPs) (Scheler et al., 2016). By combining CYPs with the diTPS we could generate novel products and are now combining related diTPS which produce similar labdanoid diterpene skeletons with a set of CYPs from various plant species that are known to act on labdane-diterpenes. Progress in the combinatorial engineering of plant labdanoid diterpenes will be presented.

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Scheler, U., et al. (2016) Elucidation of the biosynthesis of carnosic acid and its reconstitution in yeast. *Nature communications,* 7, 12942.

INVITED TALKS

Metabolic engineering and elicitation strategies to produce stilbenoids in plant cell cultures

IT4-1

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Stilbenes are naturally scarce high-added-value phytochemicals with chemopreventive, pharmacological and cosmetic properties. Cell cultures of the plant species able to synthesize them, mainly *Vitis* sp., have been used for the biotechnological production of these scarce compounds. An effective strategy consists in the elicitation of wild grapevine cell cultures, which leads to a vast stilbene resveratrol accumulation in the extracellular medium, with the combination of methylated beta-cyclodextrins and methyl jasmonate being the most successful elicitor treatment. Other bioproduction strategies include engineering the metabolisms of bacterial, fungal and plant cell systems. For the last five years we have explored the combination of both strategies, i.e. elicitation of metabolically engineered plant cell cultures with the aim of rationally managing the stilbenoid metabolism. In this talk we will present examples of (1) production of resveratrol in non-producing species, (2) diversification of stilbenoid end products through hydroxylation and methylation metabolic reactions on the accumulated resveratrol so to produce piceatannol and pterostilbene, (3) stimulation of extracellular accumulation, through a combination of elicitation and metabolic engineering of cell suspensions.

New Fatty Acids discovered for the first time in *Vitex agnus-castus*

IT4-2

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Vitex agnus-castus is a very well-known shrub for the biological effects of its extracts. These effects are mostly dedicated to non-polar compounds such as terpenoids. Different methods of extraction have been used, from which different pure compounds were isolated and tested for their biological effects. Lately we published [1] that subcritical CO_2 extraction is very selective for terpenoids and a greater percentage of rotundifuran has been found in this extract compared

to all other methods, including the supercritical CO_2. Here we will present that this method has allowed us to isolate, for the first time, not only for the *Vitex* species, but also for the whole *Lamiaceae* family, some new fatty acids. The FAME analysis of the extracts showed that the main fatty acid of the lipid fractions is linoleic acid (66-70%), an essential fatty acid of the omega-6 family. The second fatty acid is oleic acid (ca. 10%), and saturated fatty acids are ca. 8% of the total fatty acid composition. We also identified for the first time in this family some C18 branched-chain fatty acids and 12-phenyl fatty acids and 13-phenyl fatty acids.

Keywords: *Vitex agnus-castus*, branched-chain fatty acids, 12-phenyl fatty acids

References:

[1] Altin Mele; Harro Lentz; Ardita Mele; Elisabeth Feizlmayr; Sokol Abazi; Rudolf Bauer; The Journal of Supercritical Fluids, 2013, 79, 123-126

Do plant volatiles reflect taxonomy?

IT4-3

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Plants synthesize and emit many different volatile organic compounds (VOCs), which are very significant for plants physiological and ecological adaptation, their existence, and, in some cases, composition and biosynthetic pathways of VOCs correspond to evolutionary trends. According to their biosynthetic origin and chemical structure, volatile organic compounds can be grouped into terpenoids, fatty acid derived C6-volatiles and derivatives, phenylpropanoid aromatic compounds, as well as certain alkanes, alkenes, alcohols, esters, aldehydes, and ketones, in few cases, as sulfur compounds and as furanocoumarins and their derivatives. Many of them are contained in essential oils. Qualitative and quantitative composition of VOCs of closely related taxa and influence of environmental effects on their synthesis and emission are significant for discovering their function and evolutionary importance. Because of these important roles, VOCs are under strong selection and their biosynthetic pathways are genetically determined and evolutionarily conserved for the benefit of the organism (taxon). In some cases taxonomic treatments using morphology and molecular genetic markers have faced difficulties in discerning relationships within the taxa. For all these reasons, VOCs can be important for chemotaxonomy of plants, a scientific discipline (Chemical systematics of plants, *sensu lato*) dealing with chemical evidence (occurrence and distribution of specialized metabolites) as useful characters in the plant taxonomy (theory and practice of plant classification). Phytochemistry of VOCs can be successfully used in plant taxonomy, especially at lower taxonomic levels (genus, species, subspecies etc.). Also, since the chemotaxonomy is hybrid discipline between botany and chemistry of natural compounds, chemotaxonomy needs full synergistic approach from chemists and plant taxonomists, manifesting itself in a better understanding of each other.

Keywords: volatile organic compounds (VOCs), essential oils, phytochemistry, plant taxonomy

The authors thank to the Ministry of Education, Science and Technological Development of the Republic of Serbia for the financial support (Grant No. 173029).

The *Cistus creticus* Terpene Synthase Gene Family

IT4-4

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Cistus creticus, a common plant in the Mediterranean basin, is a rich source of plant specialized metabolites, mainly terpenoids and phenylpropanoids. Selected compounds of these classes possess proven antibacterial, antivirus, cytotoxic and anticancer properties. This presentation will focus on terpenoid metabolism and biochemistry of two subspecies, *Cistus creticus* subsp. *creticus* and *Cistus creticus* subsp. *eriocephalus*, studied through chemical and transcriptomic analyses. Mining of the transcriptomic libraries generated from fruit and trichomes of intact and wounded young leaves, through phylogenetic analyses, revealed that the family of *C. creticus* terpene synthases (TPS) consisted of 5 monoterpene, 4 sesquiterpene, and 4 diterpene synthases (3 type II labdane diterpene synthases and 1 type I diterpene synthase). Among those, the following were fully functionally characterized: a monoterpene synthase producing mainly α-terpineol, 4 sesquiterpene synthases catalyzing the synthesis of β-caryophyllene, β-farnesene, germacrene B and D; 3 type II labdane diterpene synthases, namely copal-8-ol diphosphate synthase and two labda-7,13-dien-15-yl diphosphate synthases; a type I diterpene synthase, namely *ent*-kaurene synthase. Additionally, we have identified two prenyltransferases, able to synthesize GGPP, the precursor of all diterpenoids and 4 terpenoid-modifying enzymes from the BAHD family of alcohol acetyltransferases (ACTs). One of the identified ACTs was shown to catalyze the acetylation of both, labda-13(E)-ene-8α,15-diol and labda-7,13(E)-dien-15-ol. The above-mentioned TPSs exhibited differential gene expression patterns in various tissues, developmental stages and stresses, suggesting different functions in plant metabolism.

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Terpenes and Terpenoids: Linking Bioactivity, Opportunities and Challenges

IT4-5

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Terpenes and terpenoids (TT) are the most numerous and structurally diverse groups of natural products. They are playing vital roles in plant physiology and are responsible for important functions in cellular membranes. Their various activities (antimicrobial, antiparasitic, antiinflammatory, antiallergenic, antihyperglycemic, antispasmodic, antitumoral, immunomodulatory, etc.) have been extensively reviewed. They are divided into several subgroups according to their structures and biological functions. One of the most diverse and active group is that of the essential oils. Studies have confirmed that they display a wide array of pharmacological properties. The

other group is sesquiterpene lactones isolated from numerous plant families, especially *Asteraceae*. Investigation of structure-activity relationships helps the understanding of the bioactivity of germacranolides, eudesmanolides, elemanolides, and guaianolides, as compounds responsible for the biological potential. TT are mainly investigated in plants, but fungi also produce a variety of these compounds (indole-diterpenes, phytohormones, lanostane triterpenes, etc.). The present work will highlight diverse TT biological activities which receive greater attention in medical fields, mainly due to their immunomodulatory and antitumor effects. Special attention is given to the mechanisms involved in the proposed beneficial effects on human health, in order to point them out as candidates for new and safe drugs discovery. TT activities could provide useful alternatives and new **opportunities** in current scientific and medical battle against widespread and frequent diseases of our time. Further research efforts are needed to validate the usefulness of TT, either alone or in combination with existing therapies, or even inspire bioactivity-guided discovery of semi-synthetic agents as a **challenge**.

SELECTED TALKS

Enzymatic behavior of edible berries – "Beroxidases"

ST4-1

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Berries are universally recognized as a good source of essential vitamins and minerals, as well as diverse phytochemicals which rank them into health benefit food. Today's literature abounds with data on chemical composition of berry fruits related to the nutritional benefits such as carbohydrates, vitamins, minerals, dietary fibers and antioxidant polyphenols. However, not many papers were dealing with enzymatic antioxidants in edible berries (strawberry, raspberry, blackberry and blueberry). Our investigation is mainly focused on various oxidases in fresh berry fruits where they could cause significant quality deterioration, primarily loss of color and texture as well as the formation of undesirable brown pigments affecting the overall acceptability by consumers. With regard to their role in the phenolic metabolism, peroxidase (POD; EC 1.11.1.7) and polyphenol oxidase (PPO; EC 1.14.18.1) are considered to be the primary enzymes responsible for phytochemicals' degradation leading to fruit ripeness. Consequently, POD and PPO have a great influence on overall fruit quality, which is limited by either enzyme or substrate concentrations. Likewise, changes in POD and PPO activities trigger alterations in protein content and other metabolites such as organic acids, sugars and ascorbic acid. Changes in enzyme activities related to the content of substrates play an important role in nutrient quality definition of fruits, which implies that all of these changes jointly contribute to the quality of the final product. A better knowledge of the factors that influence the action of POD and PPO is imperative to control and manipulate its detrimental activity in fruit products.

Keywords: fruit quality, peroxidase, polyphenol oxidase, polyphenols, proteins

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 173040).

Inhibition of satellite RNA associated cucumber mosaic virus infection by essential oil of *Micromeria croatica* (Pers.) Schott

ST4-2

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The essential oil was isolated from *Micromeria croatica* (Pers.) Schott, an endemic plant species of the Dinarides mountain range. The plant material was collected at localities on Mt. Velebit (localities Bojinac, Bačić kuk and Stupačinovo) in Croatia. GC and GC-MS analysis identified *β*-caryophyllene and caryophyllene oxide as the main oil components. Essential oil, β-caryophyllene and caryophyllene oxide, were tested for antiphytoviral activity on local and systemic host plants infected with satellite RNA associated cucumber mosaic virus (CMVsat). Simultaneous inoculation of CMVsat with essential oil or with the dominant components of oil, and the treatment of plants with essential oil or with the dominant components of oil prior to virus inoculation, resulted in a reduction in the number of lesions on leaves of the local host plants. Regardless of whether oil or oil compounds were inoculated simultaneously with the virus, or applied prior to virus inoculation, the virus concentration was reduced in the systemically infected host plants. The results showed a correlation of the antiviral activity of the essential oil with the main oil components: activity of the oil from Bojinac positively correlated with *β*-caryophyllene, while activity of the oils from Bačić kuk and Stupačinovo correlated with caryophyllene oxide. Gene expression assay showed that treatment with essential oil changed the level of alternative oxidase (*Aox*) gene expression in infected Arabidopsis plants. The involvement of *Aox in* the defence pathways against pathogens indicates a connection between the essential oil treatment, *Aox* gene expression and the development of viral infection.

Keywords: *Micromeria croatica*, essential oil, antiphytoviral activity, *β*-caryophyllene, caryophyllene oxide

This work was supported by the Ministry of Science, Education and Sports of the Republic of Croatia (Grant Nos. 006–0000000–3178 and 177–1191192–0830).

Structure elucidation of a new alkaloid and other 11 known compounds isolated from *Gymnospermium* species

ST4-3

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Gymnospermium scipetarium and *Gymnospermium maloi*, part of *Berberidaceae* family, are two newly discovered endemic plants of Albania [1, 2]. Very little has been published on their chemical composition [3]. In Albania, *G. maloi* is known as a poisonous plant, because calves fed with these plants die. The family *Berberidaceae* is well known for its alkaloids, which have various biological effects. During this work we were able to isolate and elucidate for the first time a new alkaloid. In order to investigate the presence of minor compounds, a large scale extraction was performed allowing further isolation and elucidation of other 11 compounds including sophoridine and matrine using different techniques such as: UV, UHPLC, TOF, HPLC semipreparative, HPLC/ MS, Orbitrap and cryoprobe NMR and also the databank of known botanic compounds (DNP). Biological activity was evaluated *in vitro* on the human chronic myeloid leukemia cell line K562. The overall biological results suggest that, in our experimental culture conditions, the crude extract of *G. maloi* and its fractions exert a clear-cut biological effect of cell growth inhibition on human chronic myeloid leukemia K562 cells.

Keywords: *Gymnospermium*, NMR, Orbitrap, K562

References:

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POSTER PRESENTATIONS

Salvia brachyodon Vandas - essential oil composition, antioxidant and antimicrobial activity

PP4-1

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In the present work, the essential oil of rare and endemic aromatic plant *Salvia brachyodon* Vandas (*Lamiaceae*) was analysed and evaluated for its biological properties. *S. brachyodon* is Dinarian endemic species which grows on sunny and dry rocky places (Montenegro, BIH, Croatia). The essential oil composition was determined using GC-FID and GC-MS techniques. Essential oil analysis showed that the most abundant group of compounds were oxygenated monoterpenes (58.8%), followed by monoterpene hydrocarbons. The dominant compound in the oil was 1,8-cineole (40.5%). Also, camphene (9.2%), α-pinene (8.7%), borneol (7.5%), camphor (6%) and β-pinene (5.7%) were present in significant amounts. Antioxidant properties of the essential oil were determined using two radical scavenging tests, DPPH (2,2-diphenyl-1-picrylhydrazil) and ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid). Results obtained by DPPH assay revealed that IC_{50} value of the solution was 0.33 mg mL^{-1}. In ABTS test, essential oil showed strong radical scavenging potential with1.05 mg VitC g^{-1} of dry extract. Antibacterial activity was determined by microdilution method and obtained results were compared to commercial antibiotic and mycotic. The essential oil proved to be effective against tested bacteria and *Enterobacter cloacae*, *Salmonella typhimurium* and *Escherichia coli* were the most sensitive strains, while the most resistant was *Listeria monocytogenes*. The oil possessed antifungal activity where *Aspergillus versicolor* showed maximum sensitivity towards tested strains. This result suggests that *S. brachyodon* may be considered as a new natural source of antioxidants and antimicrobial substances, taking into account its endangered status and commercialization only through cultivation.

Keywords: *Salvia brachyodon*, essential oil, antioxidant, antibacterial activity

The authors are grateful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for the financial support Grants No. 173029 and 173032.

Diploid and tetraploid *Centaurium erythraea* Rafn: a comparative study of regenerative *in vitro* potential and biosynthetic capacity

PP4-2

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Polyploidy, or duplication of the entire genomes, is a widespread mechanism in plants which can lead to changes in cytological, biochemical, physiological and developmental characteristics, including conspicuous changes in morphology and metabolite production. In this study, we examined the impact of ploidy level on regenerative *in vitro* potential and biosynthetic capacity of C. *erythraea plants*. Ten genotypes of diploid (2x) and autotetraploid (4x) centaury, with ploidy levels confirmed by flow cytometry, were randomly selected from *in vitro* pool of seedlings maintained on hormone-free half-strength MS medium (½ MS). Root explants were excised either from basal, middle or apical root zone of 3 month-old plants. *In vitro* morphogenesis was induced on ½ MS medium. The frequency of shoot regeneration and the average number of shoots per root explant were recorded after 5 weeks in culture. Regeneration frequency was genotype dependent, but not affected by explant ploidy level. Regeneration efficiency declined from the apical to the basal root segment. Regenerated shoots were transferred onto ½ MS medium for rooting. Iridoid glycosides (loganic acid, loganin), secoiridoid glycosides (secologanin, sweroside, swertiamarin, gentiopicrin) and xantones (decussatin, eustomin, methylbellidifolin, desmethyleustomin) were quantified in shoots and roots of two month-old plants using targeted UHPLC-qqqMS analysis. Interestingly, diploid plants showed higher biosynthetic capacity for the production of secoiridoids and xanthones in both shoots and roots. Furthermore, two month-old shoots of 2x genotypes produced higher shoot and root biomass than 4x plants. Results highlight a great potential of diploid *C. erythraea* genotypes for biotechnology-based sustainable production of secoiridoids.

Keywords: *Centaurium erythraea*, ploidy level, morphogenesis *in vitro*, secoiridoids, xanthones

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

Xanthone accumulation in *Gentiana utriculosa* L. hairy roots and regenerated plants

PP4-3

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Gentiana utriculosa is an annual plant, 5-30 cm in height, with deep azure-blue flowers. It has wide distribution in the mountains of Central Serbia. The aerial parts of *G. utriculosa* are characterized by the presence of xanthone-*O*-glycosides with 1,3,7,8-oxidation pattern along with xanthone and flavone-*C*-glucosides. Hairy root culture of *G. utriculosa* was established using *Agrobacterium rhizogenes* A4M70GUS. During the cultivation of hairy roots, spontaneous regeneration of

shoots occurred. Decussatin-1-*O*-primeveroside and its corresponding aglycon decussatin were found as dominant xanthones in hairy roots lines 4, 9, 12, and 13 and in control roots. Along with these xanthones, HPLC analysis revealed the presence of xanthone-*C*-glucoside mangiferin, gentiakochianin-1-*O*-primeveroside, 1,8-dihydroxy-3-methoxy-7-*O*-primeveroside and aglycons gentiakochianin and gentiacaulein mostly in lower amounts. Hairy root line 9 accumulated up to 2.5-fold higher content of decussatin-1-*O*-primeveroside and decussatin compared to plants from nature and nontransformed ones. The plants regenerated from these superior hairy roots produced more than 4.5-fold higher amount of aglycon decussatin, while production of glycoside was at the similar level as in control plants. Contrary to hairy roots, where mangiferin was detected only in line 13, this compound was detected in all hairy root-derived shoots but in lower content than in controls.

Keeping in view that xanthone decussatin was recently suggested as potential hepatoprotective and antiulcerogenic agent, *G. utriculosa* hairy roots and transgenic plants could be considered as a favorable system for sustainable production of this valuable compound.

Keywords: *G.utriculosa*, hairy roots, decussatin

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. OI173015).

Inter- and intra-population variability in secondary metabolite content of *Gentiana pneumonanthe* L.

PP4-4

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In this study, an organ-dependent distribution of secondary metabolites such as secoiridoids (swertiamarin, gentiopicrin and sweroside) and polyphenolics (mangiferin, isovitexin and isoorientin) in individuals of *Gentiana pneumonanthe* was investigated. Six populations of marsh gentian from Mt. Divcibare were selected; four populations were located in meadow communities, one in peat meadow, and one at the edge of pine forest. Field study was conducted in September 2017, in the phase of late flowering and seed formation. Seven individuals from each population were sampled. From each individual, aboveground part was separated to fruit/seed part, and stem part. Belowground part (rhizome) was harvested partially to ensure the regeneration of plant. Plant parts were analyzed separately for the content of secondary metabolites using HPLC/DAD method. Results showed that fruit part in all populations contained secoiridoid sweroside as the major compound. On the other hand, in the stems phenolic compounds dominated, while the content of secoiridoids was very low. Among the phenolics in the stems, xanthone mangiferin was detected in the highest amount. Results also showed that rhizomes of *G. pneumonanthe* populations contained very high amount of secoiridoid gentiopicrin, which was 10-fold higher than swertiamarin and sweroside content. In the rhizomes, polyphenolics were detected only in trace amounts.

