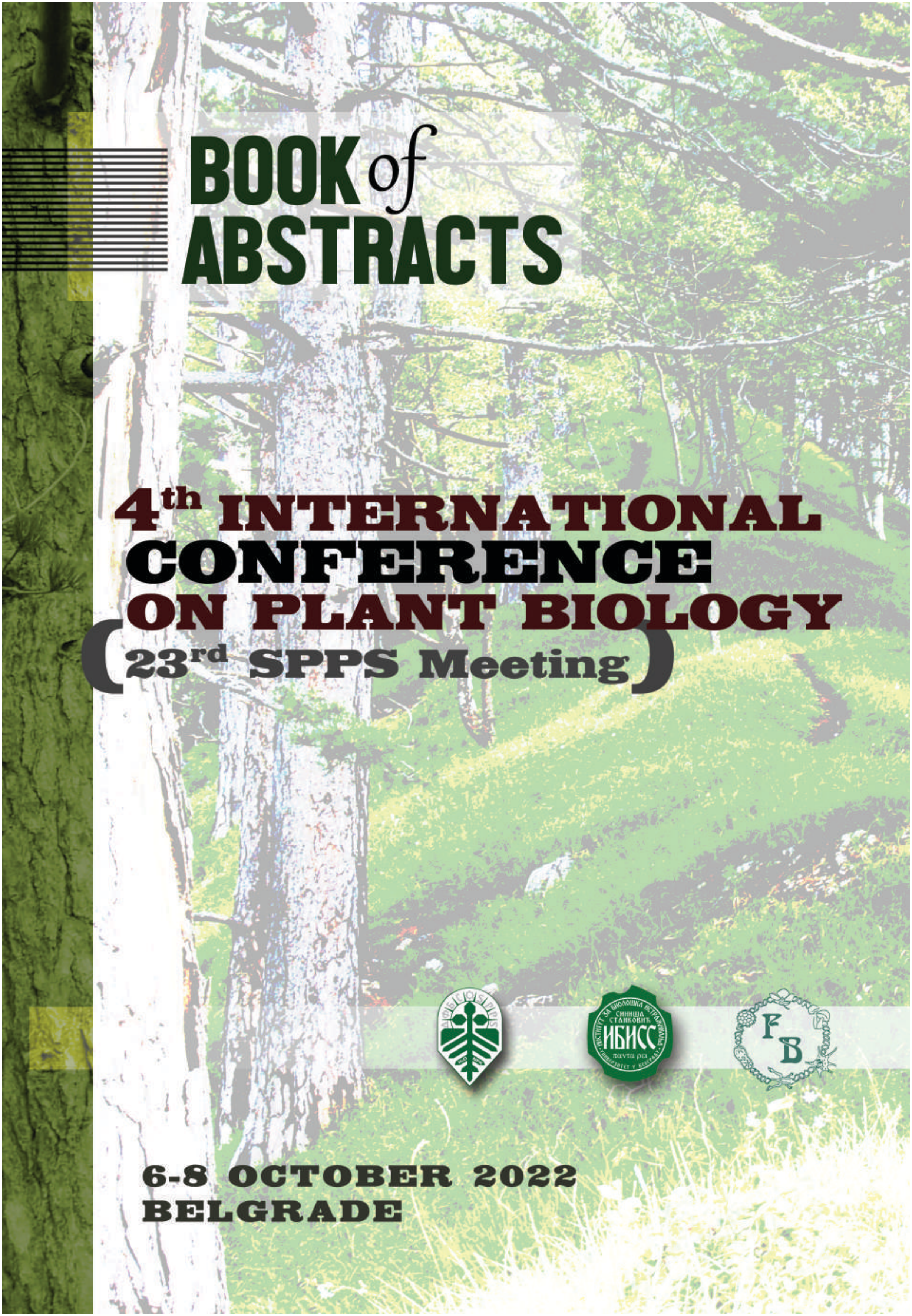

BOOK of ABSTRACTS

4th INTERNATIONAL CONFERENCE ON PLANT BIOLOGY

(23rd SPPS Meeting)

6-8 OCTOBER 2022
BELGRADE

Serbian Plant Physiology Society

Institute for Biological Research "Siniša Stanković"
National Institute of Republic of Serbia, University of Belgrade

Faculty of Biology, University of Belgrade

4th International Conference on Plant Biology

(23rd SPPS Meeting)

6-8 October 2022, Belgrade

CIP - Каталогизација у публикацији - Народна библиотека Србије, Београд

581(048)

INTERNATIONAL Conference on Plant Biology (3 ; 2018 ; Belgrade)

[Book of Abstracts] / 3rd International Conference on Plant Biology [and] 22nd SPPS Meeting, 9-12 June 2018, Belgrade ; [organized by] Serbian Plant Physiology Society [and] Institute for Biological Research "Siniša Stanković", University of Belgrade [and] Faculty of Biology, University of Belgrade ; [editor Branka Uzelac]. - Belgrade : Serbian Plant Physiology Society : University, Institute for Biological Research "Siniša Stanković" : University, Faculty of Biology, 2018 (Zemun : Alta Nova). - 188 str. : ilustr. ; 24 cm

Tiraž 30. - Registar.

ISBN 978-86-912591-5-0 (SPPS)

1. Društvo za fiziologiju biljaka Srbije. Sastanak (22 ; 2018 ; Beograd)

2. Institut za biološka istraživanja "Siniša Stanković" (Beograd)

а) Ботаника - Апстракти

COBISS.SR-ID 264415756

4th International Conference on Plant Biology
(23rd SPPS Meeting)
6-8 October, Belgrade

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Publishers Serbian Plant Physiology Society
Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, University of Belgrade
Faculty of Biology, University of Belgrade
Editor Milica Milutinović
Graphic design Dejan Matekalo
Prepress Marija G. Gray
Printed by Alta Nova, Zemun
Print run 30 pcs

Suported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia

PROGRAMME

THURSDAY 6TH OCTOBER

12:00-18:00	*Registration*
12:00-14:00	*NEPETOME project workshop (Science Fund of the Republic of Serbia, #Grant No 7749433): "Methodologies for the iridoid diversity investigation within the genus Nepeta" (Botanical Garden "Jevremovac")*
18:00-22:00	*Welcoming cocktail and Celebration of SPPS jubilee (Botanical Garden "Jevremovac")*

FRIDAY 7TH OCTOBER

09:00-09:15	*Opening Ceremony*

SECTION 2 · PLANT STRESS PHYSIOLOGY

Chairs: Jelena Dragišić Maksimović & Tamara Rakić

09:15-10:00	*Keynote:* Mondher Bouzayen *Uncoupling fruit softening from fruit ripening: a paradigm shift of thinking*
10:00-10:30	*Plenary lecture:* Miroslav Lisjak *Growth conditions may affect the nutritional quality of wheatgrass (Triticum aestivum L.)*
10:30-11:00	*Plenary lecture:* Hermann Heilmeier *The functional role of non-essential elements in the root zone: how interactions between essential and non-essential elements shape the chemical rhizosphere environment*
11:00-11:30	*Coffee break*
11:30-11:50	*Invited talk:* Zsófia Bánfalvi *Regulation and function of GIGANTEA genes in Solanum tuberosum cultivar 'Désirée'*
11:50-12:10	*Invited talk:* Ingeborg Lang *Drought or heavy metals – investigating the abiotic stress tolerance in bryophytes*
12:10-12:30	*Invited talk:* Biljana Kukavica *Flooding and antioxidative response in plants*
12:30-12:50	*Invited talk:* Sonja Milić Komić *Distinctive regulation of different phenolics biosynthesis by high light and UV-B in three basil varieties*
12:50-13:05	*Selected talk:* Mariana Stanišić *What happens with phloretin in plants? – Phloretin real-time effects and post-treatment metabolism in treated Arabidopsis seedlings*
13:05-13:20	*Selected talk:* Danijela Arsenov *Fullerenol (C60(OH)24) as a potent stress alleviator against drought and trace-element toxicity in Alliaria petiolata (M.Bieb.) Cavara et Grande*
13:20-14:00	*Poster session*
14:00-15:30	*Lunch break*

SECTION 1 · PLANT GROWTH, DEVELOPMENT, METABOLISM AND NUTRITION

Chairs: Ivana Maksimović & Slavica Ninković

15:30-16:00	*Plenary lecture:* Panagiotis Kalaitzis *A prolyl-4-hydroxylase and Arabinogalactan proteins are involved in relocation of tomato abscission zone*
16:00-16:30	*Plenary lecture:* Marjorie Guichard *State-dependent protein interaction networks of a central regulator of plant growth and metabolism*
16:30-16:50	*Invited talk:* Václav Motyka *Hormonome and role of desiccation in somatic embryogenesis of conifers*
16:50-17:20	*Coffee break*
17:20-17:40	*Invited talk:* Julien Pirrello *Transition to ripening in tomato fruit needs genetic reprogramming initiated in gel tissue*
17:40-18:00	*Invited talk:* Guido Grossmann *Robust yet adaptive - morphogenesis and growth regulation in roots*
18:00-18:20	*Invited talk:* Jan Fíla *The beta-subunit of nascent polypeptide associated complex plays a role in flowers and siliques development of Arabidopsis thaliana*
18:20-18:35	*Selected talk:* Kiril Mishev *The interaction network of the plant NudC family protein NMig1*
18:35-19:15	*Poster session*

SATURDAY 8TH OCTOBER

09:00-10:00	*SPPS Assembly*

SECTION 4 · ECOLOGY, GENETICS AND EVOLUTION OF PLANTS

Chairs: Branislav Šiler & Sanja Manitašević Jovanović

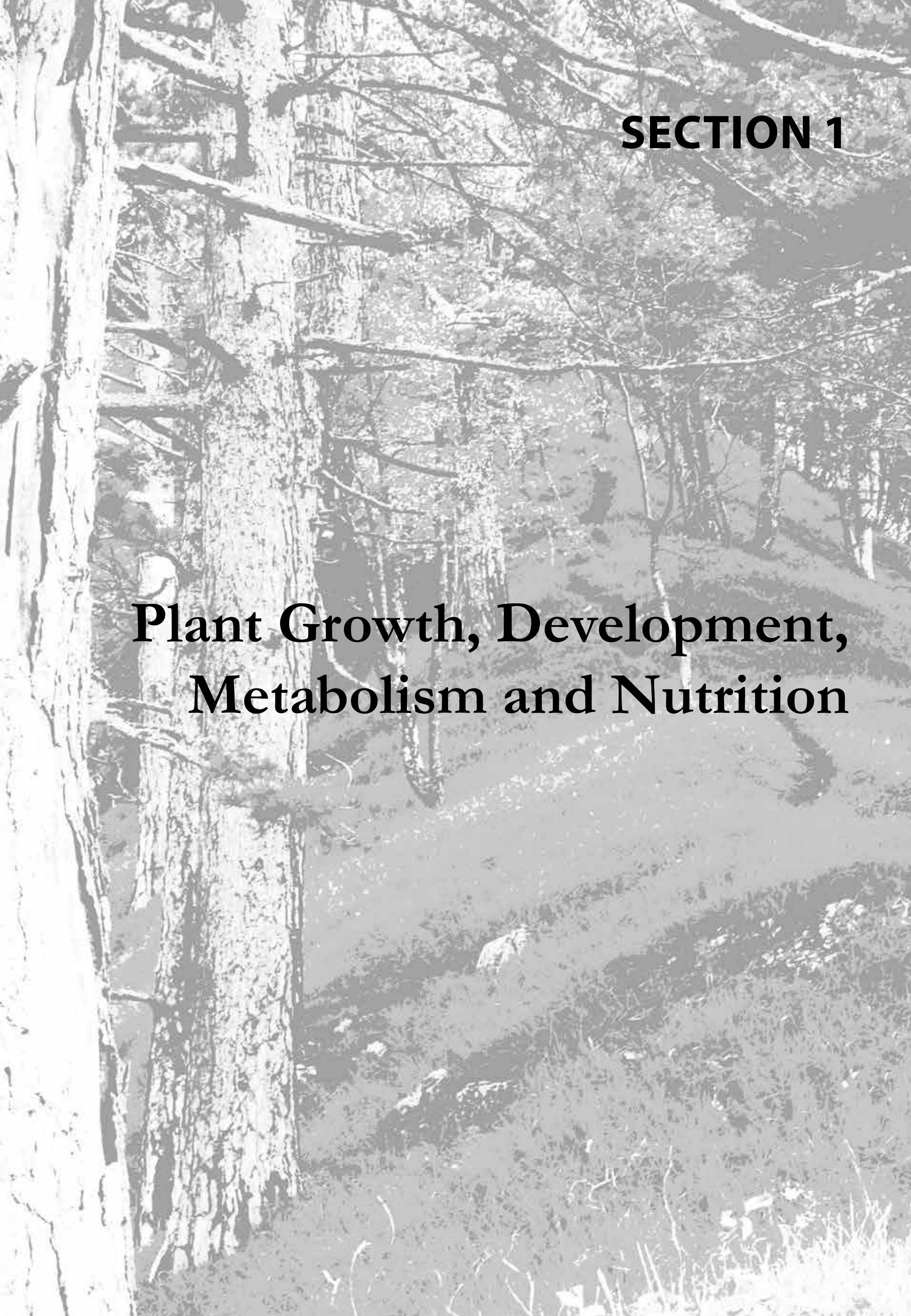
10:00-10:30	*Plenary lecture:* Velemir Ninković *Plant signaling and behavior mediated via volatiles*
10:30-11:00	*Plenary lecture:* Janez Kermavnar *Impacts of forest management on plant functional traits and ecological conditions in the Dinaric fir-beech forests (Slovenia)*
11:00-11:30	*Coffee break*
11:30-11:50	*Invited talk:* Ksenija Jakovljević *Ecophysiology of metal-hyperaccumulation in plants: what do we know so far?*
11:50-12:10	*Invited talk:* Jelena Milojević *Elucidation of the mechanism underlying somatic embryo induction in spinach*

12:10-12:30	*Invited talk:* Miroslava Zhiponova *Catmint (Nepeta nuda L.) Phylogenetics and Metabolic Responses in Variable Growth Conditions*
12:30-12:50	*Invited talk:* Neda Aničić *Progress in disentangling the diversity of iridoids within the genus Nepeta: surprising biosynthetic and evolutionary insights*
12:50-13:05	*Selected talk:* Denitsa Teofanova *Distribution, host range, and genetic variability of the holoparasitic genus Cuscuta in Bulgaria*
13:05-13:20	*Selected talk:* Katarina Hočevar *Variation in Hsp70 and Hsp101 levels in response to experimental warming in Iris pumila L.: an open-topped chamber experiment*
13:20-14:00	*Poster session*
14:00-15:30	*Lunch break*

SECTION 3 · APPLICATION IN AGRICULTURE, PHARMACY AND FOOD INDUSTRY

Chairs: Ana Ćirić & Ana Marjanović Jeromela

15:30-16:00	*Plenary lecture:* Angelos K. Kanellis *Aroma formation in Vitis vinifera grape berries*
16:00-16:30	*Plenary lecture:* Ekaterina-Michaela Tomou *Metabolomic strategy for detecting herbal products' differentiations and potential adulteration*
16:30-16:50	*Invited talk:* Mila Grahovac *Essential oils and hydrolates in control of plant pathogens*
16:50-17:20	*Coffee break*
17:20-17:40	*Invited talk:* Carla Vog *Determination of elements, isotopes and organics in plants with high local resolution by mass spectrometric methods*
17:40-18:00	*Invited talk:* Milan Mirosavljević *Integrating physiological traits in local small grains breeding program*
18:00-18:20	*Invited talk:* Nada Ćujić Nikolić *Chokeberry, from natural polyphenol resource to promising functional foods and pharmaceuticals*
18:20-18:35	*Selected talk:* Ana Pantelić *Late embryogenesis abundant (LEA) proteins in Ramonda serbica Panc identification, classification and structural characterization*
18:35-18:50	*Selected talk:* Dejan Stojković *Supercritical fluid extraction of Chicory reveals its antimicrobial, antibiofilm and wound healing potentials*
18:50-19:15	*Poster session*
19:15-19:30	*Closing Ceremony*
20:00-00:00	*Gala Dinner*



SECTION 1

Plant Growth, Development, Metabolism and Nutrition

A prolyl-4-hydroxylase and Arabinogalactan proteins are involved in relocation of tomato abscission zone

PL1-1

Andreas Perrakis[1,7], Dusan Denic[1], Dimitrios Kaloudas[1], Anezakis Nikos[1], Konstantinos N. Blazakis[1], Eleni Giannoutsou[2], Craita E. Bita[1], Myrto Rizou[1], Afrodite Krokida[1], Mohamed Kouhen[1], Athina Lazaridou[3], Khansa Mekkaoui[1], Samia Belaidi[1], Zeina El Zein[1], Mohab Khalil[1], Lamia Ezzat[1], Maria Kosma[1], Anna G. González[1], Aline Monzer[1], Dimitra Papantoniou[1], Antri Varnava - Tello[4], Mondher Bouzayen[5], Ioannis-Dimosthenis S. Adamakis[2], Azeddine Driouich[6], Costas G. Billiaderis[3], Nicolas Kalogerakis[7], Panagiotis Kalaitzis[1]

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The detachment of organs is controlled by highly regulated molecular mechanisms. The position of the tomato abscission zone (AZ) is defined by the ratio of the proximal to distal part of the pedicel. In this study, the ratio was altered due to a shift in the position of the AZ which was attributed to shorter and longer pedicels of SlP4H3 RNAi and OEX lines due to changes on cell division and expansion in AZ and distal part. This might be associated with LM2- and JIM8-AGPs which increased in OEX and decreased in RNAi lines throughout the pedicel. The JIM13 AGPs were downregulated in the flower AZ of OEX lines, pointing to a role on abscission regulation. Arabinogalactan proteins (AGPs) were identified which showed physical interaction with SlP4H3 while VIGS-induced suppression resulted in shorter distal pedicel indicating involvement in the shift of the AZ closer to the subtending flower. Moreover, ethylene-induced flower abscission was accelerated in the RNAi lines and delayed in OEX lines, while exactly the opposite response was observed in the red ripe fruit AZs. This was partly attributed to alterations in the expression of cell wall hydrolases. Overall, these results indicate that P4Hs might regulate molecular and structural features of cell walls in the AZ as well as abscission progression by regulating the structure and function of AGPs.

Keywords: tomato, abscission, prolyl 4 hydroxylase

Acknowledgment: This work was supported by the funded projects n-TOMATOMIKS and PLANT-UP

State-dependent protein interaction networks of a central regulator of plant growth and metabolism

PL1-2

Marjorie Guichard[1], Vanessa Fuchs[1], Sven Gombos[2], Anna Denzler[3], Daša Wernerová[1], Philipp Denninger[3], Waltraud X. Schulze[2], Guido Grossmann[1]

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Plants need to constantly adapt their growth and metabolism to their changing environment. At the molecular level, central regulators of these mechanisms are proteins that belong to the Small GTPase family. They are molecular switch proteins cycling between an active form associated with the plasma membrane of cells and a cytosolic inactive form. Using the root hair of *Arabidopsis thaliana* as a model, we identified the Guanine nucleotide Exchange Factor 3 (GEF3) to be important for polarizing the small GTPase ROP2 and defining the root hair polar domain. But additional evidences highlight the complexity of ROP/GEF regulation at different levels. Therefore, discovering activity state-specific interactors of such central regulators is a way to better understand how growth and metabolism are co-regulated in plants. The discovery of interacting proteins in plants benefits from the recent advances made with biotin ligase-based technologies. The biotin ligase acts as a molecular stamp that "labels" nearby proteins with biotin. By genetically encoding a fusion between a biotin-ligase and the active or inactive forms of ROP2, we were able to label in vivo interacting proteins localized in most of the cellular compartments, including the plasma membrane. Beyond discovery of individual interactors, the sets of proteins identified in the proximity of ROP2 in its different activity states enables us to determine which biological processes and signaling pathways are preferentially associated with each state or ROP2. Such network-level analyses will help us to uncover the dynamic molecular landscape that coordinates cellular responses and allow plants to thrive under changing environmental conditions.

Keywords: ROP2, protein interaction network, *Arabidopsis thaliana*

Acknowledgment: This project is funded by the Deutsche Forschungsgemeinschaft (DFG, GR 4559/4-1, GR 4559/5-1) and funds by Germany's Excellence Strategy (EXC-2048/1, project ID 390686111) to Prof. Dr. Guido Grossmann. We thank Dr. Julie Carnesecchi Institut de Génomique Fonctionnelle de Lyon, Ecole Normale Supérieure de Lyon) for German Research Foundation her helpful advice.

Hormonome and role of desiccation in somatic embryogenesis of conifers

IT1-1

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Somatic embryogenesis (SE) is a developmental process in which somatic cells dedifferentiate and divide to initiate embryogenic development. Therefore, it is very promising for conifer breeding. To date, SE techniques have been developed for most conifer species. Using the advanced HPLC-ESI-MS/MS method and somatic Norway spruce (*Picea abies*) embryos as experimental models, the changes in the profiles and concentrations of endogenous phytohormones such as auxins, cytokinins (CKs), abscisic acid, jasmonates and phenolics (salicylic acid, benzoic acid) were analysed during proliferation, maturation, desiccation, and germination. Our results showed close correlations between endogenous phytohormone profiles and specific developmental steps of the embryo. They also provided evidence for the first time of the involvement of some phytohormone forms such as the non-indole auxin phenylacetic acid, *cis*-zeatin- and dihydrozeatin-type CKs, and salicylic acid in SE and provided the most comprehensive overview of phytohormone profiles in somatic *P. abies* embryos currently available. Particular attention was paid to the desiccation stage, which is assumed to be a key step in the process of spruce SE. The specificity of desiccation treatment was determined by comparing the hormonome in desiccated somatic embryos with those during prolonged maturation as well as with zygotic embryos and will be discussed at the conference.

Keywords: desiccation, hormonome, phytohormone, *Picea abies*, somatic embryogenesis

Acknowledgment: This work was supported by Czech Science Foundation (19-12262S) and the Ministry of Education, Youth and Sports from European Regional Development Fund-Project “Centre for Experimental Plant Biology” (No. CZ.02.1.01/0.0/0.0/16_019/0000738).

Transition to ripening in tomato fruit needs genetic reprogramming initiated in gel tissue

IT1-2

Ximena Chirinos[1,2], Shiyu Ying[1,2], Elie Maza[1,2], Anis Djari[1,2], Farid Regad[1,2],
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Ripening is the last stage of the developmental program in fleshy fruits. During this phase fruits become edible and acquire their unique sensory qualities and post-harvest potential. Although our knowledge of the mechanisms that regulate fruit ripening has improved considerably over the past decades, the processes that trigger the transition to ripening remain poorly deciphered. While so far, most transcriptomic profiling of tomato fruit ripening has mainly focused on the changes occurring in pericarp tissues between Mature Green and Breaker stages, our study addresses the changes between Early Mature Green and Late Mature Green stages, in gel and pericarp separately. The data show that the shift from inability to initiate ripening to capacity to undergo full ripening requires a massive transcriptomic reprogramming that takes place first in the locular tissues before extending to the pericarp. Genome-wide transcriptomic profiling revealed the wide diversity of transcription factor families engaged in the global reprogramming of gene expression and identified those specifically regulated at Mature Green stage in the gel but not in pericarp, thereby providing new targets towards deciphering the initial factors and events that trigger the transition to ripening. Our data unveil the antagonistic roles of ethylene and auxin during the onset of ripening and show that auxin treatment delays fruit ripening via impairing the expression of genes required for System-2 autocatalytic ethylene production that is essential for climacteric ripening.

Keywords: Tomato, ripening, gel, auxin, ethylene

Robust yet adaptive - morphogenesis and growth regulation in roots

IT1-3

Christian-Frederic Kaiser[1,2], Marjorie Guichard[1,2], Milan Župunski[1], Enric Bertran Garcia de Olalla[3], Daša Wernerová[1], Jagriti Shrivastava[3], Claire E. Stanley[4], Alyona E. A. Minina[5], Guido Grossmann[1,2,3]

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PTailored devices for microscopy of biological samples have massively expanded the technical possibilities of how we cultivate, observe and experimentally interact with our model systems. Microfluidics, structured microdevices, and advances in 3D printing have led to an explosion of creative approaches to live imaging of plant-environment interactions, providing deeper insights into mechanisms of acclimation, infection, and symbiosis. Yet we are only at the dawn of an era of synthetic microenvironments that will fundamentally change the way we study—and engineer—plant-environment interactions and inter-organismal networks. In this presentation, I will review the last decade of microdevice technologies in plant science, highlight achievements and discuss technical challenges and future potential of microenvironmental engineering.

Keywords: Root-environment interactions, live cell imaging, microfluidics, synthetic biology

Acknowledgments: I am grateful to all current and past members of the Grossmann lab and to the Deutsche Forschungsgemeinschaft (DFG) for funding. Further financial support from the Excellence Cluster CellNetworks (Heidelberg), the Cluster of Excellence in Plant Sciences (CEPLAS, Düsseldorf) and the Collaborative Research Consortium CRC1208 (HHU Düsseldorf) is gratefully acknowledged.

The beta-subunit of nascent polypeptide associated complex plays a role in flowers and siliques development of *Arabidopsis thaliana*

IT1-4

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Angiosperm flower development together with male gametophyte development represent important processes of plant reproduction, which are controlled by a common activity of plethora genes. In *Arabidopsis thaliana* genome, there are five genes encoding the NACα-subunit, and two genes encoding the NACβ-subunit. The double homozygous mutants of both NACβ genes (*naβ1nacβ2*) were acquired by a conventional cross of two publicly available T-DNA insertion lines. These double homozygous mutants showed several phenotypic traits different from the Columbia-0 wild type plants, such as delayed development, lower chlorophyll content in its leaves, abnormal number of flower organs, and abnormally short siliques that carried a lower number of seeds. The *nacβ1nacβ2* phenotype was clearly complemented by either NACβ1 or NACβ2 protein from *A. thaliana* or alternatively by its homologue from liverwort *Marchantia polymorpha*. The functional redundancy of *A. thaliana* NACβ1 and NACβ2 was reflected in their high level of protein sequence homology, which reached 90%. Both NACβ1 and NACβ2 proteins had the same subcellular localization – cytoplasmic and nuclear. The NACβ1 and NACβ2 promoters were active in plethora organs, such as leaves, flowers, cauline leaves, pollen grains, and siliques together with seeds. In search of the *nacβ1nacβ2* phenotype origin, several subsequent analyses were performed, for instance the transcriptome and proteome of the *nacβ1nacβ2* flower buds were identified and analysed. Several genes included in stress responses, male gametophyte development, and photosynthesis showed significantly different abundances in the *nacβ1nacβ2* plants. Then, the NACβ1 and NACβ2 interactomes highlighted interactions of the studied proteins with ribosomal subunits, and with parts of outer membrane complexes responsible for protein sorting to mitochondria and chloroplasts. Based on the acquired data, we hypothesize that NACβ paralogues in *Arabidopsis thaliana* play a vital chaperone role in protein sorting to chloroplasts and/or mitochondria.

Keywords: flower development, translation, fruit development, photosynthesis, protein sorting

Acknowledgment: The authors gratefully acknowledge the financial support from Czech Ministry of Education, Youth and Sports (LTC20050), Czech Science Foundation (19-01723S), and European Regional Development Fund-Project "Centre for Experimental Plant Biology" (No. CZ.0 2.1.01/0.0/0.0/16_019/0000738.)

The interaction network of the plant NudC family protein NMig1

ST1

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The Nuclear distribution C (NudC) proteins are evolutionary conserved across eukaryotes and share a core CS domain that renders their chaperone activity. Members of this protein family are highly expressed in proliferating cells and are implicated in nuclear migration, mitotic spindle formation, and cell cycle progression. In *Arabidopsis*, the NudC family comprises BOB1 and NMig1, two small heat shock proteins (HSP) that are still barely investigated. BOB1 has an essential role in embryo and root development. In turn, NMig1 is a positive regulator of root growth and is involved in the process of adaptation to abiotic stress conditions. At the intracellular level, NMig1 has strong cytoplasmic, nuclear and plasma membrane localization. Here, we examined the protein interactome of NMig1 in search for novel NudC-dependent cellular pathways in plants. Immunoprecipitation experiments with overexpressing 35S::NMig1:GFP *Arabidopsis* seedlings followed by LC-MS/MS analysis revealed the presence of almost 500 proteins with statistically significant enrichment when compared to the control 35S::GFP line. Functional clustering of the enriched protein accessions based on gene ontologies showed extensive NMig1 interaction with proteins with chaperone functions. Another over-represented cluster comprised proteins associated with hormonal responses, such as auxin transporters and enzymes involved in ethylene biosynthesis. Interactions with components of the cytoskeleton were also observed. To validate some of the interactions, we performed bimolecular fluorescence complementation assays (BiFC) after transient expression of recombinant proteins in tobacco leaves. The obtained results uncovered the direct involvement of NMig1 in a set of structural and functional determinants of the plant cell homeostasis.

Keywords: NudC proteins, NMig1, *Arabidopsis*, interactomics

Acknowledgment: This work was financially supported by the Bulgarian National Science Fund, Grant No. DN11/8/15.11.2017, and initiated under the joint research project between the Research Foundation – Flanders (FWO), Belgium, and the Bulgarian Academy of Sciences, Grant No. VS.035.10N.