Keywords: *G. pneumonanthe*, secondary metabolite, HPLC

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Investigation of radical scavenging potential and total polyphenolic and flavonoid content of *Inonotus obliquus* extracts: EPR and UV-Vis study

PP4-5

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Chaga (*Inonotus obliquus*) is a black parasitic fungus inhabiting primarily the live trunks of mature *Betula* trees. Due to its various biological activities, it is widely used in European and Asian folk medicine for treating several health conditions. In this paper, scavenging potential towards ˙OH and DPPH radicals along with evaluation of total polyphenolic and flavonoid content of Chaga extracts were investigated. Chaga samples were collected at Vitosha mountain near Sofia, Bulgaria. Active components were extracted in water by reflux (Chaga : water = 1 : 15 w/w), followed by the evaporation of solvent to dryness. Dry extracts were dissolved in water to the final concentration of 1 mg mL^{-1}. Due to its reliability and high sensitivity, electron paramagnetic resonance (EPR) spectroscopy was used for testing anti-˙OH and anti-DPPH radical activity of Chaga. Total polyphenolic and flavonoid content was determined using standard UV-Vis spectrophotometric assays. Chaga extracts showed significant anti-radical activity towards ˙OH and DPPH radicals (49.24 ± 0.40% and 80.24 ± 0.34%, respectively), which was in correlation with the level of polyphenolic compounds (120.54 mg gallic acid equivalents per g of dry extract) and flavonoids (589.48 mg quercetin equivalents per g of dry extract), which are well-known as radical scavengers. According to the best of our knowledge, this is the first study which represents proof of the high antiradical scavenging potential of Chaga extracts obtained by EPR.

Keywords: Chaga, EPR, hydroxyl radical, polyphenols

Antimicrobial potential of *Helichrysum italicum* (Roth) G. Don essential oil

PP4-6

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Helichrysum italicum (Roth) G. Don (*Asteraceae*) is the Mediterranean evergreen plant, which has been used in traditional medicine since ancient times. Recently, commercial use of *H. italicum* essential oil is getting wider, due to the many beneficial features regarding its biological activity. In this research, antimicrobial activity of *H. italicum* essential oil was tested against ten reference strains of bacteria and fungi, by disc diffusion method. To assess the antimicrobial properties of essential oil, we used Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591, *Bacillus subtilis* ATCC 6633 and *Enterococcus fae-*

calis ATCC 29212 and Gram-negative bacteria: *Salmonella abony* ATCC 6017, *Salmonella enterica* serovar Enteritidis ATCC 31194, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 25922 and extended-spectrum *β*-lactamase (ESBL)-producing E*scherichia coli* ATCC 35218, as well as the yeast *Candida albicans* ATCC 1023. Antimicrobials ampicillin, streptomycin and nystatin were used as a positive control. All antimicrobial assays were performed in four different concentrations of investigated essential oil (100%, 75%, 50%, 25%). The obtained results showed that *H. italicum* oil was effective against all tested Gram-positive bacteria, especially in case of MRSA, where 50% concentration of oil caused the widest inhibition zones, and standard antibiotics did not exhibit any activity. Antifungal effect on *C. albicans* was observed for all investigated concentrations of oil. Antibacterial activity against Gram-negative bacteria was not recorded in this study. The results of this study indicate versatile antimicrobial potential of *H. italicum* essential oil, depending on oil concentration, as well as on type of microbial pathogen.

Keywords: *Helichrysum italicum*, essential oil, antimicrobial activity

Antimicrobial properties of *Lavandula angustifolia* Mill. essential oil

PP4-7

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Recently, there has been increasing attention in the medicinal plants as potential natural antioxidants, antimicrobial, antiviral and anticancer agents. Lavender (*Lavandula angustifolia* Mill.) is an aromatic and medicinal member of *Lamiaceae* family with carminative, antispasmodic, expectorant, anti-rheumatic, relaxant, sedative, antiinflammatory properties. Antimicrobial activity of lavender essential oil was determined by disc-diffusion method. Antimicrobials ampicillin, streptomycin and nystatin were used as a positive control. Various concentrations of the essential oil (100%, 75%, 50%, 25%) were used for antimicrobial assays. The screening of antibacterial and antifungal activity was evaluated against four Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, methicillin-resistant Staphylococcus aureus (MRSA) ATCC 33591, Bacillus subtilis ATCC 6633, *Enterococcus faecalis* ATCC 29212 and five Gram-negative bacteria: *Salmonella abony* ATCC 6017, *Salmonella enterica* serovar Enteritidis ATCC 31194, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 25922, extended-spectrum *β*-lactamase (ESBL)-producing *Escherichia coli* ATCC 35218 and yeast *Candida albicans* ATCC 1023. In our study, all tested microorganisms were extremely inhibited by lavender essential oil. Lavender essential oil showed the highest antibacterial effects on *S. aureus*, MRSA and *B. subtilis*: on the other hand, the smallest inhibition zone was recorded for *S. enterica* serovar Enteritidis. Also, lavender oil demonstrated a good efficiency agains yeast *C. albicans*. The results of antimicrobial assay revealed that tested Gram-positive bacteria were more susceptible to investigated essential oil than Gram-negative bacteria.

Keywords: *Lavandula angustifolia* Mill., antimicrobial activity, disc-diffusion method.

Phenolic profiles of June bearing and everbearing strawberries

PP4-8

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The delicious fruits of strawberry (*Fragaria×ananassa* Duch.), consumed in both fresh and processed form, are an important source of health promoting compounds. Amongst them, strawberries possess considerable quantities of various phenolics. For this study, twelve strawberry cultivars, 9 June bearing ('Roxana', 'Arosa', 'Joly', 'Asia', 'Alba', 'Jeny', 'Laetitia', 'Garda', 'Premy'), and 3 ever-bearing types ('Albion', 'Capri' and 'Irma'), were evaluated for anthocyanin, phenolic acid and flavonoid content using HPLC-MS. Dominant anthocyanin form in all cultivars was pelargonidin-3-glucoside with the highest values detected in 'Asia' and 'Joly' cultivars (487 and 464 μg g^{-1} FW, respectively). 'Joly' also stands out for acylated pelargonidin-3-glucoside and prunin content, since the highest values of pelargonidin-3-rutinoside and ellagic acid were detected in 'Laetitia' (33 and 43 μg g^{-1} FW, respectively). Uppermost on the flavonoid list was 'Asia' which was the richest in p-coumaroyl hexose and its derivate (87 and 64 μg g^{-1} FW, respectively), followed by 'Joly' dominating in kaempferol 3-glucuronide and kaempferol 3-coumaroyl glucuronide. The identification of phenolic compounds revealed some interesting differences correlated to the cultivar: June bearing cultivars 'Asia' and 'Joly' were distinguished from others as the richest in terms of identified phenolic compounds. Branding the cultivars with optimal phenolic composition empowers their consumption for human health and wellbeing.

Keywords: anthocyanin, flavonoids, phenolic acid, HPLC-MS

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GC-MS analysis of commercial essential oils of basil

PP4-9

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Basil (*Ocimum basilicum* L.) is an aromatic plant used for thousands of years as a culinary and medicinal herb by virtue of its positive therapeutic effects. Essential oil content may vary depending on leaf maturity, genetic and environmental factors (UV-B, temperature, etc.).

In this study we compared the composition of three essential oils of basil commercially available on Serbian markets using Gas Chromatography with Mass Spectrometry (GC-MS) as the method of choice for analysing volatile compounds. Analyzed oils (A, B, C) differed in their qualitative and quantitative composition. The most prominent component of oil A was phenylpropanoid estragole with the peak area of 70% normalized to Total Ion Chromatogram (TIC), followed by monoterpene linalool with 20% presence. Estragole is suspected to be carcinogenic and terato-

genic for humans while the other component, *trans*-anethol, is found to show an anti-fertile activity. We found that oils B and C, in contrast to oil A, were enriched with *trans*-anethol (42% and 38%, respectively). Besides, the oil B contained eugenol (4%), α-terpinyl acetate (5%) and eucalyptol (3%) in significant quantities. The oil C had a similar composition as B with an addition of limonene (4%), citronellal (3%) and eucalyptol (3%). However, the content of eugenol, an efficient antioxidant with antioxidant activity comparable to butylated hydroxytoluene (BHT) and α-tocopherol, was reduced. Taking into account that basil essential oil is widely distributed, some precautionary and legislative measures regarding comprehensive analysis of its dominant components need to be considered.

Keywords: basil, GC-MS, estragole, *trans*-anethol, eugenol

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Fatty acids profile of buckwheat (*Fagopyrum esculentum* Moench.) seeds grown under different conditions

PP4-10

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Organic farming has become an increasingly popular form of production during recent decades. This type of sustainable agriculture enables the production of health-safe and quality foods through the preservation of the environment, soil, biodiversity, agro-ecosystems, along with the improvement of the health of plants, animals and humans. Buckwheat (*Fagopyrum esculentum* Moench.), as an alternative grain, is increasingly important in the organic production system, due to expressed nutritional values. Fatty acids (FAs), especially unsaturated (UFAs), are important in human diet. Bearing in mind the fact that the chemical composition of seeds grown under two production systems can significantly differ, the aim of this paper was to examine the content of fatty acids in organic and conventional buckwheat seeds collected during 2016 season. The fatty acid content was determined by the GC-FID method and expressed as relative percentage of the total FAs. According to the obtained results, ten different fatty acids were detected in seed samples. Predominant fatty acid detected in both samples was oleic acid (C18:1n-9;ω-9). Oleic acid content in conventionally and organically grown buckwheat seed was 53.42% and 52.02%, respectively. Palmitic acid (C16:0) was the second most abundant FA (conventional - 18.6%; organic - 24.01%). Behenic acid was detected only in conventionally grown seeds (0.25%). Saturated fatty acids (SFAs) were more abundant in organic buckwheat seeds (32.62%) compared to conventional (27.2%).Unsaturated fatty acids (UFAs) were predominant in both types of seeds, with 72.8% in conventional and 67.4% in organic buckwheat.

Keywords: organic farming, seed, buckwheat, SFAs, UFAs

Polyphenolic and flavonoid content in *Aronia melanocarpa* L. fruit extracts influenced by different solvents

PP4-11

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Polyphenols and flavonoids are two diverse groups of secondary metabolites in plants. They belong to the bioactive compounds with strong positive impact on human health. Fruit of *Aronia melanocarpa* L. is one of the berries types with the most pronounced antioxidative properties, which makes it an important part of a healthy diet. Due to chemical differences, effective extraction procedure for bioactive compounds from plant material can be significantly influenced by solvent type and concentration. The aim of this work was to determine total phenolic content (TPC) and total flavonoid content (TFC) after application of three different solvent systems: 100% methanol (MeOH), 80% MeOH and 50% ethanol (EtOH). TPC and TFC were determined by standard spectrophotometric methods: Folin-Ciocalteu and aluminum-chloride methods. TPC results are expressed as miligrams of gallic acid equivalent (GAE) per gram of dry weight of samples (DW). Results for TFC are expressed as milligrams of quercetin equivalents (QE) per gram of DW (mg QE g^{-1}). The obtained results for total phenolics were: 14.3 mg g^{-1} GAE (100% MeOH), 13.7 mg g^{-1} GAE (80% MeOH) and 9.1 mg g^{-1} GAE (50% EtOH). TFC values in different solvent system were: 5.2 mg g^{-1} QE (100% MeOH), 5.6 mg g^{-1} QE (80% MeOH) and 6.2 mg g^{-1} QE (50% EtOH). According to obtained results, it can be concluded that phenolic and flavonoid content in fruit extracts is dependent on applied solvent. Solvent extraction efficiency of polyphenols was as follows: 100% MeOH> 80% MeOH > 50% EtOH. On the other side, TFC values exhibited a totally opposite trend.

Keywords: *Aronia melanocarpa* L., polyphenols, flavonoids, extraction, solvent

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Volatile components of endemic species *Centaurea melanocephala* Pančić (*Asteraceae*)

PP4-12

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Centaurea melanocephala Pančić (Syn. *Centaurea candelabrum* Hayek & Košanin) is an endemic species of Serbia. The volatile components were isolated from fresh capitula of *C. melanocephala* by simultaneous distillation extraction, using Likens–Nickerson type apparatus. The oil was analyzed by gas chromatography–mass spectrometry (GC-MS). This is the first report of essential oil composition of *C. melanocephala*. In total, 57 compounds were detected of which 55 were iden-

tified, representing 97.9% of the total oil. The essential oil was characterized by the high presence of sesquiterpenes (54.9%), of which sesquiterpene hydrocarbons and oxygenated sesquiterpenes are represented with 22.9% and 32%, respectively. The dominant components were (*E*)-caryophyllene (11.19%), caryophyllene oxide (10.79%), 1,3,8-*p*-menthatriene (6.53%), and *τ*-muurolol (6.45%). 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) represent 14.1% of the essential oil. (*E*)-Caryophyllene and germacrene D are dominant components of the essential oils in most of *Centaurea* species. However, the essential oil composition of *Centaurea* species shows a wide range of variability, especially in main compounds. Since the biosynthesis of *Centaurea* essential oil is not influenced by environmental conditions, future investigation of volatile components of *Centaurea* species from Balkan Peninsula will be directed to their taxonomic validation.

Keywords: *Centaurea melanocephala*, *Asteraceae*, essential oil

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Secondary metabolite production in differentiated and non-differentiated plant cell tissue and organ cultures of *Inula britannica*

PP4-13

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Inula britannica is a biregional element (Euro-Siberian and Irano-Turanian regions), widely distributed in Western Europe and Turkey, extending eastward to China through Iran and Pakistan. It is an important plant species used in Traditional Chinese Medicine (TCM) and Kampo Medicines. Along with *Inula japonica*, it is known as 'Xuan Fu Hua' in TCM. The anti-inflammatory, anti-bacterial, anti-hepatitic, and anti-tumor activities of extracts and isolated compounds of the species have been established. Shoot cultures were initiated from surface sterilized explants of the plants collected in Bulgaria. Then, different types of *in vitro* cultures of the plant were developed. Plant growth regulators and vitamin modification treatments in the shoot cultures of the plant led to the development of *in vitro* culture systems for targeting sesquiterpene lactones and phenolic and flavonoids production. The development of conventional genetically non-modified roots in liquid culture led to an enhancement of the production of polyphenolics, as compared with the roots of the whole *in vitro* grown plant. Light regime, as compared to dark growth conditions, and NAA, as compared to IBA were shown to enhance polyphenolic production in the root cultures of *Inula britannica in vitro*. The present findings might further be used for the targeted scale-up delivery of different phytopharmaceuticals in the different types of *I. britannica in vitro* culture systems.

Keywords: *Inula britannica* tissue culture, different *in vitro* culture types, sesquiterpene lactones, polyphenolics production

This work was supported by Grant No. DN 09/11 of the Bulgarian Science Fund.

Composition and antibacterial activity of essential oils of *Laserpitium latifolium* and *L. siler* (*Apiaceae*)

PP4-14

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Essential oils obtained by hydrodistillation from aerial parts of two *Laserpitium* species (*Apiaceae*), wild growing in Romania (*L. latifolium* L.) and Serbia (*L. siler* L.), at flowering stage, were analyzed for their chemical composition and antibacterial activity against different food spoilage bacteria. The results obtained by GC-FID and GC-MS techniques revealed that some components such as *α*-pinene, *trans*-verbenole and *trans*-pinocarveol were predominant in both analyzed oils. In addition, yellowish *L. latifolium* oil was rich in *β*-pinene, sabinene and myrcene, while significant percentage of limonene, verbenone and p-mentha-1,5-dien-8-ol was identified in pale blue *L. siler* oil. Furthermore, tested *Laserpitium* species showed moderate antibacterial activity in applied microdilution method. The results indicated that *L. siler* exhibited bactericidal effectiveness in the range of 12.5 mg mL^{-1} to 25 mg mL^{-1} (MBCs) and it possessed stronger inhibitory effect on bacterial strains in comparison to *L. latifolium* oil. The most sensitive bacterium was *Enterococcus faecalis* (ATCC 19433) (MBCs=50 mg mL^{-1} and 12.5 mg mL^{-1} for *L. latifolium* and *L. siler*, respectively). Other food contaminators, *Enterobacter cloacae* (human isolate), *Listeria monocytogenes* (NCTC 7973) and *Salmonella typhimurium* (ATCC 13311), showed similar sensitivity to tested agents (MBCs=25 mg mL^{-1} for *L. siler* and MBCs=50 mg mL^{-1} for *L. latifolium*).

Keywords: *Laserpitium latifolium*, *L. siler*, essential oil, antibacterial activity

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Phenolic compounds in ethanol extracts of different plant organs of *Inula oculus-christi* L.

PP4-15

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Inula oculus-christi L. (family *Asteraceae*), grows naturally in Asia, Central Europe and the Balkan Peninsula. Sesquiterpenoids, flavonoids and phenolics as major secondary metabolites contribute to a variety of biological activities of this perennial species. In this research, an emphasis is given

to the analysis of phenolic compounds in ethanol extracts of leaves, flowers, stems, rhizomes, and roots of *I. oculus-christi*. The content of total phenolics (TPC) and total flavonoids (TFC) in extracts of different plant parts of *I. oculus-christi* was determined. Significantly high TPC and TFC were observed in extract of flowers, while the extracts of stems and roots had the lowest content of phenolic compounds. UHPLC–LTQ/orbitrap/MS analysis was performed for separation, identification, and quantification of the major phenolic components in *I. oculus-christi* extracts. A total of 46 compounds detected belong to three structurally distinct groups: 1) hydroxycinnamic acid derivatives (14 compounds); 2) flavonoid glycosides (21 compounds); and 3) flavonoid aglycones (11 compounds). Dominant hydroxycinnamates were derivatives of caffeic acid, esterified with quinic or aldaric acids. The relative abundance of these compounds was higher in extracts of flowers, leaves and rhizomes. From the flavonoid glycoside group, flavone (nepetin, luteolin, apigenin, and chrysoeriol) and flavonol (quercetin, patuletin, kaempferol, isorhamnetin, and 3,3'-dimethylquercetin) derivatives were found in tested samples. Flavonoid aglycones were the most abundant compounds in extracts of flowers. Observed differences in phenolic content highlight the possibility of targeted collection and application of different *I. oculus-christi* extracts considering previously determined biological activities.