POSTER PRESENTATIONS

Sesquiterpene lactones content in lettuce cultivars affected by microbiological fertilisers and seasons

PP1-1

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The principal sesquiterpene lactones (lactucin, lactucopicrin, and its dihydro forms), known as contributors to bitter taste, were measured in green and red coloured lettuce using Ultra-performance liquid chromatography. Six lettuce cultivars ('Kiribati', 'Murai', 'Aquino', 'Gaugin', 'Aleppo' and 'Carmesi') were grown in a greenhouse experiment using microbiological fertilisers (EM Aktiv, Vital Tricho, and their combination) during three successive growing seasons (autumn, winter, and spring). The major sesquiterpene lactone was lactucopicrin with the highest level found in cultivar 'Carmesi' (0.65 mg/g dry weight) in autumn with the combination of fertilisers. The concentrations of lactucin, dihydrolactucin and dihydrolactucopicrin ranged from 0.001-0.085, 0.003-0.015, and 0.001-0.056 mg/g dry weight, respectively, and were not found in all lettuce samples. Generally, red cultivars showed higher content of lactones compared to green. Application of Vital Tricho and the combination of fertilisers led to an increased level of lactucopicrin, in some cultivars, in the range of 77-800% compared to unfertilised plants. Higher levels of lactucopicrin were found in the autumn trial compared to spring and winter. The present study suggests that genotype, fertilisers and season jointly affected the quantity of sesquiterpene lactones with emphasis on Vital Tricho, and/or the combination of fertilisers.

Keywords: Lactucopicrin, Lettuce, Microbiological fertiliser, Season, Sesquiterpene lactones

Acknowledgment: The authors are grateful to the company Iceberg Salat Centar, Belgrade, Serbia, for technical support during experiments. Also, this work was supported by the Serbian Ministry of Education, Science and Technological Development under Contracts No. 451-03-68/2022-14/200053 and 451-03-68/2022-14/200216.

Effect of seeds priming with green- synthesized magnetite nanoparticles on plant development and photosynthetic function in pea

PP1-2

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Iron is an essential nutrient for plants that is required for vital processes like photosynthesis and respiration. Nevertheless, there are only a few studies on the effect of iron oxide nanoparticles on seed germination, plant development and photosythetic activity. In the present work we study the effect of green-synthesized nanoparticles of Fe3O4 (magnetite), coated with laurel extract, on seed germination, plant development and photosynthesis of hydroponic cultures of garden pea plants. Magnetite particles were suspended in polyethylene glycol (PEG) 6000 solution in concentration range of 0.0125 g/L – 0.2 g/L and were used for seed priming for 6 hours. The results showed that the applied magnetite treatments do not affect significantly seed germination. After a period of 13 days the total biomass, survival index, total chlorophyll, flavonoid content, and nitrogen balance index were also not changed, as compared to PEG-only treated variants. The leaf temperature, however, was found to increase proportionally to the applied nanoparticle's concentrations. Chlorophyll fluorescence imaging demonstrates preserved photosynthetic capacity for all studied treatments. Higher number of open reaction centers of photosystem II was found for 0.025, 0.05 and 0.2 g/L treatments that resulted in higher quantum yield of this photosystem and lower extent of non-photochemical quenching of chlorophyll *a* fluorescence. In conclusion, pea seeds priming with green-synthesized magnetite nanoparticles exerts significant effect on plant leaf temperature and photosynthetic function.

Keywords: magnetite, iron oxide nanoparticles, seeds priming, germination, photosynthesis

Acknowledgment: This study is supported by the Bulgarian National Science Fund, grant number КП-06-Н36/8/13.12.2019

Pea seeds priming with stabilized Pluronic-P85 polymer micelles affects seed germination and photosynthetic function

PP1-3

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Nano-sized materials are under intensive research in the field of plant biology and agriculture due to their potential to affect (directly or as carriers of specific molecules) plant growth and development in low concentrations, thus avoiding environmental pollution. In the present work, we explore the effect of nano-sized stabilized micelles composed of poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol) (Pluronic-P85) triblock copolymer on seeds germination process, plant growth and photosynthetic function of garden pea plants. The tested seeds are primed with nanoparticles in the concentration range 0.0008 – 0.04 % (w/v) and their development is followed for a period of 13 days. The highest tested concentration is found to increase the rate of absorbtion of stabilized Pluronic-P85 solution during seed imbibition and to affect variety of growth and photosynthesis related parameters. The treatment with 0.04 % enhances the germination process and increases the number of developed plants on soil substrate, and thus the plants survival index. The photosystem II functionality and its ability for photoprotection is preserved. However, lower mean average number of open photosystem II centers and higher mean number of open photosystem I centers is detected. The presented results demonstrate that seeds priming with 0.04 % stabilized Pluronic-P85 micelles exerts positive effect on seeds germination, and strongly suggest that the nanoparticles are transferred to the photosynthetic organelles. Thus, the examined micelles might be used as cargo structures for substances with beneficial physiological effects.

Keywords: stabilized polymeric micelles, seeds priming, germination, photosynthesis

Acknowledgment: This work is supported by the Bulgarian National Science Fund, grant number КП-06-Н36/8/13.12.2019

Determination of mineral nutrient concentrations of lettuce and endive in response to pyrophyllite application in greenhouse production

PP1-4

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In the last decade, there has been an increasing interest in the possibility of use of different kinds of alumosilicate minerals in order to improve soil productivity. The use of pyrophyllite in agriculture is still not sufficiently researched, and recent data indicate its good potential to increase the capacity of soil to retain and slowly release nutrients. Lettuce (*Lactuca sativa* L.) and endive (*Cichorium endivia* L.) seedlings produced from nursery production up to the stage with 4-5 fully developed leaves were planted in pots with a volume of 1 L of nutrient substrate "Fantazija" (Gramoflor GmbH & Co., Vechta, Germany). The plants were set up in a randomized block system and initially divided into several different groups depending on the composition of the substrate, i.e. additional fertilizers and pyrophyllite, which are part of the substrate. Determination of physiological parameters included the analyses of relative water content in the leaves, fresh weight, dry weight and average yield. Analyses of metal concentrations (Cu, Zn, Fe, Ca and Mg) were performed using atomic absorption spectrophotometry. The obtained results indicate pyrophyllite application significantly increase Cu and Zn concentrations in lettuce leaves, as well as Fe concentration in endive leaves, even though its beneficial effect depends on the ratio to the fertilizer. In addition, an increase in the proportion of pyrophyllite in the fertilizer negatively affected Ca content in both tested plant species. The possibility of reducing the use of fertilizers by adding pyrophyllite to the substrate was in focus of this research.

Keywords: Pyrophyllite, Lettuce, Endive, Mineral nutrients

Acknowledgment: This work was supported by the Government of Republic of Srpska, Ministry for Scientific and Technological Development, Higher Education and Information Society, Project No. 19.032/961-121/19.

Micromorphology and histochemistry of leaf trichomes of *Nepeta cyrenaica* (Lamiaceae) propagated *in vitro*

PP1-5

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In this work, we studied the micromorphological and histochemical characteristics of leaf trichomes of *Nepeta cyrenaica* Quézel & Zaffran (Lamiaceae), an endemic and aromatic species of the Libyan flora. Fresh leaves were collected from five-week-old *in vitro* propagated plants, and subsequently analyzed using light and scanning electron microscopy. Long non-glandular trichomes are predominantly distributed on the edges of the leaves, while both adaxial and abaxial surfaces were covered by glandular trichomes, including peltate, short-stalked, and two morphotypes of long-stalked capitate trichomes. In both peltate and capitate trichomes, positive reactions for the lipids (Sudan dye) and terpenes (Nadi reagent) were observed. Secretory cells of mature peltate trichomes showed a positive reaction to pectin/polysaccharides (Ruthenium red, PAS reagent, and Toluidine tests). Ruthenium red had also stained all capitate, while the PAS reaction was positive only in short-stalked trichomes. Toluidine tests revealed the presence of phenols or carboxylated polysaccharides in the secretory cells of all glandular trichome types, but ferric chloride test and UV autofluorescence confirmed the presence of phenols only in the peltate and short-stalked capitate trichomes. As a result of Dragendorff test showed, alkaloids were detected only in the peltate trichomes secretory cells. The results obtained in this study presented the first insight into micromorphology and secretion of *N. cyrenaica* leaf trichomes, which is of special importance from an applicative point of view considering the glandular trichomes as the main bioactive compounds producing structures.

Keywords: *Nepeta cyrenaica*, glandular trichomes, histochemistry, micromorphology, *in vitro* propagation

Acknowledgment: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (No. 451-03-68/2022-14/200178; 451-03-68/2022-14/200007).

What are the functions of the S2P2 protein in *Arabidopsis thaliana*?

PP1-6

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Site-2-proteases (S2Ps) are a large family of metalloproteases which mediate intramembrane proteolysis. S2Ps were firstly identified in human and later in other organisms. In plants, first S2P homologs were described in *Arabidopsis thaliana*. They are known to be involved in cell division, polar organelle biogenesis and stress responses. The S2P2 is a highly hydrophobic, integral membrane protein, which belongs to the S2Ps. These proteases are known to be ATP-independent zinc metalloproteinases whose characteristic feature is a presence HExxH motif in one of their transmembrane domain. The motif is responsible for binding of the zinc ion in the catalytic center of the enzyme. The S2Ps are considered to participate in regulatory intramembrane proteolysis process (RIP) by releasing of membrane-anchored transcription factors and anti-σ factors. Little is known about the functions of S2P2. From the limited information in the literature it appears that this protein accumulates in the leaves during longer periods of darkness and may be involved in shaping the plant's resistance to cold. In this study, were used homozygous *Arabidopsis thaliana*, insertion lines from Nottingham Arabidopsis Stock Center: SALK_071288 (s2p2-1) and SALK_046599C (s2p2-2) and wild-type plants for comparison. The homozygosity of the mutants was previously confirmed. The course of the developmental phases of wild-type plants and S2P2 mutants was compared on the basis of BBCH scale. S2P2 mutants: sprout later (phase 0.1), later leaves emerge, bloom later (phase 6.0) and have a smaller overall rosette surface Comparative analyzes of the ultrastructure of chloroplasts, using a TEM microscope, were also carried out. This analysis shows disturbances in the number and structure of thylakoids, a difference in the size of plastoglobulins and a disturbance in starch metabolism. The level of free radicals under stressful conditions was also compared, using the method of staining leaves with DAB reagent. This study showed the accumulation of more free radicals in S2P2 mutants, which leads to the conclusion that the S2P2 protein protects the plant against stress factors.

Keywords: proteolysis, proteases, S2P2, *Arabidopsis thaliana*

Phytohormone homeostasis in *in vitro* grown kohlrabi: the effect of cytokinins and sucrose

PP1-7

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This study fathoms a comprehensive hormone profiling of four growth stages of kohlrabi (*Brassica oleracea* var. *gongylodes*) seedlings *in vitro* (T1-T4), including the effects of cytokinins, CKs (*trans*-zeatin, *trans*Z; thidiazuron, TDZ), and high sucrose concentration (6% and 9%). Depending on the type of investigated phytohormone and its metabolite, individual changes were observed on a time scale, showing specific hormone profiles. A significant increase in the content of endogenous zeatin nucleobases and gibberellin GA20 was detected at T2 stage (plantlets with two emerged leaves). However, levels of jasmonic acid, JA-isoleucine, indole-3-acetic acid (IAA) and indole-3-acetamide significantly increased as development progressed (T3), whereas content of most of analysed IAA metabolites decreased. Supplementing growth media with CKs induced *de novo* shoot formation during seedling development, and both CK and sucrose treatments caused significant changes in levels of most of the phytohormone groups at each developmental stage compared to control. Principal component analysis indicated that sucrose treatments, particularly 9%, had a stronger effect on the content of endogenous hormones than the CK treatments. The difference between two CK treatments proved to be remarkable at T1, T2, and T3 stages, while at the last stage difference was much smaller. The joint application of CKs and high sucrose concentration showed diverse relations depending on the metabolite, growth stage and amount of sucrose used. The most striking increase was induced in T4 for zeatin nucleobases when *trans*Z and 9% sucrose were applied. Our results indicate specific patterns of metabolic balance during kohlrabi *in vitro* development and regeneration, revealing a complex interplay of different phytohormonal groups with CKs and sucrose.

Keywords: kohlrabi, *in vitro* development, sucrose, cytokinin, phytohormone profiling

Acknowledgement: This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia through Grant Agreement with University of Belgrade- Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia No. 451-03-68/2022-14/200007, the Czech Science Foundation (Research Grants No. 19-12262S and 19-13103S) and the Ministry of Education, Youth and Sports of the Czech Republic from European Regional Development Fund- Project "Centre for Experimental Plant Biology" (No. CZ.02.1.01/0.0/0.0/16_019/0000738).

Trichostatin and dimethyl sulfoxide enhance somatic embryogenesis from root apices of spinach

PP1-8

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Epigenetic modifications of chromatin play a pivotal role in regulation of expression of genes involved in somatic embryo (SE) induction. Hence, the compounds which affect DNA-histone interaction may trigger somatic embryogenesis. Trichostatin (TSA) is a potent inhibitor of histone deacetylases, whose activity leads to increased histone acetylation, thereby affecting gene expression. To explore epigenetic control of SE regeneration from root apices (1 cm) of spinach seedlings, the explants were cultivated on media supplemented with 0, 0.1, 0.5, 1 or 5 µM TSA + 0, 1, 10 or 20 µM α naphthaleneacetic acid (NAA) + 0 or 5 µM gibberellic acid (GA_3). The explants were exposed to TSA for 1, 7 or 14 days, and subsequently subcultivated on TSA free medium of the same composition. TSA was dissolved in dimethyl sulfoxide (DMSO), whose final concentration in all media, including TSA-free controls, was 0.05%. TSA was not sufficient to induce SEs either alone or in combination with NAA or GA_3. SE regeneration was observed only from the explants cultivated on media supplemented with 10 or 20 µM NAA + 5 µM GA_3. In both combinations, TSA promoted somatic embryogenesis, but longer TSA treatment was needed with 10 µM NAA than with 20 µM NAA for efficient SE induction. The highest embryogenic response was attained with 0.1-0.5 µM TSA. DMSO also significantly improved SE induction, probably by enhancing NAA and GA_3 intake into the plant cells. The results indicate a significant role of epigenetic control in SE induction in spinach.

Keywords: Dimethyl sulfoxide, Trichostatin, root apices, *Spinacia oleracea*, somatic embryogenesis

Acknowledgment: Ministry of Education, Science and Technological Development of the Republic of Serbia supported this work through contract No. 451-03-68/2022-14/200007.

Enhanced metabolism of gibberellins is involved in the induction of somatic embryogenesis from root apices of spinach

PP1-9

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Gibberellic acid (GA_3) and α-naphthaleneacetic acid (NAA) are essential for efficient somatic embryo (SE) induction from root apices of spinach seedlings, while neither is sufficient to provoke an embryogenic response. To gain insight into the role of gibberellins (GAs) in SE induction, the levels of bioactive GAs, their precursors and inactivation products, were determined in embryogenic and non-embryogenic explants, cultivated on medium supplemented with 20 µM NAA + 5 µM GA_3 and 20 µM NAA, respectively, for 24 h and 7 days, and in control explants - root apices that were frozen immediately after isolation. The content of GAs was determined using ultra-high performance liquid chromatography coupled to tandem mass spectrometry. The levels of biosynthetic GA precursors GA_{44}, GA_{19} and GA_{20} were 7–18-fold higher in embryogenic explants, and only 1.1–2.8-fold higher in non-embryogenic explants compared to the control. In embryogenic explants, the levels of bioactive GA_1, GA_3, GA_4 and GA_7 increased 843-, 50433-, 60- and 136-fold, respectively, after 24 h, and remained high after 7 days of treatment. In non-embryogenic explants, only a transient increase of GA_3 level (22-fold) was observed after 24 h of treatment. Expectedly, the products of GA2ox inactivation (especially GA_8) were significantly higher in embryogenic than in non-embryogenic and control explants. The results evidence a positive impact of enhanced GA metabolism on the acquisition of embryogenic competence, confirming their important role in SE induction and indicating that the 13 hydroxylation pathway is a prevalent pathway of bioactive GA synthesis in embryogenic explants.

Keywords: Gibberellins, root apices, *Spinacia oleracea*, somatic embryogenesis

Acknowledgement: Ministry of Education, Science and Technological Development of the Republic of Serbia supported this work through contract No. 451-03-68/2022-14/200007 and the European Regional Developmental Fund Project 'Centre for Experimental Plant Biology' No. CZ.0 2.1.01/0.0/0.0/16_019/0000738 is further acknowledged.

Paclobutrazol and GA_3 synergistically promote somatic embryogenesis from root apices of spinach

PP1-10

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Gibberellic acid (GA_3) is indispensable for somatic embryo (SE) induction from root apices of spinach. To elucidate its role in SE induction, paclobutrazol (PAC), an inhibitor of gibberellin biosynthesis, was used. Root apices (1 cm) isolated from SE-derived, *in vitro*-cultivated plants of a previously selected line with high embryogenic capacity were used for experimentation. The explants were cultivated on media containing 20 µM α-naphthaleneacetic acid (NAA) + 0 or 5 µM GA_3 + 0, 1, 2.5, 5 or 10 µM PAC. NAA alone induced SE regeneration in 89% of the explants, but with only 3.5 SEs per explant, while the explants cultivated on media supplemented with NAA+GA_3 or NAA + GA_3 + 2.5 µM PAC regenerated at 100% with 17.7 and 34.6 SEs per explant, respectively. However, in the absence of GA_3, higher levels of PAC were needed: 5-10 µM PAC + NAA for 100%-response and 15-22.2 SEs per explant. To get insight into the NAA-GA_3-PAC interaction, expression of genes encoding the key enzymes that catalyze the final step of bioactive GA biosynthesis (GA20-oxidase and GA3-oxidase) and degradation (GA2-oxidase) was analyzed in the explants during 28 days of SE-induction period, using quantitative real-time PCR. In the explants with high embryogenic capacity, the expression levels of *SoGA20* ox and *SoGA3* ox were significantly lower, and *SoGA2-ox1* and *SoGA2-ox3* significantly higher than in those with low embryogenic capacity, cultivated on medium with NAA alone, during the whole SE induction period, indicating that NAA-GA_3-PAC interaction enabled the acquisition of embryogenic capacity by impacting GA metabolism.

Keywords: paclobutrazol, somatic embryogenesis, gibberellins, gene expression, *Spinacia oleracea*

Acknowledgment: Ministry of Education, Science and Technological Development of the Republic of Serbia supported this work through contract No. 451-03-68/2022-14/200007

Light-mediated modulation of growth status and antioxidant capacity in *Nepeta nuda* L.

PP1-11

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Light is a major factor controlling plant metabolism, growth and phytochemical potential. The different light spectra and intensity modulate secondary metabolism and antioxidant capacity in plants. Antioxidants not only prevent active biomolecules in cells from oxidation, but also influence in plant growth and development. For our experiment three different light qualities and photosynthetic photon flux density (PPDF) were examined: white (W) light (80 µmol $m^{-2}s^{-1}$; fluorescent) as a control, and a combination of blue and red light (blue:red:far red 15%:75%:10%) with high intensity – BR (240 µmol $m^{-2}s^{-1}$; LED), and with low intensity – BRS (40 µmol $m^{-2}s^{-1}$; LED). *In vitro*-cultivated catmint plants (*Nepeta nuda* L.) were grown under controlled environmental conditions for 5 weeks. The BR light enhanced biomass production, whereas BRS light promoted shoot elongation compared to W control. Cultivation of *in vitro* plants under the different light quality and intensity indicated that these variable light conditions altered the content of bioactive compounds, such as aesculin, FA, rosmarinic acid, cirsimaritin, naringenin, rutin, isoquercetin, epideoxyloganic acid, and chlorogenic acid. Cultivation of catmint under different light regimes provoked changes in chlorophyll, carotenoids and anthocyanins content. Estimated changes in Ferric Reducing Antioxidant Power (FRAP), 2,2'-diphenyl-1-picrylhydrazyl (DPPH) activity, total flavonoids, and total soluble sugars content showed fluctuation between treatments. This study highlights the importance of light composition and intensity for plant growth and the bio-productivity modulation of *N. nuda* phenolics and related antioxidant parameters.

Keywords: antioxidants, biomass, *in vitro*, LED, phytochemicals

Acknowledgment: This work was financially supported by the Bulgarian National Science Fund (BNSF), Grant No. KP-06-N56/9/12.11.2021. Infrastructure support was provided by Grant BG05M2OP001-1.002-0012 "Sustainable utilization of bio-resources and waste of medicinal and aromatic plants for innovative bioactive products" co-financed by the European Union through the European Structural and Investment Funds, as well by the Bulgarian Ministry of Education and Science, through Operational Program Science and Education for Smart Growth 2014–2020. We thank to METAGAL OOD for providing the light cameras.

Some new aspects of mode of action of brassinosteroids in plants

PP1-12

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Brassinosteroids (BRs) are known to have a crucial role in development of a plants. But, the main goal of contemporary investigations of action of these phytohormones are concentrated on mechanisms of their action, particularly with a special emphasis on the study of BRs receptors and BRs signaling pathways and similar aspects of molecular action of BRs, mainly by methods of molecular biology. In contrast to the mechanism of action, the mode of actions of certain chemicals in the plants is defined as the totality of the effects that a chemicals cause the effect on metabolism, growth and development of plants. In this sense, we studied the effect of some BRs on diferent plant species in their different developmental stages, both studying the impact of BRs on energetic changes and the chemical composition of plants, based on our findings in that area after 2015. In our works, we found a complete parallelism of enthalpy and entropy, as factors that determine the Gibbs free energy as a measure of the chemical potential of a system, here corn seedlings treated by different doses of BRs. We also found that BRs affect the content and ratios of various metabolites and elements in corn seedlings and soybean plants. These findings were extended by our new investigations of the parallelism of thermodynamic changes during the germination of corn seeds with possible mechanisms of their growth, and the parallelism of these processes with the metabolism of various carbohydrates in seeds of two corn hybrids during the mentioned changes. The last mentioned findings will be discussed in this paper.

Keywords: brassinosteroids, mode of action, plant energetics, plant metabolism

Acknowledgment: Different grants, donated by the Ministry of Education, Science and Technological Development of Serbia were used in the conducted research.

Cytokinins enhance the metabolic activity of *in vitro* grown catmint (*Nepeta nuda* L.)

PP1-13

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The *in vitro* micropropagation allows to investigate the biological potential of medicinal plants under controlled conditions and testing of phytoeffectors that may regulate plant productivity. Here, we explored the metabolic changes in response to exogenously applied plant growth regulators (PGRs) as the cytokinins 6-benzylaminopurine (BAP), kinetin (KIN) and thidiazuron (TDZ), on *in vitro* grown catmint *Nepeta nuda* L. subsp. *nuda*. The impact of cytokinins in *in vitro* conditions was estimated by comparison with *ex vitro* adapted plants grown in nature. The application of high concentrations (1 mg/l) of all the three cytokinins significantly enhanced the biomass by intensive callus formation. While BAP and KIN induced de novo regeneration of shoots from callus, TDZ completely inhibited the formation of new plantlets. All the three cytokinins triggered several folds' enhancement of the total phenolic compaunds and respective antioxidant activity compared to the untreated control. The content of the volatile iridoid 4a-α,7-β,7a-α-nepetalactone was substantially enriched in *in vitro* conditions as only TDZ caused significant reduction compared to the other *in vitro* variants. The overall level of phenolics and nepetalactone in cytokinin-treated *in vitro* plants was found higher than in flowers and leaves of *ex vitro* adapted plants grown in nature. We concluded that in *N. nuda* the tested cytokinins, especially BAP and KIN, induce indirect shoot organogenesis where enhanced metabolic activity as growth and synthesis of phytochemicals occurred.

Keywords: benzylaminopurine (BAP), kinetin (KIN), thidiazuron (TDZ), phenolic antioxidants, volatiles

Acknowledgment: This work was financially supported by the Bulgarian National Science Fund (BNSF), Grant No. KP-06-N56/9/12.11.2021. Infrastructure support was provided by Grant BG05M2OP001-1.002-0012 "Sustainable utilization of bio-resources and waste of medicinal and aromatic plants for innovative bioactive products" co-financed by the European Union through the European Structural and Investment Funds, as well by the Bulgarian Ministry of Education and Science, through Operational Program Science and Education for Smart Growth 2014–2020.

Influence of different light sources on growth and chlorophyll content in pepper and melon

PP1-14

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LED light sources are increasingly replacing standard fluorescent lamps FL, and have acquired an important role in plants growing under artificial lighting. We studied the effect of light sources with different color temperature, cold white (CW - 6500K) and warm white (WW - 4000K) LEDs and FL, on development of pepper and melon plants. Pepper and melon seeds were sown in containers filled with sterile substrate and their development from germination was monitored. Plant were incubated in a growth chamber at 24 ºC under a 16h/8h photoperiod. The light intensity of 49 PPFD was provided in all light treatments. Control plants were grown under natural lighting (16h/8h) photoperiod in a greenhouse at 20±4 ºC. After 8 weeks of growing in growth chamber and greenhouse the growth parameters (plant height, number of leaves, root length, plant and root mass), as well as the chlorophyll content, were determined. While plant height and mass of pepper were higher under FL lights, LED light provided better growth of melon. Also lower content of chlorophyll that was detected under FL light was more pronounced in melon than in pepper. It has been observed that CW lamps, both LED and FL are more suitable for both cultivars than WW lamps, because they supported the development of plantlets with better fitness, abundant roots and green mass accumulation. Growth in the chamber under controlled conditions was significantly higher than in the greenhouse.

Keywords: light source, growth, chlorophyll content, pepper, melon

Acknowledgment: The present work was supported by the Ministry of Science of the Republic of Serbia (Grant No. 451-03-68/2022-14/200216).

Ploidy profiling of *Centaurium erythraea* germplasm representing the diversity of the central Balkans: *in vitro* screening for highly productive diploid and tetraploid genotypes

PP1-15

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Centaurium erythraea Rafn (common centaury) is an important medicinal plant species whose various health effects have been ascribed to its key specialized bioactive metabolites (mostly to secoiridoids and xanthones). Assessment of species diversity through the analysis of ploidy level and genetic variation is important for the selection of high-yielding genotypes for sustainable production of pharmacologically important specialized metabolites. In this work, we scored ploidy level of *C. erythraea* germplasm stored in *ex situ* seed collection containing seeds of more than 40 populations originating at locations across the central Balkan Peninsula. Furthermore, we selected two neighboring populations, one of the diploid and one of the tetraploid genome background, and compared growth parameters and productivity among and within diploid and tetraploid genotypes. Plants belonging to the diploid population showed different growth patterns as compared to tetraploid plants after one month of growth under *in vitro* conditions. To evaluate the content of iridoids (loganin and loganic acid), secoiridoids (secologanin, sweroside, gentiopicrin, and swertiamarin), and xanthones (demethyleustomin, methylbellidifolin, eustomin, and decussatin) in diploid and tetraploid genotypes, a targeted UHPLC/DAD/(±)HESI-MS^2 analysis was performed for methanol extracts of shoots and roots, separately. One-month-old shoots of all diploid genotypes contained significantly higher amounts of total iridoids and xanthones than tetraploids, primarily due to high amounts of sweroside, decussatin, and methylbellidifolin. The observed variation both among and within diploid and tetraploid genotypes should be taken into consideration when estimating strategies for biotechnological improvement and for unraveling molecular background of specialized metabolites biosynthesis.

Keywords: *Centaurium erythraea*, diploid, tetraploid, iridoids, xanthones

Acknowledgment: This work is financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/200007).

Modulation of growth and metabolic composition of *in vitro* cultured *Nepeta nuda* by cytokinins

PP1-16

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Nepeta nuda is herbaceous plant species from the family Lamiaceae with perennial life cycle and wide natural distribution across Eurasia. It is known for its ability to produce various secondary metabolites, including antioxidants, essential oils and other bioactive compounds with potential applications in the fields of medicine, pharmacy and cosmetics. Cytokinins (CK) are a major class of plant hormones with key functions in the development of virtually all known groups of land plants. We aimed to investigate the effects of CK on growth and metabolic composition of *in vitro* cultured *N. nuda*. The most affected morphometric parameters were the number of plants per explant and the dry weight. CK caused a dramatic increase in both, with higher concentrations having the strongest effect and inducing callus formation. There was a clear drop in the content of plastid pigments as lower CK concentration produced the strongest inhibitory effect. In respect to metabolites, the reducing sugars and the phenols showed an increase, however, the reducing sugars appear to be affected the most in the lower CK concentration range while the exact opposite is true for the phenols and the respective total antioxidant activity. The flavonoid content is clearly lower upon CK treatment, but it does not depend on CK concentration. The overall data demonstrate that the growth and metabolic composition of *N. nuda* are strongly affected by addition of CKs and indicates that this class of hormones can be successfully used for modulation of the plant's physiology.