Keywords: *Inula oculus-christi*, UHPLC-Orbitrap MS, hydroxycinnamic acid derivatives, flavonoid glycosides, flavonoid aglycones

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Elicitation effects of methyl jasmonate on secoiridoid production in shoot culture of centaury (*Centaurium erythraea* Rafn)

PP4-16

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Centaurium erythraea (centaury), a plant with a long tradition of medicinal use, contains bioactive secoiridoid glucosides (SGs). The exogenous application of methyl jasmonate (MeJA) may elicit a response similar to stress, and is used in the present study to induce SG accumulation and expression of SGs' biosynthesis associated genes in centaury's aerial parts. UHPLC-MS/MS analyses have indicated that the addition of 250 μM MeJA to the medium has caused overproduction of almost all investigated secondary metabolites. The most notable change was detected for swertiamarin production, which was several fold-higher in MeJA-treated plants. For the purpose of determining the key genes of MeJA response, we performed a qPCR analysis of all genes potentially involved in the secoiridoid biosynthetic pathway. After five days of MeJA treatment, an increase of relative gene expression was detected for *G8O*, *8HGO7*, *IO*, *7DLGT*, *7DLH2*, *SLS* and *CPR1*. On the other hand, in centaury plants subjected to MeJA treatment for ten days, there was no significant increase in gene expression. For *GPPS* and *LAMT*, decreased expression levels were detected, when

compared to control plants. Patterns of SGs biosynthetic gene expression are highly correlated to SGs' profiles during MeJA-elicitation treatments, giving clear evidence that the identified genes are a part of SGs' biosynthetic routes. All the results indicate that MeJA is a suitable elicitor that leads, through gene expression changes, to increased flux through the pathway and production of swertiamarin, sweroside and gentiopicrin, which can be used for future production of secoiridoids with the aid of biotechnological processes.

Keywords: *Centaurium erythraea*, gene expression, methyl jasmonate, secoiridoid glucosides, UHPLC-MS/MS analysis

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Total phenolic content and peroxidase activity in Salanova lettuce

PP4-17

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Lettuce (*Lactuca sativa* L.) belongs to a group of leafy vegetables commonly used as a salad in human nutrition. As minimally processed food product, it is an important source of vitamins, minerals and various antioxidative compounds. In our greenhouse experiment, two Salanova lettuce cultivars ('Aquino' RZ- green and 'Gaugin' RZ- red multi-leaf butterhead) were grown in the fertile soil during three successive seasons (autumn, winter and spring). The aim of the study was to examine the effect of different microbiological fertilizers (EM Aktiv, Vital Tricho and combination of EM Aktiv and Vital Tricho), cultivars and growing seasons on the total phenolic content and peroxidase activity. Our results showed that in all seasons red cultivar 'Gaugin' had higher total phenolic content compared to green cultivar 'Aquino'. Their highest level was measured in autumn with fertilizer Vital Tricho (549.14 μg GAE g^{-1} FW). The elevated phenolic status of red versus green cultivar was further strengthened by its higher level of peroxidase with the highest activity measured in spring with combination of fertilizers EM Aktiv and Vital Tricho (0.43 U mg^{-1} prot). The higher peroxidase activity and content of UV-absorbing phenolics, as plant-borne substrates of these enzymes, in the red cultivars indicate that the red leaves are better equipped to combat oxidative stress. Consequently, red cultivar 'Gaugin', with increased synthesis of health-promoting antioxidants, could be considered as functional food in human diet.

Keywords: lettuce, microbiological fertilizers, phenolics, peroxidase

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 173040).

Characterization of chemically modified pectins as novel material for various applications

PP4-18

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Pectin is a natural biopolymer contained in the plant cell wall. Ability of pectin to form hydrogels has been widely investigated for various purposes from food industry to biomedical applications. The goal of our study was to create modified pectin with improved gelling properties, that could produce stable covalently cross-linked hydrogels. Aromatic groups were introduced into the previously oxidized pectin chains in a reductive amination reaction with tyramine and cross-linking was achieved through enzyme reaction with soybean peroxidase in the presence of hydrogen peroxide. Characterization confirmed that a series of obtained tyramine-pectins (oxidized from 2.5 to 20 mol%) had different degrees of modification. UV spectra confirmed the presence of tyramine group with the absorbance peak at 275 nm. Chemical shifts of modified pectin in ^{1}H NMR spectra correspond to newly formed functional groups. The presence of C-C in-ring stretching vibrations peaks at 1518 and 1417 cm^{-1} in FTIR spectra of modified pectin confirmed the presence of aromatic rings. Increased nitrogen percentage in elemental analysis and additional ionizable group on the titration curve of tyramine-pectin proved the introduction of positively charged amino group. Surface morphologies also showed certain differences under SEM-EDS. Tyramine-pectin has been successfully tested as a carrier for enzyme immobilization, but potential applications of this material could be in tissue engineering, drug delivery or wound healing.

Keywords: pectin, tyramine, hydrogel

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. 173017.

Antioxidative potential of high-altitude *Plantago atrata* plants

PP4-19

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Plants produce a large variety of metabolites that assure their antioxidant potential against the cell damaging effect of reactive molecules generated due to developmental and environmental factors. *Plantago atrata*, also known as mountain plantain, belongs to the *Plantaginaceae* family and grows as a high-altitude mountain perennial plant that is subjected to extreme conditions, including increased ultraviolet light, low temperature, etc. Because of its habitat, this plant could

provide a valuable knowledge about the mechanism of antioxidative response. In accordance with our aim, we investigated *in vivo* plants collected in Bulgaria at mountain Vitosha (2200 m) during the period of flowering, as well as fully developed plants grown *in vitro* under controlled environment. Extracts from *in vivo* and *in vitro P. atrata* leaves were fractioned by sequential solubilization in non-polar (chloroform) and polar (methanol) solvents and then methanol extracts were subjected to Gas Chromatography–Mass Spectrometry analysis of the polar metabolite content (aminoacids, sugars, organic, phenolic acids). The methanol extracts were also evaluated regarding the total quantity of phenolics, flavonoids, antioxidant activity and reducing sugars. In accordance with the growth conditions, the data showed specific composition of identified primary and secondary metabolites. In both variants of plant cultivation, significant correlation was observed between the content of phenolics and sugars and the antioxidant activity; however, a strong reduction in all parameters was observed during *in vitro* cultivation. Our work provides information about antioxidative compounds with role in the adaptation of high-altitude mountain plant species.

Keywords: *Plantago atrata, in vivo, in vitro,* GC-MS, antioxidants

Physiological drought alters nepetalactone metabolisam in *Nepeta rtanjensis* leaves

PP4-20

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Major constituents of *Nepeta rtanjensis* Diklić & Milojević: *trans*,*cis*-nepetalactone and its dehydrogenation product dehydronepetalactone, are synthetized and accumulated in glandular trichomes. Interestingly, dehydronepetalactone is the major monoterpenoid in fresh leaves of *N. rtanjensis*, while the amount of this compound dramatically decreases in dry leaves. Furthermore, dehydronepetalactone has been previously identified in *Nepeta* species mainly in the cases when essential oils and extracts prepared from fresh plant material were analysed. All this lead us to presume that nepetalactone metabolism is reprogrammed during the process of leaf dehydration. Here we present for the first time an insight into the molecular background of constitutive nepetalactone biosynthesis in leaves of *N. rtanjensis* and its alterations under physiological drought stress, which was experimentally induced *in vitro* by exposing plants to PEG 8000 (3 MPa) for 1, 3 and 6 days. Leaves of PEG-treated and of non-treated plants were collected and subjected to gene expression analysis and to metabolic profiling. Putative genes encoding enzymes for intermediate steps of nepetalactone biosynthetic pathway (*GPPS*, *GES*, *G8O*, *8HGO*, *IS1* and *IS2*) were mined from *N. rtanjensis* leaf transcriptome. Although majority of analysed genes were significantly down-regulated during the process of leaf dehydration, PEG-induced physiological drought induced no significant changes in nepetalactone content, while dehydronepetalactone content was slightly decreased. The possible key enzymes controlling the nepetalactone biosynthetic-flux could be *GPPS*, *G8O*, and *IS1*, which showed stable expression levels during dehydration. Stressed plants

most likely maintain nepetalactone content stable by lowering both its biosynthesis and degradation, which results in decreased dehydronepetalactone content in leaves, and thus in altered nepetalactone/dehydronepetalactone ratio.

Keywords: nepetalactone, dehydronepetalactone, nepetalactone biosynthetic pathway genes, physiological drought

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Salvia sclarea L. essential oil as possible natural antimicrobial and antigenotoxic agent

PP4-21

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Clary sage (*Salvia sclarea* L.) belongs to genus *Salvia* (family *Lamiaceae*). This cultivar is also known as a "clear-eye" since its seeds are traditionally used to easily remove foreign objects from the eye. The essential oil obtained from the plant aerial part is widely used as an antiseptic, antidepressant, antispasmodic, carminative, and aphrodisiac. The aim of this study was to determine the pharmacological potential of selected essential oil, obtained by means of steam distillation, according to screened antimicrobial and antigenotoxic activity. The antimicrobial activity was assessed using the microdilution method against ten ATCC standardized microorganisms, nine bacterial strains (of which six G+ and three G-) and one fungi. The *in vitro* protective effect of the essential oil from *S. sclarea* against hydroxyl radical-induced DNA damage was also evaluated. The obtained MIC values pointed out good antimicrobial potency of tested essential oil against *Bacillus subtilis* (0.3125 μg μL^{-1}), *Bacillus cereus* (0.3125 μg μL^{-1}), *Enterococcus faecalis* (10 μg μL^{-1}), *Staphylococcus aureus* (25 μg μL^{-1}), *Staphylococcus epidermidis* (1.56 μg μL^{-1}), *Micrococcus lysodeikticus* (50 μg μL^{-1}), *Escherichia coli* (50 μg μL^{-1}), *Pseudomonas aeruginosa* (10 μg μL^{-1}), *Salmonella enteritidis* (10 μg μL^{-1}), *Candida albicans* (6.25 μg μL^{-1}). Antigenotoxic activity was dose-dependent, decreasing with higher dosages in a concentration range from 25 to 400 μg mL^{-1}. Conclusively, examined oil may be characterized as a potential therapy against infections caused by *Bacillus* strain as well as a supplement in cancer treatments as healthy cells protector.

Keywords: essential oil, *Salvia sclarea*, antimicrobial activity, antigenotoxic potential

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. III43004) and Progetti di Ricerca di Università 2015, Sapienza Università di Roma (Grant Nos. C26A15RT82 and C26A15J3BB).

Antimicrobial and antigenotoxic activity of *Ocimum basilicum* L. essential oil

PP4-22

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The essential oil from *Ocimum basilicum* L. (fam. *Lamiaceae*), obtained by means of steam distillation, was examined in order to determine its pharmacological potential by virtue of antimicrobial and antigenotoxic activity. Commonly called as sweet basil, considered oil is used worldwide as food flavor as well as in folk medicine for the treatment of headaches, diarrhea, coughs, constipation, warts, and kidney malfunctions; *O. basilicum* leaves are known for antiseptic properties as well as for the ability to destroy harmful bacteria in food; in the pharmaceutical industry, oil is used as an outstanding natural preservative. Herein, the antimicrobial activity of *O. basilicum* essential oil was determined according to microdilution method against six G+ and three G- bacterial strains, and one fungal species. Also, the protective effect of the essential oil on DNA damage induced by hydroxyl radical was investigated by evaluating several concentrations (25, 50, 100, 200, and 400 μg mL^{-1}). The tested essential oil showed good antimicrobial activity according to obtained MIC values against *Bacillus subtilis* (1.25 μg μL^{-1}), *Bacillus cereus* (0.078 μg μL^{-1}), *Enterococcus faecalis* (10 μg μL^{-1}), *Staphylococcus aureus* (10 μg μL^{-1}), *Staphylococcus epidermidis* (2.5 μg μL^{-1}), *Micrococcus lysodeikticus* (10 μg μL^{-1}) (G+); *Escherichia coli* (2.5 μg μL^{-1}), *Pseudomonas aeruginosa* (50 μg μL^{-1}), *Salmonella enteritidis* (50 μg μL^{-1}) (G- bacteria); *Candida albicans* (0.3125 μg μL^{-1}) (fungus). DNA protective activity of essential oil was in a concentration-dependent manner and decreased with increasing concentrations. Conclusively, examined oil may be characterized as a potential therapy against infections caused by *Bacillus* strain as well as a supplement in cancer treatments as healthy cells protector.

Keywords: essential oil, *Ocimum basilicum*, antimicrobial activity, antigenotoxic potential

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. III43004) and Progetti di Ricerca di Università 2015, Sapienza Università di Roma (Grant Nos. C26A15RT82 and C26A15J3BB).

UHPLC-Orbitrap MS characterization of total phenolics in French marigold (*Tagetes patula* L.) extracts

PP4-23

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Aqueous, ethanol and methanol extracts of aerial parts of ornamental French marigold (*Tagetes patula* L.), which consist mostly of flavonoids, have been reported to exhibit different biological activities, including cytotoxic effect on various human cancer cell lines. However, some of the most potent flavonoids characteristic for the genus *Tagetes*, such as quercetagetin and patuletin, are rare in nature. Thus, optimization of extraction process together with employment of sensitive techniques and detection equipment should be of interest in gaining information about marigold biologically active compounds. In the present study, separate extracts of leaves and flowers were obtained from dry plant material by overnight incubation in methanol, dichloromethane or hot (60 °C) water, followed by ultrasonic bath maceration. UHPLC–LTQ/orbitrap/MS, employed for separation and identification, revealed the presence of 68 different compounds in total, belonging to phenolic acids (20 compounds), flavonoid glycosides (37 compounds), and flavonoid aglycones (11 compounds). The highest number of compounds was detected in methanol extracts, i.e. 55 in flowers and 60 in leaves, while after the extraction in dichloromethane only 23 compounds were detected in leaves and 24 in flowers. In both leaves and flowers, the most diversified were flavonoid glycosides with quercetin and patuletin in 3, 7 or 3,7 di-*O*- derivativeforms. Aglycon patuletin was detected in all analyzed extracts, while quercetagetin was detected only in methanol and dichloromethane extracts of flowers. Obtained data confirmed *T. patula* extracts as a rich source of compounds with biological and potential pharmacological values.

Keywords: *Tagetes patula* L., UHPLC-Orbitrap MS, flavonoids, extracts

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Phenolic compound contents and antioxidant activity of various *Hypericum* spp. collected from the Republic of Macedonia

PP4-24

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The aim of this study was to evaluate the contents of total phenolics (TP) and flavonoids (TF), as well as the antioxidant activity (DPPH) in methanolic extracts of leaf, stem, flower and aerial parts from fourteen *Hypericum* taxa: *H. perforatum* var. *perforatum* L., *H. perforatum* var. *angustifolium* DC.,

H. perforatum var. *microphyllum* DC., *H. barbatum* var. *barbatum* Jacq., *H. barbatum* var. m*acedonicum* (Boiss. & Orph.) Boiss., *H. olympicum* var. *olympicum* L., *H. dimoniei* Vel., *H. cerastoides* (Spach) N.K.B.Robson, *H. annulatum* Moris, *H. montbretii* Spach, *H. richeri* subsp. *grisebachii* (Boiss.) Nyman, *H. rumeliacum* var. *rumeliacum* Boiss., *H. tetrapterum* Fries and *H. maculatum* subsp. *immaculatum* (Murb.) Fröhl. originating from the Republic of Macedonia. Flower and aerial part extracts from *H. perforatum* var. *perforatum*, *H. perforatum* var. *angustifolium*, *H. tetrapterum*, *H. richeri*, *H. barbatum* var. *macedonicum* and *H. maculatum* were shown to be the richest source of TP and TF compared to other tested species. The strongest DPPH scavenging activities were noticed in flower and aerial part extracts of *H. perforatum* var. *perforatum*, *H. perforatum* var. *angustifolium*, *H. tetrapterum*, *H. cerastoides*, *H. rumeliacum* and *H. maculatum*, as well as in leaf extracts from *H. barbatum* var. *macedonicum*, *H. olympicum*, *H. dimoniei* and *H. tetrapterum*. Present results revealed significant positive correlations between TP, TF and DPPH, indicating that phenolics and flavonoids are the major contributors to the antioxidant properties of tested *Hypericum* taxa. This study suggested that Macedonian *Hypericum* species represent promising source of phenolic compounds that could be used as natural antioxidants in food and pharmaceutical industry.

Keywords: antioxidant activity, flavonoids, *Hypericum* spp., phenolic compounds

Bioaccumulation abilities of different parts of *Iris pumila* L.

PP4-25

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The presence of certain elements in the soil controls their bioavailability to the plant. Uptake of elements depends on their concentration, solubility of occurring form and soil properties. Also, the propensity of the plant for the bioaccumulation process will condition the transfer of ions of elements from the soil to the various parts of the plant. Sometimes good bioaccumulation properties of plants can be used for their application for bioremediation of polluted areas.

The objective of the present study was to determine the concentrations of selected elements in the rhizome and above-ground parts of *Iris pumila* grown in Deliblato Sands (Serbia) in relation to its surrounding soil. Potassium and selenium contents (mg $kg^{-1}$of dry matter) were determined by ICP-OES method after digestion of samples. The concentration, transfer and accumulation of some elements from the soil to rhizome and above-ground parts were evaluated in terms of Biological Absorption Coefficient (BAC): $c_{plant\ part}/c_{soil}$. The concentration of K+ ions in soil, rhizome, above-ground vegetative parts and flower were: 1,845.3 mg kg^{-1}, 1,307.0 mg kg^{-1}, 4,461.4 mg kg^{-1} and 2,875.1 mg kg^{-1}, respectively. The presence of selenium as a trace element was confirmed both in the soil (0.112 mg kg^{-1}) and in the plant parts (rhizome - 0.156 mg kg^{-1}; above-ground vegeta-

tive parts- 0.135 mg kg^{-1}; flower - 0.122 mg kg^{-1}). The obtained results confirmed that the above-ground parts accumulated potassium ($BAC_{vegetative}$= 2.42; $BAC_{flowers}$= 1.56). Also, all plant parts have shown the ability to bond selenium from soil in significant quantities ($BAC_{rhizomes}$= 1.38; $BAC_{vegetative}$= 1.20; $BAC_{flowers}$= 1.08). This suggests that this specie have potential in phytoremediation.

Keywords: bioaccumulation; *Iris pumila*; Deliblato sands; phytoremediation

This study is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. 173025.