Keywords: 6-benzylaminopurine, morphometry, pigments, reducing sugars, phenolic antioxidants

Acknowledgment: This work was financially supported by the Bulgarian National Science Fund (BNSF), Grant No. KP-06-N56/9/12.11.2021. Infrastructure support was provided by Grant BG05M2OP001-1.002-0012 "Sustainable utilization of bio-resources and waste of medicinal and aromatic plants for innovative bioactive products" co-financed by the European Union through the European Structural and Investment Funds, as well by the Bulgarian Ministry of Education and Science, through Operational Program Science and Education for Smart Growth 2014–2020.

Changes in secondary metabolites content in wheat (*Triticum aestivum* L.) seedlings caused by seed priming and cold stress

PP1-17

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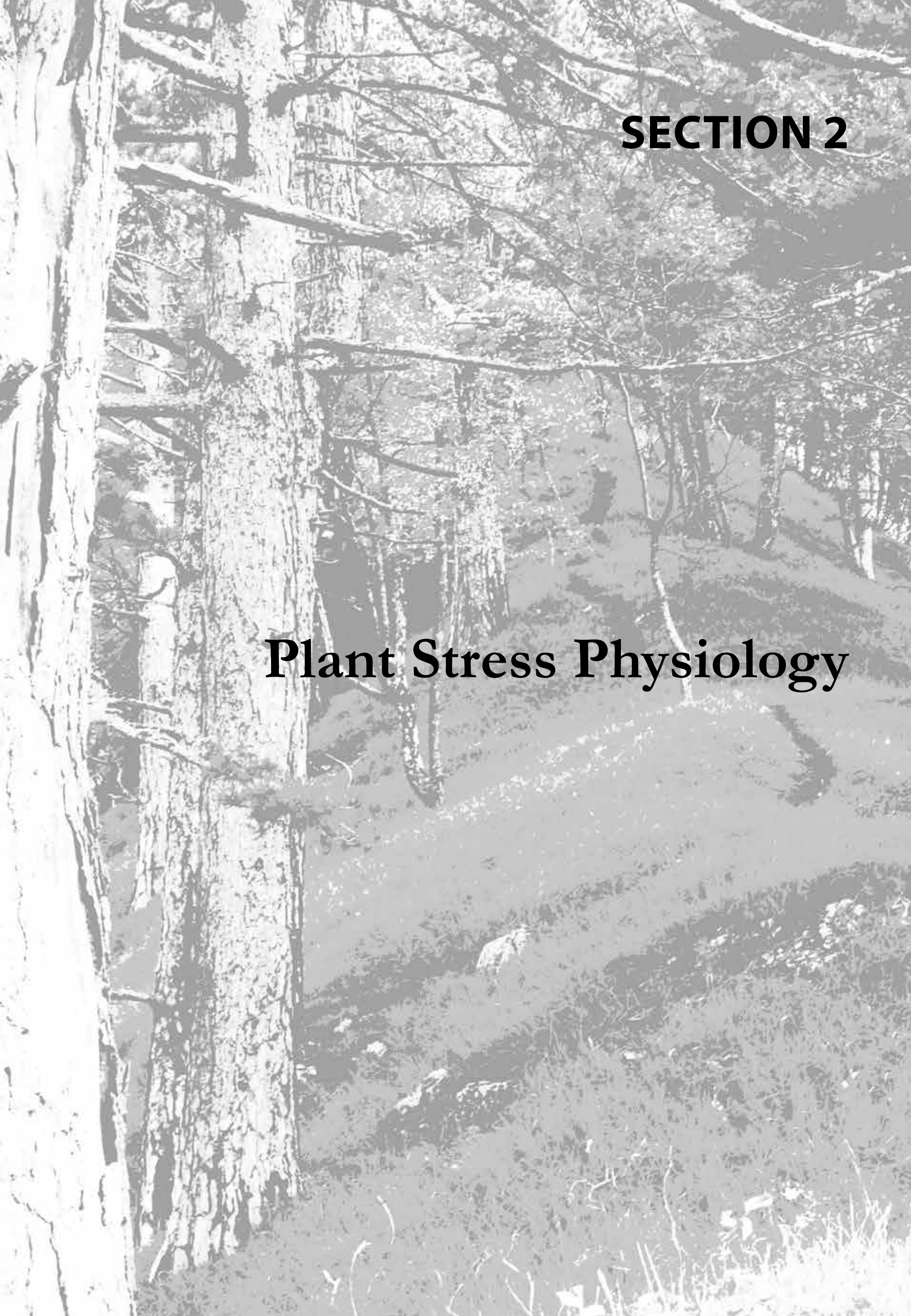
Crops are exposed to adverse environmental conditions throughout their life cycle, and abiotic stresses, such as cold stress, can negatively affect the main physiological and biochemical processes in plants. Seed priming is a promising technique applied to improve seed germination and seedling growth under various environmental conditions. The main goal of this work was to investigate whether the priming of wheat (*Triticum aestivum* L.) seeds could induce the synthesis of secondary metabolites in wheat seedlings and protect wheat against cold stress. Different priming agents were tested including phytohormones (GA_3 and IAA), salt solutions (KNO_3 and $MgSO_4$), hydrogen peroxide (H_2O_2), ascorbic acid (AA) or distilled water (H_2O). Primed and unprimed seeds were germinated and grown under 10 °C for ten days. HPLC analysis confirmed that the application of priming significantly increased the concentration of phenolic compounds in wheat seedlings compared to those which are grown from unprimed seeds. The most prominent effects were achieved by H_2O and GA_3 as priming agents. In general, in seedlings developed from primed seeds chlorogenic acid, catechin, and 4-hydroxy benzoic acid are present in significantly higher concentration. Similarly, the significant presence of flavonoids such as rutin, naringin, and quercetin was also confirmed. Having in mind the importance of secondary metabolites in the adaptation of plants to abiotic factors, the obtained results indicate that the application of seed priming could be regarded as an economical and promising approach for mitigation of harmful effects of cold stress in wheat seedlings.

Keywords: wheat, secondary metabolites, phenolic acids, flavonoids

Acknowledgment: This investigation was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant no: 451-03-68/2022-14/200122).

SECTION 2

Plant Stress Physiology



KEYNOTE

Uncoupling fruit softening from fruit ripening: a paradigm shift of thinking

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Fruit softening is classically considered as a ripening-associated process and the main way to control softening consists, so far, in slowing down ripening which has detrimental impact on sensory qualities. We recently showed that *SlMBP3*, a MADS-box transcription factor, is a master regulator acting at very early pre-ripening stages and its expression is required for locular gel formation. *KO* mutation of *SlMBP3* gene results in All-flesh phenotype with absence of gel tissue formation giving rise to an "*aubergine-like*" inner tissue in tomato fruit. Remarkably, *SlMBP3-KO* lines exhibit enhanced fruit firmness from early pre-ripening stages throughout advanced stages of ripening. Consistently, SlMBP3 overexpression results in extreme fruit softening starting at very early pre-ripening stages. Combined RNA-seq and ChIP-seq approaches revealed that misexpression of *SlMBP3* impairs locular gel formation through massive transcriptomic reprogramming at initial phases of fruit development. It is noteworthy that dual KO mutation of MBP3 and AGL11, two members of the same class D clade, results in severe detrimental phenotypes affecting plant growth, fruit size and seed formation, thereby precluding any potential use in breeding strategies. Our findings uncover a master regulator of fruit texture and softening and provide new leads to circumvent losses of sensory quality associated with incomplete fruit ripening in tomato and possibly other fleshy fruits.

Keywords: Fruit firmness, MADS-box, RNAseq, ChIP-seq, Tomato

Acknowledgment: This research was supported by the EU H2020 project "TomGEM 679796" and "Fondation Jean Poupelain" France.

PLENARY LECTURES

Growth conditions may affect the nutritional quality of wheatgrass (*Triticum aestivum* L.)

PL2-1

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Young shoots of wheat (*Triticum aestivum* L.), called wheat grass, contain numerous phytochemicals that are essential to humans such as fatty acids, enzymes and antioxidants. As a rich source of chlorophyll, vitamins, minerals, and unlike wheat grain it doesn't contain gluten, it's becoming increasingly popular as a food supplement in people's daily diet. Young plant seedlings can also be rich in bioavailable forms of zinc and selenium, the key players in health and immunity. This study examined the genotypic specificity of 100 varieties and 5 wild relatives of wheatgrass, grown in fully controlled conditions, regarding the content of biologically active compounds that positively affect human health. Based on the obtained results, 9 varieties were sown in open field conditions and biofortified with zinc and selenium. From the harvested biofortified grain, wheatgrass has been grown in controlled conditions. The influence of the term of cutting and different growth conditions, on the content of chloroplast pigments, phenols, flavonoids, flavanols, phenolic acids, ascorbic acid, proline, lipid peroxidation, metal chelating capacity and antioxidant activity of fresh wheatgrass juice, have been examined. The variety of wheat, biofortification and the oscillation of ecological parameters during growth, as well as the intensity and duration of irradiation, the temperature and water availability, significantly influenced the examined parameters. The research revealed which varieties and growth conditions can be recommended for home cultivation, in order to maintain the optimal nutritional value of this functional food supplement.

Keywords: functional food, food supplement, home cultivation, wheatgrass

Acknowledgment: This research is part of the project: „Genotypic specificity of the wheatgrass (Triticum aestivum L.), highly nutritional food supplement", UIP-2017-05-4292, funded by Croatian science foundation, led by PhD Andrijana Rebekić.

The functional role of non-essential elements in the root zone: how interactions between essential and non-essential elements shape the chemical rhizosphere environment

PL2-2

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Root carboxylate release is an essential strategy of plants to access sparingly soluble nutrients and avoid element stress from deficiency to excess. Although carboxylate release is initially regulated by cellular nutrient supply levels and predominantly targets the acquisition of essential mineral nutrients, the resulting chemical changes in the rhizosphere must be generally considered as non-element-specific. That means while nutrient deficiency triggers a shift in metabolism towards elevated carbon release, the compounds released do not only solubilize the nutrients but also mobilize a number of non-essential elements in the root-zone impacting their chemical speciation and availability to the plants. As the availability of non-essential elements raises, the mobile elements can adjust and interfere with nutrition-related rhizosphere processes, including carboxylate release and root-microbe signalling. The rationale behind this hypothesis is based on results from three greenhouse experiments and one field-scale experiment targeting the interactions between deficiency-induced root activity, the functional rhizosphere microbiome and the availability of non-essential elements (silicon, aluminium, REE). Nutrient deficiency altered the amount and composition of carboxylates and increased the availability of non-essential elements. The presence of mobile non-essential elements, in turn, significantly affected the amount and composition of root exudates, altered the functional rhizosphere microbiome, and consequently, the nutritional state of the plants. These findings suggest a fundamental role of non-essential elements during nutrient acquisition of plants, have major implications for our understanding of plant adaptations to highly mineralized or nutrient-impoverished soils and highlight the need to consider physiological impacts of elements on whole plant level.

Keywords: bioavailability, plant nutrition, root exudates, root microbiome

Acknowledgment: OW was supported in the framework of the project Phalaris 2 (Grant Number 22018913) financed by the Federal Ministry of Food and Agriculture, Germany. Further OW acknowledges funding from a PROCOPE mobility scholarship and a postdoctoral habilitation scholarship funded by the German Academic Exchange Service (DAAD).

INVITED TALKS

Regulation and function of *GIGANTEA* genes in *Solanum tuberosum* cultivar ’Désirée’

IT2-1

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GIGANTEA (GI) is a plant-specific, circadian clock-regulated, nuclear protein involved in diverse processes from flowering to stress responses. However, in potato (*Solanum tuberosum* L.), only its role in tuber initiation has been reported thus far. Thus, the aim of our work was the detailed study of the regulation and function of *GI* in potato. To achieve this goal a search for the *Arabidopsis GI* homologue was carried out and two transcript variants located on chromosomes 4 and 12 (*StGI.04* and *StGI.12*) were identified. *In silico* characterisation and expression analysis of the two genes revealed that their regulation is partially different. While osmotic stress, cold stress, and heat lead to up-regulation of *StGI.04*, the same stresses down-regulate *StGI.12* and while *StGI.04* is expressed in each organ, *StGI.12* is not expressed in flower. Thus, we presumed that the function of *StGI.04* and *StGI.12* are at least partially different. To test this hypothesis, the expression of *StGI.04* in commercial potato cultivar ‘Désirée’ was repressed. The highest level of repression reached around 50%. This level did not influence tuber formation and yield but did cause a reduction in anthocyanin content of tuber skin. Transcriptome analysis of leaves revealed that, as with *GI* in other plant species, *StGI.04* influences the expression of the key genes of the circadian clock, flowering, starch synthesis and stress responses. Experiments to isolate knock out mutants using the CRISPR/Cas9 system to get more information on the function of *GI* genes in potato are in progress.

Keywords: potato, transcriptome, anthocyanin

Acknowledgment: The authors are grateful to M. Kiss for the excellent technical assistance. This study was supported by the National Research Development and Innovation Office, Hungary, Grant No. NN_124441 and by the Stipendium Hungaricum Scholarship program.

Drought or heavy metals – investigating the abiotic stress tolerance in bryophytes

IT2-2

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Bryophytes are evolutionary models to investigate the settlement of plants on land. Very successfully, they inhabit almost every corner on earth, from antarctica to the rain forest. Most bryophytes lack a cuticle and are therefore quickly affected by drought. It is thus even more surprising that they survive in harsh environments and grow on bare rock or sites with metal contamination and high solar irradiation. By investigating plant anatomy, physiology and subcellular features of bryophytes, we are looking for the clues that render bryophytes so tolerant to abiotic stress factors. We apply high resolution 2D- and 3D-microscopy, specific staining techniques and elemental analysis at the scanning electron microscope and synchrotron. The cell shape and the cell wall play a major role in metal tolerance. Moss species with long cells and thick cell walls are more tolerant than species with isodiametric cell shapes and thin walls. Additionally, we found species-specific tolerance to heavy metals. Very recent experiments at the synchrotron show that some metals are evenly distributed on the leaf surface, some others – like manganese – are locally adsorbed to specific cells. With respect to drought tolerance, desiccation experiments clearly show cytorrhysis with species-specific adaptions to rehydration times. Additionally, we noticed differences in physiological parameters of the investigated bryophyte species and a varying ability to shrink by diminishing cell size. We are now looking at the subcellular level and analyze the changes of cortical microtubules and the endoplasmic reticulum in cells with reduced turgor pressure.

Keywords: dehydration, metal adsorption, moss, turgor

Acknowledgment: This work was supported and expanded from a bilateral project SRB15/2018 between Serbia and Austria within the international "Scientific and Technical Cooperation" program of the OeAD (Austrian Agency for Education and Internationalisation).

Flooding and antioxidative response in plants

IT2-3

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Recent climate changes, caused by anthropogenic gas emissions, affect global warming, leading to an increase in air temperature and changes in other climate elements, causing pronounced droughts and floods. According to the RCP (representative concentration pathway) 8.5 climate scenario, in the future we can expect even more extreme droughts, but also more intense precipitation that can cause flash floods (fluvial, torrential and urban). The impact of flooding on plants is intense: flooding reduces O_2 availability leading to hypoxia and even anoxia. In addition, flooding negatively affects energy metabolism by reducing the level of ATP synthesis, the process of photosynthesis, plant growth and yield. Although it reduces the level of O_2, flooding in plants leads to increased production of ROS and oxidative stress. Plants have developed numerous morphological, physiological and biochemical adaptations that enable them to cope with the stress caused by flooding. An important defense mechanism against flooding is antioxidative metabolism (enzymatic and non-enzymatic). Our work aimed to evaluate antioxidative metabolism in two genotypes of maize and two genotypes of wheat exposed to short-term flooding. We elaborated roles of antioxidative enzymes (superoxide dismutase, catalase, Class III peroxidase) and non-enzymatic antioxidants (phenolic compounds and glutathione) in response to flooding stress, with the aim to detect flooding-resistant genotypes of maize and wheat.

Keywords: maize, wheat, non-enzymatic antioxidants, enzymatic antioxidants, genotypes

Acknowledgment: This work was funded by the Ministry of Scientific and Technological Development, Higher Education and Information Society of the Republic of Srpska (Project No. 19.032/961-104/19 and 19.032/961-95/19). The work is also supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-68/2022-14/200007).

Distinctive regulation of different phenolics biosynthesis by high light and UV-B in three basil varieties

IT2-4

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Three varieties of basil (*Ocimum x citriodorum*, *Ocimum basilicum* var. Genovese and *Ocimum basilicum* var. *purpurascens*) were used to examine the effect of different PAR intensities (100, 400 and 1400 µmol photons $m^{-2}s^{-1}$), as well as different UV-B/PAR ratios, on leaf phenolics accumulation, and components of the ascorbate-glutathione cycle. Phenolic compounds represent the most abundant class of secondary metabolites, and their function in plants involves protection against numerous abiotic stresses, role in growth and development, flowering, reproduction and seed dispersion. A preferential accumulation of HCAs over Flav, induction of class III POD activity and decreased ascorbate content were characteristic responses to pro-oxidative effect of the high UV-B/PAR ratio in all three basil varieties. The most remarkable result was the lack of accumulation of epidermal UV absorbing substances, which appeared to be a crucial photoprotective mechanism in sunlight. The contrasting effect of ecologically relevant UV-B radiation on basil plants, pro-oxidative vs. acclimative, was determined by the quality and intensity of the background light. Acclimation of basil to high light comprises a number of processes, among which are the accumulation of epidermal flavonoids and total leaf phenolics, antioxidant response (increase in Asc and GSH) and lack of downregulation of PSII upon increasing light intensity. Among varieties that we used as model system in this study, purple basil, with the highest constitutive amount of anthocyanidins, showed the least induction of epidermal flavonoids and lack of light effect upon transfer from initial 50 to 250 µmol photons $m^{-2}s^{-1}$.

Keywords: basil varieties, phenolics, high light, leaf anthocyanins

Acknowledgment: This research was funded by the Ministry of Education, Science and Technological Development, the Republic of Serbia (Contract No. 451-03-68/2022-14/200053, 2022).

SELECTED TALKS

What happens with phloretin in plants? – Phloretin real-time effects and post-treatment metabolism in treated Arabidopsis seedlings

ST2-1

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Phloretin, phenolic compound found exclusively in Apple trees, possesses bioherbicidal potential towards other plant species. Our recent studies emphasized phloretin phytotoxic action towards model plant Arabidopsis [*Arabidopsis thaliana* (L.) Heynh.] as a consequence of disturbed auxin homeostasis in roots and disrupted normal leaf cell function due to chloroplast ultrastructural changes and decreased chlorophyll a and b levels. Given the known phloretin reactivity that results from its flexible dihydrochalcones structure, this study aimed to elucidate phloretin direct effects on the root plasma membrane, as well as phloretin post-treatment metabolism in Arabidopsis plants. Electrophysiological measurements were performed to assess real-time response of root plasma membrane to exogenously applied phloretin. Exposing Arabidopsis roots to a 250 μM phloretin resulted in rapid and significant depolarization of the root membrane potential with amplitude of about 47mV that confirmed high sensitivity of Arabidopsis to phloretin treatment. Reverse-phase liquid chromatography with mass spectrometry (LC-MS) was used to evaluate post-treatment metabolism of phloretin in Arabidopsis seedlings grown on 500 μM phloretin enriched medium for 10 days. Analyses revealed that phloretin underwent intensive glycosylation within plant tissues. Dominant peaks from treated plants showed a palette of phloretin derivatives mainly mono- and di-glycosides. Their quantity was significantly higher than in control plants, suggesting high uptake and metabolic interconversion of phloretin as an attempt of phloretin detoxification and/or preparation for vacuolar sequestration.

Keywords: electrophysiological measurements, liquid chromatography with mass spectrometry, membrane potential, phloretin, phytotoxicity

Acknowledgment: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract number 451-03-68/2022-14/200007.

Fullerenol ($C_{60}(OH)_{24}$) as a potent stress alleviator against drought and trace-element toxicity in *Alliaria petiolata* (M.Bieb.) Cavara et Grande

ST2-2

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Trace element (TE) pollution of soil combined with drought stress can cause severe environmental alterations, thus presenting an ongoing challenge on a global scale. Carbon nanomaterials, including fullerene and its water-soluble derivatives fullerenols are attracting increasing attention due to their exquisite applicative potential in modulating plant response under stress condition. Thus, we tested fullerenol (FNP) as a potential anti-drought and biostimulant agent, aiming to provide insights into TE accumulation and tolerance mechanisms of *Alliaria petiolata* grown on multi-metal contaminated soil combiend with drought stress. The FNP was applied in a foliar spray manner (100 and 200 nM concentration), and its effect was tested by analysing plant biomass, photosynthetic performances, chlorophyll fluorescence, cadmium (Cd), lead (Pb) and zinc (Zn) accumulation, as well as oxidative stress markers. The decrease in plant biomass production induced by TE and drought effects was lessened by FNP addition. The beneficial effect of FNP in preserving the efficacy of photosynthetic apparatus by elevation of chlorophyll content and increased photochemical efficiency was evident under multi-stress condition. Further, FNP diminished negative effect of water stress by decreasing proline content in leaves and roots, regardless of the applied concentration, compared to non FNP-treated plants. The same trend was noticed in MDA content, indicating the favorable effect of FNP on drought tolerance. Interestingly, FNP induced elevation of Zn and Pb accumulation in above-ground plant parts in polluted soil, respectively under multi-stress condition, with no significant changes in Cd level. Observed results indicate that FNP can mitigate oxidative stress thus facilitating phytoremediation process.

Keywords: phytoremediation, nanotechnology, drought stress, heavy metal tolerance, antioxidative response

Acknowledgment: This work was supported by the French National Research Agency [ECOPOLIS ANR-20-CE22-0010] and the Pays de Montbéliard Agglomération [PIA3 Territoire d'Innovation]. D.A. received a post-doc grant from the Région Bourgogne Franche-Comté.

POSTER PRESENTATIONS

Effects of high metal concentrations on antioxidant enzymes activities in *Typha latifolia* grown in mine and flotation tailings ponds

PP2-1

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Typha latifolia (cattail) occurs naturally in a variety of wetlands and shallow waters. It also grows spontaneously in the shallow waters of mine and flotation tailings ponds, which are characterized by extremely high concentrations of metals. The main objective of this study was to examine the accumulation and translocation of metals in the plants and to evaluate the effects of elevated metal concentrations on the activity of antioxidant enzymes in cattail roots and leaves. Metal concentrations in plant roots were positively correlated with their concentration in sediment, whereas metal concentrations in leaves were significantly lower than in roots due to their relatively low translocation to the aboveground plant parts. The activities of superoxide dismutase, catalase and glutathione reductase were highest in the roots of plants from Robule, possibly as a result of ultra-acidic reaction of the substrate. The statistically significant higher activities of catalase, ascorbate peroxidase, and guaiacol peroxidase in the plants' leaves from Rudnik could be a consequence of the synergistic effect of elevated concentration of Pb and Zn in plant leaves. The obtained results suggest that the high metal concentration in tailings triggered oxidative stress in cattail which led to an increase in the activities of the studied antioxidant enzymes, especially in the roots. This study provided useful information on plant metal tolerance and suggests that cattail could be a potentially useful macrophyte for application in phytoremediation of outflow water from mining processes heavily contaminated with metals, as a part of constructed wetlands.

Keywords: cattail, phytoremediation, metal tolerance, oxidative stress

Acknowledgment: This work was supported by the Serbian Ministry of Education, Science and Technological Development (Grant No. 451-03-68/2022-14/200178).

Evaluation of ROS production in selected bryophytes during salt stress via EPR spectroscopy

PP2-2

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Plants exposed to salt stress are characterized by specific metabolic, physiological and anatomical changes that result from signaling pathways. One of the most common groups of intermediates produced by stress in plants are reactive oxygen species (ROS). Besides harmful effects of ROS, they play a significant role in plant growth and development. In this study, we used EPR spectroscopy with the aim to identify and quantify ROS and to explore possible patterns of antioxidative defense mechanisms. Three bryophyte species that showed different adaptation strategies to salt stress (non-halophyte moss *Physcomitrella patens*, and moss-halophytes *Entosthodon hungaricus* and *Hennediella heimii*) were exposed to different NaCl concentrations for 3 weeks. Subsequently, for the first time, spin-trap EPR spectroscopy was applied using DEPMPO for the detection of ROS in whole plants *in vivo*. Whereas hydroxyl radical ($^{\bullet}$OH) production was high in *P. patens* control plants, it decreased in all mosses when treated with NaCl. In general, bryo-halophytes produced less $^{\bullet}$OH radicals compared to *P. patens*. However, *E. hungaricus* expressed pattern similarity with *P. patens* compared to *H. heimii*. Ascorbil radical (Asc$^{\bullet-}$) was also detected, and differences between non-halophyte and bryo-halophyte were evident. Alongside with great production of $^{\bullet}$OH *P. patens* also produced Asc$^{\bullet-}$ in control plants. On the contrary, bryo-halophytes have produced Asc$^{\bullet-}$ exclusively when treated with high concentration of NaCl, which may be due to high activity of antioxidative mechanisms, but also decreased photosynthetic activity. It is evident that tested species have diverse mechanisms of antioxidative defense, suggesting different survival strategies to salt exposure.

Keywords: EPR, mosses, ROS, salt stress

Acknowledgment: We acknowledge the support of the Ministry of Education, Science and Technological Development (Grant. No. 451-03-68/2022-14/200178).

How ABA affects total phenolic compounds in bryophytes during NaCl stress?

PP2-3

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Phenolics are very potent non-enzymatic component of bryophyte's antioxidative mechanisms and often have a key role in scavenging reactive oxygen species. In this study, three bryophyte species with different adaptive strategies to salt, were selected (namely non-halophyte *Physcomitrella patens* and bryo-halophytes *Entosthodon hungaricus* and *Hennediella heimii*) to investigate how ABA affects total phenolic content (including phenolic acids, flavonoids and coumarins). Bryophyte species were studied during salt stress to reveal localization of phenolic accumulation in protonema and phylloid cells. The greatest concentration of total phenolics (phenolic acids, flavonoids and coumarins included) was detected in *E. hungaricus*, compared to *P. patens* and *H. heimii*. However, when ABA was applied, total phenolics decrement occurred. Control plants displayed high concentration of flavonoids that decreased significantly in ABA treated *E. hungaricus.* Nevertheless, bryo-halophytes maintained higher concentration of phenolics than non-halophyte *P. patens* during exposure to 50 mM NaCl, suggesting that total phenolic content was not influenced by mild stress in those species. Conversely, during extreme stress (250 mM NaCl) all phenolics decreased except coumarins in *P. patens*, indicating their protective role during salt stress. Compared to other two species, *H. heimii* maintained stable concentration of phenolic acids, suggesting different strategy towards salt stress. UV autofluorescence undoubtedly showed presence of phenolics in cytoplasm of phylloids and protonemata. Besides, ABA treatment leads to cell walls fluorescence, especially in *P. patens* and *H. heimii*, indicating accumulation of phenols in cell walls. Phenolic compounds are clearly important component of investigated bryophytes contributing their survival of salt stress.

Keywords: ABA, NaCl, stress, mosses, phenolics

Acknowledgment: We acknowledge the support of the Ministry of Education, Science and Technological Development (Grant. No. 451-03-68/2022-14/200178).

Histochemical detection of Cu, Zn, and Pb in *Phragmites australis* growing in flotation tailings ponds

PP2-4

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Flotation tailings are highly contaminated with potentially toxic metals and represent a very unfavorable environment for most plant species. However, some plants have developed strategies to cope with these conditions successfully. *Phragmites australis* (common reed) inhabiting the Pb, Zn and Cu mine in Rudnik Mountain (central Serbia) were investigated in this study. The main objective of this research was to analyze metal concentrations in plant parts and localize potentially toxic metals in plant tissues. Pb, Cu and Zn in plant parts were detected histochemicaly by sodium rhodizonate solution (pH neutral) and zincon, and by SEM-EDX. The results showed that Pb and Zn were mainly accumulated in the cell walls, while Cu was mainly stored in the cell vacuole. In roots, Pb and Zn were detected in the cell walls of exodermis, sclerenchyma and pith cells. The presence of Cu was detected in all tissues except in the parenchyma cells of the root cortex. In leaves, Pb and Zn were accumulated in the cell walls of the sclerenchyma cells, while Zn was also stored in the phloem cells. Copper was detected in the cells of phloem and bundle sheath. The obtained results suggest that *Phragmites australis* metal tolerance is also based on effective metal immobilization in its tissues, reducing the potentially harmful effects of accumulated metals on plant vital functions.

Keywords: common reed, phytoremediation, histochemical staining, metals immobilization

Acknowledgment: This work was supported by the Serbian Ministry of Education, Science and Technological Development (Grant No. 451-03-68/2022-14/ 200178).