Organ-specificity and genotype-dependency of secoiridoid glucosides' constitutive biosynthesis in *Centaurium erythraea* Rafn

PP4-26

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Plant species *Centaurium erythraea* Rafn (fam. *Gentianaceae*) is characterized by the presence of secoiridoid glucosides (SGs) as dominant secondary metabolites. The SG biosynthetic pathway has not yet been fully elucidated, despite the great pharmacological importance of this species. Here, an insight into the SG biosynthesis is achieved by comparing chemical profiles and secoiridoid-related gene expression patterns of different *C. erythraea* genotypes and plant organs. The results revealed that leaves are the main site of secoiridoid biosynthesis and accumulation in *C. erythraea*. The key function in the secoiridoid glucoside biosynthetic pathway has been assigned to genes encoding GES, G8O, 8HGO, 7DLGT and 7DLH2, while for *SLS* and *CPR* a potential biosynthetic-flux regulatory role has been determined. The correlation between the levels of these genes' expression and SG content is evident in different plant organs. Also, the analysis of SG high- and low-productive genotypes of *C. erythraea* points out that chemical variability existing at intra-species level is, at least partially, determined by the different patterns of expression of SG-related genes in different genotypes. Taking into consideration the biological activity of secoiridoid glucosides, not only is the information obtained in this study of importance for further SG biosynthesis elucidation, but it also shows a great potential for future biotechnology-based sustainable production of these valuable metabolites.

Keywords: *Centaurium erythraea* Rafn, secoiridoid glycosides, qPCR, UHPLC-MS/MS

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Volatile compounds of hemicryptophytes - *Veronica montana* L.

PP4-27

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Forty species of the genus *Veronica* L. (*Plantaginaceae*) have been described in Croatia, eight of which belong to Central European hemicryptophytes: *V. aphylla* L., *V. austriaca* L. ssp. *austriaca*, *V. paniculata* L., *V. saturejoides* Vis. ssp. *saturejoides*, *V. scardica* Griseb., *V. teucrium* L. ssp. *crinita* (Kit.) Velen., *V. teucrium* L. ssp. *pseudochamaedrys* (Jacq.) Nyman and investigated species *V. montana* L. Species *V. montana* was collected from Tisovac near Crni Lug in Gorski kotar (Croatia), in the July 2016. Water distilled volatile compounds (VC) from aerial parts of investigated plant have been analyzed by GC and GC/MS (Adams, 2007) using VF-5ms capillary column and the peaks appeared from 18 to 65 minutes. The total yield of VC was 0.1%, based on dry weight of the samples. Fifteen compounds were determined, representing 89.9% of the total VC, which were characterized by a high concentration of oleic acid (43.8%), hexadecanoic acid (30.9%) and by two sesquiterpene hydrocarbons, caryophyllene oxide (7.2%) and germacrene D (5.2%). According to a literature review, GC-MS studies have been performed only for *Veronica thymoides* subsp. *pseudocinerea* and the most abundant constituent was hexatriacontene (21%) (Ertas et al., 2015). In our previous research in the oil of V. spicata one of the most abundant hydrocarbon compounds was heptacosane (Dunkić et al., 2015). The present study gives additional knowledge about secondary metabolites of the genus *Veronica*.

Keywords: *Veronica montana*, volatile compounds, oleic acid, hexadecanoic acid

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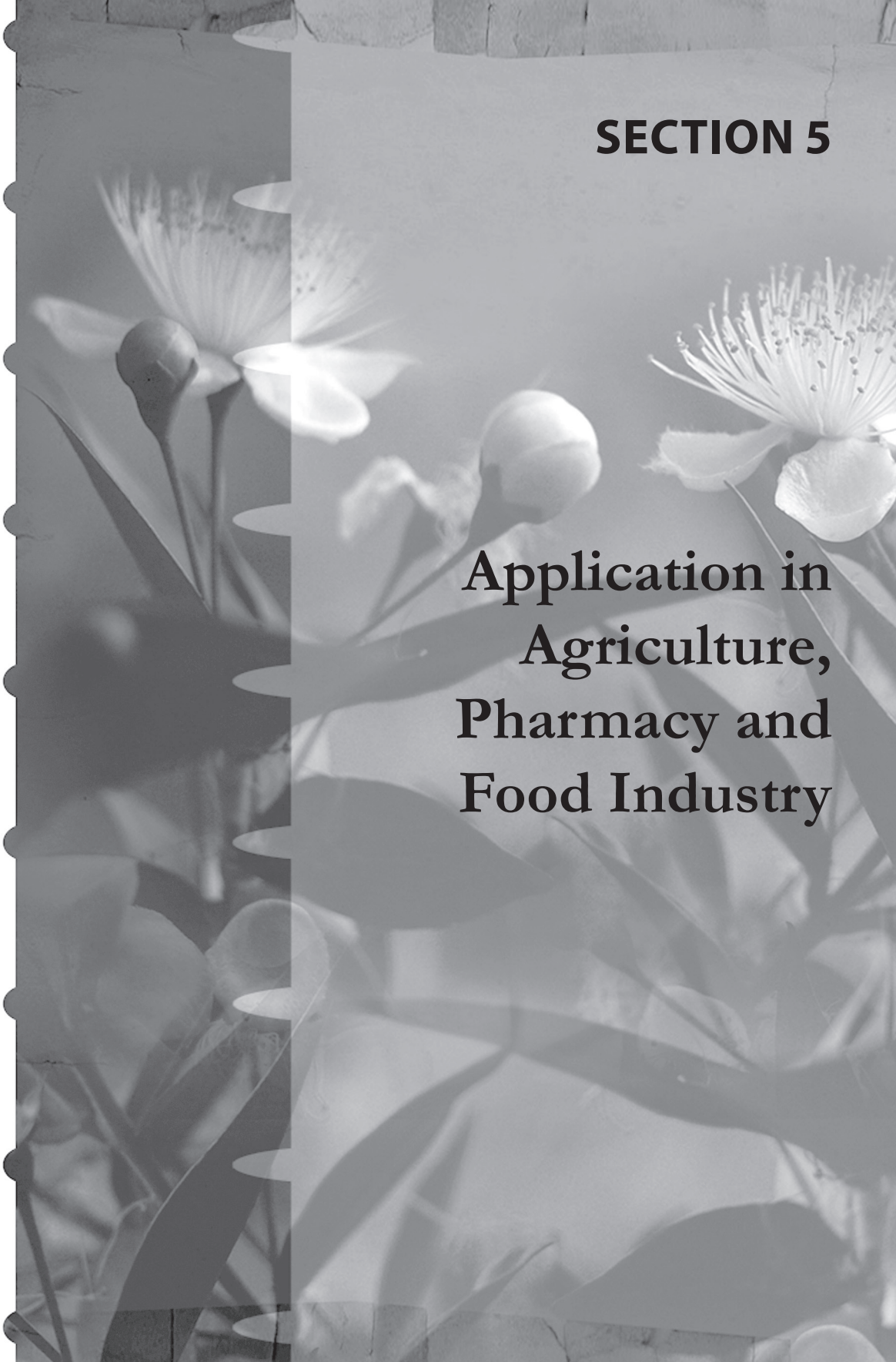

SECTION 5

Application in Agriculture, Pharmacy and Food Industry

PLENARY LECTURES

New factors controlling fruit development: epigenetic modifications associated with the fruit set transition in tomat

PL5-1

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Developmental shifts are associated with major genetic reprogramming in all living organisms. The flower-to-fruit transition, so-called fruit set, is triggered by flower pollination and this process is essential for plant reproductive success, seed formation and crop yield. The shift from flower to fruit provides a remarkable example of developmental transition that is associated with massive transcriptomic reprogramming. Epigenetics is emerging as a major mechanism regulating plant growth and adaptation to changing environment. Histone marking and DNA methylation are the main epigenetic modes for genetic reprogramming; however, their respective contribution to the fruit set-associated transcriptomic reprogramming is unknown. To gain insight into the mechanisms underlying this process, we combined genome-wide transcriptomic profiling, ChIP-sequencing and DNA bisulfite sequencing approaches. Our data indicate that global changes in gene expression are mostly associated with histone marks repositioning, whereas changes in DNA methylation concern a very minor fraction of differentially expressed genes. Moreover, the expression levels of genes essential for fruit setting such as those related to hormone metabolism, cell division, and embryo development, highly correlated with their H3K9ac or H3K4me3 marking status but not with change in DNA methylation. These findings seemingly contrast with previous reports indicating that DNA methylation plays a prominent role in reprogramming key genes during the ripening transition. In this respect, global transcriptomic reprogramming underlying the fruit set and fruit ripening transitions appears to rely on different modes of epigenetic regulation.

New breeding technologies for fruit trees

PL5-2

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Many studies have focused on manipulating levels of secondary metabolites in fruit through both conventional breeding and GM approaches. However, New Breeding Technologies (NBTs) offer ways of providing large changes in consumer traits (in addition to grower traits) if the public will accept the resulting fruit. Annualization of woody perennials has the potential to speed the breeding and production of fruit crops and rapidly improve horticultural species. Kiwifruit (*Actinidia chinensis*) is a recently domesticated fruit crop with a short history of breeding and tremendous potential for improvement. We used CRISPR/Cas9- mediated manipulation to target mutation of

CENTRORADIALIS (CEN)-like genes. Targeting these genes transformed a climbing woody perennial, which develops axillary inflorescences after many years of juvenility, into a compact plant with rapid terminal flower and fruit development. These changes have made kiwifruit amenable for accelerated breeding, indoor farming and cultivation as an annual crop. Using these plants, crossing has begun targeting higher levels of anthocyanins and carotenoids for future cultivars.

Keywords: CRISPR-Cas9, editing, flowering

INVITED TALKS

Food and Pharmacy Application of Anthocyanins Originating from Colored Grains

IT5-1

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Anthocyanins have been suggested as promising dietary compounds with an important role in human health due to their high antioxidant capacity. They are natural pigments responsible for red, purple and blue colors in plant kingdom. However, the use of numerous potential food plants as commercial sources of anthocyanin-based colorants is limited by availability of raw material and by economic considerations. Colored grains can be rich sources of anthocyanins. Cereal processing can generate a large amount of anthocyanin-rich by-products at low cost given that anthocyanin pigments are located in outer layers of the grain, which could be separated. In this study, the composition of anthocyanins in grain of blue popping maize, deep purple maize, purple wheat and black soybean was determined. The relationship between acylated and non-acylated forms of anthocyanins and their stability in alkaline condition has also been studied. In addition, their current and potential use as a natural food colorant, functional food ingredient and dietary supplement in food and beverage industry, as well as pharmacy is discussed. Ten anthocyanins were identified in blue popping maize, two of which are isomers of cyanidin-3-(malonylglucoside) and three of cyanidin-3-(dimalonyl-β-glucoside). In deep purple maize, purple wheat and black soybean, seven, nine and three anthocyanins have been identified, respectively. Acylated forms of cyanidin derivatives, as predominant anthocyanins, accounted for about 98%, 29%, 71% and 0% of the total content, respectively. According to our research, grain anthocyanin extracts, lyophilized and spray dried microencapsulates of anthocyanins, as well as colored cereals' flours gave good results in the production of bread, cookies, breakfast cereals, juices and smart packaging.

Keywords: anthocyanin composition, stability, natural colorants, functional food ingredients, health benefit

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant Nos. TR 31069 and OI 173040).

Microbial-based inputs: opportunities and challenges for sustainable and resilient agricultural productions

IT5-2

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The interest and market for microbial-based biofertilizers and biopesticides is increasing worldwide due to improved production technologies, deeper knowledge about the mechanisms of activity of the inocula, isolation and selection of new beneficial strains, and the trend in reducing the impact of agricultural practices on the environment. Different kinds of soil microorganisms belonging to several taxa colonizing the rhizosphere or the plant tissues can be utilized for the production of these bioproducts. However, their application in agricultural practice is still hindered by several factors. The main reasons derive from not always consistent results (derived from the still limited understanding of the relationships among microorganisms and between them and the plants), problems in identifying and tracking the inoculated strains in the field, as well as the technology of production. After an excursus on the key microorganisms utilized to improve plant productivity and their mechanisms of action, a review of the factors affecting the efficacy of these bio-products on crop productivity will be presented. In particular, factors related to the effect of farmers' practices on products' efficacy, the formulation options (multifunctional products) and the persistence and traceability of inoculants in soil will be considered, since they are important to assure the future wider use of bio-products. Finally, the legal issues related to quality control and definition of biofertilizers will be considered. The data will be presented to discuss the role of biofertilizers and biopesticides in developing an integrated and sustainable agricultural management system.

Keywords: biofertilizers, biopesticides, plant growth promoting microorganisms

Old problems, new tools - Integrated approach to oil crop breeding

IT5-3

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Oil crop breeding and selection is a continual process designed to increase yield levels and improve resistance to biotic and abiotic stresses. Breeders have been successful in producing a large number of varieties using conventional breeding methods which vary depending on the species. Development of biotechnology, notably genetic transformations, and appearance of new techniques such as genome editing, paved the way for more efficient trait introduction. The last

three decades have also seen tremendous advances in the evolution of marker systems and the respective detection platforms. The most common application of these marker systems in oil crop breeding is marker assisted backcross breeding for gene introgression, as well as mapping of agronomically important traits. Novel approaches in genotyping and phenotyping enabled more efficient data collection for identification of quantitative characters and elucidation of the genetic basis of agriculturally important traits. Old and new tools found their place in oil crop breeding. However, there is still room for improvement, especially in data collection and integration. That is why further efforts should be made at better combining of phenotypic and -omics data and their integration into the breeding process and identification of traits and markers of real practical value for the breeders.

Keywords: breeding, phenotyping, molecular markers, oilcrops

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. TR31025, Provincial Secretariat for Higher Education and Science of Vojvodina, Grant No. 114-451-2126/2016-03, Serbian-German bilateral cooperation project - No. 451-03-01732/2017-09/3 and COST Action CA 16212.

Plant terpenes and bioplastics

IT5-4

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Plants produce a large variety of highly functionalized terpenoids, in which the presence of, for example, partially unsaturated rings and carboxyl groups provides handles to use these compounds as feedstock for biobased commodity chemicals. Methylperillate, a monoterpenoid present in *Salvia dorisiana*, is useful for this purpose, as it carries both a ring and a methylated carboxyl group. By two mild chemical steps it can be converted to terephthalic acid, a precursor for PET plastic. In this work, we identified type VI-like trichomes from *Salvia dorisiana* as the site of biosynthesis and storage of methylperillate. mRNA from purified trichomes was used as a source to identify four genes that constitute the pathway towards methylperillate. This pathway includes a (-)-limonene synthase, a limonene 7-hydroxylase, and a perilla alcohol dehydrogenase. We also identified a terpene acid methyltransferase, perillic acid *O methyltransferase* (OMT), with homology to salicylic acid OMTs. Transient expression of these four genes, in combination with a geranyl diphosphate synthase to boost precursor formation, resulted in the reconstitution of the methylperillate pathway in *Nicotiana benthamiana*. This demonstrates the potential of these enzymes for metabolic engineering to produce a feedstock for biobased commodity chemicals.

Data Science in Biosystems

IT5-5

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Data science has witnessed an unprecedented spreading in various fields, from hotel booking, through seat allocations in airplanes, autonomous driving, to industrial intelligence and many other examples where data are used to train systems to explain complex phenomena. Various fields of certain similarity and overlap like pattern recognition, machine learning, artificial intelligence etc. are today commonly referred to as Data Science. Due to extreme complexity and lack of sufficient data from biosystems, there were not that many examples of successful applications in biosystems. As we are getting more and more data, we are getting the opportunity to explain complex models from the data in a novel way in different areas of biosystems – medicine, biology, agriculture, farming etc. Apart from the overview of data science applications in biosystems, we will give the insight in concrete state-of-the-art examples of application of Data Science in different fields of biosystems as well as perspectives for the future.

SELECTED TALKS

Prospects of cabbage leaf debris use in the control of *Fusarium* wilt of pepper

ST5-1

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In intensive agricultural production with narrow rotations, *Fusarium oxysporum*, a plant pathogen causing wilt disease of pepper, frequently reaches high density in soil, thus threatening production profitability. Reducing inoculum level below the critical threshold is very demanding because of extremely hardly resting, long-lasting chlamydospores. Soil disinfection with methyl bromide was the most effective way to control this pathogen for many years. However, the ban of methyl bromide use increased the interest in control alternatives. Many studies have shown that glucosinolates, biologically active secondary metabolites of *Brassicaceae* family of plants, and their derived products negatively affect plant pathogenic bacteria and fungi, insects, nematodes, and weeds. The glucosinolates occur in plants simultaneously with an endogenous *β*-thioglucosidase enzyme that is responsible, after tissue disruption (e.g. cut, ground or chewed), for the hydrolysis of glucosinolates into numerous biologically active products. The aim of this research was to estimate the prospects of cabbage leaf debris use in the control of *Fusarium* wilt of pepper. Fresh cabbage leaves were blended and incorporated in sterile substrate 7 days before inoculation of pepper plants at 5-7-leaf stage. Conventional fungicide captan and biofungicide based on *Trichoderma asperellum* were used for comparison. The assessment was performed 25 days after inoculation; the degree of wilting, plant growth parameters and the length of necrosis were determined. Fresh cabbage leaves were highly effective in *Fusarium* wilt control (91.3% compared to control), indicating a great potential for their use in the *Fusarium* wilt management.

Keywords: *F. oxysporum*, glucosinolates, methyl bromide alternatives

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Effects of tansy essential oil on fitness and digestion process of gypsy moth larvae

ST5-2

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Larvae of gypsy moth (*Lymantria dispar* L.) are major defoliators of deciduous forests and urban environment. The biological means of regulation of moth population density were incorporated along with conventional treatment in the integrative management concept. The effects of tansy (*Tanacetum vulgare* L.) essential oil (EO) to gypsy moths were monitored through the effects of residual contact and digestive toxicity on the mortality and development in the second instar larvae, but also through the growth and feeding indices of the fourth instar larvae. Although EO presence didn't cause residual contact toxicity or significantly affect larval mortality, tested oil notably elongated developmental time by delaying the molting moment and reduced percentage of molted larvae. On the other hand, EO digestion caused significant mortality in the second instar larvae (72% compared to control, at the end of experiment). Digestion of food enriched with tansy oil decreased the efficiency of the diet, and led to reduced mass gain, and therefore induced the delay of the molting moment, or completely stopped molting into the third instar larvae. Besides that, EO reduced the daily mass gain (0.06 mg mg^{-1} day^{-1} compared to 0.3 mg mg^{-1} day^{-1} in control) and the food consumption rate (0.61 mg mg^{-1} day^{-1} compared to 1.23 mg mg^{-1} day^{-1} in control) in the fourth instar larvae and caused decrease of the conversion rate of digested food to larvae body mass (10.62% compared to 26.05% in control). Despite that, food assimilation rate to larvae body mass was not significantly decreased, which moderated negative effects of EO digestion as well as reduced food intake.

Keywords: *Tanacetum vulgare* L., essential oil, *Lymantria dispar* L., mortality, toxicity

This work is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 173015)

Advantages and limitations of phytogenic feed additives

ST5-3

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Phytogenic feed additives (PFA) are a wide range of bioactive plant secondary compounds and metabolites with positive effects on animal health and performance. Lately, PFA have gained a lot of attention in livestock industry because they are considered to be a safe and efficient alternative

to antibiotics used as growth promoters, which were banned in the EU in 2006. The use of PFA also supports the concept of 'clean, green and ethical' animal industry, where "clean" stands for decreasing the use of antibiotics in treatments, "green" for decreasing mortality, animal waste and ammonia emission and "ethical" for improving animal welfare. PFA benefits in livestock industry are explained by numerous biological effects of plant secondary compounds (PSC): antibiotic, antioxidant, quorum sensing inhibiting, anti-inflammatory, digestive fluids stimulating, etc. Nevertheless, PFA use in animal industry is bellow expectation because *in vivo* effects on animal health and performance are not consistent and stability of PSC in feed production and in storage conditions is low. Explanation for ambiguous PFA biological effects relies on the fact that the mutual interaction of PSC is poorly understood as well as their mode of action and their bioavailability in gastrointestinal tract of animals. We argue that the first step in optimizing success rate of PFA is to understand the factors that influence production of PSC, such as plant genetics, plants growth conditions, developmental stage at harvest and processes of extracting active compounds. Increased research focus on determination of factors that influence biological effects of PFA is essential in helping livestock industry controlling quality and effects of PFA on animal production.

Keywords: phytogenic feed additives, quality control, biological effects

POSTER PRESENTATIONS

Chemical composition and allelopathic effects of *Vitex agnus-castus* L. essential oil on lettuce and radish seedling growth

PP5-1

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Vitex agnus-castus L. essential oil has a very wide range of applications and represents a very interesting object for the researches. For the purpose of the research, the commercially obtained essential oil of this plant species was used. The analysis included examination of the chemical composition and allelopathic effect on lettuce and radish seedlings. The chemical analysis included quantification of total phenols and Gas chromatography-mass spectrometry (GC/MS) analysis, while allelopathic effect was evaluated through the inhibitory effect of different oil concentrations (10, 20 and 30 μM mL^{-1}) on length of lettuce and radish seedlings and germination percentage. A relatively high concentration of total phenols and 79.70% of the components were quantified by GC/MS. By analysis of allelopathic properties, it was found that essential oil of *V. agnus-castus* showed inhibitory effect on the germination process for both analysed plant species. Lower concentrations of oil were stimulating for elongation of root while higher concentrations were inhibitory with phytotoxic effect on lettuce and radish seedlings.

Keywords: essential oil, phenols, GC/MS analysis, phytotoxicity, allelopathy

The composition of fatty acids and tocopherols in wheat bran oil

PP5-2

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Wheat is a significant component in human diet. The health effects of whole kernel utilization have been associated with their unique phytochemical compounds distributed in the endosperm, germ and bran fractions. The wheat brans represent rich source of tocopherols and essential fatty acids, such as linoleic acid (18:2n6) and linolenic acid (18:3n3). The aim of this study was to analyse tocopherol and fatty acid contents in bran oil of 17 wheat cultivars in order to identify genotypes with higher nutritional value. The oil was extracted from wheat bran using classical Rushkovsky method. Normal phase high performance liquid chromatography (NP-HPLC) with fluorescence detection was used for identification and quantitation of tocopherols. Trimethylsilyl esters of fatty acids were determined from wheat bran oils by using gas chromatography with a flame ionisation detector (GC-FID). Total tocopherol content ranged from 40.86 mg kg^{-1} (cv. NS rana 5) to 124.85 mg kg^{-1} (cv. Partizanka), with average content of 22.9 mg kg^{-1} α- tocopherols, 8 mg kg^{-1} β-tocopherols and 47 mg kg^{-1} γ- tocopherols. Cvs. Partizanka and NS 40S were identified as genotypes with significantly higher than average contents of all tocopherols. The content of 18:2n6, oleic and 18:3n3 acids were within the ranges of 55.85-73.05%, 9.09-24.82% and 3.63-7.81% of the total fatty acids, respectively. Our results showed that contents of both tocopherol and essential fatty acids (18:2n6 and 18:3n3) varied significantly in the investigated cultivars, suggesting that it is feasible to breed wheat cultivars with increased levels of beneficial phytochemicals for human health.

Keywords: wheat, bran oil, phytochemicals, nutritional value

This work is a part of the project TR31066 supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Antioxidant and antimicrobial activities of native and *in vitro* propagated *Micromeria croatica* (Pers.) Schott (*Lamiaceae*)

PP5-3

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The genus *Micromeria* Bentham (*Lamiaceae*) includes more than 70 species that are generally considered as aromatic since they produce considerable quantities of essential oils, which exhibit antimicrobial and antioxidant activities. *Micromeria croatica* (Pers.) Schott, an endemic Illyric-Balca-

nic species, was previously reported to possess considerable *in vitro* antioxidant activity. In view of the potential pharmacological value of this species, the present study was initiated to evaluate the antioxidant and antimicrobial activity of *in vitro* cultured *M. croatica*, in order to compare its biological activity with wild-growing plants. Antioxidant activity of methanolic extracts obtained from *in vivo* and *in vitro* plant material was evaluated using DPPH, ABTS and total reducing power Fe^{3+} to Fe^{2+} *in vitro* assays. Their total phenolic content and total flavonoid content were also determined. DPPH scavenging activity of methanolic extracts from wild-growing and micropropagated plants, similar to that of the reference compound BHT when applied at the same concentration, renders both extracts potent antioxidants. ABTS radical scavenging activity and total reducing power of *in vitro* grown *M. croatica* were considerably higher compared to native plant material, likely due to an increased content of total phenolics in plants grown *in vitro*. The antimicrobial activity of *M. croatica* methanolic extracts was investigated using broth microdilution method. Among six bacterial strains tested, *Bacillus cereus* and *Staphylococcus aureus* were the most sensitive microorganisms. Extracts obtained from *in vitro* cultures generally exhibited greater antibacterial potential, compared to wild-growing plants. Our results indicate that *in vitro* culture conditions affect both antioxidant and antimicrobial activity of *M. croatica*.

Keywords: antimicrobial activity, antioxidant activity, *Lamiaceae*, *Micromeria croatica*, micropropagation

This research was financially supported by The Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant Nos. 173015, 173030, 172047.

Allelopathic effects of essential oils of chosen representatives of *Thymus* L. genus on germination of *Ailanthus altissima* (Mill.) Swingle, an invasive species

PP5-4

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Allelopathy is a common biological phenomenon by which one organism produces chemicals that affect growth, development and survival of other organisms and because of that it represents an interesting field of research. For the purpose of this research, commercially purchased essential oils of *Thymus vulgaris* L. and *Thymus serpyllum* L. were used for germination test of *Ailanthus altissima* (Mill.) Swingle. The analysis included evaluation of allelopathic effect of different concentrations of essential oils (100, 200 and 300 μg mL^{-1}) and thymol (100, 200 and 300 μg mL^{-1}) as a component of these essential oils, on germination percentage of tested species. It was found that both essential oils had an inhibitory effect on the selected species (the highest recorded percentage of germination was 29%), whereas the essential oil of *T. serpyllum* showed stronger inhibitory effect (100% inhibited germination). It was found that thymol did not have significant inhibitory effect on seed germination of tested invasive species, which suggests that isolated compounds are not as effective as complex essential oil.

Keywords: allelopathic effect, essential oils, thymol

Towards pharmacological potential of edible lichen *Evernia prunastri* growing in the mountain region of southern Serbia

PP5-5

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Evernia prunastri (L.) Ach. (*Parmeliaceae*), edible lichen known as oak moss, is traditionally used for making breads by Egyptians. The lichen was tested *in vitro* in this study and linked with *diabetes mellitus* therapy (enzyme inhibition: anti-amylase and anti-glucosidase activities), Alzheimer's and Parkinson's disease treatment (enzyme inhibition: anti-cholinesterase and anti-tyrosinase activities), oxidative stress relief (antioxidant activity: free radical scavenging (DPPH and ABTS), reducing power (CUPRAC and FRAP), metal chelating and phosphomolybdenum assay) and bacterial infections treatment using *in vitro* antibacterial assay. We have determined total phenolic and flavonoid contents in ethyl-acetate (EtOAc) extract of *E. prunastri*. The effects were assessed for investigating biological profiles of *E. prunastri* EtOAc extract and describing the species as functional food. It was shown that *E. prunastri* possessed all biological activities investigated. The extract exhibited inhibitory effects on the activity of enzymes linked to *diabetes mellitus* and those linked to Alzheimer's disease; antioxidant activity and significant antibacterial activity against pathogenic and food contaminant bacterial species. The EtOAc extract of *E. prunastri* only failed in tyrosinase enzyme inhibition. This study showed that edible lichen *E. prunastri* might be classified as functional food with numerous health-beneficial effects in a dose dependent manner.

Keywords: *Evernia prunastri*, phenols, flavonoids, enzymes inhibition, antioxidant activity, antimicrobial activity

This work has been supported by the Serbian Ministry of Education, Science and Technological Development for financial support (Grant No. 173032).

Influence of bio- and magneto-priming on flax seedlings growth

PP5-6

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Flax (*Linum usitatissimum*) is an important food and fiber crop, due to its nutritional and nutraceutical qualities. Flax seeds have been consumed for at least 6,000 years, which makes it one of the world's first cultivated crops. Seed priming with living bacterial inoculum, termed biopriming, involves the application of plant growth-promoting rhizobacteria. The present study was con-

ducted to investigate the effect of growth-promoting rhizobacterium *Bacillus amyloliquefaciens* on the growth of flax seedlings under different strengths of magnetic field. Seedlings were soaked in bacterial suspension and bacteria were allowed to colonize. After ten minutes of soaking, the seedlings were exposed to magnetic field of 60 and 90 mT for 24 h, in dark under laboratory conditions. Untreated seedlings were used as controls. Shoot and root length, number and length of newly formed lateral roots and biomass production were measured after 1, 3, 5, 7 and 10 days. Results indicate that the combinations of magnetic field and plant growth-promoting bacteria gave half- to two-fold better results than bacteria or magnetic field alone. The best result was achieved in seedlings treated with *Bacillus amyloliquefaciens* and 90 mT magnetic field. This work showed a significant impact of bio- and magneto-priming on the improvement of flax seedling growth and biomass productivity.

Keywords: *Bacillus amyloliquefaciens*, *Linum usitatissimum*, static magnetic fields

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant Nos. 173015, TR 31080 and III 45003).

Effect of seed magneto-priming on flax (*Linum usitatissimum*) seed germination and subsequent seedling growth

PP5-7

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In the context of seed technology, the use of magneto-priming, as a method for increasing plant production, offers advantages in comparison to conventional treatments with chemical substances. Magneto-priming is a cheap, non-invasive technique, based on the application of magnetic fields (MF) and described as eco-friendly, with proved beneficial effects on seed germination and subsequent plant biomass yield. In this study, seeds of flax (*Linum usitatissimum*), placed on moist filter-paper, were treated with 100 mT MF strength, with exposure time of 0, 24, 48, 72, 96 and 120 h and kept in dark under laboratory conditions. Seeds that were not exposed to magnetic field were used as controls. Efficacy of MF on seed germination and seedlings height, shoot and root length and biomass was evaluated. The MF promoted seed germination rate by 10%-40 %, depending of treatments, compared to control. The growth parameters were better in seedlings exposed to all magnetic treatments, with an increase of up to 60% over the control. MF treatment of 24 h had a 50% better effect than 120 h MF treatment. In addition, the best biomass yield of acclimated plants was achieved after the shortest magnetic exposure treatment. This study suggests a positive effect of stable MF on flax seed germination and subsequent growth of seedlings. Therefore, magnetic field may be useful in organic agriculture in replacing the usage of plant growth regulators.

Keywords: *Linum usitatissimum*, seed germination, static magnetic fields

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Antioxidant activity, total phenolic content and allelopathic effects of *Juniperus communis* essential oil

PP5-8

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There is a great demand for different medical and aromatic plants in the world. Nowadays their use is limited to pharmaceutical and cosmetic industry. However, by exploring the biochemical properties of essential oils, their usage could expand further to medicine, ecology and agriculture. The aim of this research was to examine antioxidative properties, total phenolic content and allelopathic effects of the commercially purchased *Juniperus communis* essential oil on lettuce (*Lactuca sativa*) and radish (*Raphanus sativus*) seeds. The antioxidant potential of the extract was determined using DPPH free radical scavenging method. Antioxidant activity was expressed as IC_{50} value. The total phenolic content was determined using Folin Ciocalteu reagent. The IC_{50} value of *J. communis* essential oil was 20.30 mg mL^{-1}, while the total phenolic content was 0.06 mg GAE mL^{-1}. The inhibitory effect of different essential oil concentrations (10, 20 and 30 µg mL^{-1}; water was used as a control) on the germination rate and the root and seedling length was also analysed. *J. communis* essential oil did not cause noticeable variations in germination percentage in seeds of both tested species. The average germination rate for all treatments was above 82%. According to these results, the *J. communis* essential oil at applied concentrations did not have an inhibitory effect on the growth of both salad and radish seedlings.

Keywords: essential oil, *Juniperus communis*, allelopathy

Dry matter content in sunflower plant organs depending on the seed size

PP5-9

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The mass of the plants depends on assimilates, which are formed in the process of photosynthesis and their amount varies depending of different factors. In this study the influence of seed size on the sunflower plant dry matter content at the bud stage was examined. Natural seeds of sunflower hybrid Oliva were separated into six grades. First, seeds were separated in two grades according to the dimensions, and then seed separation according to the specific weight within these grades was carried out. The field experiment during two growing seasons was carried out at two localities. The accumulation of dry matter was observed in bud stage. The individual plant organs were analyzed – stems, leaves, petioles and buds. Data were analyzed using three-way ANOVA for a split-slit-plot model design. The dry matter content in the plant organs was significantly influenced by weather conditions in experimental years, localities, as well as the seed grades. At this stage, the highest content of dry matter was in the buds (39.22%), followed by leaves (13.86%),

stems (9.99%) and finally the petioles (7.68%). The highest dry matter content of stems was observed in grades of large and heavy seeds (10.01%-10.41%), while the lowest was observed in grades of small and light seeds (9.72%-9.93%). The results obtained for leaves were the same as the results for stems, while the dry matter content of petioles and buds was reversed. Also, the results of this study indicate that there is no regularity in the amount of dry matter collected by the seed grades of the tested hybrid.

Keywords: sunflower, seed size, plant organ, dry matter content

This research is part of the Project TR 31025: Development of new varieties and production technology improvement of oil crops for different purposes, funded by The Ministry of Education, Science and Technological Development of the Republic of Serbia.

Comparison of seed germination and seedling growth of wild and cultivated sunflower

PP5-10

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The physiological parameters of the seeds were monitored in 4 annual wild sunflower species and 7 genotypes of cultivated sunflower. Seed germination and parameters of growth (seedling mass and length of seedling shoots and roots) were tested under laboratory conditions using the standard laboratory test. Wet sterilized filter paper was used as the germination medium. The emergence in field conditions of cultivated sunflower was also tested. Field emergence is determined by counting the plants between the phase of well-developed first pair and the beginning of development of the second pair of leaves. The obtained results indicate differences in seed germination of wild (averaged 73%) and cultivated sunflower (averaged 95%), where the germination of cultivated sunflower was expected to be higher. Comparing the germination of cultivated sunflower in laboratory and field conditions, it is noted that the germination was lower in the field (averaged 72%), and that in laboratory conditions it was uniform in all genotypes, while significant differences were observed in the field. After analyzing the results of growth parameters measurements, the differences between the length of wild sunflower seedling roots (1.5-2.5 cm) and shoots (1.7-3.1 cm) and the length of cultivated sunflower seedling roots (3.4-9.0 cm) and shoots (3.9-6.9 cm), as well as within the studied groups, were observed. However, in the case of seedling dry mass, the differences were observed between genotypes but not among the studied groups. Furthermore, it was noticed that some wild sunflower genotypes created twice as much amount of dry matter (6.0-26.0 g per plant) as compared to cultivated genotypes (4.28-11.17 g per plant).

Keywords: wild and cultivated sunflower, germination, length of seedlings roots and shoots, dry matter

This research is part of the Project: Anatomic characterization of wild sunflower collection as a potential genepool for cultivated sunflower breeding in Vojvodina (Grant No. 114-451-2126/2016-03), funded by The Provincial Secretariat for Higher Education and Scientific Research, APV.

Aggressiveness of *Monilinia* spp. towards detached plum fruit

PP5-11

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Stone fruit species are by far the most important for total fruit production in Serbia, with plum being the most important. Over the last five years, total annual plum production amounted to over 500,000 tons, placing Serbia among the three top producers in the world and the first in Europe. However, plum is severely affected by brown rot disease that occurs every year, resulting in significant losses. *Monilinia laxa* and *Monilinia fructigena* are widely distributed brown rot causal agents, with first reports of their presence and significance in Serbia originating from the middle of the 20th century. *Monilinia fructicola*, the most destructive pathogen of *Monilinia* spp., has recently been introduced into Serbia. Its spreading in stone fruit orchards has already been observed. The aim of this research was to compare aggressiveness of newly introduced *M. fructicola* with well-established *M. laxa* and *M. fructigena* species towards plum fruit, in order to assess the potential of *M. fructicola* to repress and replace the other two species in Serbian plum orchards. Unwounded and wounded fruits of two plum cultivars at three developmental stages were inoculated with *M. fructicola*, *M. laxa*, and *M. fructigena*. Seven days after inoculation, the fruits were visually examined for symptoms of brown rot. A CART model, describing the probability of infection establishment was constructed. The results showed that wounding of fruits, developmental stage of fruits and species of the pathogen had significant effects on the incidence of brown rot, while the effect of cultivar was not significant.

Keywords: brown rot, stone fruit, disease incidence

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Coriander essential oil in battle against *Staphylococcus aureus* and *Candida albicans*

PP5-12

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Increased prevalence of antimicrobial-resistant strains in immunocompromised patients is a major clinical challenge for the treatment of *Staphylococcus aureus* and *Candida albicans* infections, and it has prompted the search for potent, novel antibacterial drugs or complementary agents against resistant pathogens which with new targets or novel mechanisms, distinct from

currently used antimicrobial therapies. Therefore, we examined coriander essential oil (*Coriandrum sativum* L.) aimed to determine its activity against growth and biofilm production of oral isolates *S. aureus* and *C. albicans*. Coriander oil was evaluated for antibiofilm activity against *S. aureus* and *C. albicans*, as well as against polymicrobial biofilm produced by these strains. Commercially available antimicrobial agents, amoxicillin and ketoconazole were used as controls. Chemical analysis of essential oil showed that the main component of oil is linalool (69.6%). All tested strains had similar sensitivity to coriander oil. The essential oil exhibited good antimicrobial activity and reduced biofilm formation in a dose dependent manner. Considering that *S. aureus* and *C. albicans* are leading opportunistic bacterial and fungal pathogens, this makes the tested coriander essential oil a significant antimicrobial and antibiofilm agent. Even though antimicrobials proved to be more effective against the tested microorganisms than the essential oil, considering their side effects and misuse, data indicating potent naturally sourced activity seems to be of great importance.