Enzymatic component of antioxidative system in succulent plant *Tacitus bellus* as a response to hemibiotroph *Fusarium verticillioides* infection *in vitro*

PP2-5

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Fungi and plants interact in different ways, creating a scale of associations. Hemibiotrophic fungi represent the most interesting group, as they use sequential biotrophic and necrotrophic infection strategies. The co-evolution of plant and fungal life-styles has not been well characterized. We present changes of enzymatic component of antioxidative system in succulent plant *Tacitus bellus* that specifically correspond to subsequent phases (spore germination, biotrophic phase, and necrotrophic phase) of hemibiotroph *Fusarium verticillioides* infection. *T. bellus* response to *F. verticillioides* spore germination was characterized by transient increase in catalase (CAT), but decrease in superoxide dismutase (SOD) and peroxidase (POD) activity. During biotrophic phase of *F. verticillioides* infection, when hyphae spread intercellularly in epidermal and mesophyll tissue, host antioxidative system was suppressed. The transition from biotrophic to necrotrophic phase (inter and intracellular colonization and sporulation) triggered the host plant cells to create a highly defensive environment: CAT, SOD and POD activities were significantly stimulated, slowing, or even currently arresting, colonization of *T. bellus* mesophyll cells. CAT, showing the most pronounced activity increase, could be suggested as the main enzyme responsible for slowing the progression of necrotrophic phase of *F. verticillioides* growth. However, contrary to host CAT and SOD which isoenzyme profile didn't change, new highly acidic POD isoforms replaced the two mildly acidic isoforms, suggesting their specific role in slowing the progression of infection. Presented results add to knowledge of events and mechanisms related to hemibiotrophic fungi pathogenicity in succulent plants grown under high relative humidity, similar to conditions in greenhouse.

Keywords: *Tacitus bellus*, *Fusarium verticillioides*, fungal leaf infection, antioxidative enzymes

Acknowledgment: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (contract no. 451-03-68/2022-14/ 200053; 451-03-68/2022-14/ 200178; 451-03-68/2022-14/ 200007.

Zinc provokes different morphogenesis patterns of various genotypes of the moss *Atrichum undulatum*

PP2-6

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The response to stress caused by different concentrations of zinc of three *Atrichum undulatum* genotypes (Serbian, Hungarian and German) was examined under controlled in vitro conditions. After establishing *in vitro* culture of *A. undulatum*, the plants were exposed to long-term (4w) and short-term (2 and 24h) stress by using zinc acetate dehydrate salts. Additionally, biochemical analysis (photosynthetic pigments concentration), as well as quantitative analyses (measurement of zinc content in cauloids and phylloids) were performed. The results obtained for morphogenetic parameters (survival and index of multiplication) may indicate different survival strategies of the specific genotype depending on the duration of stress. Short-term stress did not affect survival of Serbian and Hungarian genotypes as well as the concentration of chlorophylls. However, concentration of total chlorophylls decreased in long-term stress. The concentration of total carotenoids in plants exposed to long-term stress decreased with the increase in the concentration of zinc in the substrate, indicating that plants, in addition to carotenoids, use other enzymatic and non-enzymatic protection mechanisms to keep the plants alive. During long-term stress, the highest zinc content in all three genotypes was recorded in cauloids and phylloids of the plants grown on the medium with the highest zinc concentration. Finally, based on morphogenesis parameters, physiological parameters, as well as detection adoption of zinc in phylloids and cauloids, it can be concluded that the Serbian and the Hungarian genotype share similar patterns, while the German genotype differs in overcoming stress. Thus, the genotypic plasticity is also influencing the stress reaction of mosses.

Keywords: moss, salt stress, zinc acetate dehydrate

Acknowledgment: We acknowledge the support of the Ministry of Education, Science and Technological Development (Grant. No. 451-03-68/2022-14/200178).

Flooding induced changes in photosynthetic pigments and oxidative status in leaves of pea plants

PP2-7

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Soil flooding, nowadays, is one of the most common abiotic stresses that negatively affect plant growth and crop yield. Flooding reduces the intensity of photosynthesis, has negative effects on energy metabolism, favours the generation of reactive oxygen species (ROS) and the induction of oxidative stress. The consequence of oxidative stress is lipid peroxidation (LP) and an increase in the concentration of malondialdehyde (MDA) as indicators of LP. The aim of this paper was to analyse the impact of flooding on the concentration of photosynthetic pigments and oxidative parameters (MDA and H_2O_2) in pea plant leaves. Pea plants were exposed to flooding for 3 and 7 days, during which the oxygen concentration decreased by 44% compared to the beginning of the treatment. Extraction of MDA and H_2O_2 was done in an acidic medium, using trichloroacetic acid, while the photosynthetic pigments were extracted in acetone. Concentration of measured parameters was determined spectrophotometrically. The results showed that the flooding led to an increase in the chlorophyll a and total chlorophyll concentration. During flooding, the H_2O_2 concentration did not change significantly while an increase in MDA concentration was measured. Increased MDA concentration indicates the effect of flooding on cell membranes and induction of lipid peroxidation. The obtained results confirm the influence of flooding on photosynthetic pigments and oxidative parameters in pea leaves. In the future, research on the impact of flooding on pea plants should take into account the different duration of the flooding as well as the developmental stage of the plants.

Keywords: flooding, pea plant, lipid peroxidation, H_2O_2, photosynthetic pigments

Antioxidant defence system in pea plants under short-term waterlogging stress

PP2-8

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Optimal water supply is an important factor for plant growth and development. However, excess water in the soil can cause reduce in oxygen levels, thus resulting in hypoxia. This study aimed to evaluate the influence of short-term flooding and consequent oxygen deficiency on non-enzymatic (total phenolic and flavonoid content) and enzymatic (Class III peroxidases (POD, EC 1.11.1.7)) antioxidants in pea plants exposed to flooding stress for 3 and 7 days as well as in plants after 15 days of post-submergence recovery. Folin-Ciocalteu reagent was used for total phenolic content determination, while total flavonoid content was measured using the aluminum chloride colorimetric method. The evaluation of the antioxidant activity of plant ethanolic extracts was done using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Obtained results showed that compared to the control, 7-day flooding caused significant changes in the ability of the leaf extract to remove the DPPH radical and in the activity of PODs in the leaves. In flooded plants, after recovery, significant changes relative to control were detected in flavonoid content and the ability to remove DPPH radical in the leaves, and the content of total phenolics in the leaves and root. The results were discussed in terms of the importance of antioxidant protection systems, especially non-enzymatic, in the plant's response to flooding.

Keywords: flooding, phenolic compounds, flavonoids, antioxidant activity, peroxidases

Efficient generation of *CRISPR/Cas9*-Mediated Knockouts of Two Highly Homologous Intrinsically Disordered Proteins AtDSS1(I) and DSS1(V) and their partial characterization

PP2-9

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DSS1 is a small acidic protein, highly conserved across different species. As a member of the intrinsically disordered protein family, DSS1 interacts with different protein partners, thus forming complexes involved in diverse biological functions such as: DNA repair, regulation of protein homeostasis, mRNA export, etc. The *Arabidopsis thaliana* genome contains two highly homologous *DSS1* genes, *AtDSS1(I)* (At1g64750) and *AtDss1(V)* (At5g45010), embedded within chromosomes I and V, respectively. This study was designed with the aim to identify phenotypic or functional differences between *AtDSS1(I)* and *AtDSS1(V)*, as well as to decode their potential role in oxidative stress. We have generated several single *dss1(I)* and *dss1(V)* mutant lines of both of Arabidopsis *DSS1* genes by using the CRISPR-Cas9 technology. Mutant lines with larger indels (*dss1(I)Δ25* and *dss1(V)*+18) in homozygous state were selected for further characterization. The mutant lines were phenotypically described during plant development, and their sensitivity to oxidative stress was analyzed. The phenotypic characterization revealed that *dss1(I)* and *dss1(V)* mutant lines differed in root and stem length, as well as in rosette area size. Plants with disrupted *AtDSS1(V)* gene showed reduced survival rate and increased level of oxidized proteins in comparison to WT plants when exposed to oxidative stress induced by hydrogen peroxide. These results suggest DSS1(V) protein could be a important player in the molecular mechanisms underlying in plant abiotic stress responses.

Keywords: Arabidopsis, oxidative stress, protein homeostasis, genome editing, intrinsically disordered proteins

Recovery of photosynthetic apparatus of resurrection plant *Haberlea rhodopensis* from drought- and freezing-induced desiccation

PP2-10

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Haberlea rhodopensis is an unique desiccation tolerant angiosperm that can also survive winter frost. Similar to drought, temperatures below -6 °C induce dehydration of plants, and they endure the harsh winter conditions in desiccated state. Moreover, the recovery of plants during the initial phase of rehydration is a serious stress for plants but less investigated. In this study, the recovery of photosynthetic activity during first hours of rehydration of *H. rhodopensis* from drought- and freezing-induced desiccation were investigated. The water uptake during the initial 15 h was slow thus preventing cellular damages. The results showed faster recovery of quantum efficiency of PSII in plants rehydrated after freezing stress (RAF) compared to plants rehydrated after drought stress (RAD) and the most significant differences between them were evident after 9–15 h of rehydration. Following rehydration, PSI activity recovered faster compared to PSII and in contrast to PSII, its activity was higher in RAD compared to RAF plants. During the first hours of rehydration, prominent alterations in energy transfer between photosynthetic complexes occurred as revealed by 77 K fluorescence of isolated thylakoids. High proportion of thermal energy dissipation in dry plants and during the first hours of rehydration protects them from photooxidation. The most obvious change in thylakoid complexes was the increase in the proportion of the PSI-LHCII complex, which started around 70% RWC. The oligomerization of Lhc-m was observed from the beginning of the recovery period. Some increase in the proportion of PSII-s complexes started around 20-30% RWC.

Keywords: desiccation tolerance, rehydration, chlorophyll fluorescence, 77 K fluorescence spectra, pigment-protein complexes

Acknowledgment: This work was supported by the Bulgarian National Science Fund, Ministry of Education and Science (Project КП-06-Н21/8).

Antioxidant defense during recovery of resurrection plant *Haberlea rhodopensis* from drought- and freezing-induced desiccation

PP2-11

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In this study, the total antioxidant activity, free-radical scavenging activity, the content of flavonoids and the contribution of nonenzymatic (ascorbate, glutathione) and enzymatic antioxidants (superoxide dismutase, catalase, glutathione reductase, glutathione S-transferase) in the first hours of recovery of the resurrection plant *Haberlea rhodopensis* from drought- and freezing-induced desiccation were assessed. The initial stage of recovery after desiccation is critical for plants, but less investigated. Our results showed that the onset of rehydration led to increase of total antioxidant activity, free-radical scavenging activity and content of flavonoids of *H. rhodopensis* plants, but they decreased below values of those of air-dried leaves after complete recovery in both type of plants. Ascorbate content remained high during the first 9 h of rehydration of desiccated plants and declined when the leaves water content significantly increased. The glutathione content remained high at the first hour of rehydration and then strongly decreased. The changes in ascorbate and glutathione content during recovery from drought- and freezing-induced desiccation showed great similarity. To better understand the alterations in the activity of antioxidant enzymes, their isoenzyme patterns were determined. At the beginning of rehydration (1–5 h), the activities of antioxidant enzymes were significantly increased or remained as in dry plants. During 7–24 h of rehydration, certain differences in the enzymatic responses between the two plant groups were registered. The maintenance of a high antioxidant activity and upregulation of individual enzyme isoforms indicated their essential role in protecting plants from oxidative damage during the onset of recovery.

Keywords: rehydration, drought stress, low temperature, non-enzymatic antioxidants, antioxidant enzymes

Acknowledgment: This work was supported by the Bulgarian National Science Fund, Ministry of Education and Science (Project КП-06-Н21/8).

Mechanisms of desiccation tolerance in *Ramonda serbica* Panc.: transcriptomic, proteomic, metabolomic, and photosynthetic aspects

PP2-12

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Ramonda serbica Panc. is a resurrection plant species that can survive desiccation for a long period and fully resume metabolic functions upon rewatering in a very short period, even within 48 h. The goal of this study was to identify key candidates and pathways involved in desiccation tolerance in *R. serbica*. To achieve this, systems biology approach combining transcriptomics, proteomics, and analysis of specific metabolites was employed. In addition, FTIR analysis of the cell wall polymers and a detailed analysis of the photosynthetic electron transport (PET) chain were performed. In total, 1192 different protein groups were quantified by TMT-based comparative quantitative proteomics. Among them, 408 protein groups showed a statistically significant difference in abundance between hydrated (HL) and desiccated leaves (DL). Upon desiccation, the majority of proteins related to photosynthetic processes were less abundant, while chlorophyll fluorescence measurements implied shifting from linear photosynthetic transport (PET) to cyclic electron transport (CET). The amounts of H_2O_2 scavenging enzymes, including ascorbate-glutathione cycle components, catalases, peroxiredoxins, Fe-, and Mn- superoxide dismutase (SOD) were reduced in DL. However, four Cu/ZnSOD isoforms, three polyphenol oxidases, six germin-like proteins (GLPs), and 22 late embryogenesis abundant proteins (LEAPs; mainly LEA4 and dehydrins), were desiccation-inducible. Desiccation-induced cell wall remodelling by changes in cell wall polymer composition might be linked with pectin demethylesterification and GLP-derived H_2O_2/$HO^\bullet$. Our study demonstrated that desiccation tolerance in *R. serbica* is a complex, species-specific process orchestrated by several metabolic pathways and regulatory networks acting at the transcript, protein, metabolite and physiological levels.

Keywords: cell wall remodelling, cyclic electron transport, germin-like proteins, late embryogenesis abundant proteins, polyphenol oxidase

Acknowledgement: This research was funded by the Science Fund of the Republic of Serbia-RS (PROMIS project LEAPSyn-SCI, grant no. 6039663) and by the Ministry of Education, Science and Technological Development, the Republic of Serbia (Contract No. 451-03-68/2022-14/200042, 2022).

Differential gene expression of lncRNA and mRNA of maize seedlings under chilling stress

PP2-13

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Transcriptome analysis has been widely applied in studies trying to elucidate the molecular mechanisms by which plants adapt to their environment. In consideration of ongoing climate changes, it is very important to get as much information as possible about the pathways and genes included in the plant response to abiotic stress since crop productivity is seriously negatively affected. Maize is one of the most important crop species and one of the promising approaches for avoiding yield losses caused by drought stress is earlier sowing. By applying this strategy, maize plants are exposed to lower than optimal temperatures in the early developmental stages. With the aim of exploring differential gene expression under chilling stress conditions and thus contributing to the enlightenment of mechanisms underlying maize response to stress, lncRNA (long non-coding RNA) and mRNA of maize seedlings were subjected to Next Generation Sequencing (Illumina platform). Two maize inbred lines (tolerant and sensitive) were chosen for the experiment. Samples were taken from five days old seedling stage (grown under control and stress conditions) 6h and 24h from the stres begining. After sequencing, bioinformatic analysis of generated data was done. Results of the differential gene expression in these two types of RNA will be presented.

Keywords: maize, lncRNA, mRNA, DEGs, chilling stress

Acknowledgment: This study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, grant number 451-03-68/2022-14/200040

The content of photosynthetic pigments in *Phragmites communis* (Cav.) Trin. ex Steudel., *Salvina natans* L. (All.), *Utricularia vulgaris* L. and *Ceratophyllum demersum* L. during the growing season in the Bardača fishpond area (Republic of Srpska)

PP2-14

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Aquatic macrophytes form an important part of freshwater ecosystems and play different roles in their structure and function. It is known that their occurrence and distribution depend on water temperature and transparency, nutrient content, conductivity, pH, chemical composition, pH and water circulation. Therefore, the aim of the research was to determine the influence of physical and chemical characteristics of water on the content of photosynthetic pigments in aquatic macrophytes of the Necik pond, at the Ramsar site Bardača (Republic of Srpska). The content of chlorophyll (a+b) during the growing season (Juny-October) in *Phragimtes communis* ranged from 0.231-1.145 mg/gFW, in *Salvina natans* from 0.061-0.541 mg/gFW, in *Utricularia vulgaris* from 0.063-0.626 mg/gFW and in *Caratophyllum demersum* ranged from 0.063-0.626 mg/gFW. Research has shown that floating and submerged macrophytes had a lower a/b ratio compared to emergent hydrophytes, which is most likely due to stress caused by increased light intensity and high temperature. Seasonal variations in the content of photosynthetic pigments indicate that the species *Phragimtes communis* is more tolerant to stress caused by the influence of various abiotic factors, while *Salvina natans* is much more sensitive. Spearman's rank correlation coefficient between different physical and chemical parameters of water quality and the concentration of macrophytes photosynthetic pigments showed a significant influence of certain abiotic factors (temperature, transparency and nutrient content) on the content of photosynthetic pigments in leaves of *Phragmites communis*, *Salvinia natans*, *Utricularia vulgaris* and *Ceratophyllum demersum*.

Keywords: aquatic macrophytes, photosynthetic pigments, stress, eutrophication

The effect of sodium nitroprusside on antioxidative enzymes activity of centaury (*Centaurium erythraea* Rafn) under stress conditions caused by sodium chloride *in vitro*

PP2-15

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Common centaury (C*entaurium erythraea* Rafn) is a medicinal plant used in traditional medicine since ancient times. Centaury inhabits dry pastures, roadsides and fields from lowlands to mountain vegetation. Considering that centaury can also be found on moderately saline habitats in nature, it was interesting to investigate the effect of exogenously applied sodium nitroprusside (SNP), as a donor of nitrogen monoxide (NO), on antioxidative enzymes activity of centaury shoots grown under stress caused by sodium chloride (NaCl) *in vitro*. Centaury shoots were grown on ½MS nutrient media containing graded concentrations of SNP (0, 50, 100 and 250 μM). After three weeks of pretreatment, shoots were further transferred to ½MS nutrient media supplemented with NaCl (0 or 150 mM) and/or SNP (0, 50, 100 or 250 μM) during one week treatment. After four weeks, the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) was determined spectrophotometrically. Thus, through 14 different treatments, the effect of NaCl and SNP on biochemical response of centaury shoots was investigated. The results obtained in this work showed that NaCl decreased the activity of SOD, CAT and POX. On the other hand, exogenous application of SNP increased SOD, CAT and POX activity in centaury shoots after four weeks. It can be concluded that the effect of SNP on increased tolerance of centaury shoots to salinity is reflected through the changed activity of enzymatic components of antioxidant defence.

Keywords: centaury, reactive oxygen species, nitric oxide, salinity

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract number 451-03-68/2022-14/200007.

Chilling induced stress sRNAs in maize plants in early stages of development

PP2-16

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Small non-coding RNAs (sRNAs) have an important role in regulating plant gene expression, both in developmental processes and stress responses, through RNA interference or silencing. sRNAs respond to environmental stressors in manners dependant on the type of stress, tissue and genetic background. Herein, their role in chilling stress response is examined in young maize seedlings (*Zea mays* L.) of two inbred lines of contrasting chilling tolerance, using high-throughput sequencing methods. Understanding this response is critical since one of the most promising strategies of avoiding maize yield loss due to climate change is earlier sowing, during periods of suboptimal temperatures. Sterilized seeds of the two lines, marked as LT (tolerant), and LS (sensitive), were germinated in the dark for five days (optimal conditions), after which the 5-d old seedlings were exposed to chilling conditions for 24h (temperature: 10/8 °C; photoperiod: 12/12h). Samples for RNA isolation were taken after 6h and 24h of treatment. After the cDNA library preparations were finished, single-end 50 bp sequencing was performed (Illumina® Novaseq 6000). sRNAseq analysis was performed using a custom pipeline that included quality control, mapping, known miRNA alignment and novel miRNA prediction and quantification. This is an ongoing research and a selection of results will be presented. On average 81% of sRNA reads were succesfully mapped and belong to the class of microRNAs (miRNAs). Some of the sRNAs appear to be differentialy regulated depending on the genotype and the chilling conditions, but more research is needed to verify their potential role in this process.

Keywords: maize, abiotic stress, chilling, NGS, sRNAs

Acknowledgment: This study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, grant number 451-03-68/2022-14/200040

Effect of moderate water deficit on biochemical and physiological parameters of tomato leaves and fruits

PP2-17

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Drought affected growth and productivity of vegetables by inducing different changes in plants, which consequently reduces the yield and its quality. The aim of this work was to evaluate the effect of moderate drought stress on physiological and biochemical parameters of the cultivated tomato genotype Stupicke. Plants were grown in the greenhouse and exposed to water deficit from anthesis of 2nd flower truss until fruit harvesting (red-ripe stage). Control plants were optimally irrigated, while treated plants were exposed to drought and soil humidity was maintained around 25% of maximum WHC. Drought-induced reduction of leaf growth parameters was followed by changes in stomata conductance and photochemical efficiency. The results of biochemical analysis indicated that the soluble sugar content decreased under drought conditions, while organic acid level and vitamin C was increased. The significant decrease in fruit diameter and dry matter content was accompanied with stable sugars and organic acid content which implicates the maintenance of main fruit taste components under water deficit. The drought also affected the level of other important nutritional components by the increase of the total carotenoid and vitamin C content. Accumulation of the vitamin C in leaves and fruits are also a response to secondary, oxidative stress under prolonged drought, while the increase of ABA content in both organs is related to drought adaptive responses. These results indicated the potential of this genotype for breeding programs to create new drought-resistant tomato lines with the stable or improved nutritional fruit quality under variable environmental conditions.

Keywords: tomato, moderate drought, leaf growth, fruit quality, breeding programs

Acknowledgment: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grants No. 451-03-68/2022-14/200116 and 451-03- 68/2022-14/200216).

Photosynthetic performance of *Arabodopsis thaliana*, wt and lut2 mutant, after treatment with two stress factors – low temperature and high light illumination

PP2-18

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The response of lutein-deficient mutant *lut2* of *Arabidopsis thaliana* to combined treatment for 6 days with two stress factors – low temperature at high light illumination and after recovery at control conditions was evaluated in comparison with the wild type. Obtained results indicated that under control growth conditions the net photosynthetic rate, oxygen evolving activity, effectiveness of NPQ, qP and non-regulated energy dissipation were lower in the mutant, while the cyclic electron transport through PSI operated faster than in the wild type. The contribution of alternative electron flows, PGR5- and PTOX-dependent, for the effectiveness of photosynthetic performance of wt and *lut2* after treatment with two stress factors was evaluated by application of two specific electron transport inhibitors - antimycin A and octyl gallate, respectively. The lutein-deficient mutant *lut2* demonstrated lower electrolyte leakage, lower excitation pressure and higher effectiveness of PSII performance in comparison with the wt during treatment indicating lower susceptibility to applied stress conditions in the mutant. The lower values of Φ_{NPQ} and its main component qP in *lut2* during treatment pointed out that the non-photochemical mechanisms of energy dissipation in LHCII were not responsible for the higher stress tolerance of *lut2*. It was supposed that the higher capacity for cyclic electron flow through PSI and PSI-dependent energy quenching could serve as efficient photoprotective mechanism thus providing higher tolerance of the mutant to combined stress treatment.

Keywords: abiotic stress, carotenoid mutant, energy partitioning, photoprotection, photosynthetic performance

Acknowledgment: This work was financially supported by Bulgarian Science Fund under project КП-06-Н26/11. The seeds of the wt and mutant lut2 of Arabidopsis thaliana were a obtained from Prof. R. Bassi.

Alterations in primary photosynthetic reactions in lut2 mutant of *Arabidopsis thaliana* as a response to combined treatment with low temperature and high light

PP2-19

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Performance of the primary photosynthetic reactions at exposure for 6 days to combined stress – low temperature and high light illumination of whole plants of *Arabidopsis thaliana*, wild type and lutein-deficient *lut2* mutant, was investigated. Special attention was paid to recovery of plants at control conditions. The response of wt and *lut2* to both stress factors was evaluated by changes in net photosynthesis, photochemistry and energy distribution and interaction between two photosystems. The alterations in oxygen evolving capacity were evaluated by registration of flash-induced oxygen yields and kinetics of initial oxygen burst. The effect of exposure at low temperature and high light on the fluidity of thylakoid membranes isolated from wt and *lut2* plants was assessed. During exposure to combined action of two stress factors the antenna size of photosystem I and photosystem II were affected as revealed by 77K fluorescence emission and excitation spectra. Obtained results about net photosynthetic rate, photochemical activity of photosystem II, activity of oxygen evolving complex and fluidity of the lipid phase of thylakoid membranes indicated that *lut2* was more negatively affected by the combined stress treatment in comparison with the wt. The grana-situated photosystem II complexes were more sensitive to applied stress. However, the lutein-deficient mutant showed higher ability to recover the photosynthetic performance on return of plants to control conditions. In conclusion it was shown that the lack of lutein, the most abundant xanthophyll in higher plants, lead to higher sensitivity to low temperature at high light, provided better recovery than in the wild type.

Keywords: 77 K fluorescence, carotenoid mutant, fluidity, oxygen evolution, photosynthetic performance

Acknowledgment: This work was financially supported by Bulgarian Science Fund under project КП-06-Н26/11. The seeds of the wt and mutant lut2 of Arabidopsis thaliana were a obtained from Prof. R. Bassi.

The effect of silicon on the photosynthetic characteristics of three species from family Brassicaceae exposed to NaCl

PP2-20

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Over 6% of the world's soil contains high concentrations of salt and every year about 2 million hectares of agricultural soil worldwide are degraded due to increased salinization. Global climate change enhances this negative phenomenon in world agriculture. As the presence of Si is known to increase tolerance to abiotic and biotic stress under certain circumstances, this study aimed to assess the possibilities of using silicon to mitigate the negative effects of salt on oilseed rape (*Brassica napus* L.), black (*Sinapis nigra* L.) and white mustard (*Sinapis alba* L.). The impact of silicon (final conc. 2 ml/L) on the growth and photosynthetic activity of plants exposed to the increased salt concentrations (50 mM and 100 mM NaCl added to Hoagland nutrient solution) and growing in semi-controlled conditions of a greenhouse, was analyzed. Treatments with Si increased the mass of plants (root, stem, leaf) in all three species compared to the treatments with 50 and 100 mM NaCl alone, and in black and white mustard also compared to the control. The addition of Si to the nutrient solution containing 50 mM NaCl alleviated the effect of salts on the concentration of photosynthetic pigments only in oilseed rape. In white mustard, the photochemical activity of photosystem II significantly differed from non-Si treatments, and control. It increased by 42% in the presence of Si and 50 mM NaCl, and by 40% in the presence of Si and 100 mM NaCl compared to the solution without Si, but with the same concentration of NaCl.

Keywords: *Brassica napus* L., *Brassica nigra* L., *Sinapis alba* L., silicon, sodium chloride

Acknowledgment: We thank the Ministry of Education, Science and Technological Development of the Republic of Serbia for financial support (451-03-68/2022-14/200117).

The role of the sink tissue in variegated *Pelargonium zonale* under excess excitation energy pressure: morphologic, photosynthetic and metabolic study

PP2-21

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In the global warming era, we are facing extreme changes in environmental conditions such as high and low temperatures, high light and ultraviolet radiation intensity, as well as drought and increased CO_2 levels. The effects of these factors on plants are often interrelated and usually result in a disturbed balance between the amount of energy received and the ability to process it. In order to balance energy input and prevent photooxidative damage, plants have evolved multiple mechanisms for energy dissipation and photoprotection. To efficiently dissipate the excess excitation energy (EEE), the additional electron sinks, such as photorespiration, biosynthesis of phenolics and nitrate reduction are stimulated. Variegated leaves of *Pelargonium zonale* have been proven to be a suitable model system for examining 'source–sink' interactions within the same leaf, providing the same microenvironment conditions, unlike common root-shoot studies. The aim of our study was to investigate carbon and nitrogen reallocation between photosynthetically active (green leaf sectors) and photosynthetically inactive (white leaf sectors) under EEE pressure induced by low temperature in combination with high light intensity. Besides the amino acids and phenolics analyses, we monitored morphological and photosynthetic parameters of *P. zonale* leaves. Our results revealed higher content of free proteogenic amino acids in the white leaf sectors than in the green ones. Moreover, EEE triggered increased polyphenol synthesis, namely anthocyanins, already on the fourth day of the experiment. Also, the leaf fresh/dry weight (FW/DW) ratio was significantly lower in plants exposed to EEE, indicating possible cell wall stiffening.

Keywords: Amino acids, anthocyanins, high light intensity, 'source–sink' interactions, variegated plants.

Acknowledgements: This research was funded by the Ministry of Education, Science and Technological Development, the Republic of Serbia (Contract No. 451-03-68/2022-14/200042, 2022).

The usage of silicon fertilisation in order to mitigate the oxidative stress and to improve the resilience of barley subjected to drought

PP2-22

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Drought causes huge agricultural and economic losses worldwide. Silicon (Si) is considered a beneficial element for plants. It mitigates stress caused by salinity, drought, and high and low temperatures by promoting antioxidant production. However, the underlying mechanisms are still not elucidated. We investigated Si fertilisation effect on photosynthetic parameters, trichome number, leaf optical properties, as well as profiles of amino acids and polyphenols in barley exposed to water shortage. Silicon was applied in three growth stages: (i) before the flag leaf emergence; (ii) prior to the grain filling phase; (iii) at the grain filling phase start. Drought negatively impacts photochemical efficiency, stomatal conductance, photosynthetic pigment content, and leaf reflective and transmissive properties. Si application between flag leaf emergence and grain filling had the strongest effect on light reflectance. Among all analyzed phenolics, saponarin was the most abundant in all samples, irrespective of water regime and Si supply. Caffeoyl ester was the only hydroxycinnamic acid showing significant accumulation with the latest applied Si compared to no added Si upon drought. The major amino acids in barley leaves were glutamate, glutamine, aspartate, asparagine, and serine. Aspartate content was the highest in leaves exposed to drought without Si addition, while lysine was the most accumulated in the leaves supplemented by Si at the grain filling phase start. Proline was 2.5 times more abundant in the leaves exposed to drought regardless of Si treatment. Taken together, although Si did not mitigate drought stress effects, its effect was dependent on the barley growth phase prior to supplementation.

Keywords: amino acids, proline, polyphenols, optic leaf properties, photosynthesis

Acknowledgement: This work was funded by the Bilateral project between the Republic of Serbia and the Republic of Slovenia (ID No: 337-00-21/2020-09/2) and the Ministry of Education, Science and Technological Development, Republic of Serbia (451-03-68/2022-14/200042, Contract No. 2022). This research was funded by the Slovenian Research Agency (Javna agencija za raziskovalno dejavnost RS) through the young researcher grant (Anja Mavrič Čermelj) and program group P1-0212 (Plant biology).

Physiological and antioxidant defense mechanisms of *Nepeta nervosa* plants against polyethylene glycol (PEG)-mediated drought stress

PP2-23

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Drough-stress conditions lead to excessive production of reactive oxygen species (ROS) and oxidative damage in plants. Plants display various defense mechanisms for survival, including the activation of antioxidant enzymes and the production of low molecular weight non-enzymatic antioxidants like phenolics. In this work, roots of four weeks old *Nepeta nervosa* plants were exposed to PEG solutions with osmotic pressure of −3 MPa for one, three and six days. Fresh weight (FW) and relative water content (RWC) of *N. nervosa* plants were significantly reduced compared to the corresponding control - up to 68% (FW) and up to 35% (RWC). In leaves, the content of rosmarinic and chlorogenic acids, dominant phenolic compounds, as well as of less abundant ferulic and caffeic acids, was dependent on the duration of PEG-induced drought stress. The content of rosmarinic acid in control plants achieved an average value of 18.27 µg/mg FW, while at PEG-treatments it increased by 355% (3 days of PEG-treatment). Among all examined antioxidative enzymes in leaves of PEG treated *N. nervosa* plants, superoxide dismutases (SOD) were the most active (an increase of 853% on day 6). PEG treatment also caused an increase in peroxidase (POX) activity in leaves of N. nervosa plants (108% increase on day 3). Since catalase (CAT) activity was significantly decreased on PEG treatment, except on day 1, it could be presumed that H_2O_2 elimination is performed mostly by POX. This work is a useful contribution to the development of a strategy for overcoming oxidative stress in *Nepeta* species, especially in arid and semiarid regions.

Keywords: water stress, antioxidant enzymes, *Nepeta nervosa*, phenolic acid

Acknowledgment: This work is financed by the Science Fund of the Republic of Serbia, Program IDEAS (project NEPETOME, No. 7749433), and is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/200007).

Production of phenolic compounds and antioxidant activity in *Hypericum perforatum* L. hairy roots elicited with salicylic acid

PP2-24

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The aim of this study was to investigate the effect of different concentrations of salicylic acid (SA: 50, 100 and 250 µM) on the growth, production of total phenolics (TP), flavonoids (TF), flavan-3-ols (TFA) and xanthones (TX), as well antioxidant capacity (DPPH and CUPRAC) in hairy root (HR) cultures of *Hypercum perforatum* L. during the period of post-elicitation (day 1, 4, 7, 14, 21 and 28). Present results demonstrated that exogenous application of 50 µM SA slightly decreased fresh biomass of HR (17%), while 100 and 250 µM SA markedly reduced biomass production (82-83%) compared to control HR cultures. All tested SA concentrations significantly increased production of TP, TF and TFA in elicited HR compared to control HR during the early post-elicitation period (day 1-day 4). At the end of post-elicitation period (day 28), SA treatments showed inhibitory effect on phenolic contents in elicited HR compared to control HR. During the entire post-elicitation period, TX contents in elicited cells were significantly decreased in comparison to control cultures indicating that elicitation with SA could not exceed the immense biosynthetic capability of HR for secondary metabolite production. The antioxidant capacity assays exhibited significantly positive correlation with TP, TF and TFA production suggesting that these phenolic compounds have a great contribution to the antioxidant activity of HR. Therefore, it could be assumed that SA mediated certain type of stress conditions and the enhancement of phenolic contents appear to have a potential role in the antioxidant defense strategy of *H. perforatum* HR cultures.

Keywords: Elicitation, Hairy roots, *Hypericum perforatum* L., Phenolic compounds, Salicylic acid

Overexpression of DSS1 protein in *Arabidopsis thaliana*

PP2-25

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Intrinsically disordered protein DSS1 interacts with different proteins and takes roles in diverse biological processes due to its flexible structure. DSS1 involvement in maintenance of genome stability is the only function well studied in plants. It has been shown that yeast Dss1 (Sem1) is proteasome regulatory and structural subunit. Additionally, it seems DSS1 is involved in elimination of oxidized protein through the proteasome system by targeting damaged proteins in the process called "DSSylation". *Arabidopsis thaliana* genome contains two highly homologous *AtDSS1(I)* and *AtDSS1(V)* genes. We approached in silico analysis of primary DSS1(I) and DSS1(V) amino acid sequences using the PSIPRED algorithm. Seven different amino acids between these two isoforms were observed. DSS1(V) has one amino acid less and only one significant shift of amino acid polarity - from glutamine to leucine at position 44. Our specific ambition is to elucidate the potential role of *DSS1* gene in protein damage control and to add one more piece of puzzle to complicated mechanisms of protein homeostasis in plant cells. We used Gateway cloning technology to generate transgenic arabidopsis overexpressing DSS1 lines. The coding region of *DSS1* was cloned into Gateway-compatible plant expression vector (pEarlyGate 100) under CaMV35S promoter, plus BASTA gene as selection marker. Efficient Agrobacterium-mediated plant transformation was applied and next T1 generation was selected by BASTA herbicide. Level of overexpressed *DSS1* was analyzed in T2 by Real-time PCR.

Keywords: DSS1, Gateway cloning, *Arabidopsis thaliana*

Acknowledgment: This research was supported by the Science Fund of the Republic of Serbia, #6473515, OxInDisPro.

French marigold essential oil affected DNA methylation in potato

PP2-26

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Different stressors from the environment can prime defense and enable enhanced responses to subsequent stress exposure. It has been proposed that induced epigenetic changes, on the level of DNA and histones, could be involved in this response. Plant essential oils (EOs) have shown promising prospects as novel priming inducers. Still, little is known about their potential to induce epigenetic alternations. In this study, we examined the effect of the French marigold (*Tagetes patula* L.) EO on DNA methylation of potato (*Solanum tuberosum* L.). Potato plants were exposed to EO for 3 consecutive days for 8h each day, and samples were collected 10 days after EO removal. Whole-Genome Bisulfite Sequencing (WGBS) was used to identify differentially methylated (DM) DNA sequences among control and EO-exposed samples. Promoters, exons, introns, 3'UTR, 5'UTR, and intergenic regions, each at a differential methylation ratio 0.1 to 0.25 between control and treated samples were analyzed. The average methylation level of the whole genome was 72.29% and 69.23% in control and exposed samples, respectively. Differential DNA methylation has predominantly occurred in the CG context compared to CHH and CHG. In general, hypomethylated sequences outnumbered hypermethylated ones. Functional annotation clustering of differentially methylated genes distinguished 6 affected KEGG pathways – endocytosis (sot04144) and protein processing in the endoplasmatic reticulum (sot04141) among others. The expression of DM genes associated with these pathways, as well as different defense responses in potato (GO:0006952), will be analyzed subsequently in potato plants challenged with different abiotic and biotic stress conditions.

Keywords: DNA methylation, potato, French marigold, essential oil, priming

Acknowledgment: This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract number 451-03-68/2022-14/200007

Morpho-physiological and ultrastructural changes of Arabidopsis seedlings treated with phloretin

PP2-27

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Phloretin is a phenolic compound unique for apple (*Malus* × *domestica* Borkh.) species. Our research group recently reported phytotoxic potential of phloretin towards other plant species such as model plant Arabidopsis. Since sugars in *in vitro* growth media could modulate plant metabolism, we analyzed phloretin effects on Arabidopsis seedlings grown on the medium without sucrose and myoinositol (WSM) but enriched with different concentrations of phloretin (0 to 1500 µM). Phloretin treatment on WSM induced seedlings growth retardation at much lower concentrations compared to sucrose and myoinositol enriched medium. Inhibition of primary root and leaf growth, and lateral root emergence increased with both phloretin concentration and treatment duration (maximal 85.2%, 30.4% and 46.5% at 1500 µM phloretin at 15 days after germination - DAG, respectively). Prolonged cultivation (4 weeks) at phloretin concentrations over 500 µM induced complete growth stagnation and necrosis of the leaves. Transmission electron microscopy reviled relocation of chloroplasts within mesophyll cells, shape change into spherical form, as well as ultrastructural changes in thylakoid organization in leaves of 10 DAG-old seedlings treated with 250 or 500 µM phloretin. Significantly decreased number of starch grains in chloroplasts and chlorophyll a and b content in leaves reflected impaired function of chloroplasts due to ultrastructural changes. Phloretin also induced cell wall undulation and folds that resulted in irregularly shaped mesophyll cells. The presented study revealed that beside roots that had been previously emphasized as primary targets of phloretin action, leaves were also significantly affected by phloretin treatment, especially in the aspect of photosynthetic apparatus.

Keywords: bioherbicide, *Malus* × *domestica* Borkh., phloretin, phytotoxicity, ultrastructural changes

Acknowledgment: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract number 451-03-68/2022-14/200007.

Phloretin, dihydrochalcone from apple tree, bursts antioxidant capacity of tested Arabidopsis seedlings

PP2-28

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Apple species are exclusively rich source of dihydrochalcones phloretin and phloridzin, phenolic compounds with elusive role in planta but suggested auto-allelochemical features related to 'apple replant disease' (ARD). Our recent study emphasized phytotoxic action of phloretin towards other plant species such as model plant Arabidopsis as a consequence of disturbed auxin homeostasis in roots and altered chloroplast structure and function. Since elevated production and accumulation of reactive oxygen species (ROS) in plant cells is the one of the most common effect of allelochemicals, this study aimed to analyze the antioxidant status of Arabidopsis seedlings treated with phloretin. Total phenolic content (TPC) and total antioxidant capacity (TAC), as well as antioxidant enzymes activities were determined spectrophotometrically in treated and control seedlings at 5th and 10th day after germination (DAG). TAC increased c.a. 280% and 200% relative to control at 5th and 10th DAG respectively, presumably due to highly elevated TPC in treated seedlings (c.a. 150%). At the same time, the activity of antioxidant enzymes significantly decreased. The activity of catalase (CAT) was reduced by c.a. 40% and guaiacol peroxidase (POD) activities by 93% and 47% at 5th and 10th DAG, respectively. The activity of superoxide dismutase (SOD) was reduced by 55% and 18% at 5th and 10th DAG, respectively. The burst of antioxidant capacity of phloretin treated Arabidopsis seedlings resulted in significantly decreased antioxidant enzymes activities presumably due to strong antioxidant properties of absorbed phloretin. However, phloretin inhibition of antioxidant enzymatic activity due to its direct interaction with enzymes is object of further investigation.

Keywords: antioxidant enzymes, *Malus × domestica* Borkh., phloretin, phytotoxicity, total phenolic content

Acknowledgment: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract number 451-03-68/2022-14/200007.

CROPINNO – Introducing multi-omics tools for improved crop stress resilience

PP2-29

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Agriculture is one of the "victims" of climate change and one of the most severely affected sectors. It has become evident that as the climate changes, crop production strategies must change as well, including primarily adaptations through breeding and crop management. In the future, it is expected that integrative approaches that combine -omics technologies by using bioinformatic tools will facilitate the identification of target genes and markers for complex traits and facilitate crop adaptation to the changing environment. Within its activities, Twinning project CROPINNO will implementat and validate different phenotyping and multi-omics tools in breeding for improved stress resilience. Sunflower is chosen as a model crop for validation of different tools and approaches since it is considered as potential model crop for adaptation to a changing environment. Activities within CROPINNO are aimed at: i) pre-screening sunflower genotypes from IFVCNS collection using for biotic and abiotic stress resilience using different phenotyping methods; ii) study of effects of drought on sunflower plants at chromatin and transcriptional level; iii) performing whole genome SNP analysis in order to develop SNP-based markers for drought stress resilience; iv) performing integrated data analysis and comparative bioinformatics for drought responses in order to unveil possible direct correlations between stress-induced genes transcriptional variation and histone modification levels and design of networks of candidate genes for sunflower drought tolerance. Models, tools and know-how developed on sunflower will be transferred and implemented in the breeding programs of other main field crops at IFVCNS and Western Balkans region.

Keywords: crops, multi-omics, resilience, breeding

Acknowledgment: This work is supported by the Ministry of Education, Science and Technological Development of Republic of Serbia, grant number 451-03-68/2022-14/ 200032, by the Science Fund of the Republic of Serbia, through IDEAS project "Creating climate smart sunflower for future challenges" (SMARTSUN) grant number 7732457, by Center of Excellence for Innovations in Breeding of Climate-Resilient Crops - Climate Crops, Institute of Field and Vegetable Crops, Novi Sad, Serbia, and based upon work from COST Action EPI-CATCH (CA19125), supported by COST (European Cooperation in Science and Technology). www.cost.eu.

Effects of elevated temperature on the organ-specific expression of superoxide dismutase gene family in potato, *Solanum tuberosum* L.

PP2-30

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Potato, *Solanum tuberosum* L., is the leading vegetable crop in the world and is highly vulnerable to elevated ambient temperatures. Since high-temperature stress causes the accumulation of reactive oxygen species (ROS) in plant cells, investigations regarding major enzymatic components of the potato antioxidative system are of importance. Superoxide dismutases (SODs) are metalloenzymes that play an important role as primary ROS scavengers. Previously, we conducted a detailed in silico analysis and characterization of the potato SOD gene family. In the present study, we analyzed the expression of potato SOD-encoding genes, three *StCu/ZnSODs*, one *StMnSOD* and four *StFeSODs*, in different organs of potato cv. Désirée plants at optimal growth conditions (20/17 °C, 70% humidity) and during mild heat stress (30/20 °C, 70% humidity) on the 1st, 7th and 21st day of treatment. Our results revealed that cytosolic *StCu/ZnSOD1*, chloroplast *StCu/ZnSOD2* and *StFeSOD3*, and mitochondrial *StMnSOD* were characterized by a high level of expression in potato organs, especially leaves and stolons. Conversely, chloroplast *StFeSOD1* and *StFeSOD4* expression levels were very low in all investigated organs. Under mild heat stress, the significant increase in the abundance of most *StSODs* transcripts was mainly observed in leaves and stolons after 7 days of the treatment. Interestingly, transcript levels of all eight potato *StSODs* were the lowest in developing tubers compared to other organs, regardless of temperature treatment. Our findings provide insight into the modulation of organ-specific expression of eight potato *SODs* by elevated temperature and may contribute to a better understanding of potato antioxidant response to mild heat stress.

Keywords: potato, antioxidant response, superoxide dismutase, heat stress

Acknowledgment: This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract number 451-03-68/2022-14/200007.

Phytohormone Profiling of the Potato Plants Exposed to French Marigold Essential Oil

PP2-31

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French marigold (*Tagetes patula* L.) is a common companion plant for many vegetable crops because of its repellent effects on herbivorous insects and other pests. However, little is known about the effects of its essential oil (EO) volatiles on crops physiology. Given the importance of phytohormones in integrating signals and regulating vital processes in plants, this study focused on phytohormone profiling of potato (*Solanum tuberosum* L.) plants exposed to French marigold EO for different time periods (4h, 8h, and 12h). Results of LC-MS analysis showed alterations in accumulation of different phytohormones and/or their metabolites after exposure to EO, with the most pronounced change observed for stress-related phytohormones: ABA's metabolite ABA-glucose ester, ethylene precursor 1-aminocyclopropane-1-carboxylic acid, and jasmonic acid. In most of the analyzed phytohormone groups similar response pattern was observed – an increase in levels after short time exposure (4h), followed by a decrease to control values after prolonged exposure (8h and 12h). In order to present changes in activity of the genes included in metabolism of phytohormones after 8h long exposure, a detailed analysis of data from a previous microarray study was performed. These results revealed jasmonic acid biosynthetic pathway as the most affected one, with the highest number of altered transcripts and with one of the genes from this pathway (12-*oxophytodienoate reductase 1-like*) having highest expression rate. In the future, the data obtained from this comprehensive analysis may help to understand the effects of EO on the physiology and defense strategy of exposed plants, and subsequently predict plant responses.

Keywords: potato, French marigold, essential oil, volatiles, phytohormones

Acknowledgement: This work was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Contract No. 451-03-68/2022-14/200007), the Czech Science Foundation (Grant No. 19-12262S) and the Ministry of Education, Youth and Sports of the Czech Republic from European Regional Development Fund - Project "Centre for Experimental Plant Biology" (No. CZ.02.1.01/0.0/0.0/16_019/0000738).

Alterations in specialized metabolism and antioxidant capacity of *Nepeta sibirica* L. as induced by two *Trichoderma* sp.

PP2-32

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Trichoderma spp. have been extensively explored and used in agriculture due to their well-known biological control mechanisms. Fungi of the genus *Trichoderma* produce secondary metabolites which affect plant metabolism by stimulating the production of defense-related compounds and increasing the antioxidant capacity through enhanced polyphenol content and elevated activity of antioxidant enzymes, a result of the excess production of reactive oxygen species. In this work, *Nepeta sibirica* plants were grown in vitro on solid medium treated with two *Trichoderma* strains (*T. viride* and *T. harzianum*). After three and six days of treatment, leaves were metabolically profiled for major phenolic acids (chlorogenic, caffeic, and rosmarinic acid), and iridoids content (cis,trans-nepetalactone and 1,5,9-epideoxyloganic acid), in parallel with the expression analysis of nepetalactone biosynthetic genes and regulatory genetic elements-transcription factors (TFs). Both fungal strains induced changes in phenolic acids production, while only *T. harzianum* induced elevated levels of iridoids. Biosynthetic genes GPPS, and IS, as well as TF MYC2, were the only genes with expression levels not affected by the treatments with micro fungi. The response of antioxidant enzymes in *N. sibirica* leaves was also studied. The most active antioxidant enzyme following the infection with *T. viride* and *T. harzianum* was peroxidase (POX). Catalase (CAT) and superoxide dismutase (SOD) activity were also affected by the treatments. Results indicate a possibility of using *Trichoderma* infection in *N. sibirica* to elicit the production of biologically active defense compounds.

Keywords: *Trichoderma*, antioxidant activity, *Nepeta sibirica*, phenolic acid, nepetalactone biosynthetic pathway genes, iridoids

Acknowledgment: This work is financed by the Science Fund of the Republic of Serbia, Program IDEAS (project NEPETOME, No. 7749433), and is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/200007).

Metabolomic profiling of "living fossil" plants: a tool for understanding plant-environment interactions

PP2-33

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A "living fossil" can be defined as a plant that lived in ancient times and still survives on Earth today. They are characterised by their extraordinary ability to adapt to different environmental conditions. It is known that various plant metabolites play an important role in plant-environment interactions. Therefore, we hypothesise that metabolic profiling of "living fossils" can contribute to the understanding of their survival abilities. In recent years, we have performed targeted and untargeted metabolic profiling using a multi-platform method that included HPLC-DAD-MS /MS, GC-MS and MALDI imaging as part of several projects. During the presentation, metabolic profiling results of *Psilotum nudum* (L.) Beauv., *Equisetum* sp. and *Ginkgo biloba* L. will be presented and discussed.

Keywords: living fossil plats, metabolic profiling, *Psilotum nudum* (L.) Beauv., *Equisetum* sp. and *Ginkgo biloba* L.

Acknowledgment: Current research on this topic is supported by Croatian Science Foundation project "Biflavonoids role in plants: Ginkgo biloba L. as a model system" under the grant no. UIP-2019-04-1018.

The effect of 2-chloroethylphosphonic acid foliar application on stem yield components and sex xpression in fiber hemp ’Marina’

PP2-34

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Quantitative variations in the sex ratio significantly affect the industrial hemp (fiber hemp) cultivation, since the production is closely related to the reproductive biology of the species. Both dioecious and monoecious varieties are cultivated, but only monoecious ones enable simultaneously mechanized harvesting of stalks and grains. 2-chloroethylphosphonic acid (Ethrel®) is a synthetic plant growth regulator that affects sex expression and suppresses stem elongation and plant growth. Thus, four various dosages of Ethrel® were applied as foliar sprays to fiber hemp plants cv. Marina, twice- in stage of flower primordia and, two weeks later in stage of flower formation. The trial was set up in Bački Petrovac at experimental field of the IFVCNS, as completly randomized block design in three replicates. Sex ratio in the control plots was 37.11 to 62.89% in favor of femail plants. Although the ratio significantly changed with increase of applied Ethrel dosages (47.84 to 52.15 at maximal applied Ethrel concentration, 2.5 ml l^{-1}), the total grain yield significantly decreased only if plants were treated with the highest dosage, 748.04 kg ha^{-1} compared to 836.81 kg ha^{-1} in control plots. Treated plants had shorter stems (276.78 cm and 233.84 cm in control and plants treated with 2.5 ml l^{-1} of Ethrel, respectively) with lower fiber content. The fiber content in these stems was by 8% lower (20.10%) compared to the stem fiber content of the control plants (28.25%). The applied Ethrel treatments had no statistically significant effect on the hemp stem diameter.

Keywords: *Cannabis sativa* L., Ethrel®, plant sex ratio, yield, oxidative stress

Acknowledgment: The research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant number:451-03-68/2022-14/200032) and Provincial Secretariat for Higher Education and Scientific Research, Autonomous Province of Vojvodina, Republic of Serbia (grant No 142-451-2160/2022-01).

Antioxidant system response of *Chenopodium murale* L. seedlings to phytotoxicity of *Sorghum halepense* L. rhizome extract

PP2-35

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The use of bioherbicides offers the possibility to suppress harmful weeds by ecologically acceptable methods and thus increase crop productivity. Progress in understanding the mechanisms of bioherbicides action and weed defense response mechanisms, could help in defining novel weed control strategies. This work highlights the bioherbicide potential of rhizomes of *Sorghum halepense* L., characterized by a high content of phenolic acids, especially *p*-hydroxybenzoic acid (pHB A) and chlorogenic acid (CHL A). Methanol extract of *S. halepense* rhizome (SHR), and its major bioactive principles, displayed phytotoxic effects against *Chenopodium murale* L., which is a common weed of fields and roadsides. Besides reduction in *C. murale* seed germination, rhizome extract of *S. halepense* applied in the concentrations of 0.01, 0.1, 1 and 2 mg/ml, induced changes in the activity of antioxidant enzymes in seedlings. Catalase (CAT) activity was increased, while peroxidase (POX) and superoxide dismutase (SOD) activities were generally decreased, with a few exceptions (increased POX and SOD activities at 0.1 mg/ml at 0.01 mg/ml SHR, respectively), suggesting that CAT is the major responsible for the elimination of reactive oxygen species generated during the exposure of *C. murale* seedlings to SHR. Application of CHL A induced changes in the activities of antioxidant enzymes, similar to those observed on SHR treatments. The treatment with pHB A did not significantly change the activity of POX and SOD, while CAT activity was significantly increased at all applied concentrations. These data highlight the phytotoxic potential as a highly efficient strategy of *S. halepense* to invade new habitats.

Keywords: Sorghum halepense, *Chenopodium murale*, rhizome extract, phenolic acid, antioxidative enzimes

Acknowledgment: This work was supported by Ministry of education, secience and technological development of Republic of Serbia, IBISS Work Program for 2022, Contract Number: 451-03-68/2022-14/200007.

The effect of phloretin on root growth and antioxidant activity in selected plant species *in vitro*

PP2-36

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Phloretin is phenolic compound specifically produced in apple tree species and secreted into the soil as a response to biotic and abiotic factors. Since our previous results indicated roots as the primary site of phloretin phytotoxic action, this study focused on assessment of phloretin effects on the root growth of weed (*Chenopodium murale* and *Lotus corniculatus*), crop (*Solanum tuberosum*) and woody species (*Castanea sativa* and *Malus × domestica* Borkh.). Significant decrease in root biomass production in liquid growth medium enriched with 500 µM phloretin was observed both after 2 and 4 weeks of treatment in all tested species. The percentages of roots growth inhibition were as follows: *Chenopodium murale* (95% and 89,1%), *Lotus corniculatus* (69,4% and 81,8%), *Solanum tuberosum* (88,4% and 84,7%), *Castanea sativa* (85,6% and 100%) and *Malus × domestica* Borkh. (65,3% and 87,4%, respectively). Total antioxidant capacity (TAC), total phenolic content (TPC), as well as antioxidant enzymes activities were determined spectrophotometrically in treated and control *Chenopodium murale* roots after 4 weeks of phloretin treatment. TAC increased 44,1% presumably due to elevated TPC (25,8%) in treated roots. However, there were no significant differences in catalase (CAT), guaiacol peroxidase (POD) and superoxide dismutase (SOD) activities in control and treated roots after 4 weeks of treatment. Increased antioxidant activity in treated roots is presumably the consequence of known phloretin antioxidant potential.

Keywords: antioxidant activity, phloretin, phytotoxicity, root growth, total phenolic content

Acknowledgment: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract number 451-03-68/2022-14/ 200007.

Wheat (*Triticum aestivum* L.) seed pre-sowing increasing resistance of seedlings to cold stress

PP2-37

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Seed priming is a pre-sowing method used to increase crop viability under adverse environmental conditions. In this paper, various priming agents (hormopriming, halopriming, osmopriming, and hydropriming) were tested to evaluate whether the pre-sowing method could contribute to the resistance of wheat (*Triticum aestivum* L.) seedlings to cold stress (10 °C) conditions. Compared to the control plants (grown from non-primed seeds) stimulatory effects on protein synthesis under cold stress in wheat seedlings were achieved after halopriming with $MgSO_4$. All priming agents significantly reduced malondialdehyde (MDA) content under cold-stress conditions and increased cell membrane stability with the most prominent effect achieved by hormopriming (GA_3 and IAA). In the case of antioxidant enzyme activities, pre-sowing effects depended on applied priming agents. Generally, priming decreased the level of superoxide-dismutase (SOD) and ascorbate-peroxidase (A-POX) under cold-stress conditions compared to the non-primed seedlings. On the other hand, the activity of CAT, G-POX, and P-POX was significantly higher under cold-stress conditions and after seed priming. As in the case of MDA level, the most favourable priming agents for the increased activities of antioxidant enzymes were GA_3 and IAA. In general, the results show that hormopriming and halopriming treatments have the best effect on improving the tolerance of wheat seedlings to abiotic stress caused by low temperature. By activating germinative processes, seed priming strengthens the antioxidant capacity under cold stress conditions by increasing the activity of certain enzymes and reducing lipid peroxidation products.

Keywords: cold stress, wheat, priming

Acknowledgment: This investigation was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant no: 451-03-68/2022-14/200122).

Comparative study of physiological, biochemical and morphological parameters in two tomato genotypes, wild type cv. Ailsa Craig and its ABA-deficient mutant *flacca*

PP2-38

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The objective of this study was to determine the constitutive differences in physiological, biochemical and morphological parameters between two tomato genotypes with different levels of abscisic acid (ABA) – wild type Ailsa Craig (WT) and ABA-deficient mutant *flacca*. Due to reduced ABA biosynthesis, *flacca* mutant is a suitable model system for investigating the influence of endogenous leaf ABA level in response to drought in plants. Within this research, plants were grown under controlled conditions at 800 µmol $m^{-2}s^{-1}$, until the end of the vegetative phase when samples were taken. The accumulation of the most abundant soluble sugars, sorbitol, phenolic compounds, and ascorbate in the leaves, as well as the cell walls compounds, were analyzed. Lower constitutive ABA content in *flacca* was accompanied by two times higher stomatal conductance and similar leaf water potential. Higher content of phenolic compounds (HBAs, HCAs, flavonoids) was determined in WT plants, which was in contrast with the elevated accumulation of the epidermal flavonoids in *flacca*. Larger accumulation of sorbitol in WT, and of the most abundant soluble sugars (glucose, fructose and sucrose) indicate that *flacca* accumulates lower content of osmolytes which was opposite to the condition at low light. However, an alternative mechanism related to cell wall modulation imposed its importance in the development of plant acclimation mechanisms under stressful environmental conditions in tomato deficient in ABA. On the other hand, an elevated ascorbate redox state in *flacca* indicates a higher sensitivity to oxidative stress of the mutant compared to WT even in optimal environmental conditions.

Keywords: tomato, *flacca* mutant, phenolic compounds, cell wall, ascorbate

Acknowledgment: This research was funded by the Ministry of Education, Science and Technological Development, the Republic of Serbia (Contract No. 451-03-68/2022-14/200053, 2022, Contract No. 451-03-68/2022-14/200116, 2022).