Keywords: coriander oil, *Staphylococcus aureus*, *Candida albicans*

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Synergistic interactions among pizza ingredients

PP5-13

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Pizza is one of the most popular foods in the world and a common fast food in Europe and North America. The most famous pizzas usually contain tomato and champignons, as well as oregano and basil as spices. The aim of the present study was to examine total phenolic content and antioxidant activity of both individual and combined extracts of main pizza ingredients in order to investigate possible synergistic effects in their combinations. Tomato concentrate was extracted using hexane and hot water, and the obtained extracts were dissolved in DMSO and mixed to obtain tomato extract (T). Fresh champignons (FCh), both fresh and dry oregano (FO, DO) and basil (FB, DB) were extracted using hot distilled water. Subsequently, the combined extracts were prepared by mixing the individual extracts as following: 50%T + 40%FCh + 10% spice, and 50%T + 40%FCh + 5% spice (FO/FB) + 5% spice (DO/DB). Total phenolic content (TPC) of extracts was measured using Folin-Ciocalteu reagent, while antioxidant activity was evaluated using total reducing power (TRP) assay. The interactions were determined using combination index (CI), where CI>1.1 was evaluated as synergism. The extracts of DO and FO showed the highest TPC (>90 mg GAE g^{-1}) and TRP (>1400 μmol AAE g^{-1}). The extracts showed synergistic interactions in their combinations, with the highest CI indices for combination TFChFBFO and especially for TFChDBDO. The obtained findings indicate that the conventional usage of fresh or dry spices in pizza enhances the beneficial effects of tomato and champignons.

Keywords: pizza, spices, antioxidant, phenolics, synergism

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Phenolic content, antioxidant and antineurodegenerative activities of *Thymus serpyllum* and *T. vulgaris* cultivated in Serbia

PP5-14

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Genus *Thymus* L. (thyme) belongs to the *Lamiaceae* family and consists of about 215 species of herbaceous perennials and small shrubs. Thyme has a long history of being used in traditional medicine for treatment of various diseases in the form of tea, tincture or syrup. Our current study was aimed to examine the antioxidant and antineurodegenerative potentials of *Thymus serpyllum* and *T. vulgaris* from Serbia. Crude extracts were prepared using 70% methanol, 70% ethanol and hot distilled water, and were diluted to the concentrations of 0.1 mg mL^{-1}, 0.25 mg mL^{-1} and 0.5 mg mL^{-1}. All the activities were measured spectrophotometrically, including the total phenolic (TP), flavonoid (TF) and flavonol (TFl) contents. DPPH, ABTS, FRAP and β-carotene bleaching assays were used for the assessment of the antioxidant activity, whereas the antineurodegenerative activity was evaluated using acetylcholinesterase (AChE) and tyrosinase (TYR) inhibition assays. The results showed that at the concentration of 0.1 mg mL^{-1}, *T. serpyllum* extracts were richer in TP content, whereas *T. vulgaris* extracts showed slightly higher concentrations of TF and TFl. Additionally, at the same concentration, both methanolic and ethanolic extracts of T. vulgaris exhibited stronger effects in all of the antioxidant assays compared with *T. serpyllum*. All the presented results were concentration dependent. Moreover, both plants showed similar rate of enzyme inhibition (from 54.50–73.38% in AChE and from 60.64–65.86% in TYR assay at the concentration of 0.1 mg mL^{-1}). The given results indicate that both species cultivated in Serbia are potent antioxidant and antineurodegenerative agents.

Keywords: thyme, antioxidant activity, antineurodegenerative activity, phenolic content

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Nepeta nuda tincture Vs. Listerine – who wins the battle against oral pathogens?

PP5-15

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Antimicrobial activity of *Nepeta nuda* L. (*Lamiaceae*) tincture and Listerine against a selected group of oral pathogenic microorganisms (4 bacterial species and 9 fungal strains) was explored. The activity of *N. nuda* tincture and Listerine to inhibit the formation of biofilm has been compared as well as their potential to demolish biofilm that is already established. Furthermore, based on *in vitro* experiments, the amount of *N. nuda* tincture and swishing time necessary for reaching better or equivalent antimicrobial effect as Listerine were predicted. Chemical constituents in *N. nuda* tincture regarding phenolic compounds were determined by LC-DAD/ESI-MSn. Both Listerine and *N. nuda* tincture possessed good antimicrobial potential, and acted on biofilms on two different developmental stages. When dealing with bacteria that have biofilm forming ability, *N. nuda* tincture would be highly recommended since it showed much better potential to inhibit formation of biofilms. For application via swishing, both mouthwashes should be used for 30 s when dealing with selected microorganisms in general and for 60 s (*N. nuda* tincture, 100 mg mL^{-1}) when dealing with bacterial biofilms. Recommended volume of liquid for swishing is 20 mL. Chemical profiling showed rosmarinic acid and verminoside as the most dominant phenolic compounds present in the *N. nuda* tincture. So, who wins this battle? The authors could conclude that both mouthwashes stand shoulder to shoulder when it comes to exhibiting antimicrobial potential, and since Listerine has been in wide use for long time period, and it would be an attractive challenge to put some competition on the market.

Keywords: mouthwash, oral microorganisms, chemical constituents, biofilms

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Strains of green microalgae as a promising feedstock for commercial fatty acid production

PP5-16

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Microalgae have received a lot of attention as a source of high-lipid feedstock to be used for the production of renewable energy in the form of biodiesel or other types of fuel, such as bioethanol, biobutanol and biogas. In addition, many microalgal strains produce significant amounts of essential fatty acids (such as ω3- and ω6-polyunsaturated fatty acids) which may have large importance in nutrition, cosmetics, and pharmacy. Recent investigations demonstrated that a group of freshwater green microalgae – desmids (*Zygnematophyceae*, *Streptophyta*) might have a significant potential for the commercial fatty acid production. Over 20 desmid strains were grown in standard cultivation conditions to assess their biomass and fatty acid content and productivities during the growth phases. The desmid strains investigated demonstrated the moderate values of biomass productivity (around 0.08 g CDW L^{-1} d^{-1}), being the highest in *Staurastrum boreale* (0.14 g CDW L^{-1} d^{-1}). The six desmid strains belonging to *C. crenatum* var. *boldtianum*, *C. meneghinii*, *C. regnellii* var. *pseudoregnellii*, *C. leave*, *Staurastrum boreale* and *S. punctulatum* had the exceedingly high total fatty acid contents (>200 mg g^{-1} CDW). The high amounts of linoleic and palmitic acids, and the low amount of oleic acid influenced the characteristics of desmid biodiesel, which partly satisfied the demanded standards for biodiesel. Hence, the improvement of the desmid biodiesel is recommended by the enhancement of the desmid growth conditions and/or by the partial hydrogenation. In addition, palmitic, linoleic and hexadecadienoic acids from desmid extracts can be utilized in cosmetics, pharmacy, medicine, and in industry.

Keywords: green microalgae, *Cosmarium*, *Staurastrum*, fatty acids, biodiesel

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Evaluation of anticancer activity of *Plectranthus* spp. extracts

PP5-17

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The genus *Plectranthus* has been widely used in traditional medicine to treat numerous diseases, including cancer. In this study, 31 extracts obtained from 16 *Plectranthus* spp. with medicinal potential were evaluated for their anticancer properties. The cytotoxic effects of all extracts were assessed in non-small cell lung carcinoma cell line NCI-H460. Five most promising *Plectranthus* spp. extracts (*P. aliciae, P. japonicus, P. malvinus, P. stylesii* and *P. strigosus*) were additionally tested for growth inhibition activity in multidrug resistant (MDR) cell lines with P-glycoprotein overexpression: NCI-H460/R (non-small cell lung carcinoma) and DLD1-TxR (colorectal adenocarcinoma), and compared to their sensitive counterparts, NCI-H460 and DLD1. *P. strigosus* acetonic extract was shown to be the most active. Parvifloron D, a diterpene identified in this extract, was tested in NCI-H460 and NCI-H460/R cells, as well as normal human embryonic bronchial fibroblasts (MRC-5) to evaluate its selectivity against cancer cells. It displayed the same efficacy in sensitive and MDR cancer cells, implying that parvifloron D is not a substrate for P-glycoprotein. Flow-cytometric analysis revealed that while parvifloron D is not exported via the P-glycoprotein, it does not possess the potential to inhibit this transporter's activity in NCI-H460/R cells. This study provides valuable information on the use of the *Plectranthus* genus as a source of therapeutically useful compounds against cancer cells including those with MDR phenotype, as well as compounds potentially responsible for their activity such as abietane diterpene parvifloron D. Additionally, the bioactivities of several *Plectranthus* spp. not previously described are reported.

Keywords: *Plectranthus*, anticancer activity, parvifloron D

Anticancer properties of the abietane diterpene 6,7-dehydroroyleanone from *Plectranthus madagascariensis*

PP5-18

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Natural compound-based green chemistry has been the focus of current nanobiotechnology research to improve efficacy and safety of medicines through targeted drug delivery. The *Lamiaceae* family, widely used in traditional medicine, is a well-known source of natural compounds with anticancer properties. A wide spectrum of bioactive diterpenes have been isolated from the genus *Plectranthus*, including 6,7-dehydroroyleanone (DHR), a abietane found in the essential oil of *P. madagascariensis*. The biological activity of DHR was investigated in P-glycoprotein-overexpressing multidrug resistant (MDR) non-small cell lung cancer cell line (NCI-H460/R), its sensitive counterpart (NCI-H460) and normal human embryonic bronchial epithelial cells (MRC-5). DHR showed significant growth inhibition and slight selectivity against cancer cell lines when compared to normal cells. The results also confirmed that DHR is not a substrate for P-glycoprotein as it does not interfere with its activity. This diterpene was incorporated into Hybrid nanoparticles in order to increase treatment efficacy and delivery selectivity. Anticancer properties of this nanosystem were compared to the activity of DHR alone. Additionally, to address the problem of multidrug resistance, this nanosystem was tested against cancer cells with MDR phenotype. Coupling of DHR with Hybrid nanoparticles additionally improved the cytotoxicity of DHR, decreasing the IC_{50} value 8-fold in NCI-H460 cells and 5-fold in NCI-H460/R cells. These findings imply that combining natural products, such as DHR, with nanoparticles could be considered as a promising anticancer strategy with potential to overcome drug resistance.

Keywords: *Plectranthus*, 6,7-dehydroroyleanone, cytotoxicity, multidrug resistance, nanotechnology

Relationships among bryophytes and plant pathogenic fungi – a case study on *Monilinia laxa*

PP5-19

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Bryophytes are a group of higher plants spread all over the world. It is widely accepted that they have a very few predators having in mind their peculiar chemical contents. However, it is known that they cohabit with many different organisms giving them shelter or serving as a host. With the aim to study the biotic interactions of selected bryophyte species and pathogenic ascomycete fungus namely *Monilinia laxa*, we isolated the fungus and cultured it *in vitro*. Eight bryophyte species, both liverworts and mosses, were selected to test relationship with this fungus. The fungus inhabits stone-fruit trees both in wild and orchards, and thus we also chose some epiphytic bryophyte species growing on tree barks as well. Various extract types from these selected bryophyte species were tested *in vitro* on *M. laxa* and generally no significant interaction could be noticed suggesting that bryophyte either suppress or enhance the development of *M. laxa*. However, during these tests an interesting feature among bryophytes has been documented. The moss species *Polytrichum formosum* and *Cinclidotus fontinaloides* extracts had negative effects on *M. laxa* growth as compared to control treatment. The former one is terrestrial and the latter one is aquatic moss species, and commonly they do not cohabit in native stands with tested fungus as epiphytic bryophytes do. Thus, the mosses *P. formosum* and *C. fontinaloides* have potential to treat brown rot disease which is very serious disease of many stone fruit trees difficult to fight with. Moreover, they exhibit biopesticide potential as they are not harmful for the environment. Further study on these moss species and their biotechnical approach are in progress.

Keywords: mosses, *Monilinia laxa*, biotic relationships, biopesticide

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Agrobacterium-mediated genetic transformation of *Viola cornuta* L. "Lutea Splendens" with capsanthin-capsorubin synthase gene

PP5-20

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Viola cornuta L. 'Lutea Splendens' is a perennial ornamental plant with small yellow flowers that naturally grows in the Pyrenees in Spain and France. To develop novel cultivars with orange and red flower colors, we performed *Agrobacterium tumefaciens* (LBA4404)-mediated transformation with the binary vector pWBVec10a/P35S::Llccs::TNos that harbored capsanthin-capsorubin synthase (*Llccs*) gene from *Lilium lancifolium* under the control of CaMV35S constitutive promoter and the nopaline synthase (Nos) terminator. Capsanthin-capsorubin synthase catalyzes the conversion of anteraxanthin and violaxanthin, two yellow ubiquitous 5-6-epoxy-xanthophylls, into capsanthin and capsorubin, two red xanthophylls, respectively. Starting with hypocotyl explants, we developed a transformation protocol with 0.3% shoot regeneration efficiency. Histochemical assay for β-glucuronidase (GUS) activity showed *uidA* reporter gene expression in all putative *Llccs*-transgenic shoots. The presence of *Llccs* transgene, hygromycin phosphotransferase (*hpt*) selectable marker gene and *uidA* (GUS) reporter gene in all putative *Llccs*-transgenic lines were confirmed by PCR analysis. This is the first report on *Agrobacterium*-mediated genetic transformation of *V. cornuta* L. with the aim to introduce desirable traits into this species.

Keywords: horned pansy, flower color, capsanthin, capsorubin, carotenoid biosynthesis

This study was supported by the Grant No. TR31019 from the Ministry of Education, Science and Technological Development of the Republic of Serbia.

A wild plant as rich source of biologically active components and potential supplements to food products

PP5-21

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Sambucus nigra L., commonly known as elderberry, is wild growing plant from *Capriofoliaceae* botanical family. This plant is widely recognized for its extraordinary biologically active components and beneficial effect on human health. Therefore, elderberry is one of the medicinal plants of particular interest, suitable for pharmaceutical and food purposes [1]. The investigation includ-

ed collection of plant material and the preparation of extracts, using modern extraction techniques such as, microwave and ultrasound, as well as using two solvents (water and 50% ethanol) in sample to solvent ratio of 1:30. In the prepared extracts, the total content of phenols and flavonoids, antioxidant and enzyme inhibitory activities were analyzed. Antioxidant potential was analyzed using several different *in vitro* antioxidant assays: FRAP (Ferric Ion Reducing Antioxidant Power) IC_{50} = 2.024 ± 0.26 µmol eq Trolox mL^{-1} extract, NO (nitric oxide radicals) IC_{50} = 73.55 ± 6.23 µg mL^{-1} and metal chelating 10.38 ± 0.12 mg EDTAE g^{-1} extract. Total phenolic and total flavonoid content were determined spectrophotometrically. Enzyme inhibitory effects were tested against lipase (41.01 ± 1.19 mg OE g^{-1} extract) and tyrosinase (29.83 ± 1.43 mg KAE g^{-1} extract). Obtained results revealed strong antioxidative and inhibitory enzymatic activity, as well as a high content of total phenols and flavonoids. Thanks to the high biological activity, flower extracts might be used as supplements for various food products. Due to its pharmacological activity, *S. nigra* flowers are potentially excellent component of functional food.

Keywords: elderberry, biological activity, functional food, extraction techniques [1] Mikulic-Petkovsek et al. (2016). Journal of Food Chemistry 200: 134-140.

Plant growth promoting characteristics of the genus *Azotobacter*

PP5-22

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Selected strains of plant growth promoting (PGP) microorganisms represent a promising tool in sustainable agriculture relied on soil biological processes. PGP influence manifests through improvement of plant nutrition, stimulation of plant growth and immune response, and inhibition of plant pathogens. The main group of diverse plant growth promoting mechanisms is directed towards increasing the availability of nitrogen and phosphorus in the soil. *Azotobacter* sp. was mainly recognized as free-living bacteria involved in nitrogen fixation, but other plant growth promoting mechanisms have lately occupied more attention. The aim of the present research was to characterize the main plant growth promoting abilities of *Azotobacter* sp. with the specific emphasis on phosphate solubilisation process. The ability of *Azotobacter* sp. to solubilise inorganic phosphorus was tested by tracking the diameter of colonies and transparent zones on media with Ca_3PO_4 (NBRIP). The dynamics of phosphate solubilisation was monitored by pH changes in liquid NBRIP media. Enzymatic activity of *Azotobacter* sp. was tested by API ZYM test. *Azotobacter* sp. showed prominent potential to improve the availability of phosphorus in soil. The phosphate solubilisation index determined after two weeks was 3.4. The enzymatic profile of *Azotobacter* sp. showed positive reactions for both acid and alkaline phosphatase. *Azotobacter* sp. has significant plant growth promoting potential which is expressed through plant nutrition improvement. Besides its nitrogen fixation activity, *Azotobacter* sp. is capable to increase amount of available phosphorus in the soil using two different mechanisms: solubilisation of inorganic phosphate and mineralization of organic phosphorus compounds.

Keywords: plant growth promoting bacteria, *Azotobacter*, phosphate solubilisation

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The effect of different genotypes and growing seasons on the content of nitrate and vitamin C in lettuce leaves

PP5-23

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Lettuce is a cool season crop, grown worldwide and important vegetable usually used as a salad. It is rich in vitamin C, which has significant role as antioxidant, but lettuce can also accumulate toxic compounds like nitrates. The purpose of this study was to examine the effect of different lettuce genotypes and growing seasons on the content of nitrate and vitamin C in the outer and inner leaves. Six cultivars ('Kiribati' RZ, 'Murai' RZ, 'Aquino' RZ, 'Gaugin' RZ, 'Aleppo' RZ, 'Carmesi' RZ) were grown in the greenhouse experiment during autumn, winter and spring. Vitamin C was determined spectrophotometrically at 550 nm and nitrate colorimetrically at 420 nm. Vitamin C ranged between 3.64-12.41 mg per 100 g FW in autumn, 3.68-7.34 mg per 100 g FW in winter and 3.05-7.72 mg per 100 g FW in spring. Cultivar 'Carmesi' had the highest level of vitamin C in the outer leaves (9.15 mg per 100 g FW) and 'Gaugin' in the inner leaves (12.41 mg per 100 g FW), both in autumn. Nitrate content ranged between 258.77-908.2 mg kg^{-1} FW in autumn, 296.62-977.64 mg kg^{-1} FW in winter and 42.74-450.74 mg kg^{-1} FW in spring. Cultivar 'Carmesi' had the highest level of nitrate in the outer leaves (977.64 mg kg^{-1} FW) and 'Aleppo' in the inner leaves (701.45 mg kg^{-1} FW), both in winter. Nitrate content in all seasons and genotypes stayed within the limit of EC regulation for protected lettuce. Generally, vitamin C was allocated to the inner and nitrate to the outer leaves. Results showed that genotype and growing season can affect the content of vitamin C and nitrate in lettuce leaves.