Evaluation of the effect of water deficit on proline content and lipid peroxidation in leaves of two tomato lines

PP2-39

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Water deficit can be a trigger of physiological and biochemical changes in leaves, that could be important for overall plant adaptive response and it can affect tomato yield and quality. This experiment was conducted to evaluate the effects of water deficit on proline content and lipid peroxidation in leaves of two tomato lines (M7 and R83). Our previous research has shown that there are differences in the biochemical characteristics of both lines during the reproductive phase. Accordingly, it is to be expected that there will be differences even in the early stages of plant development. Tomato plants were grown in a glasshouse and two water regimes were applied (optimal field capacity and water deficit - reduction of water for 70% compared to control), during three weeks after planting of seedlings. Our results showed that proline content and lipid peroxidation in the tomato leaves were fluctuated according to water content in the substrate, and it also depended on the genotype. The content of proline was significantly higher in the leaves of both tomato lines exposed to water stress. Similar to the content of proline, the lipid peroxidation was significantly higher in tomato leaves of the M7 line in contrast to the R83 line, where no significant differences were found. Also, water stress was a significant effect on plant height, number of leaves and shoot fresh weight of both tomato lines. Reduction of these parameters was much higher in the M7 line compared to the line R83.

Keywords: tomato, proline content, lipid peroxidation, optimal field capacity, water deficit

Acknowledgment: This work was supported by the Serbian Ministry of Education, Science and Technological Development under Contracts No. 451-03-68/2022-14/200216 and 451-03-68/2022-14/200116.

UV-B radiation-elicitated biosynthesis of iridoids in leaves of *in vitro* grown *Nepeta nuda* L.

PP2-40

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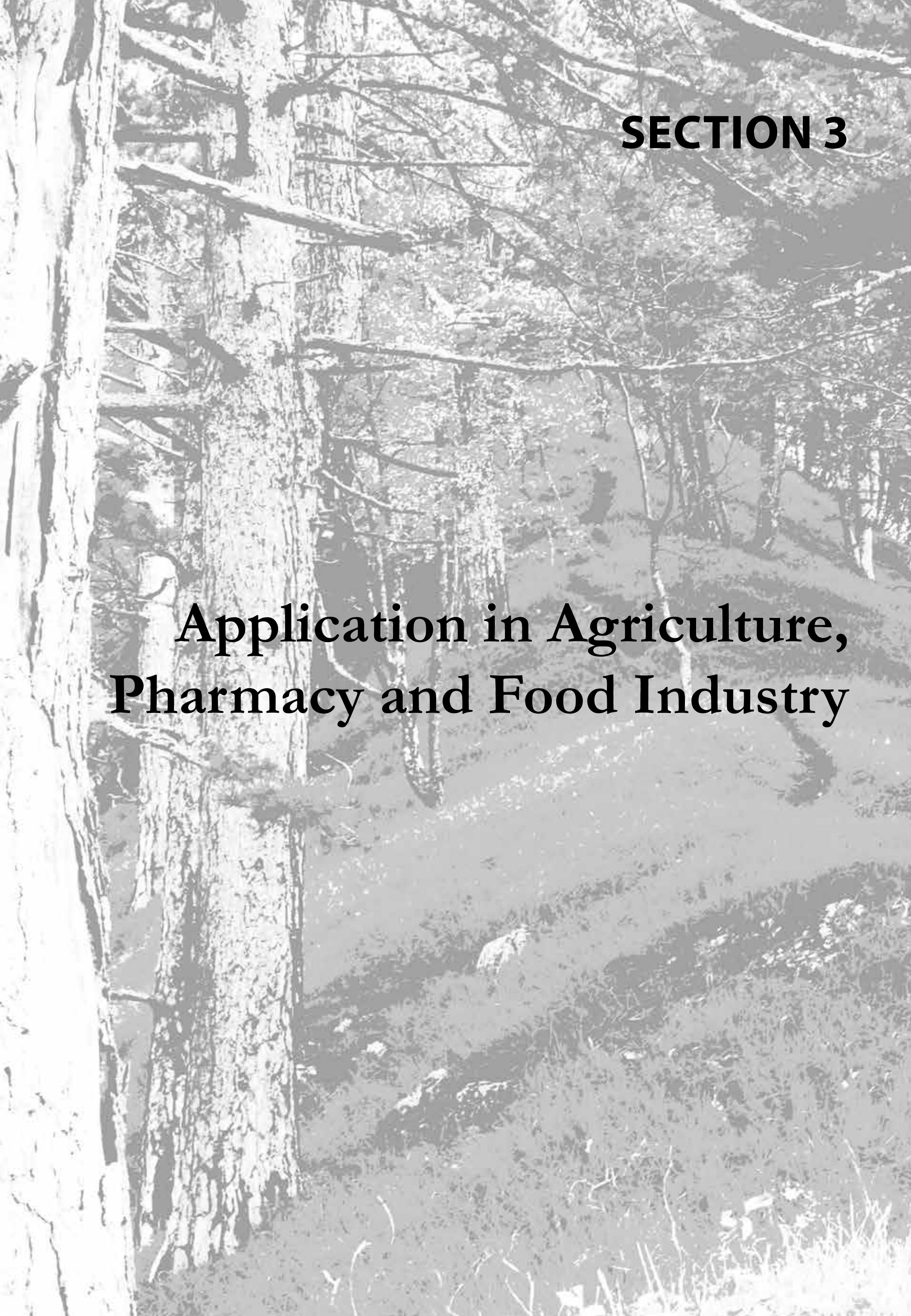
Because of their sessile lifestyle, plants have been constantly facing ultraviolet (UV-B) radiation, a type of abiotic stress which affects their overall growth, development, physiology, and metabolism. Qualitative and quantitative content of specialized metabolites, chemical compounds that allow plants to adapt to specific conditions of the environment, is also affected. *Nepeta nuda* L. (fam. Lamiaceae) is characterized by a significant amount of iridoids, monoterpenoid compounds with prominent pharmacological effects. The aim of this study was to detect changes in the content of major iridoids (1,5,9-*epi*deoxyloganic acid (1,5,9-*e*DLA), nepetalactone (NL), and dehydronepetalactone (DNL)) in *N. nuda* plants after exposure to UV-B radiation (during 30 and 60 min), in parallel with expression analysis of genes participating in the biosynthetic pathway of iridoids (*NnGAPDH*, *NnGPPS*, *NnGES*, *NnG8O*, *Nn8HGO*, *NnIS*, *NnNEPS*). After both UV-B treatments, the gene *GPPS* showed the highest increase in gene expression, relative to control plants, while the gene *G8O* showed the highest decrease in expression level. At the same time, UHPLC/DAD/(±)HESI-MS^2 analysis revealed an increase in the concentration of 1,5,9-*e*DLA and DNL following the exposure of plants to UV-B radiation for 60 minutes. In the case of a 30-minute treatment, there was an increase in the concentration of *e*DLA, while the concentration of DNL was reduced, relative to non-treated plants. The present study brings novel insights into the molecular background of iridoid biosynthesis in UV-B stressed plants, which could help in optimizing the elicitation procedures and thus accumulation of bioactive compounds in *N. nuda*.

Keywords: *Nepeta nuda*, UV-B radiation, nepetalactone, epideoxyloganic acid, dehydronepetalactone

Acknowledgment: This work was supported by Ministry of education, secience and technological development of Republic of Serbia, IBISS Work Program for 2022, Contract Number: 451-03-68/2022-14/200007.



SECTION 3

Application in Agriculture, Pharmacy and Food Industry

PLENARY LECTURES

Aroma formation in *Vitis vinifera* grape berries

PL3-1

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Aroma, an important attribute of wine quality, is a mixture of compounds constituting the so-called "bouquet" that is synthesized in ripe grape berries and partially during the vinification and aging processes. It must be noted, however, that most of the wine aroma precursors derive from grape berries that might be altered by fermentation and/or maturation conditions; its complexity and genetic basis is poorly understood. To approach this intriguing theme, we compared the chemical and transcriptomic analyses in mid-ripe stage grape berries of six Greek wine varieties (Assyrtiko, Malagouzia, Moschofilero, Roditis, Xinomavro and Agiorgitiko) and in selected clones exhibiting high and low aroma profiles based on wine tasting criteria. In the present work, the chemical and RNA-seq data were analyzed by applying all recent bioinformatic tools, (PCA, Volcano plot, Heat Maps, Go enrichment and KEGG analysis) to visualize the differences/similarities among and within the varieties and to propose functional biosynthetic pathways, as well as to pinpoint genes participating in the aroma formation in ripening grape berries. Among these genes, the following were functionally characterized by expressing them either in yeast or by *in vitro* assays: raspberry ketone synthase (RZS1), contributing to the formation of 4-(4-Hydroxyphenyl) butan-2-one (raspberry ketone), coniferyl alcohol acetyl transferase (CAAT) and eugenol synthase (EGS1), both participating in the synthesis of eugenol, all belonging to the phenylpropanoid pathway, R-linalool synthase (RLin), linalool/nerolidol synthase (LinNer), and P450-CYP76F14, a wine lactone synthase participating in the biosynthesis of precursors of wine lactone, namely E)-8-hydroxylinalool and E)-8-carboxylinalool, glutathione S transferases (GST3, GST4), και vanillin synthase (VAN).

Keywords: *Vitis vinifera*, aroma, genes, Greek wine varieties

Acknowledgment: This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship, and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T1EDK-03719/ HELLENOINOS). We thank S. Petropoulos and K. Bakasietas for sampling the grape berry tissues and Dr. E. Sarrou for GC-MS analyses.

Metabolomic strategy for detecting herbal products' differentiations and potential adulteration

PL3-2

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Plant extracts consist of unique and convoluted mixtures of bioactive natural products with complicated and unpredicted chemical patterns. Metabolomics has mainly improved the dereplication process of crude plant extracts, providing the total metabolite fingerprinting of plant extracts, and detecting differences between metabolites[1]. One of the most popular applications of metabolomics in the field of natural products is for the quality control and safety of phytomedicines[2]. Among the many analytical techniques, Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR) spectroscopy are widely used in analyses based on metabolomics [2,3]. In current years, herba *Sideritis* is considered one of the most economically interesting medicinal plants since mountain tea is one of the main exported Greek aromatic herbs to global markets. *Sideritis* plants (mountain tea in English, "τσάι του βουνού" in Greek) are rich in terpenoids (e.g., diterpenes and iridoids) and polyphenols (i.e., flavonoids, phenylethanoid glycosides, phenolic acids) to which great pharmacological activities are attributed[4-9]. In this study, LC-MS/MS, GC-MS, and NMR spectroscopy have been used to detect the metabolic profiling of different mountain tea samples (*Sideritis* species) from Greece and Cyprus.

Keywords: mountain tea, *Sideritis* plants, metabolomics, metabolic profiling, adulteration

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INVITED TALKS

Essential oils and hydrolates in control of plant pathogens

IT3-1

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Under conditions of intensive plant production and intensive international trade with plant material, coupled with climate changes, plant pathogens became one of the major limiting factors in food and feed production. Besides these extremely favorable conditions for reproduc-tion and dissemination of plant pathogens, resistance development to available control tools and ecotoxicological properties of these tools show that plant pathogen control has never been more challenging. Development of alternative, eco-friendly control tools against phytopatho-gens, became a necessity of contemporary agriculture.

Essential oils are mixtures of complex volatile compounds. They are synthesized through secondary metabolic pathways in many plant species. Essential oils are normally extracted by hydro-distillation or steam distillation of plant tissues. Hydrolates are co-products of the distillation formed in significantly higher quantities than the target substance. Hydrolate represents aromatic water containing approximately 0.1% essential oil compounds. Essential oils play important roles in plants helping them trigger defense mechanisms, acting as antimicrobi-als and as signal molecules to communicate with other plants and beneficial insects. These properties made essential oils and their hydrolates an important part of alternative control tools against plant pathogens. There are many data suggesting high antimicrobial activity and effective control of plant pathogens by essential oils and hydrolates, *in vitro* and *in vivo*. Also, there are commercially available products based on these substances – biofungicides and bio-bactericides already in use in plant protection. There is no doubt that advantages of biocontrol tools are numerous, but there are also certain limitations when it comes to essential oils and their hydrolates.

Keywords: essential oils, hydrolates, biocontrol, phytopathogens

Acknowledgment: This research is funded by the Ministry of Education, Science and Tech-nology of the Republic of Serbia (contract number 451-03-68/2020-14/ 200117).

Determination of elements, isotopes and organics in plants with high local resolution by mass spectrometric methods

IT3-2

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In order to assess the status of a plant information about nutrition and growth conditions, deseases, deficiency symtoms or metabolic processes are helpful, as well as knowledge about the uptake, transportation and storage of macro- or micronutrients, or the release of small organic compounds, like fragrants or protecting chemicals. If these informations will be obtained from samll areas of different plant components on the micrometer scale and concentrations of the elements or compounds under investigation are low as well, application of mass spectrometric methods is the best choice. With these techniques sample material is removed and ionized by laser photons with a subsequent analysis in mass spectrometers of different types and mass resolution. For quantification suitable reference materials have to used – either bought or synthesized - which match the average composition of the plant tissue under investigation. Another crucial point is to find a propper sample preparation which will not destroy or affect sample composition and/or construction.

The presentation will focus on element and isotope analysis for quantification of nutrient uptake and storage by laser ablation ICP-MS on the micrometer scale (e.g. for lead and beryllium in corn and cress), and the determination of active organic ingredients in trees of different plant species by MALDI combined with high resolution FT-ICPMS.

Keywords: trace elements, isotopes, Laser ablation ICP-MS, µXRF, MALDI-MS

Integrating physiological traits in local small grains breeding program

IT3-3

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Wheat yield has continuously been increasing over the last century, as a result of improvement in crop production technology and breeding progress. However, annual genetic gains in cere-als are between 0.5–1.0 percent, showing stagnation in many regions across the world. In or-der to increase grain yield genetic progress, different strategies based on genomics data or physiological traits have to be integrated in breeding activities. Physiological approach in con-ventional wheat breeding is based on the crosses of parents with desirable complex physiolog-ical traits, and progeny selection according to important physiological traits. During the past ten years, different physiological strategies have been applied in regular breeding activities at the Institute of Field and Vegetable Crops, Novi Sad, Serbia. First of all, small grains gene bank at the Institute has been screened according to different physiological traits, such as NDVI values, infrared temperature and chlorophyll index. Some physiological analysis, such as chlorophyll fluorescence or stomatal conductance, have been identified as labour and time consuming, restricting their application in regular breeding activities at the Institute. Further-more, each growing season several crosses of parents bearing desirable complementary physiological traits are made and progenies with increased NDVI and chlorophyll index are selected. Currently, several new wheat "physiological" lines have been included in preliminary multilocation testing, with high chance to be released as potential new cultivars to the market during the next two or three years. Further integration of physiological breeding with high throughput phenotyping and genomic selection could be promising strategies for additional small grains yield improvement.

Keywords: grain yield increase, crop physiology, wheat

Chokeberry, from natual polyphenol resource to promising functional foods and pharmaceuticals

IT3-4

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Chokeberries, as one of the richest plant sources of phenolics, represent a natural nutraceu-ti-cals, which play important role in human nutrition due to their antioxidant potential. Pro-cessing of chokeberry juice generates waste by-product still containing high amounts of poly-phenolics, which can be extracted. Problem with polyphenols extract instability can be solved by microencapsulation techniques. Encapsulation of chokeberry extracts using different carriers (maltodextrin, skimmed milk, gum arabic, alginate, inulin) by two different tehniques, electrostatic extrusion and spray drying was employed. Microencapsulated systems were examined (FTIR, SEM, particle size, zeta potential, moisture content). For investigation of microencapsulated polyphenols bioavailability, their digestive stability and release from food matrix, *in vitro* simulated digestion method was employed. The release profiles from mi-crobeads were investigated determining the total polyphenols and anthocyanins, as well as individual phenolics. Our result showed that stability of polyphenols, especially anthocyanins could be improved using different microencapsulation tehnologies. Both microencapsulation techniques were suitable for particles production, as efficient systems for the prolonged poly-phenols delivery and increased stability. Since the subject of this examination is current plant species, chokeberry extract effects on blood pressure were examined for the first time through *in vivo* monitoring of systemic and regional hemodynamic and biochemical parameters in the model of essential hypertension. A four week extract administration in spontaneously hypertensive rats significantly reduced systolic and pulse pressure, associated with increased diuresis. Chokeberry extracts and prepared microbeads, due to their antioxidant potential, represent a promising food additive for incorporation into dietary suplements or functional food.

Keywords: chokeberry, polyphenols/anthocyanins, microencapsulation, digestion, hyperten-sion

Acknowledgment: Ministry of Education, Science and Technological Development of the Republic of Serbia and project leader Katarina Šavikin.

SELECTED TALKS

Late embryogenesis abundant (LEA) proteins in *Ramonda serbica* Panc identification, classification and structural characterization

ST3-1

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An ancient resurrection plant *Ramonda serbica* Panc. is able to survive a long desiccation period and reestablish metabolic activity upon watering. A hallmark of desiccation tolerance in the resurrection species is the accumulation of protective late embryogenesis abundant proteins (LEAPs). These intrinsically disordered proteins (IDPs) may stabilize the correct structure of proteins and membranes during cellular dehydration. The aim of our study was to assess LEA genes' expression levels in hydrated (HL) and desiccated leaves (DL) and to identify, characterise, and estimate the potential role of *R. serbica* LEAPs in desiccation tolerance. In total, 318 LEAPs from HL and DL were identified and classified into the seven LEA protein family groups ranging from LEA1-LEA5, seed maturation proteins (SMPs), and dehydrins (DEH). Analysis of the physicochemical properties, motif architecture, secondary structure, homology, and phylogenetic relationships demonstrated that *R. serbica* LEAPs greatly differed among the LEA family groups. The most abundant LEA2 proteins (mostly downregulated upon desiccation) exhibited lower hydrophilicity and propensity to fold into organised globular domains. Oppositely, hydrophilic LEA4 proteins tended to form amphipathic, A-type, α-helices. Most of desiccation-upregulated *LEA* genes encoded highly disordered DEH1, LEA1, LEA4.2, and LEA4.3 proteins. While dehydrins might chelate metals and bind DNA under water deficit, other ID LEAPs (e.g. LEA1, LEA3, LEA4) might participate in forming intracellular proteinaceous condensates or adopt amphipathic α-helical conformation, enabling them to stabilise desiccation-sensitive proteins and membranes. Taken together, possible functions of LEAPs are discussed with significant implications on drought tolerance improvement of crops grown in arid areas.

Keywords: 3D protein structure modelling, de novo transcriptome assembly, intrinsically disordered proteins, liquid-liquid phase separation, secondary structure prediction

Acknowledgment: This research was funded by the Science Fund of the Republic of Serbia-RS (PROMIS project LEAPSyn-SCI, grant no. 6039663) and by the Ministry of Education, Science and Technological Development, the Republic of Serbia (Contract No. 451-03-68/2022-14/200042, 2022).

Supercritical fluid extraction of Chicory reveals its antimicrobial, antibiofilm and wound healing potentials

ST3-2

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Chicory (*Cichorium intybus* L.) is an important industrial crop cultivated mainly to extract the dietary fiber inulin. This study aimed to investigate antimicrobial, antibiofilm and wound healing potentials of the extract obtained by supercritical fluid extraction (SFE). Antibacterial activity of extract ranged from 0.25 to 4.00 mg/mL regarding minimal inhibitory concentra-tions (MICs), while minimal bactericidal concentrations (MBCs) were noted within the range of 0.5 to 8.00 mg/mL. The extract has shown promising antifungal potential with MICs rang-ing 0.125- 0.75 mg/mL and MFCs 0.25-1.0 mg/mL against *Candida* species. Incubation with the extract has reduced the ability of both *C. albicans* and *P. aeruginosa* to attach to the sur-face and begin the process of biofilm formation. Concentration equal to previously deter-mined MIC has almost completely (>90% inhibition) reduced the biofilm biomass formed by *P. aeruginosa*. Even twice as lower concentrations (0.5 MIC) has limited the biofilm forming ability and induce more than 80% inhibition in *P. aeruginosa* as bacterial biofilm model or-ganism. Impact on fungal biofilm was less profound and MIC concentration was able to re-duce the biofilm formation for less than 60%. HaCaT (human immortalized keratinocytes cell line) cells were treated with IC_{20} (24 h) concentration of chicory SFE extract. It is estimated that SFE extract has slight effect on wound closure in keratinocyte monolayer, when com-pared to untreated control. Since, Chicory SFE fraction possessed excellent antibacterial po-tential against *E. coli* and *K. pneumoniae* and antifungal activity against *Candida* species these results are considered very important in the future inventions.

Keywords: Chicory, extracts, antimicrobial activity, wound healing, antibiofilm

Acknowledgment: This work was conducted as part of the CHIC project (H2020-NMBP-BIO-2017) with financial support received from the EU Horizon 2020 research and innova-tion program under grant agreement no. 760891.

POSTER PRESENTATIONS

Determination of some microelements in organic and conventional produced maize seeds (*Zea mays*) using inductively coupled plas-ma optical emission spectrometry (ICP-OES)

PP3-1

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Organic agricultural production can be most easily defined through its goals: the production of healthy and high-quality food as a result of the preservation of the environment, soil and its fertility, biodiversity, agro- and ecosystem. Sustainable food production is gaining global importance due to numerous consequences caused by conventional agriculture. For many years, numerous studies have shown that there is a difference in the mineral composition be-tween organic and conventional crops and their products. The aim of this study was to deter-mine the existence of differences in the content of microelements (Cu, K, Li, Mn, Na, S and Se) in organically and conventionally produced maize seeds (*Zea mays* L., Rumenka hybride, produced in Maize Research Institute, Zemun Polje), during 2016. The determination of these elements was conducted by using Inductively Coupled Plasma Optical Emission Spectrome-try (ICP-OES) with previous digestion of samples and results were expressed as mg/kg of dry weight (DW). The obtained results revealed that the lowest content in analyzed seeds was for selenium (0.10 mg/kg DW, conventional seed). In conventional maize seed, higher quantity of Cu (1.53 mg/kg DW) and Na (9.26 mg/kg DW) was determined, while organic maize seed contained higher content of K (119.08 mg/kg DW), Li (2.47 mg/kg DW), Mn (2.92 mg/kg DW), S (925.68 mg/kg DW) and Se (0.17 mg/kg DW) compared to conventionally grown sample.

Keywords: maize seed, conventional growing system, organic growing system, ICP-OES.

Acknowledgment: Authors are grateful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for support through contract no. 451-03-68/2022-14/200116.

In vitro analysis of antidiabetic potential of ethanol extracts from selected medicinal plants

PP3-2

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Type 2 diabetes mellitus (T2DM) is characterized by a high level of post prandial blood glucose, which consequently lead to oxidative stress. An important therapeutic approach in the treatment of T2DM is inhibition of α-amylase and α-glucosidase. Synthetic pharmaceuticals, such as acarbose, are effective in therapy, but they also show harmful side effects. Therefore, there is an increasing focus on finding natural plant metabolites, such as phenolic compounds, with the possibility of use in therapy T2DM. The aim of our work was to examine *in vitro* an-tidiabetic and antioxidant activity of selected medicinal plants from the Republic of Srpska: *Salvia officinalis*, *Trifolium pratense*, *Agrimonia eupatoria*, *Cichorium intybus* and *Vinca minor*. Antidiabetic activity was determined in ethanol leaf extracts by measuring the degree of inhibition of α-amylase and α-glycosidase activities, while antioxidant activity was deter-mined by DPPH (1,1-diphenyl-2-picrylhydrazyl), phosphomolybdenum and β-carotene bleaching methods. The obtained results indicate that the ethanol extracts of *T. pratense* and *A. eupatoria* have the lowest IC_{50} values for α-glucosidase inhibition (2.25 ± 0.89 and 2.88 ± 0.06 $\mu g_{\text{phenolic compounds}}$/mL, respectively), while the lowest IC_{50} for α amylase was measured for A. eupatoria (26.57 ± 3.09 $\mu g_{\text{phenolic compounds}}$/mL). *A. eupatoria* also had the highest antioxi-dant capacity measured as ability to remove DPPH radicals and can be considered as plant with the potential for use in T2DM therapy. Bioactive properties of *A. eupatoria* could be as-cribed to high content of flavonols (rutin and isoquercetin) and flavones (isoorientin and vi-texin). *T. pretense* is rich in ferulic acid and in quercetin derivatives.

Keywords: α glucosidase, α amylase, antioxidative activity, phenolic compounds

Acknowledgment: This work was funded by the Ministry of Scientific and Technological Development, Higher Education and Information Society of the Republic of Srpska (Project No. 19.032/961-104/19 and 19.032/961-95/19). The work is also supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-68/2022-14/200007).

Volatiles of *Dittrichia viscosa* (L.) Greuter – Biological Activities of Essential Oil and Hydrosol

PP3-3

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Biologically active compounds from natural sources were studied in a readily available aro-matic wild shrub widely distributed in the Mediterranean region, the species *Dittrichia viscosa* (L.) Greuter (Asteraceae). We investigated a phytochemical profile of *D. viscosa* by GC-MS and HPLC analysis of the lipophilic fraction (essential oil) and the water fraction (hydrosol). GC-MS analysis identified 1,8-cineole, caryophyllene oxide, *α*-terpenyl acetate, and *α*-muurolol as the major constituents of the essential oil, while in the hydrosol *p*-menth-1-en-9-ol, *1,8*-cineole, linalool, *cis*-sabinene hydrate, and a-muurolol were the major volatile constituents. HPLC analysis identified *3,4*-dihydroxybenzoic acid as the predominant com-pound among phenolic compounds in the hydrosol composition. The antimicrobial potential of both extracts was evaluated against thirteen opportunistic pathogens associated with common skin and wound infections and emerging food spoilage microorganisms. The antimicrobial activity of the essential oil suggests that the volatiles of *D. viscosa* could be used as novel antimicrobial agents. The antiproliferative results of *D. viscosa* are also novel findings that showed promising activity against three cancer cell lines: HeLa (cervical cancer cell line), HCT116 (human colon cancer cell line), and U2OS (human osteosarcoma cell line). The results presented are also the first report of significant antiphytoviral activity of hydrosol against tobacco mosaic virus infection. The results show that *D. viscosa* is much more than a weed plant, with the potential in crop protection, as a natural disinfectant and natural anticancer agent.

Keywords: Dittrichia viscosa, volatiles, antiviral, antimicrobial, anticancer activity

Toward alternative herbicides: ethanol and indole-3-acetic acid in the suppression of plant growth

PP3-4

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Modern agriculture relies heavily on the use of synthetic herbicides: however, although efficient, these chemicals are also harmful to the environment. Therefore, there is an ever-increasing need for alternative herbicides: ethanol and indole-3-acetic acid (IAA) are among the candidates. Ethanol can be cheaply produced from a vast variety of substrates in large-scale fermentation processes. Moreover, in the last 2 decades, its applicability as an alternative, biosustainable herbicide is also being recognized. Ethanol has a well-known phytotoxic effect, although its mechanisms of actions are still poorly understood. IAA, a plant phytohormone, exerts its negative effect when applied in higher doses. Each plant has different sensitivity to ethanol and IAA. This property can be used to suppress the growth of harmful species (e.g. weeds) while stimulating the growth of desired cultures. In this study, we tested the effects of IAA and ethanol on duckweeds (*Lemna minor* L.). We measured non-enzymatic parameters of oxidative stress and multiplication rates of duckweeds exposed to IAA and to ethanol, respectively. Our results showed that higher concentrations of IAA (>1 mg l^{-1}) suppress the growth of duckweeds while even small amounts of ethanol (0.5% and 1% v/v) were sufficient to reduce the growth and biomass production of duckweeds. In conclusion, ethanol and IAA can exert significant negative effects on plant growth, at least in part by increasing oxidative stress in plants, and can therefore be potentially used as low-cost, eco-friendly herbicides.

Keywords: herbicides, IAA, ethanol, duckweed

Acknowledgment: This research was founded by Ministry of education, science and technological development of the Republic of Serbia, Contract No. 451-03-68/2022-14/20007. Authors would also like to thank dr Branka Vinterhalter for her support.

Activities of Class III peroxidase and polyphenol oxidase in fruits of selected tomato genotypes from Gene Bank of Republic of Srpska

PP3-5

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Tomato (*Solanum lycopersicon* L.) genotypes stored within the Gene Bank of the Republic of Srpska are traditional landraces well adopted to local agro-climatic conditions, with good nu-tritional quality and better organoleptic features of the fruit compared to modern commercial varieties. Class III peroxidases (POD) and polyphenol oxidases (PPO) play important roles in plant growth and development but also in the defense responses. The aim of our research was to assess the activities of POD and PPO and isoenzyme profiles in the fruits of 10 tomato genotypes from the Gene Bank of the Republic of Srpska. Enzymatic activities were meas-ured spectrophotometrically, and by analyzing isoenzyme profiles on native PAGE and isoe-lectrofocusing electrophorograms. For the first time, the biochemical characterization of POD and PPO in tomato fruits from the territory of the Republic Srpska was performed. The re-sults showed that the highest activities of POD and PPO were in the genotypes GB01129 and GB01126 (POD 0.933 and 0.477 µmol mgprot^{-1} min^{-1}, respectively and PPO 0.063 and 0.055 µmol mgprot^{-1} min^{-1}, respectively). Isoelectrofocusing showed the presence of anionic POD and PPO isoforms in tomato fruits of all genotypes. Exploring natural diversity as a source of novel alleles is important in order improve tomato productivity, nutritional value and tolerance to stress.