Keywords: lettuce, season, vitamin C, nitrates

In search for the new bio-fungicide: grow suppression of fungal pathogen - gray mold disease (*Botrytis cinerea*)

PP5-24

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Gray mold disease caused by the necrotrophic fungus *Botrytis cinerea* affects many plant species, including crops. It can be found on over 200 dicotyledonous and couple of monocotyledonous plants, and cause significant damage in viticulture, horticulture, and production of vegetables and small fruit crops. It can infect mature or senescent tissues, plants prior to harvest, or even seedlings. It is conventionally treated by various groups of fungicides which can be harmful for environment and can induce resistance in *B. cinerea*. Thus, with the aim to search for bio-treatment of gray mold disease, we isolated *B. cinerea* from strawberry fruits and tested extracts from various

selected bryophyte species (both moss and liverwort species). The extracts of bryophytes were made in two different ways (methanol and ethanol extracts) with the aim to get various compounds set from each of the selected bryophyte species. The obtained crude bryophyte extracts were tested *in vitro* for their potential to affect *B. cinerea*. The results showed that all bryophyte extracts inhibited the growth of *B. cinerea*. However, ethanolic extract of the pleurocarp moss *Isothecium alopecuroides* was the most effective in growth inhibition of *B. cinerea* under *in vitro* conditions. Further investigations are ongoing, having in mind that these results already have huge potential in development of biopesticide for this pathogenic fungus.

Keywords: *Botrytis cinerea*, plant pathogen, bryophytes, biopesticide

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant Nos. 173024 and 173030), and Serbia-Montenegro bilateral project.

Application of different methods for measuring carotenoid status in maize grain

PP5-25

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Maize grain carotenoids are among the most abundant in cereals, exhibiting considerable diversity in the composition profiles. In this experiment, HPLC-DAD was used for lutein and zeaxanthin (predominant carotenoids) and β-carotene (proVA carotenoid) determination in flour of whole maize kernel. In addition, Raman spectroscopy, as a non-destructive method for pretreatment-free and rapid *in situ* screening of carotenoids status in different kernel regions, was applied. Six yellow maize inbred lines (L1-L6) were evaluated. After the spectral normalization, deconvolution was performed on three individual bands in three observed regions on kernel longitudinal section (aleurone cell layer, floury and vitreous endosperm), for all genotypes. It was found that the mean fitted area and intensity of three main carotenoid bands were the highest at 1520 cm^{-1}, medium at 1155 cm^{-1} and the lowest for the band at 1007 cm^{-1}, as spectral features used to identify carotenoids and quantify relative carotenoid concentration. Both the highest values for mean band fitted area and intensity observed in the floury endosperm, and the lowest in the aleurone cell layer, were recorded in the same regions for all genotypes evaluated. L1 and L2 exhibited the highest values, while L3 and L4 exhibited the lowest values for the average and total band area. Significant and negative correlation between carotenoid content quantified by HPLC-DAD and bands area fit for vitreous endosperm region obtained by Raman spectroscopy (-0.847; $p \leq 0.05$), and between carotenoid content and total bands area (-0.898; $p \leq 0.05$) indicated the competition for carbon supplies reflected through increased starch reposition over carotenoids accumulation.

Keywords: β-carotene, endosperm, lutein, Raman spectroscopy, *Zea mays* L.

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The screening of selected *Lamiaceae* species for antioxidant activity in relation to phenolic content of plant extracts

PP5-26

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This study presents the results of total phenolic and flavonoid content, as well as antioxidant activity of the ethanolic extracts of seven species from *Lamiaceae* family (*Leonurus cardiaca* L., *Lamium album* L., *Marrubium vulgare* L., *Micromeria juliana* (L.) Benth. ex Rchb., *Stachys recta* L., *Vitex agnus-castus* L. and *Melissa officinalis* L.). Total phenolic content is expressed in terms of gallic acid equivalents, GAE (mg GA g^{-1} extract). Its values are the following: *Leonurus cardiac*a – 60.50, *Lamium album* – 71.19, *Marrubium vulgare* – 74.64, *Micromeria juliana* – 104.28, *Stachys recta* – 128.39, *Vitex agnus-castus* – 133.06 and *Melissa officinalis* – 199.12 mg GA g^{-1} extract. The concentration of flavonoids is expressed in terms of rutin equivalent, RuE (mg Ru g^{-1} extract) and its values are: *Leonurus cardiaca* – 41.51, *Lamium album* – 49.14, *Marrubium vulgare* – 64.07, *Micromeria juliana* – 43.30, *Stachys recta* – 78.12, *Vitex agnus-castus* – 101.39 and *Melissa officinalis* – 168.83 mg Ru g^{-1} extract. Obtained results of the antioxidant activity of the extracts, expressed as IC_{50} values, ranged from 131.15 μg mL^{-1} (minimal value) for *Leonurus cardiaca* to 21.56 μg mL^{-1} (maximal value) for *Melissa officinalis*. A significant relation was observed between the investigated parameters of phenolic content and antioxidant activity. According to our research, the species *Melissa officinalis, Vitex agnus-castus, Stachys recta* and *Micromeria juliana* showed significant antioxidant activity and can therefore be regarded as promising candidates for natural plant sources with high levels of biologically active compounds.

Keywords: *Lamiaceae species*, secondary metabolites, antioxidant activity

This investigation was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. III41010).

Identification and differentiation of *Ascochyta* complex fungi from field pea (*Pisum sativum* L.)

PP5-27

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Ascochyta pisi, *Dydimella pinodes* and *Dydimella pinodella* are three worldwide-distributed fungal pathogens of pea that occur singly or in combination and are sometimes referred to as the *Ascochyta* complex. They are among the most prevalent and damaging pathogens of legumes worldwide and their identification is currently based on symptoms and morphology. Since the identification based on these criteria remains difficult and uncertain, the aims of this study were to identify the pea-associated *Ascochyta* species and estimate their molecular phylogenies, through

two main approaches: (i) using sequence data from the ribosomal internal transcribed spacer regions (ITS) and elongation factor 1-alpha (EF); and (ii) using specific PCR-based marker (IGS1). Eighty nine isolates assumed to be *A. pisi, D. pinodes* and *D. pinodella* of diverse geographical origins were used. Following DNA extraction, ITS and EF were amplified in all tested isolates. The partial sequences were used for identification and clarification of intra- and inter-species relationships. The phylogenetic analysis using ITS sequences revealed that the most *A. pisi* isolates formed clusters with high bootstrap values, but differentiation between *D. pinodes* and *D. pinodella* isolates was not possible. Phylogeny based on EF sequences enabled differentiation of *D. pinodella* isolates, but *A. pisi* and *D. pinodes* could not be separated. Amplification with primers specific for IGS1 marker resulted in different amplification profiles in all three fungi, enabling their identification and differentiation.

Keywords: ascochyta, differentiation, sequence, IGS1

Optimization of reaction conditions for phenol removal in batch reactor with horseradish peroxidase immobilized within tyramine-alginate micro-beads

PP5-28

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Removal of phenolic compounds from wastewaters was previously studied using different enzymatic approaches. In the presence of hydrogen peroxide, peroxidases are able to oxidize phenol-like compounds and form non-soluble polymers that could be easily removed from aqueous phase. Horseradish peroxidase (HRP) is the most investigated peroxidase used for phenol removal from waste effluents, but it can be easily inactivated during this process by excess of hydrogen peroxide. In order to increase operational stability of the enzyme, immobilization on different materials and various peroxide delivery systems were tested. In our previous work, we studied bioinspired hydrogels based on natural cell wall polymers and enzymes, for efficient removal of phenols from water. In this work, tyramine-alginate hydrogels that we have previously developed were used for horseradish peroxidase encapsulation within micro-beads obtained in a coupled emulsion polymerization reaction. The aim of this research was to study the influence of tyramine- alginate concentration and hydrogen peroxide delivery system on operational stability and efficiency of phenol removal by immobilized peroxidase. The best result of 96% phenol removal from water solution was achieved by peroxidase immobilized within 20% (w/v) tyramine-alginate micro-beads using delivery system for hydrogen peroxide composed of 0.187 U mL^{-1} of glucose oxidase and 4 mmol L^{-1} of glucose. The reusability studies showed that these biocatalysts can be used up to five cycles with slight decrease in their catalytic performance.

Keywords: immobilization, horseradish peroxidase, phenol removal, tyramine, alginate

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Phenolic content and antioxidant activity of *Lythrum salicaria* L. aerial part and root extracts

PP5-29

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Lythrum salicaria L. (*Lythraceae*) has been traditionally used for therapeutic purposes by European nations since ancient times. The present study explores phenolic content and antioxidant activity of *L. salicaria* aerial parts (LSA) and roots (LSR) methanolic extracts. The extracts used in this study were obtained by the maceration process. Spectrophotometric determination of phenolic compounds in extracts showed high presence of total phenolic compounds in both investigated extracts. LSR was richer in the content of total phenolic compounds (326.36 mg GKE g^{-1}) and condensed tannins (255.94 mg GKE g^{-1}), while LSA had higher content of total flavonoids (43.36 mg QUE g^{-1}), flavonols (9.14 mg RUE g^{-1}) and anthocyanins (17.84 mg Cy 3 glc g^{-1}). Both studied extracts exhibited considerable total antioxidant capacity, reducing power and radical scavenging activity *in vitro*. The root extract showed better activity in all antioxidant assays. In phosphomolybdenum assay, LSR was found to be more effective antioxidant than Trolox (1255.29 mg Trolox g^{-1}), while reducing power of LSA and LSR were 438.37 and 540.83 mg Trolox g^{-1}, respectively. $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ free radical scavenging activity of extracts were comparable to the reference antioxidants, BHT and gallic acid. The radical scavenging activity of gallic acid was the most prominent in reactions with $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ with IC_{50} values up to 3 μg mL^{-1}, while IC_{50} values of LSR in these assays were 11.52 and 12.33 μg mL^{-1}, respectively. Based on the obtained results, very good antioxidant activity of *L. salicaria* root extract may be a basis for its use in pharmaceutical and food industry.

Keywords: *Lythrum salicaria*, phenolic content, *in vitro*, antioxidant activity, spectrophotometric determination

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. III43004.

Finding of parthenocarpic fruit in *Gymnocladus dioicus* (L.) K. Koch in a plantation at Fruška gora (Serbia)

PP5-30

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The genus *Gymnocladus* (*Fabaceae*) consists of five species with reproductive system described as polygamous, monoecious and dioecious. The species *Gymnocladus dioicus* (L.) K. Koch has been present in Serbia for over 150 years. Its flower sexuality was described as dioecious, but there are references stating that there are flowers that are morphologically hermaphrodite and functionally monosexual having the sterile pollen. In the area of Belgrade, a sexually reproductive tree

with morphologically hermaphrodite flowers was recorded, but it is still needed to be checked for functional androdioecy. In this paper, we point out the finding of parthenocarpic fruits in *G. dioicus* in a large number of adult trees in a plantation at Fruška Gora (Serbia). Considering that during a multiannual period a mass occurrence of parthenocarpic fruits with concurrent absence of pods with characteristic seeds for this species was recorded, there is a possibility that the plantation was raised by clonal selection. So far, the occurrence of parthenocarpic fruits in *G. dioicus* was recorded on one isolated tree in France. The analyzed parthenocarpic fruits have the characteristic shape, structure and size that are in compliance with parthenocarpic fruits from France. The pods are seedless, i.e. with undeveloped seed about 1 mm in size, with the characteristic curved tip pointed to the ventral suture, with a length of 4.6-8.9 cm (6.9 cm on average), and width of 1.8-3.6 cm (2.6 cm on average). Research of the sexual dimorphism of *G. dioicus* is important for silviculture of this allochthonous species in Serbia, because in plantations at favourable sites, the individuals of a certain sex may achieve growth that is desirable for timber production, but with limited ability of subspontaneous dispersal by vegetative means.

Keywords: *Gymnocladus dioicus* (L.) K. Koch, parthenocarpic fruit, introduction, Serbia

In vitro antidiabetic activity of *Hypericum perforatum* L. hairy root cultures

PP5-31

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Methanolic extracts of *Hypericum perforatum* L. hairy root clones (HR B, HR F and HR H) and non-transformed roots (NTR) were evaluated for total phenolic (TP) and flavonoid (TF) contents and antioxidant activities using ABTS and CUPRAC assays. The *in vitro* antidiabetic effect of plant extracts was investigated through inhibition of α-amylase (α-AMYL) and *α*-glucosidase (α-GLUC) linked to diabetes mellitus. The HR clones were established by genetic transformation of *H. perforatum* with *Agrobacterium rhizogenes* strain A4. The TP contents in HR clones ranging from 12.56 to 25.46 mg gallic acid (GA) g^{-1} DWwere significantly higher compared to NTR (9.06 mg GA g^{-1} DW). Production of TF in HR clones varied from 6.18 to 18.26 mg catechin (C) g^{-1} DW in comparison to NTR (5.61 mg C g^{-1} DW). Results for ABTS scavenging activity in HR clones were ranged from 86.96 to 205.80 μM Trolox (T) g^{-1} DW, while NTR showed the lowest value of 61.23 μM T g^{-1} DW. Also, the CUPRAC values in HR clones (77.33-170.66 μM T g^{-1} DW) were markedly enhanced compared to NTR (53.64 μM T g^{-1} DW). With respect to *in vitro* antidiabetic properties, HR and NTR extracts showed considerable and dose dependent (250, 150 and 50 μg mL^{-1}) inhibition of α-AMYL and α-GLUC activities. The HR B clone at 250 μg mL^{-1} showed the highest inhibitory activities against α-AMYL (40.5%) and α-GLUC (91.3%). Present data suggested that *H. perforatum* transgenic roots represent a promising source of phenolic compounds with antioxidant and antidiabetic properties that could be used for treatment of hyperglycemia.

Keywords: antidiabetic activity, antioxidant activity, hairy roots, *Hypericum perforatum* L., phenolic compounds.

Rubus cultivars planted in Serbia – nutritional quality and potential health benefits

PP5-32

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Flavonoids and phenolic acids are the most common phenolic compounds in small fruits with strong antioxidant capacity. Their content in fruits varies among species and cultivars, depending on environmental factors and cultivation techniques. Raspberries are among the most popular berries in the agriculture of Serbia. They are consumed as fresh fruits or processed into various products. In this work ethanolic fruit extracts of different cultivars of *Rubus* spp. ('Čačanka bestrna', 'Loch ness', 'Thornfree', 'Evergreen' and 'Darrow') were examined for their total phenolic and flavonoid content, as well as free radical scavenging potential and metal chelating activity. Total phenolic content in the extracts was determined using Folin-Ciocalteu reagent and their amounts ranged between 53.6 and 141 mg GA g^{-1}. The highest phenolic amount was found in extract of 'Čačanska bestrna'. Quantification of flavonoids was evaluated using aluminium nitrate nonahydrate. The content of flavonoids in the extracts varied from 3.26 to 5.33 mg Qu g^{-1}. The highest flavonoid content was identified in cultivars 'Darrow' and 'Thornfree' extracts. Free radical scavenging potential of analyzed extracts was determined using DPPH and ABTS tests. According to obtained results of DPPH test, extract of 'Evergreen' berries had the highest activity, with IC_{50} value of 0.06 mg mL^{-1}. In ABTS assay, the antioxidant capacity ranged from 0.07 to 1.09 mg AA g^{-1} of dry extract. The highest free radical scavenging potential was found for 'Thornfree' extract. 'Evergreen' extract possessed significant reducing capacity (0.0172 mg AA g^{-1}) and reducing power in FRAP assay (0.26 µM Fe g^{-1}). Different *Rubus* cultivars (berries) could be recommended as functional food with good antioxidant potential.

Keywords: *Rubus* cultivars, phenols, flavonoids, DPPH, ABTS, TRP, FRAP

Antioxidant properties of anthocyanins from berries of *Rubus* cultivars

PP5-33

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Anthocyanins present in some fruits have shown to play very important role in the prevention of different diseases (cancer, cardiovascular diseases, degenerative disorders, etc.). They possess mechanisms responsible for various biological activities: antioxidant, anti-proliferation, anti-inflammatory, anti-angiogenic activity etc. Antioxidant activities which can explain some of the beneficial effects of anthocyanins are investigated in the present work. The total anthocyanins from different *Rubus* fruit cultivars were isolated according to Pharmacopeia, Ph. Eur. 6. DPPH and ABTS assays were used to test radical scavenging ability of anthocyanins. FRAP and TRC, procedures

based on reducing potential, are used for determination of total antioxidant capacity of examined samples of anthocyanins ('Čačanka bestrna', 'Loch ness', 'Thornfree', 'Evergreen' and 'Darrow'). The total anthocyanin content in the examined extracts ranged between 0.02 and 0.55%. The highest anthocyanin amount was found in extract of 'Darrow'cultivar. On the other hand, the content of anthocyanins from 'Čačanka bestrna' was very low. FRAP values varied between 0.1 μmol Fe^{+2} mg^{-1} ('Thornfree') and 0.05 μmol Fe^{+2} mg^{-1} ('Evergreen'). In TRC test, 'Čačanka bestrna' sample showed the strongest metal chelating activity (0.066 mg AA g^{-1}). BHA and L-ascorbic acid used as a control in this test had higher antioxidant activity. According to the obtained values, antioxidant capacity of anthocyanins was weaker than antioxidant activity of phenol or flavonoid compounds from the same samples. The antioxidant ability of anthocyanins depends on the basic chemical structure and sugar residues. The anthocyanin content in different berry fruit varies with the cultivar, ripening and climate characteristics.

Keywords: *Rubus* cultivars, anthocyanins, DPPH, ABTS, TRP, FRAP

Allelopathic effect of *Thymus serpyllum* L. and *Thymus vulgaris* L. essential oils on germination and seedlings growth in lettuce

PP5-34

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Today, essential oils of *Thymus serpyllum* L. and *Thymus vulgaris* L. are widely represented in the commercial production due to their medical and healing properties. Presented study analysed allelopathic properties of these oils using Petri dish assay by randomized design. The essential oil effects were evaluated using three concentrations of essential oils (100 μg mL^{-1}, 200 μg mL^{-1}, 300 μg mL^{-1}). *T. vulgaris* oil at the concentration of 300 μg mL^{-1} showed a significant reduction in germination rate (only 27% seeds germinated), while for *T. serpyllum* germination rate of lettuce was reduced by 50%. Inhibitory effects of analyzed essential oils were also recorded for shoot and root length of lettuce seedlings.

Keywords: essential oils, germination, lettuce, seedlings, thyme

Toxicity of plant essential oils against fungi causing dry bubble, wet bubble and cobweb disease of cultivated mushroom

PP5-35

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Biofungicides based on plant oils have many ecological advantages in comparison with chemical fungicides. Twenty-two essential oils from Germany and Albania were assayed for inhibitory and fungicidal activity against pathogens of cultivated mushroom (*Agaricus bisporus* (Lange) Imbach) using macrodilution fumigant method. Serious crop losses in mushroom cultivation in Serbia are induced by fungal pathogens *Lecanicillium fungicola* var. *fungicola* (Preuss) Hasebrauck, *Mycogone perniciosa* (Magnus) Delacroix, *Cladobotryum dendroides* (Bull.) W. Gams & Hooz., causal agents of dry bubble, wet bubble and cobweb disease. The strongest and broadest activity against all three pathogens was shown by the two samples of mint oil (*Mentha piperita* L.) at 0.02 μg mL^{-1}, from both Germany and Albania, followed by black pine (*Pinus nigra* L.), cade (*Juniperus oxycedrus* L.), lavender (*Lavandula angustifolia* Mill.), maritime pine (*Pinus pinaster* Aiton), oregano (*Origanum vulgare* L.) The most sensitive to plant essential oils proved to be *M. pernicosa*, being susceptible to 18 oils, while 14 oils were lethal to the pathogen. Fifteen oils (lavender, cade, eucaliptus (*Eucalyptus globulus* Labilardie), etc.) inhibited the growth of *C. dendroides* and twelve oils showed fungicidal effects to the pathogen. The least sensitive pathogen was *L. fungicola* var. *fungicola*, as 13 oils (cade, eucaliptus, etc.) inhibited growth of the fungi and 11 oils were lethal. These results imply that mint oil could contribute to disease control of cultivated mushroom against important fungal pathogens after further *in vivo* experiments.

Keywords: plant essential oils; antifungal activity; cultivated mushroom

This work is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant Nos. TR 31043 and III 44008.

Toxic elements accumulation by two truffle species (*Tuber* spp.)

PP5-36

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Truffles are ectomycorrhizal fungi, primarily from the genus *Tuber*, which form underground fertile bodies. In order to form a fruiting body, it is primary that the truffle gets into symbiosis with the roots of certain plants. In addition, truffles require special conditions for successful growth and development (microclimate conditions, physical and chemical properties of the soil, the compo-

sition of vegetation). On the other hand, economic interest in these fungi is on the rise. In some countries, truffles are considered as an agricultural product, whereas in others they are considered a wild product. In the Republic of Serbia, some of the famous truffle sites are Obedska bara, Deliblatska peščara, Fruška gora, Kosmaj, Rudnik and Oplenac. The aim of this work was to determine the level of toxic elements absorption by two truffle species collected in Obedska bara area: the black truffle *T. melanosporum* (most common one) and the white truffle *T. magnatum* (rare and highly valued one). The bioaccumulation factor (BAF) which represents the ratio of the concentrations of toxic elements in the truffle and the soil was calculated. Quantitative analysis of As, Cd, Cr, Hg, Ni and Pb was performed using the analytical technique of inductively coupled plasma with optical emission spectroscopy (ICP-OES). Concentrations of As, Hg and Pb were below the limit of detection, whereas Cd had the highest concentration in both samples. Consequently, in both species the highest BAF was observed for Cd, 1.734 and 1.070 for black and white truffle, respectively. Bioaccumulation of Cr was higher in black (0.037) than in white truffle (0.025), whereas Ni was detected only in white truffle. Results indicated that truffles readily accumulated Cd.

Keywords: truffle, toxic elements, bioaccumulation, ICP-OES

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 46009). Authors acknowledge MOL Institute for ICP analysis.

Comparative evaluation of antimicrobial and antigenotoxic potential of two *Onosma* species from Turkey

PP5-37

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The methanolic extracts of aerial parts of two species from the genus *Onosma* L. (*Boraginaceae*), *Onosma sericea* Willd and *Onosma stenoloba* Hausskn. ex Riedl from Turkey, were tested for their biological potential. Antimicrobial activity of *Onosma* extacts was assessed by microdilution method using selected bacterial and fungal strains. The comet assay was performed to evaluate the *in vivo* genotoxic and protective effect of *Onosma* extracts at different concentrations (20, 40 and 80 mg mL^{-1}) against the ethyl methanesulphonate-induced DNA damage in *Drosophila melanogaster*. The *in vitro* ability of extracts to protect DNA against hydroxyl radical was estimated. The contents of total phenolics and flavonoids in the extracts were also determined by spectrophotometrical measurements. Antimicrobial activity of tested extracts was moderately pronounced, with the lowest minimal inhibitory concentration values (MIC) of 2.5 mg mL^{-1}. Extracts at higher concentrations showed the absence of genotoxicity. The antigenotoxic effect was evident after treatment with both extracts with percentage reduction over 80%. In concentration range from 25 to 400 µg mL^{-1}, the protective effects of the extracts against hydroxyl radical-induced DNA damage were dose-dependent, increasing with higher dosages. The results showed much higher content of total phenolics in *O. sericea* than in *O. stenoloba* extract (69.79 and 32.46 mg GAE g^{-1}, respectively).

Flavonoids were the dominant class of bioactive compounds in *O. sericeum* extract (52.62 mg RE g^{-1}), while they were present in *O. stenoloba* extract in much lower quantity (8.36 mg RE g^{-1}). Overall results indicate that tested *Onosma* extracts have a great potential for further biomedical investigations.

Keywords: *Onosma sericea*, *Onosma stenoloba*, phenolic content, antimicrobial activity, antigenotoxic potential

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. III43004.

Alchemilla vulgaris L.: Phytochemical profile and ameliorating effect against cisplatin-induced oxidative damage

PP5-38

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Alchemilla vulgaris L. (*Rosaceae*) is known in traditional medicine for its anti-inflammatory, diuretic and astringent properties. The aim of this study was to investigate the ameliorative effect of methanol extracts of aerial parts and roots of *A. vulgaris* (AVA and AVR, respectively) against cisplatin-induced hepatorenal and testicular toxicity. *In vivo* studies for the determination of protective effects of extracts were performed using male Wistar rats. Different groups of rats (n=5 per group) were treated orally with three doses of AVA and AVR (50, 100 and 200 mg kg^{-1} b.w., respectively) for 10 consecutive days. The same protocol was applied for ellagic acid (100 mg kg^{-1}) as a reference compound. Negative and positive (cisplatin) controls and extracts *per se* groups were also included. Toxicity was induced by intraperitoneal application of a single dose of cisplatin (7.5 mg kg^{-1} b.w.) on the 5th day. Treatment with AVA and AVR caused a significant attenuation ($p<0.05$) of the levels of serum parameters of liver, kidney, and testicular injury, compared to the cisplatin group. A significant reduction in TBARS level and a marked increase in the levels of tissue oxidative parameters were observed in all groups treated with extracts. Histopathological study revealed marked restoration of tissue morphology in groups treated with AVA and AVR. According to the UHPLC/(+/-)-HESI-MS/MS analysis, among 23 identified phenolic compounds in both extracts, well-known antioxidants ellagic acid, catechin, and catechin gallate were the dominant components. Our findings suggest that *A. vulgaris* may be considered as a remedy in the therapy of oxidative stress-related injuries.

Keywords: *Alchemilla vulgaris* L.; cisplatin; oxidative stress; ellagic acid

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant No. III43004.

Phenolic profile and neuroprotective activity of two closely-related plantain species: *Plantago lanceolata* L. and *Plantago altissima* L.

PP5-39

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Plantago lanceolata L. is highly recognized in ethnomedicine as a remedy for oral or pharyngeal irritations, dry cough, treatment of the wounds and ulcers, inflammation, gastrointestinal tract diseases, conjunctivitis and eye irritation, and numerous scientific data also support its use [1]. On the other hand, there are many other *Plantago* species which are poorly investigated. One of them is *P. altissima* L., very similar to *P. lanceolata* by its morphological characteristics. Hence, in this study, teas prepeared from these two species were analyzed by in-depth polyphenolic profile characterization, as phenolics are the main "offenders" of plants biological activities, followed by determination of neuroprotective activity. An LC-MS/MS technique was used to evaluate the quantitative content of 48 selected phenolic compounds in samples [2]. Among 19 detected phenolic compounds, in both samples, chlorogenic acid was the dominant one, while the most abundant flavonoid was luteolin. Although phenolic profiles were almost identical, some quantitative differences in phenolics between these two species were obvious. Namely, *P. altissima* had higher content of vanilic acid, while the content of flavonoid glucosides was extremely lower than in *P. lanceolata*. Neuroprotective effect was estimated through the potential of acetylcholinesterase inhibition. Statistically significant differences were not found between *P. lanceolata* (IC_{50} 2.37 mg mL^{-1}) and *P. altissima* (IC_{50} 2.00 mg mL^{-1}) activities, but, in comparison to physostigmine, a well-known AChE inhibitor, both exhibited moderate neuroprotective potential. Obtained results showed that both species present a valuable source of phenolic compounds and that phenolic profile could be possibly used to distinguish these species. Furthermore, the presented results support further investigation of *P. altissima* biological potential.

Keywords: *Plantago*, phenolic profile, acetylcholinesterase inhibition

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This study was supported by The Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. OI 172058).

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Application of natural coagulants from common oak (*Quercus robur*) for water treatment

PP5-40

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One of the most effective and simplest methods of water treatment is coagulation/flocculation. The application of chemical coagulants, specifically inorganic ones, is the most widespread; however, their usage can be potentially harmful for the environment and human health. Hence, plant-based natural coagulants could be a promising alternative for turbidity reduction. Compared to chemical coagulants, natural coagulants are safe, eco-friendly and generally toxic-free. In addition, it has been found that up to five-fold lower generation of sludge was achieved using plant-based coagulants. Due to these concerns, the aim of this study was to obtain plant-based coagulant from common oak using water or aqueous NaCl solution as the extraction solvent. Efficiencies of coagulation of whole common oak acorn fruit as well as its cotyledons and coat were evaluated at different pH values in model water at different initial turbidities. Generally, crude extracts of acorn whole fruit, cotyledons and coat displayed higher coagulation activities at higher pH values. Acorn whole fruit, cotyledons and coat are shown to be sources of components for water turbidity removal that acted more efficiently at lower water turbidities. Highest turbidity removal was achieved by crude coat extract and cotyledons supernatant, which amounted to 42% and 66%, respectively. According to the obtained results it can be concluded that acorn could be a promising source of natural coagulants for water treatment.

Keywords: common oak, natural coagulant, water treatment

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Polyphenolic and sugar profiles of apple leaves treated with metamitron (BREVIS®)

PP5-41

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Fruit thinning is relevant management practice to produce apples with high quality fruit size, red coloration, and high fruit quality parameters. Recently, Brevis® (15% metamitron) started to be used as a new secondary fruit thinner. It interferes with the photosynthetic process, curbing the production of carbohydrates used for tree growth. The stressed tree then directs the supply of

carbohydrates to shoots and leaves at the expense of fruits, resulting in a smaller number of larger fruits. The aim of this study was to determine the polyphenolic and sugar profiles in apple leaves treated with different concentrations of thinning agent (Brevis®), picked before and 3, 6, and 9 days after application. Leaf samples (apple cultivar 'Summered') were obtained within the cooperation of the Faculty of Chemistry, University of Belgrade and the Norwegian Institute of Bioeconomy Research, Norway. The polyphenolic profile was determined using ultra-high pressure liquid chromatography (UHPLC) coupled with diode array detector (DAD) coupled with TSQ Quantum Access Max triple-quadrupole mass spectrometer, whereas high-performance anion exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) was used for sugar analysis. As expected, the highest variations were noticed in sucrose, maltose, and sorbitol, while glucose and fructose seemed to be the most stable sugars during application of metamitron. Regarding polyphenolics, phlorizin, quercetin, quercetin-3-O-rhamnoside, phloretin, and naringin were the most abundant. The highest values of those polyphenolics were detected on the third day after application of metamitron due to high stress in plant. On the sixth and ninth day after application, its level gradually stabilized.

Keywords: apple leaves, UHPLC-Orbitrap MS, HPAEC-PAD, polyphenolics, sugars

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Antioxidant activity, phenolic content and allelopathic effects of laurel essential oil (*Laurus nobilis* L.)

PP5-42

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Essential oils represent complex mixtures and products of plant secondary metabolism. In this study, the commercially purchased essential oils of *Laurus nobilis* were evaluated for antioxidant activity, total phenolic content and allelopathic activity. The antioxidant potential of the essential oil was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. Extracts were mixed with DPPH solutions and absorbance was recorded using UV/Vis spectrophotometer. Antioxidant activity was represented as IC_{50} value. The extract displayed antioxidant activities, with an IC_{50} value of 24.39 mg mL^{-1}. The content of total phenolics in the extracts was determined according to the Folin–Ciocalteu procedure and calculated as gallic acid equivalents (GAE). A total phenolic content was 0.0724 mg GAE mL^{-1} oil. In addition, allelopathic effects of different concentrations of laurel essential oil (10, 20 and 30 µg mL^{-1}) on germination and early establishment of lettuce (*Lactuca sativa*) and garden radish (*Raphanus sativus*) were studied. *L. nobilis* essential oil inhibited lettuce and garden radish germination. Germination of lettuce was inhibited most strongly by 30 µg mL^{-1} extract, where germination percentage was 74%, and of radish by 10 µg mL^{-1} extract, where germination percentage was 85%. It can be concluded that laurel essential oil may have potential in reducing concentration of free radicals and controlling germination and growth of some plants, especially at the higher concentrations.

Keywords: antioxidant activity, phenolic content, *Laurus nobilis*, allelopathy

Red clover and plant growth promoting bacteria: the combination that can speed up soil remediation rate

PP5-43

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Phytoremediation is an attractive technique for solving the global problem of organic pollutants present in soil. The effectiveness of this approach can be raised up by involving physical and photochemical techniques. Recently, microbial remediation draws more and more attention. The degradation performed by microorganisms is referred as rhizoremediation. Rhizosphere microorganisms stimulate pollutant degradation and speed up mechanisms of phytoremediation. The key factor for successful rhizoremediation is the adequate choice of plant species and bacterial strain. In this study, red clover (*Trifolium pratense* L.) seeds were inoculated with several plant growth promoting bacteria (PGPB) and sown in substrate contaminated with PAHs, PCBs and organotin substances. The influence of bacteria on red clover growth (height, biomass and root length) was monitored during tree-month experimental period. The highest increase in seedling height was noted in treatments with *Bacillus amyloliquefaciens* D5 ARV and *Pseudomonas putida* P1 ARV. The same isolates, together with *Serratia liquefaciens* Z-I ARV, significantly affected biomass production. Those isolates caused total biomass increase of 70%, 48% and 33% compared to control. Root growth was positively affected by *Serratia liquefaciens* Z-I ARV. Obtained results confirmed that presence of several PGPB promote the growth of red clover and have the capacity to improve red clover remediation potential and speed up the remediation rate.

Keywords: phytoremediation, organic pollutants, red clover, plant growth promoting bacteria

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Insights on the phytochemical analysis and pharmacological activities of *Blackstonia perfoliata* (L.) Huds.

PP5-44

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Blackstonia perfoliata (L.) Huds. (Yellow wort) belongs to the *Gentianaceae* family, which contains many species widely used in traditional medicine as constituents of bitters and as ingredients in similar herbal preparations. Revealing the chemical composition and the therapeutic benefits

of *Gentianaceae plants*, the aim of this study was to identify and quantify secondary metabolites of methanolic extract of *B. perfoliata* aerial parts, as well as to evaluate its *in vitro* antioxidant and anti-inflammatory properties. Secoiridoid glycosides were detected in the highest quantities in the extract using UHPLC/DAD/(+/-)HESI-MS/MS analysis. Gentiopicrin (278.49 mg g^{-1} dry extract) was the most dominant compound among identified secoiridoids. Besides secoiridoid glycosides, HPLC analysis showed that extract also contained more than twenty different phenolic compounds. The antioxidant activities of extract were estimated using seven different methods. Considering the results of the total antioxidant capacity, *B. perfoliata* methanolic extract possessed approximately 16% of the antioxidant capacity of ascorbic acid. *B. perfoliata* extract showed better antioxidant potential with lower IC_{50} value in the determination of inhibition of lipid peroxidation compared to its $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and $NO^{\bullet}$ radical scavenging activities. At the concentration of 50 µg mL^{-1}, extract showed 19.65% of COX-1 and 48.02% of COX-2 inhibition activities. The results of the COX-2 gene expression assay in THP-1 macrophages displayed that the *B. perfoliata* extract, at the concentration of 25 µg mL^{-1}, had no significant influence on COX-2 gene expression. The obtained results open up the opportunity to deeply investigate promising anti-inflammatory properties of *B. perfoliata*.

Keywords: *Blackstonia perfoliata* (L.) Huds., secoiridoids, antioxidant activity, COX-1, COX-2

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Effect of nitrogen mineral nutrition on yield and some technological traits of sugar beet root in 2017

PP5-45

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Constant progress in plant breeding, the development of new hybrids and varieties, accompanied by climate change requires continuous review of growing technology. The most important part of plant production is fertilization, particularly with nitrogen. Nitrogen is required in great quantities for growing plants and it affects the yield of many crops. However this is very dynamic element and large nitrogen reserves cannot be stored in the soil. Considering the specificity of sugar beet production, where nitrogen, in addition to its impact on root yield, significantly affects the quality of the root, it is necessary to constantly review fertilization protocols. Effects of four levels of nitrogen fertilizers (40, 80, 120 and 160 kg N ha^{-1}) on root yield and quality of commercial sugar beet hybrids were examined in the field trials during the growing season 2017. Nitrogen fertilizers were applied before the sowing. Field trial was organised by Randomised Complete Block Design (RCBD) with four replications. There were differences between sugar beet hybrids for root yield, sugar content and alpha amino nitrogen. Unfavorable environmental conditions in 2017 reduced the effect of nitrogen fertilization, except that the high nitrogen application rates significantly increased alpha amino nitrogen level in sugar beet roots.

Keywords: growing technology, nitrogen, sugar beet

The effect of reflective mulch foil on photosynthetic efficiency of apple

PP5-46

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The reflective mulch foil is used for increasing fruit quality of different fruit crops, including apple, especially in orchards where hail nets are installed. Fruit crops under hail nets can have enhanced vegetative growth, reduced photosynthesis and lower fruit quality. Reflective mulches especially influence coloration of apple fruits through the effect on anthocyanins and flavonoids. This makes fruits more attractive to consumers, but also increases their nutrient value. In this study, the effect of reflective mulch foil on photosynthesis of apple leaves was investigated. The study was conducted in the apple orchard of Agricultural Centre Ltd. in Valpovo (Eastern Croatia) with hail nets. Two cultivars were used, Idared and Jonagold. Treatments included trees beneath which reflective foil was set, while control had regular inter-row cultivation. Photosynthetic efficiency was analyzed by measuring polyphasic chlorophyll *a* fluorescence rise (OJIP). Measurements were performed on east and west side of the trees. Results did not indicate strong influence of reflective mulch foil on PSII functioning and photosynthetic performance of apple plants. In some cases, overall photosynthetic performance index (PI_{ABS}) was increased in treated plants and slight differences in absorption of light energy, trapping, electron transport and dissipation of excitation energy were detected, but generally both treated and control plants showed high levels of photosynthetic efficiency. This might be due to favorable environmental conditions which enable the use of maximal photosynthetic capacity even without reflective mulching. Additional experiments in less favorable environmental conditions could provide better insight into possible effects of reflective mulches on photosynthesis in apple plants.

Keywords: apple, photosynthesis, OJIP, reflective mulch foil

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BIO-RAD ekskluzivni partner u Srbiji za Life Science