Keywords: *Solanum lycopersicum*, traditional landraces, POD, PPO, isoenzymes

Acknowledgment: This research was supported by COST Action CA18210 „Oxygen sensing a novel mean for biology and technology of fruit quality" (Roxy-COST).

The possibility of energy plants for phytoremediation of heavy metal contaminated sediment

PP3-6

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Industrialization and human activities have resulted in the release of various contaminants into the aquatic ecosystem. As a result of the discharge of untreated wastewater, heavy metals are often present in the sediment. Phytoremediation is the environmentally friendly process of using plants and their associated microbes for environmental cleanup due to their intensive uptake of contaminants. To assess the phytoremediation ability of different species of energy plant, pot tests were conducted. The heavy metal contaminated sediment from Begej Canal was used. Pot experiments were performed in the open field under natural weather conditions, in pots filled with 20 kg of sediment. Plants selected for pot trials were rapeseed (*Brassica napus*), white mustard (Brassica alba), hemp (*Cannabis sativa*), and sunflower (*Helianthus annuus*). Pots with rapeseed were treated with commercial products for plant growth-promoting rhizobacteria, PGPR (TrifenderPro, PanoramaBio, and BioEho). Ten weeks after sowing, harvest was performed, and the below- and above-ground biomasses were measured. The contaminated sediment did not affect plant growth and obtained biomass. Among rape-seed trials, the highest biomass was obtained in the treatment with PGPR TrifenderPro. The plant samples were digested, and the content of Pb, Cr, and Cu was analyzed. Bioaccumulation (BAF) and translocation factors (TF) were calculated. In the case of Cr, the highest BAF was obtained for rapeseed with no treatment and with TrifenderPro treatment, and hemp. In the case of Cu the highest BAF was obtained for sunflower. TF was <1, which indicates that the main mechanism of metal removal is phytostabilization, not phytoextraction.

Keywords: energy plants, heavy metals, phytoextraction, sediment

Acknowledgment: This study has funded by the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 101006912-Phy2Climate.

The allelopathic effect of moss extracts on the germination of lettuce and radish seeds

PP3-7

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Bryophytes are rich in chemical compounds that may display allelopathic effect on seed ger-mination. The aim of this study was to examine the effects of different concentration of nine moss extracts (*Abietinella abietina, Ctenidium molluscum, Dicranum polysetum, Hylocomium splendens, Isothecium alopecuroides, Leucodon sciuroides, Racomitrium elongatum, Thuidium delicatulum, T. tamariscinum*) on the germination of lettuce and radish seeds and seedlings length. Lettuce seeds were treated with different concentrations of moss extracts: 10^{-4} M, 10^{-3}, 4x10-3, $8x10^{-3}$ and 10^{-2}, whereas radish seeds were treated with the different concentrations: 10^{-4} M, 10^{-3}, $4x10^{-3}$ and $8x10^{-3}$. The percentage of germinated seeds and the length of the hy-pocotyls were measured after three and five days. High extract concentrations of *A. abietina*, *C. molluscum*, *D. polysetum*, *H. splendens* and *I. alopecuroides* showed an inhibitory effect on the germination of lettuce seeds. Besides, those extracts negatively affected the length of the lettuce hypocotyl. Contrarily, when extracts of *R. elongatum*, *T. delicatulum* and *T. tamariscinum* were applied, more than 90% of lettuce seeds germinated. Nevertheless, similar moss extracts (*A. abietina*, *D. polysetum*, *H. splendens* and *I. alopecuroides*) have also negatively affected germination of radish seeds and hypocotyl length indicating strong allelopathic effect of those moss species. Additionally, extracts of *C. molluscum*, *L. sciuroides*, *R. elongatum* and *T. delicatulum* did not affect the germination of radish seeds. Interestingly, only extract of *T. tamariscinum* showed stimulating effect on radish seed germination. Examined mosses show high allelopathic potential to seed germination and can be used in further studies as potential biopesticides.

Keywords: mosses, allelopathy, seed germination, lettuce, radish

Acknowledgment: This work was supported by the Innovation fund, the program Proof of concept (No. 5714).

Potential of *Thymus pannonicus* All. *in vitro* culture: micropropagation, rosmarinic acid production and antimicrobial activity

PP3-8

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In Serbia, citral chemotype of *Thymus pannonicus* All. (Lamiaceae) can be only found on Mt Vršačke planine, as dominant form, where is traditionally used for medicinal purposes. To overcome difficulties in harvesting due to distribution in inaccessible areas, and to prevent possible overexploitation from its natural habitat, finding the means for alternative cultivation of this plant for its prospective beneficial use in human health care was set as objective of this study. Efficient system for *in vitro* shoot propagation of *T. pannonicus* was established for the first time. Different types of auxin (indole-3-acetic acid, IAA; indole-3-butyric acid, IBA; 1-naphthaleneacetic acid, NAA) were used for testing the process of rhizogenesis *in vitro*. Best response was detected on IBA and NAA for rhizogenesis frequency and number of formed roots, respectively. Rooted plants were successfully acclimatized. *In vitro* shoots cultivated on PGR free medium (E1) and medium with 1 mg L^{-1} IAA (E2) were extracted with 80% methanol and analysed by HPLC. Presence of rosmarinic acid (RA) as dominant component was indicated in both extracts, with E2 containing higher RA amount (45.116 mg g^{-1}DW). Antimicrobial activity of extracts was tested using broth microdilution method against nine strains of laboratory control pathogenic microorganisms. Both extracts exerted significant antimicrobial activity against all tested pathogens with minimal inhibitory con-cen-trations (MIC) in the range of 6.25-50 mg mL^{-1}, while E2 was more effective against Gram positive bacteria (6.25-12.5 mg mL^{-1}). Presented results could give inducement for potential greater commercial use of this significant species in the future.

Keywords: *T. pannonicus, in vitro* micropropagation, acclimatization, rosmarinic acid, antimi-crobial potential

Acknowledgment: This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia through Grant Agreement with University of Belgrade- Institute for Biological Research "Siniša Stanković" - National Institute of Republic of Serbia No. 451-03-68/2022-14/200007 and Grant Agreement with University of Belgrade-Faculty of Pharmacy No. 451-03-68/2022-14/200161.

Comparative NMR fingerprinting and anti-inflammatory activity of endemic species from genus *Stachys*

PP3-9

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Stachys thracica Davidov, *Stachys bulgarica* Degen & Nejceff and *Stachys scardica* Griseb. are Balkan endemic species which are included in The Red Data Book of Bulgaria. Due to their conservational status little is known about their chemical composition and pharmacologi-cal potential. The aim of the present work was to perform comparative NMR profiling and anti-inflammatory activity of extracts from *in situ*, *in vitro* cultivated and *ex vitro* adapted plants. *In vitro* shoot cultures of *S. thracica*, *S. bulgarica* and *S. scardica* were successfully maintained on MS medium under controlled environmental conditions and collections of *ex vitro* adapted plants are grown on an experimental field. NMR fingerprinting allowed identification of major alterations in the metabolome of the three endemic plants during the process of *ex situ* conservation. Phenylethanoid glycosides were identified to be the main secondary metabolites in *S. thracica* and iridoids in *S. bulgarica* whereas in *S. scardica* both types of compounds were equally present. The anti-inflammatory potential of extracts from the three endemic species was tested by the hemolytic assay. For *S. thracica* and *S. scardica* the highest complement inhibition was 90% in the extracts from *in situ* wild and *ex vitro* adapted plants whereas for *S. bulgarica* the highest activity was observed for *in vitro* cultivated plants. The established collections of *in vitro* cultivated and *ex vitro* adapted plants is an alternative ap-proach for the preservation of *S. thracica*, *S. bulgarica* and *S. scardica* and may serve as an effective platform for sustainable production of pharmaceutically valuable molecules.

Keywords: *Stachys*, phenylethanoid glycosides, iridoids, anti-inflammatory

Acknowledgment: This work was financially supported by Grant BG05M2OP001-1.002-0012-C01 financed by the European Structural and Investment Funds through the "Science and Education for Smart Growth" Operational Program.

Analysis of storage proteins in a worldwide collection of pea seeds

PP3-10

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Peas (*Pisum sativum* L.) are an important source of vegetable protein for both humans and animals. However, pea seed proteins are limited in sulfur-containing amino acids (SCAA) and thus considered to be an insufficient source. The main seed proteins in pea are 7S (vicilins, convicilins) and 11S (legumins) globulins. Because 11S globulins contain more SCAA than 7S globulins, variations in the 7S/11S ratio affects the nutritional quality of pea seeds. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was used to separate seed proteins from 260 pea genotypes from around the world (the EUCLEG collection). Based on the electropherogram, 18 protein bands were densitometrically measured. Protein compositional data are intrinsically multivariate. The protein concentration of each genotype is presented in closed form i.e., sum up to 100%. Therefore, for multivariate data analysis specific statistical methodology was used. All methods used in the study were carried out in classical and robust manner that was resistant to outlier observations. The storage protein composition of the genotypes studied varied greatly; in some cases, only traces of the main components were found. The results suggest that some of the genotypes tested could be useful in various breeding programs aimed at producing agronomically viable plants, yielding high-protein seed with specific composition of storage proteins for specific food applications.

Keywords: Pea collection, Seed proteins, Storage proteins

Acknowledgment: This research has received funding from the European Union's Horizon 2020 project EUCLEG under grant agreement n°727312.

Antiherpes virus activity of water extracts from callus culture, different aerial parts of *ex vitro* grown and from whole *in vitro* grown medicinal plant *Nepeta nuda* ssp. *nuda* L.

PP3-11

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In the present study water extracts from flower and leaf of *ex vitro* grown and from callus culture and whole *in vitro* grown *Nepeta nuda* ssp. *nuda* L. was tested against replication of HHV type 1 strain F (ACV-sensitive) *in vitro*. Maximal nontoxic concentration (MNC) and cytotoxic concentration (CC50) was determined by colorimetric method (MTT assays) at the 72 hours. To determine the antiviral activity of the extracts we used MTT-based colorimetric assay for detection of HHV replication inhibition (effect was expressed as % of protection). All extracts (except for this from the whole *in vitro* grown *Nepeta nuda* ssp. *nuda* L. which does not show any antiviral potential) showed potent anti-herpesvirus activity in both used experimental arrangements (according to the time of addition of the extracts). The activity increased when water extracts was added simultaneously with the inoculation in comparison with the results obtained when extracts were added 1 h after the inoculation of cell monolayer. Selectivity for viral versus cellular molecular targets (selectivity index) was for water extracts from leaf of ex vitro grown and from callus culture and whole *in vitro* grown plant were low (below 10). Only selectivity index (SI) for water extracts from flower was ~10 (it is generally considered that biological efficacy is not due to *in vitro* cytotoxicity when SI ≥ 10 and has therapeutic significance). All extracts were tested by direct contact assay for activity against extracellular form of the virions.

Keywords: *Nepeta nuda* L., Human alphaherpesvirus (HHV), *in vitro*, ACV-resistance, antivi-ral activity

This work was financially supported by the Bulgarian National Science Fund (BNSF), Bulgarian Ministry of Education and Science, Grant No. KP-06-N56/9/12.11.2021 and Grant D01-217/30.11.2018 under the National Research Programme "Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed).

Multidrug-resistant cancer cells are sensitive to abietane diterpenoids from *Plectranthus* species

PP3-12

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Plants of the genus *Plectranthus* (Lamiaceae) are used in traditional medicine. Here, the anti-cancer effects of the three abietane diterpenoid derivatives from *Plectranthus* species are described. Compounds' effects (comp. 1 = VI31.1.1, comp. 2 = VI20.1.1, comp. 3 = RoyBz) were tested in human lung cancer cells, in sensitive NCI-H460 and chemoresistant NCI-H460/R, as well as in colon cancer cells, sensitive DLD1, and chemoresistant DLD1-TxR. The resistant cells were more sensitive than corresponding parental cells to 1 and 2 in MTT assay, with IC_{50} values ranging from 3 to 10 μM. For the most potent 3 (IC_{50} as low as 1 μM), resistant cells had up to 2 times higher IC50 values than sensitive cells. The growth inhibition effect by all three compounds was more pronounced in cancer cells, compared to normal human fibroblasts (MRC-5). Only 2 induced a significant cell death effect showing 70% non-viable cells in NCI-H460. The effect the compounds might have on the P-gp extrusion pump was also tested using doxorubicin and rhodamine 123 accumulation assays. Compounds 1 and 2 caused a significant increase in the accumulation of both P-gp substrates, doxorubicin, and rhodamine 123. The compounds isolated from *Plectranthus* showed anticancer potential in lung and colon cancer cells. Importantly, they displayed colateral sensitivity - a phenomenon when the chemoresistant cells are more sensitive to the compounds than corresponding sensitive cells. The compounds inhibited the P-gp activity implying MDR modulating potential.

Keywords: *Plectranthus*, abietane diterpenoid, anticancer effect

Acknowledgment: This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (ref. number 451-03-68/2022-14/ 200007) and by the STSM grant awarded by STRATAGEM COST Action CA17104 "New diagnostic and therapeutic tools against multidrug resistant tumours".

Transient overexpression of *β*-glucosidase in leaves of *Centaurium erythraea* Rafn reduces the content of secoiridoid glucosides

PP3-13

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Centaurium erythraea Rafn, also known as common centaury, is a rich source of pharmaco-logically active specialized metabolites among which secoiridoid glucosides (SG) predomi-nate. These compounds belong to the group of monoterpenoids, which are derived from the iridoid cyclopentan-C-pyran skeleton with *β*-D glucose linked at C1 position. Major SGs of *C. erythraea* (sweroside, swertiamarin, and gentiopicrin) are biosynthesized through general iridoid pathway via secologanin, and it has been recently proved that their catabolism starts with the deglycosylation step catalyzed by beta-glucosidases (BGlu). Two BGlu genes from *C. erythraea* (*Ce*BGlu1 and *Ce*BGlu2), with previously confirmed function, were transiently overexpressed in leaves of five-month-old diploid and tetraploid centaury plants. Agroinfiltration of *Agrobacterium tumefaciens* GV3101 strain harboring genes of interest in TMV-based overexpression pJL-TRBO vector (pJL-TRBO:*Ce*BGlu1; pJL-TRBO:*Ce*BGlu2) result-ed in transient *in planta* overexpression of *Ce*BGlu1 and *Ce*BGlu2. Metabolic profiling of secoiridoids in leaves of diploid and tetraploid *C. erythraea* genotypes revealed that amounts of sweroside, swertiamarin, and gentiopicrin, were significantly reduced in agroinfiltrated leaves, especially when *Ce*BGlu1 and *Ce*BGlu2 were co-expressed with transgene silencing suppressor p19. Diploid plants displayed more pronounced decrease in SGs content than tet-raploids. The effectiveness of gene overexpression was obviously altered by plant ploidy, and was under the control of post-transcriptional gene silencing mechanisms in the infiltrated tis-sues. Transgenes are more prone to transcriptional inactivation in *C. erythraea* tetraploids than in diploids. In conclusion, SGs-specific *β*-glucosidases could serve as a molecular target of biotechnological interest, in order to shape SG profiles of centaury and related economically important species of the Gentianaceae family.

Keywords: *Centaurium erythraea*, secoiridoid glucosides, beta-glucosidase

Acknowledgment: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia grant numbers OI173024, 451-03-9/2021-14/200007, and 451-03-68/2022-14/200007

In vitro neuroprotective activity and *in silico* studies of phenolic compounds from *Hypericum perforatum* hairy roots

PP3-14

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Hypericum perforatum hairy root (HR) clones obtained by *Agrobacterium rhizogenes* A4-mediated transformation were analyzed for their phenolic profile and *in vitro* inhibitory activi-ty against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) as key enzymes re-lated to neurodegenerative disorders. *In silico* molecular modeling was performed using Au-toDock 4.2 software to provide the interactions between selected phenolics and target en-zymes. Chromatographic analyses revealed that HR represents efficient source of hy-droxybenzoic acids, flavan-3-ols, flavonol glycosides and xanthones. The AChE inhibitory activity of HR clones at all tested concentrations varied from 23% to 32%, while BChE inhi-bition was ranged from 13% to 38%. Molecular docking study was performed with vanillic acid, epicatechin, quercetin 6-*C*-glucoside, mangiferin and γ-mangostin due to their abun-dance in *H. perforatum* HR extracts. Docking experiment on AChE revealed that γ-mangostin showed the best docking score with binding energy of -11.58 kcal·mol^{-1}. Favorable interac-tions in the AChE active site were also found for quercetin 6-*C*-glucoside, epicatechin and mangiferin. Vanillic acid showed the lowest affinity towards AChE (binding energy -4.09 kcal·mol^{-1}) due to the limited interactions within the enzyme cavity. The γ-mangostin and epi-catechin showed the best affinities to BChE active center (binding energies -10.08 and -8.55 kcal·mol^{-1}, respectively). These findings indicated that xanthones and flavonoids significantly contributed to the *in vitro* neuroprotective potential of *H. perforatum* transgenic root extracts and thus could be proposed as promising remedies in the management of Alzheimer's disease.

Keywords: Hairy roots, *Hypericum perforatum* L., Molecular docking, Neuroprotective activi-ty, Phenolic compounds

'Joly' and 'Sibilla': Two newer strawberry cultivars with better biochemical traits compared with their common parent 'Clery'

PP3-15

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The most popular cultivated strawberry is the dessert strawberry (*Fragaria×ananassa* Duch.) from which breeders have created an abundance of new improved cultivars with specific agronomic, qualitative and sensorial characteristics. In this study, two newly introduced strawberry cultivars 'Joly' and 'Sibilla' were compared with their mutual parent and commercial cultivar 'Clery' in terms of individual phenolics, sugars, and organic acids content. All forms of free pelargonidin were significantly higher in 'Joly' and 'Sibilla' (17.6 and 11.3 µg/g FW, respectively) compared to 'Clery' (3.4 µg/g FW), in which cyanidin 3-glucoside is the dominant (6.2 µg/g FW). Quercetin 3-O-glucuronide values were higher in 'Sibilla' and 'Joly' (17.4 and 11.6 µg/g FW, respectively) than in 'Clery' (2.6 µg/g FW). The highest values of *p*-coumaric acid were detected in 'Sibilla' (6.2 µg/g FW). Fructose content (3.1 g/100g FW) was the highest in 'Joly', sucrose content (0.8 g/100g FW) in 'Sibilla', while glucose content was similar in all three cultivars. The highest value of fumaric and citric acid (13.1 µg/g and 6.2 mg/g FW, respectively) was detected in 'Sibilla', while malic and shikimic acid had similar values in all three cultivars. Two newer strawberry cultivars 'Joly' and 'Sibilla' generally had higher levels of analyzed bioactive compounds compared to their common parent 'Clery'. Given that sensorial and nutritional quality of strawberry fruit is significantly affected by the ratio of sugars and organic acids, as well as their combination with phenolic compounds, the established better fruit quality of newer cultivars indicated their significant potential for commercial cultivation.

Keywords: strawberry cultivars, fruit quality, organic acids, sugars, phenolics, HPLC

Acknowledgment: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant No. 451-03-68/2022-14/200053 and 451-03-68/2022-14/200116).

Effects of different extraction solvents on chemical composition and antimicrobial activity of *Cota tinctoria* extracts

PP3-16

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Cota tinctoria (L.) J. Gay ex Guss. (Asteraceae), is called Yellow Ring in Serbia, or Jarmen. This is a biennial to perennial plant, 20-60 cm tall, with spindly or branched roots. It is known that this species was used for dyeing hair or wool, while there is no data of its use in tradi-tional medicine. It was shown that this plant exhibits some anticancer effects. This study ex-plored the influence of different extraction solvents (ethanol and water in different ratios) on chemical composition and antifungal activity of the extracts. The phenolic profile of extracts was determined by LC-DAD-ESI/MSn (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA). The effects on the following fungal species were tested: *Candida albicans* (clinical isolate), *Candida albicans* (ATCC 10231), *Candida krusei* (clinical isolate), *Microsporum gypseum* (clinical isolate), *Trichophyton rubrum* (ATCC 28188) and *Trichophyton mentagrophytes* (clinical isolate). A total of 9 compounds were identified in the extracts, with the *O*-dicaffeoylquinic acid being the most dominant compound in all of the extracts. The highest total phenolic content was recorded for the infusion of *Cota tinctoria*. The most sensi-tive species were: *T. mentagrophytes* and *T. rubrum*, while the most resistant the species was *C. krusei*. Commercial antifungals, ketoconazole and fluconazole showed better antifungal activity compared to the tested extracts. The results of this investigation showed *C. tinctoria* as good source of bioactive phenolics with good antifungal characteristics.

Keywords: *Cota tinctoria*, antifungal potential, dermatomycetes, chemical composition

Acknowledgment: This research is funded by the Serbian Ministry of Education, Science and Technological Development (Contract No. 451-03-68/2022-14/200007).

Antagonistic activity of *Trichoderma* spp. against soilborne pathogens

PP3-17

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Potential use of *Trichoderma* spp. against different soilborne plant pathogens (*Sclerotinia sclerotiorum*, *Rhizoctonia solani* and *Pythium aphanidermatum*) was investigated in this study. Thirteen *Trichoderma* spp. isolates were derived from the rhizosphere of different host plants originating from seven localities in Serbia. Based on the results of preliminary tests, us-ing a dual culture confrontation method, four *Trichoderma* spp. isolates with the best antago-nistic activity were chosen for identification and further investigation. Morphological and physiological characteristics and sequence analysis of the translation elongation factor 1-alpha (*tef 1*), showed that all antagonistic strains belong to T*richodema harzianum* species complex (ThSC). Antagonistic activity of the ThSC strains was quantified *in vitro* on PDA medium at 24°C, by a double-layer-well method. The highest antagonistic activity was achieved by T1 isolate against *P. aphanidermatum* and *R. solani*, where mycelial growth of the pathogens was completely inhibited. None of the ThSC strains was able to inhibit the growth of *S. sclerotiorum* under presented experimental conditions. To determine the mode of antagonistic activity, tree types of liquid ThSC culture filtrates were used: filtrated through cheese cloth (containing fungal spores), filtrated by syringe filters (spore free) and heated cheese-cloth-filtrate (10 min at 100°C). The research showed that *T. harzianum* strains exhibited direct my-coparasitism as the mode of antagonistic activity and that produced metabolites didn't ex-press suppressive effect on pathogenic isolates. Presented study revealed that tested isolates of ThSC, or at least T1 strain, could be effective biocontrol agent(s) against *R. solani* and *P. aphanidermatum*.

Keywords: plant pathogens, biological control, *Trichoderma harzianum*

Acknowledgment: Ministry of Education, Science and Technological Development of Republic of Serbia (contract 451-03-68/2022-14/200214).

Ascorbic acid and *α*-tocopherol content in *Rosa spinosissima* hips

PP3-18

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Wild roses are used in European and Asian traditional medicine for nutritional and therapeutic purposes. Rose hips, as aggregate fruits, are a rich source of compounds such as vitamins, phenolics and carotenoids. These fruits contain a notable amount of ascorbic acid (AA) in pericarps and *α*-tocopherol (AT) in nutlets. These micronutrients are well-known antioxidants which have a significant role in oxidative stress prevention. In this study, the AA and AT content in *Rosa spinosissima* L. fruits collected from mountains Zlatibor, Stara planina, Stolovi and Pešter plateau (Serbia) were analysed. Extraction of fully ripe fruits was performed in liquid nitrogen with 5% meta-phosphoric acid (AA) and chloroform-methanol (AT). Target compounds were separated and quantified using high-performance liquid chromatography (HPLC) coupled with a photodiode array detector (DAD). The results of the chemical analysis showed that extracts obtained from pericarp contain a notable amount of AA, where the highest level was determined in those from Stolovi Mt. (2.73 mg/g FW). Furthermore, HPLC analysis also revealed prominent AT content in rose nutlets and the highest amount (0.04 mg/g FW) was quantified in the samples collected from the Pešter plateau. The presented study showed a high level of AA and AT in fruits from all analysed localities. These results suggest that *R. spinosissima* hips could be used in the treatment of colds, flu and vitamin C deficiency.

Keywords: wild roses, *Rosa spinosissima*, fruits, HPLC, vitamins

Acknowledgement: The authors are thankful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for financial support (Grant No. 451-03-68/2022-14/200178).

Total phenolic content and antioxidant potential of *Rosa spinosissima* leaf extracts

PP3-19

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Rosa leaves are used in European and Asian traditional medicine as a source of bioactive agents that can reduce the level of free radicals and inhibit the development of diseases caused by oxidative stress. They contained antioxidative compounds such as phenolic acids, flavonoids and vitamins. This study aimed to evaluate the total phenolic content (TPC) and antioxidant activity of *Rosa spinosissima* L. leaves collected from four localities in Serbia (Zlatibor, Stara planina, Stolovi mountains and Pešter plateau). Extracts were prepared with 70% ethanol using ultrasonic-assisted extraction. TPC and antioxidant capacity of extracts were determined by spectrophotometric methods. Antioxidant activity was evaluated by DPPH, ABTS (IC_{50} mg/mL) and FRAP (µmol Fe^{+2}/mg) assays. The results showed a high level of TPC in *R. spinosissima* leaves, with the highest amount in the extract from Pešter (279.84 mg GAE/g). The highest radical scavenging capacity in DPPH and ABTS tests showed an extract from the leaves collected from Pešter (IC_{50}=0.81 and 0.16 mg/mL, respectively). This extract also possesed the highest antioxidant activity in the FRAP assay (1712.29 µmol Fe^{+2}/mg). The antioxidant capacity of tested extracts was in the range of reference compounds BHA, α-tocopherol and ascorbic acid. This study suggests that, besides the hips of *R. spinosissima*, leaves are also rich in antioxidants and should be recognised as a perspective source of natural antioxidative agents.

Keywords: *Rosa spinosissima*, leaves, DPPH, ABTS, FRAP

Acknowledgement: The authors are thankful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for financial support (Grant No. 451-03-68/2022-14/200178).

Plant derived sweeteners promote virulence of pathogenic bacteria

PP3-20

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Different sweeteners are used in diet but not much is known about different aspects of human health that they can affect. Consequently, the influence of sweeteners used in nutrition on the development of resistance and virulence of microorganisms (species of the genus *Candida*, *Pseudomonas*, *Staphylococcus*) were examined. Three plant-derived sweeteners were selected: demerara sugar (from sugar cane), stevia (isolated from *Stevia rebaudiana*), and coconut sugar (from coconut palm). An increase in the minimum inhibitory concentration of commercial antibiotics in the presence of studied sweeteners has been observed. Also, an increase in the biofilm production ability in the presence of sweeteners is noticed for all the species except *C. albicans*: demerara sugar (*P. aeruginosa* 528%, *S. aureus* 262%, *C. albicans* 64%), stevia (*P. aeruginosa* 140%, *S. aureus* 180%, *C. albicans* 79%), coconut sugar (*P. aeruginosa* 408%, *S. aureus* 307%, *C. albicans* 57%) as compared to the untreated control biofilm production (100%). Exposure of *P. aeruginosa* to demerara sugar induced increase in expression of virulence associated *lasI* and *lasR* genes. On the other hand, in *C. albicans* no significant increase in formation of hyphae in the presence of sweeteners has been observed (demerara sugar induced hyphae to 113%, stevia 72%, coconut sugar 13%, compared to the untreated control). Plant derived sweeteners increase virulence of bacterial species, but not *C. albicans*. Higher awareness and further research is needed in order to highlight the effects plant derived sweeteners could induce in pathogenic bacteria and to provide novel tools to reduce this negative aspect.

Keywords: sweeteners, virulence factors, biofilm, microorganisms

Acknowledgment: This research is funded by the Serbian Ministry of Education, Science and Technological Development (Contracts No. 451-03-68/2022-14/200007).

Antiradical activity of liposome encapsulated *Uncaria tomentosa* extracts – an EPR study

PP3-21

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Cat's claw (*Uncaria tomentosa*) is a woody vine, native to the Amazon rain forests. This plant has been used for centuries in traditional medicinal practice to treat arthritis, asthma, wounds as well as chemotherapy side effects. Scientific data show that extracts of this vine exhibit wide range of anti-inflammatory and antitumor effects. However, there are no records of any detailed study of its antiradical activity. The goal of this study was to encapsulate/incorporate *Uncaria tomentosa* extracts into DPPC model liposomes in order to enhance their bioavailability and potential pharmacokinetic effects, and to evaluate their antiradical activity towards $DPPH^{\cdot}$, $^{\cdot}OH$ and $O2^{\cdot -}$ free radical species using electron paramagnetic resonance (EPR) spectroscopy. The vine samples were collected in Amazonas, Colombia and water/hydroethanolic extracts were obtained by maceration. Liposomes (200 nm) were prepared by thin film and extrusion methods. For short-lived free radical species detection, the EPR spin-trapping technique has been used. The results show that water/hydroethanolic extracts efficiently eliminate $DPPH^{\cdot}$ (59.59/96.46 %) and $^{\cdot}OH$ radicals (47.28/95.70 %). No reduction of the $O2^{\cdot -}$ radicals signal could be observed. Upon encapsulation/integration into the DPPC liposomes, water/hydroethanolic extracts eliminated $DPPH^{\cdot}$ (49.39/44.97 %) and $^{\cdot}OH$ radicals (26.23/25.37 %). These results suggest that after encapsulation/integration of extracts, liposomes still pos-sess significant antiradical activity, which could be increased using more concentrated ex-tracts for liposome preparation. Our results indicate that water/hydroethanolic extracts of *Uncaria tomentosa* possess strong, but selective, antiradical activity, making it a promising remedy for the treatment of various health conditions related to oxidative stress.

Keywords: *Uncaria tomentosa*, liposomes, EPR

Acknowledgment: The financial support for this research was provided by the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract number: 451-03-68/2022-14/200146.

EPR evaluation of *Thymus pannonicus All.* extracts antiradical activity

PP3-22

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Thymus pannonicus All. is a perennial aromatic herb spread over Central and Eastern Europe. There are several chemotypes of this plant, most of them having thymol as a dominant com-ponent of the essential oil. The only known citral chemotype population is located in the Vršac mountains (Serbia). Due to respiratory and digestive organs' health-promoting effects combined with appealing aroma, efforts have been made in cultivating the citral chemotype. Currently, the antiradical activity of *Thymus pannonicus All.* extracts have not been studied in more detail. The goal of this study was to evaluate their antiradical activity towards DPPH˙ and ˙OH free radical species using electron paramagnetic resonance (EPR) spectroscopy. The extracts of *Thymus pannonicus All.* in water, 50% EtOH, EtOH, and MeOH were obtained by 4h maceration in 1:5 ratio (dry sample/solvent, w/v) at 40 °C. For short-lived free radical species detection, the EPR spin-trapping technique (and DEPMPO as the spin-trap) has been used. The results show that DPPH˙ has been efficiently eliminated by water (21.45%), EtOH (41.56%), 50% EtOH (81.84%), and MeOH (58.24%) extracts. On the other hand, ˙OH radi-cals have been even more efficiently eliminated from the system by water (84.54%), EtOH (89.07%), 50% EtOH (76.42%), and MeOH (95.37%) extracts. The obtained results suggest that all extracts of *Thymus pannonicus All.* used in this study possess strong antiradical activi-ty towards DPPH˙ and ˙OH. These findings confirm the considerable potential of their appli-cation in pharmacy and medicine. Their selectivity towards other biologically relevant free radical species should be further confirmed.

Keywords: *Thymus pannonicus All.*, EPR, antioxidant

Acknowledgment: The financial support for this research was provided by the Ministry of Education, Science and Technological Development of the Republic of Serbia, contract number: 451-03-68/2022-14/200146.

Phenotyping of camelina (*Camelina sativa* (L.) Crantz) response to drought stress at germination stage

PP3-23

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Camelina sativa (L.) Crantz, is a self-pollinated, annual oilseed from the Brassicaceae family, which is gaining interest worldwide due to its frost, heat, and drought tolerance. The aim of this study was to evaluate camelina germination under osmotic stress, and to identify critical soil moisture levels for successful germination establishment. A germination test was set up in controlled conditions, comparing six winter and six spring genotypes with differing seed sizes (ranging from 0.88 to 1.83 g/1000-seeds) under increasing levels of osmotic stress (0, –0.4, –0.8, –1.2, –1.4, –1.6 MPa) that was produced with polyethylene glycol (PEG). The results showed good tolerance of all camelina genotypes to drought at the germination stage. Plants remained unaffected at mild level of osmotic stress (–0.4 MPa) having no significant decrease in germination percentage compared with the control. Even at −1.2 MPa, examined genotypes still had high germination (75%). Significant differences in germination were observed be-tween biotypes, where spring biotypes performed better than winter ones. Shoot and radicle lengths were significantly diminished by imposed osmotic stress, but shoot growth seemed more impacted. In general, spring biotypes had longer shoots and radicles than winter ones. Seed size played a role in the response of camelina to drought, but it depended on biotype and stress level imposed. Camelina confirmed to withstand high levels of drought stress at germination and could be considered as more suitable oil crop than oilseed rape on marginal lands, or areas with irregular precipitation pattern.

Keywords: osmotic stress, shoot length, radicle length, seed size

Acknowledgements: This work is done in the scope of the activities of Centre of Excellence for Innovations in Breeding of Climate-Resilient Crops – Climate Crops of Institute of Field and Vegetable Crops, as well as a part of the project supported by the Provincial Secretariat for Higher Education and Scientific Research of APV Vojvodina (Project no. 142-451-2609/2021-01/02) and Ministry of Education, Science and Technological Development of Republic of Serbia, grant number 451-03-68/2022-14/ 200032.

Modern plant phenotyping in breeding programs of the Institute of Field and Vegetable Crops Novi Sad (IFVCNS)

PP3-24

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The Institute of Field and Vegetable Crops Novi Sad, National Institute of the Republic of Serbia has more than 85 years long tradition of plant phenotyping work, but first projects related to use of modern phenotyping platforms were established a decade ago. From these first steps till now, the IFVCNS become one of the leading institutions in this research area in Serbia. So far, the Institute's researchers finalised four Transnational Access to European Plant Phenotyping Network projects. In these studies, some wheat and sunflower genotypes were identified as potential sources for biotic and abiotic stress tolerance. Thus, it was found that wheat cvs. NS 40S, Subotičanka and NS Avangarda have relatively high levels of drought and salt tolerance. Use of Flower Colour Image Analysis (FloCIA) software enhanced ray florets colour determination and paved the way to more objective and accurate sunflower phenotyping, while thermal imaging was used for screening of sunflower for tolerance to *Sclerotinia* head and stem rot. Also, a collaborative work with the Department of Agri-Food Sciences and Technologies, University of Bologna, Italy have shown a good tolerance of camelina to drought at the germination stage. The Institute has a strategy for more intensive use of modern phenotyping technologies in its future research and practice. Recently approved projects: SmartSun (7732457), CROPINNO (101059784), EMPHASIS-GO (101079772) and AgroServ (101058020), funded by the National Science Foundation of Serbia and EU Commission, are aimed at institutional capacity building in the application of phenomics in crop improvement.

Keywords: plant phenotyping, phenomics, breeding, collaborative projects.

Acknowledgment: This work is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-68/2022-14/200032, by the Science Fund of the Republic of Serbia, through IDEAS project "Creating climate smart sunflower for future challenges" (SMARTSUN) grant number 7732457, by the Provincial Secretariat for Higher Education and Scientific Research of APV Vojvodina (project no. 142-451-2609/2021-01/02) and by Centre of Excellence for Innovations in Breeding of Climate-Resilient Crops - Climate Crops, IFVCNS, Serbia.

Bioherbicidal evaluation of *Sorghum halepense* (L.) rhizome extract and its bioactive components against selected weed species

PP3-25

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Nowadays, one of the priorities in agricultural production is the usage of natural bioherbicides for weed control, instead of commercial herbicides which have a strong negative effect on human health and the environment. Due to its widespread distribution and the economic losses it causes to the global economy, *Sorghum halepense* has been described as one of the world's most invasive plant and a dangerous weed. We here examined the possibility to exploit the phytotoxic and bioherbicide potential of this species against other weed species: *Amaranthus retroflexus*, *Chenopodium murale* and *Datura stramonium*. The rhizome extracts of *S. halepense* (concentration of 0.01, 0.1, 1, 2, and 5 mg/ml) notably reduce the final seed germination of *C. murale* and *D. stramonium*, especially at the highest investigated concentration, while *A. retroflexus* seed germination was not affected. Early seedling growth of *A. retroflexus was stimulated by the rhizome extract, while in C. murale and D. stramonium the values* of growth parameters *were reduced. As revealed by UHPLC/DAD/qqqMS^2 metabolic profiling of polyphenolics, major bioactive compounds in S. halepense* rhizom extract were phenolic acids: *p*-hydroxybensoic acid (*p*HB A), chlorogenic acid (CHL A), and *p*-coumaric acid (*p*CUM A). Analysis of bioherbicide potential of these compounds, applied in a series of concentrations (0.005, 0.01, 0.1, 0.2, and 0.3 mg/ml), revealed that *p*HB A, CHL A, and *p*CUM A, are the main responsible for the overall effects of *S. halepense* rhizom on selected weeds. These results highlight *Sorghum halepense* (L.) rhizomes, and their main constituents, as potent bioherbicides.

Keywords: bioherbicide, *Sorghum halepense*, *p*-hydroxybensoic acid, chlorogenic acid and *p*-coumaric acid

Acknowledgment: This work was supported by Ministry of education, secience and technological development of Republic of Serbia, IBISS Work Program for 2022, Contract Number: 451-03-68/2022-14/200007

Elicitation effects of methyl jasmonate on iridoid biosynthesis in leaves of *Nepeta rtanjensis* and *Nepeta nervosa*

PP3-26

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The genus *Nepeta* L. comprises medicinal plant species that synthetize terpenes and phenolics of therapeutic value. Considerable variability in qualitative and quantitative content of iridoid monoterpenoids has been detected within the genus *Nepeta*, which comprises iridoid produc-ing and non-producing species. *Nepeta rtanjensis* Diklić & Milojević is an endemic plant of the Balkan Peninsula, which produces both iridoid aglycones (IAs) and iridoid glucosides (IGs). *N. nervosa* Royle ex Benth., endemic to the Himalayas of Kashmir, India, and Paki-stan, is iridoid-lacking species. This study aimed to comparatively analyze the effect of me-thyl jasmonate (MeJA), an elicitor of terpene production in many plant species, on iridoid bio-synthesis at the molecular level. Iridoid content in MeJA-treated *N. rtanjensis* and *N. nervosa* plants under *in vitro* conditions was analysed in parallel with the co-expression patterns of iridoid related biosynthetic genes (*GPPS*, *GES*, *G80*, *8HGO*, *7DLGT*, *IS*, and *NEPS*), major latex protein-like gene (*MLPL*), and genes coding for different transcription factors (*MYC2*, *YABBY5*, *COI1*, and *JAZ3*). In general, the exogenous application of 250 µM MeJA for 2 h, 24 h and 72 h resulted in an increased accumulation of targeted IAs (trans, *cis*-nepetalactone and 5,9-dehydronepetalactone) and IGs (1,5,9-epideoxyloganic acid) in leaves of *N. rtanjensis*, as a result of elevated expression of the key biosynthetic genes. Interestingly, although the majority of iridoid-related biosynthetic genes and TFs were identified in transcriptome of *N. nervosa* leaves, and the expression of some of them (*NEPS* and *JAZ3*) is up-regulated follow-ing MeJA treatment, no iridoids were accumulated in leaves of *N. nervosa*.

Keywords: *Nepeta rtanjensis*, *Nepeta nervosa*, chemotypes, methyl jasmonate, iridoid biosynthesis

Acknowledgment: This work is financed by the Science Fund of the Republic of Serbia, Pro-gram IDEAS (project NEPETOME, No. 7749433), and is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/200007).

Some new aspects of mode of action of brassinosteroids in plants

PP3-27

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Brassinosteroids (BRs) are known to have a crucial role in development of a plants. But, the main goal of contemporary investigations of action of these phytohormones are concentrated on mechanisms of their action, particularly with a special emphasis on the study of BRs receptors and BRs signaling pathways and similar aspects of molecular action of BRs, mainly by methods of molecular biology. In contrast to the mechanism of action, the mode of actions of certain chemicals in the plants is defined as the totality of the effects that a chemicals cause the effect on metabolism, growth and development of plants. In this sense, we studied the effect of some BRs on different plant species in their different developmental stages, both studying the impact of BRs on energetic changes and the chemical composition of plants, based on our findings in that area after 2015. In our works, we found a complete par-allelism of enthalpy and entropy, as factors that determine the Gibbs free energy as a measure of the chemical potential of a system, here corn seedlings treated by different doses of BRs. We also found that BRs affect the content and ratios of various metabolites and elements in corn seedlings and soybean plants. These findings were extended by our new investigations of the parallelism of thermodynamic changes during the germination of corn seeds with pos-sible mechanisms of their growth, and the parallelism of these processes with the metabolism of various carbohydrates in seeds of two corn hybrids during the mentioned changes. The last-mentioned findings will be discussed in this paper.

Keywords: brassinosteroids, mode of action, plant energetics, plant metabolism

Acknowledgment: Different grants, donated by the Ministry of Education, Science and Technological Development of Serbia were used in the conducted research.

Antiviral activity of extract from *Astragalus glycyphyllos* L. ap-plied alone and in combination with acyclovir against HSV-1 and HSV-2

PP3-28

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In vitro studies of a methanolic extract obtained from the aerial part of the A*stragalus glycyphyllos* L. plant on the replication of human alphaherpesvirus type 1, strain F and human al-phaherpesvirus type 2, strain BA, were conducted. Maximum non-toxic concentration (MNC) and cytotoxic concentration (CC50) were determined by colorimetric method (MTT assay) at 72 hours. By MTT-based colorimetric assay to measure the inhibition of viral replication, the extract administered at the maximal non-toxic concentration showed 84% and 94% protection of cells inoculated with HSV-1 and HSV-2 respectively. To determine the combined antiviral effect of AGO and Acyclovir (ACV) on HSV-1 replication, we used the three-dimensional model of Prichard and Shipman. The effect of simultaneous application is additive.

Keywords: *Astragalus glycyphyllos* L., Human alphaherpesviruses (HSV), antiviral activity, combined effect

Acknowledgment: This work was supported by the Bulgarian Ministry of Education and Sci-ence (Grant D01-217/30.11.2018) under the National Research Programme "Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)"

Est veritas in vino? Antiherpes activity of wines from herbal sources

PP3-29

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In the present study alternative forms of antiviral therapy are used as antiviral agents *in vitro*. In preparation for an attempt for grape-free wine production based on alternative sources - ethnomedicine plants with antiviral properties, several herbal wines were tested for establishing a baseline for futures studies. Four types of wines were tested against replication of HHV type 1 strain F and HHV type 2 strain BA *in vitro*. *Allium sp*., *Taraxacum sp*., *Urtica sp*., and *Solanum sp*., were used as a base for the production of the wines – all from the Balchik region in Bulgaria. Maximal nontoxic concentration (MNC) and cytotoxic concentration (CC_{50}) were determined (using MTT assays). Both effect over viral replication (expressed as % of protection) and its extracellular form were determined. The impact on replication shows weak activity, as even in MNC - the values were below 50%. A virucidal effect is observed for all wine products. The activity of *Taraxacum sp.* and *Allium sp.* wine is quite weak, reaching 96% against the used viral model. For the other two products (*Urtica sp*., and *Solanum sp.*), the activity was slightly higher with a peak of 99.7% inactivation at 360 minutes of contact in the *Solanum sp.* wine. Based on this pilot research – use of sources with known antiviral activities such as *Nepeta nuda* can be effective way for developing of innovative biotechnological product with good health benefits.

Keywords: *Nepeta nuda* L., Human alphaherpesvirus (HHV), *in vitro*, non-traditional wine, antiviral activity

Acknowledgment: This work was financially supported by the Bulgarian National Science Fund (BNSF), Grant No. KP-06-N56/9/12.11.2021 and by the Ministry of Education and Science of Bulgaria

Tissue-specific distribution of antioxidants during ripening of *Solanum dulcamara* L. fruits: the redox state alterations

PP3-30

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Identifying plant species tolerant to hypoxia and determining mechanisms this tolerance is standing upon may aid in improving nutritional quality of fruits of waterlogging-intolerant crops. Berries of *Solanum dulcamara* L. (bittersweet), a Eurasian species belonging to the Solanaceae family that grows in both dry habitats and wetlands, represent a suitable hypoxia-tolerant model species to study redox processes in relation to fruit development. Using biochemical and physico-chemical analyses, we examined differences in enzymatic and non-enzymatic components of the antioxidant system of *S. dulcamara* during the transition from mature green (MG) to ripe red (RR) developmental stage. By combining untargeted (UHPLC/Orbitrap MS^n) and targeted (UHPLC-DAD MS^2) metabolomics approaches, we revealed a significant change in polyphenolic profile of *S. dulcamara* fruits during fruit ripening. In order to improve knowledge on the link between metabolism and cell redox status, Electron Paramagnetic Resonance Spectroscopy (EPRS) and Imaging (EPRI) of reactive oxygen species (ROS) in intact fruits, fruit sections and extracts, were performed in parallel with the analysis of tissue-specific distribution of antioxidants and activity of antioxidant enzymes, which demonstrated alterations in the redox state and the increase of total antioxidant capacity of bittersweet berries during ripening, especially of outer layers (pericarp) of the fruits. This knowledge could provide important information to direct research on fruit quality improvement of flood-intolerant crops that are a close relatives of bittersweet, such as tomato and eggplant.

Keywords: waterlogging, fruit, bittersweet, antioxidant system, metabolomics

Acknowledgements: This work has been supported by the networking activities "Oxygen sensing a novel mean for biology and technology of fruit quality" (CA18210) which is implemented under the COST Action "Roxy-COST" funded by the European Cooperation in Science & Technology (2019–2023), and by the Serbian Ministry of Education, Science and Technological Development of the Republic of Serbia, grant numbers451-03-9/2021-14/200007, 451-03-9/2021-14/200146, 451-03-9/2022-14/200007 and 451-03-9/2022-14/200146.

Effect of UV – B radiation on secoiridoids production in *Centaurium erythraea* Rafn leaves

PP3-31

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Studies focusing on plant specialized metabolism and enhanced production of bioactive compounds in a controlled environment are of increasing interest bearing in mind that some of those compounds are widely used. *Centaurium erythraea* Rafn produces a plethora of specialized metabolites among which secoiridoid glucosides predominate. Secoiridoids in plants serve as defense compounds against herbivores and microorganisms, which makes them highly attractive for agricultural and pharmaceutical applications. In plants, ambient UV–B radiation has been suggested to prime protective responses towards various stressors. The overall goal of our research was to reveal the effect of short-term UV–B light exposure (30 and 60 minutes) on secoiridoid metabolism in leaves of common centaury grown *in vitro*. In order to achieve this goal, expression analysis of genes involved in the secoiridoid biosynthetic path-way was performed in parallel with UHPLC/(–)HESI–MS^2 metabolic profiling of leaves. Centaury plants exposed to a UV–B light for 60 minutes displayed prominent discoloration, indicating possible chlorophyll degradation, or its reduced synthesis. Gene expression analysis of nine secoiridoid biosynthesis-related genes (*CeGPPS, CeGES, CeG80, Ce8HGO, CeIS, CeIO, Ce7DGLT, Ce7DLH, CeSLS*) was monitored. Following the UV–B treatment, a sta-tistically significant decrease in relative gene expression was detected only for *CeG80*, while metabolic profiling revealed elevated accumulation of sweroside, swertiamarin, and gentiopic-rin in both 30 and 60 min UV–B-treated centaury plants. These results suggest that UV–B light exposure can be used to stimulate accumulation of secoiridoids in centaury plants for possible extraction and application in pharmacy, agriculture and food industry.

Keywords: *Centaurium erythraea*, UV – B radiation, secoiridoid glucosides

Acknowledgment: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia grant numbers OI173024, 451-03-9/2021-14/200007, and 451-03-68/2022-14/200007

Bioactivity of different *Asplenium ceterach* L. extracts

PP3-32

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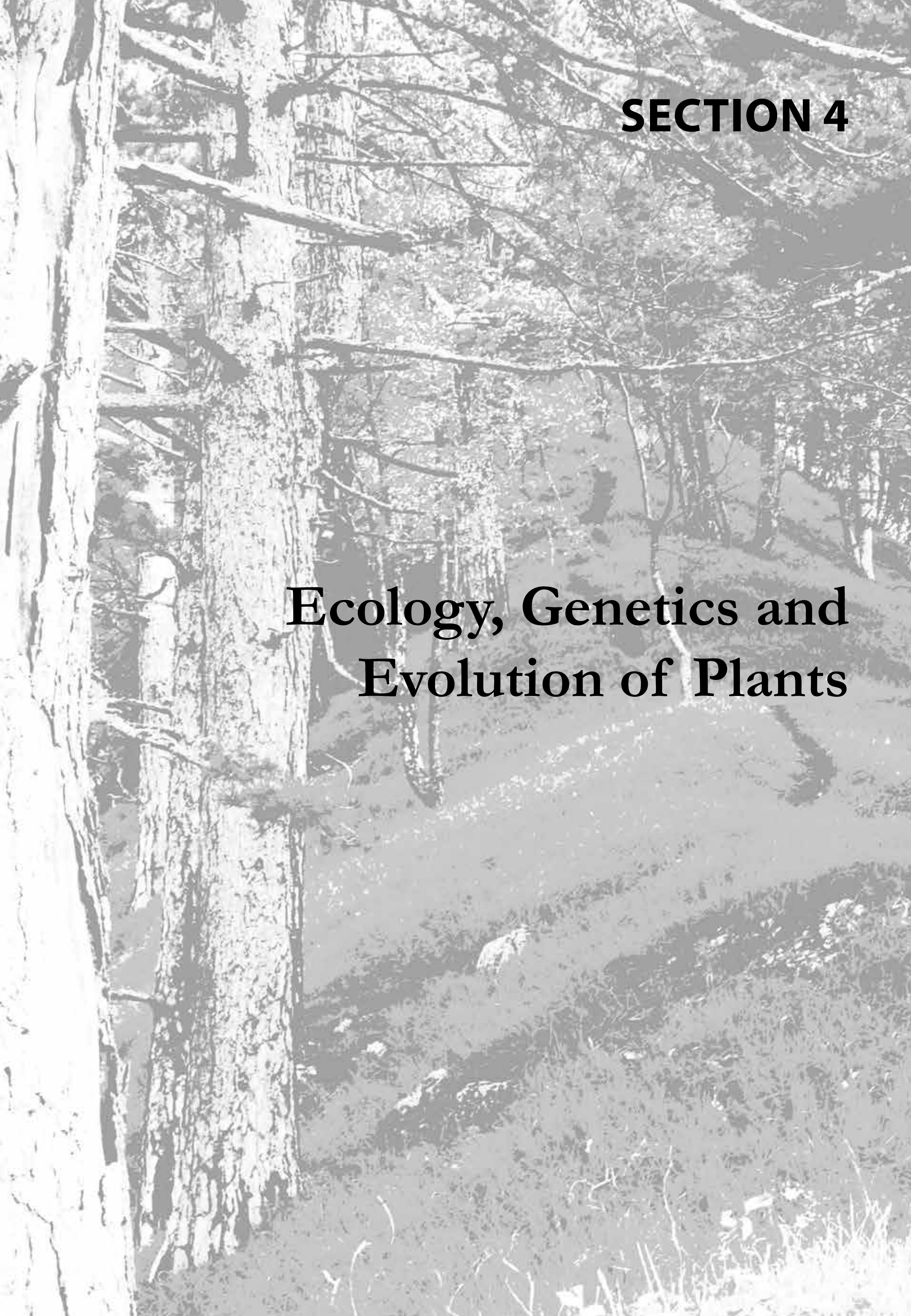
Methanol (ME) and dichloromethane (DCM) extracts of rustyback fern (*Asplenium ceterach* L.) sporophytes were analyzed for antioxidant properties by thin layer chromatography (TLC)-based bioautography, using DPPH as detection reagent, and comparatively tested for their antimicrobial and antibiofilm activity. Bioautography assay indicated that rustyback fern ME extract had significant antioxidant potential and moderate *α*-amylase activity, in comparison to the other extract used in the study. Antibacterial and antifungal activity of plant extracts against 6 bacteria and 6 fungi strains was assessed by microdilution method and the results are presented as minimal inhibitory concentration and minimal bactericidal/fungicidal concentration. Both extracts showed significant antibacterial and antifungal potential against all tested strains, although in variable degree. The Gram-negative *Escherichia coli* and Gram-positive *Bacillus cereus* were the most sensitive bacteria, whereas the most sensitive fungus was yeast *Candida krusei*. Furthermore, tested extracts have shown significant capability to prevent *Candida albicans* biofilm establishment with no significant difference between the two extracts examined, and inhibition range of 72%–74%. The results of the present study highlight *A. ceterach* extracts as a significant source of bioactive com-pounds, which support their potential use against diseases caused by multi-drug resistant microorganisms.

Keywords: rustyback fern, antimicrobial activity, antioxidative potential

Acknowledgment: This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-68/2022-14/ 200007. PLENARY LECTURES



SECTION 4

Ecology, Genetics and Evolution of Plants

PLENARY LECTURES

Plant signaling and behavior mediated via volatiles

PL4-1

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The capacity of individual plants to rapidly detect and respond to the presence of competitive neighbors is essential as it determines their growth strategy and survival. Volatile organic compounds (VOCs) provide important information about the physiological status and identity of the emitter. Plants eavesdropping to volatiles respond to the neighbors even before competition takes place, initiating specific growth readjustments that can increase their competitive capacity. Touching between plants is another cue in detection of competitive neighbors. We have demonstrated that plants respond to touch by upregulation of early defense genes and by changes in the emission of VOCs that activated the same defense genes in neighboring plants. Our studies show that effective volatile interactions occur only in specific combination of species/genotypes, indicating that plants use volatile organic compounds in the detection and adaption only to substantial competitive neighbors. This preparing for future competition mechanism affects the behavior and abundance of herbivore pests and their natural enemies giving to this phenomenon wider ecological perspective.

Keywords: adaption, coexistence, plant-plant communications, plant-insect interactions

Impacts of forest management on plant functional traits and ecological conditions in the Dinaric fir-beech forests (Slovenia)

PL4-2

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Understanding forest biodiversity responses to management disturbance contributes valuable insights for its conservation. Functional approaches are useful in such context as plant traits integrate species' evolutionary and eco-physiological information. By simulating the effects of large-scale disturbances, we analysed initial changes in environmental conditions and understory vegetation. The experimental design consisted of study sites in Dinaric fir-beech forests (Slovenia), where different cutting intensities on 0.4 ha plots were performed in 2012: control (closed stands without tree cutting), 50% cutting of stand growing stock (thinned stands) and 100% cutting (canopy gaps). Vegetation surveys and ecological measurements were conducted before and after cutting. The degree of change in environmental factors and vegetation was proportional to the intensity of cutting. Microclimatic conditions were significantly influenced by the interaction between cutting intensity and local topography associated with rugged karst terrain. Composition of plant functional traits differed significantly between control stands and canopy gaps. The proportion of annuals/biennials, grasses and sedges, tall-statured herbaceous plants, species with lighter seeds, greater dispersal ability and longer flowering duration increased after cutting. Canopy gaps and thinned stands were mainly colonized by disturbance-adapted species. We also observed that majority of typical forest plants were able to survive and that general patterns of vegetation response were driven by pre-disturbance abiotic and biotic conditions. The coexistence of typical forest species, regeneration layer of tree species and new colonists with divergent functional traits resulted in high community-level functional diversity. Study sites represent an important baseline for long-term monitoring of post-disturbance forest vegetation succession.

Keywords: cutting intensity, understory vegetation, functional composition, stand microclimate, species diversity

Acknowledgment: The research was funded by the European LIFE project „Managing forests for multiple purposes: carbon, biodiversity and socio-economic wellbeing ManFor C.BD" and the Slovenian Research Agency core funding for the Programme Groups P4-0107 and P4-0085. Janez Kermavnar received funding from the Slovenian Research Agency as part of the Young Researcher Programme (Contract No. 1000-18-0404).

INVITED TALKS

Ecophysiology of metal-hyperaccumulation in plants: what do we know so far?

IT4-1

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Hyperaccumulation is an unusual ability of plants to accumulate certain elements in their shoots at concentrations 100-1000 times higher than non-accumulator plant species in the surrounding, without causing toxicity symptoms. This unique phenomenon is favored by physiological mechanisms that allow highly efficient uptake of the element into the roots, followed by intense translocation and sequestration into the above-ground plant tissue. Although hypertolerant to anomalous metal concentrations in the substrate, hyperaccumulator plant species show different physiological responses to this type of stress. These differences become particularly apparent when the mechanisms of hyperaccumulation of the various trace elements are considered. Of particular interest are Ni, Zn, and Cd, which are the focus of research on metal hyperaccumulation, and the physiology of their accumulation in plants is presented. Response patterns to elevated metal concentrations in hyperaccumulator species are explained using two facultative serpentinophytes, *Noccaea praecox* and *N. kovatsii*, as examples. Populations of these species collected from ultramafic and non-ultramafic sites were exposed to a gradient of Ni concentrations, whereupon the total content of chlorophyll, anthocyanins, proline, as well as the intensity of lipid peroxidation and the localization of adopted Ni were analyzed. Significant differences in the degree of tolerance were found between populations from ultramafic and non-ultramafic sites.

Keywords: metal accumulation, tolerance, plant species, Brassicaceae

Acknowledgment: The Ministry of Education, Science and Technological Development of the Republic of Serbia supported this research (grant number 451-03-68/2022-14/ 200178)

Elucidation of the mechanism underlying somatic embryo induction in spinach

IT4-2

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Spinach is a dioecious plant species with complex sex determination, which limits the success of its classical breeding. Hence, a biotechnological approach has been recognized as a promising alternative. Elucidation of the mechanism underlying somatic embryo (SE) induction and improvement of its efficiency have been the main goals of many years of research in this plant species recalcitrant to *in vitro* regeneration. Results have evidenced that the interplay among auxin, gibberellins (GA) and light was crucial for the induction of somatic embryogenesis from the root apices. None of these factors can be substituted for each other and only the lines with inherited high embryogenic capacity could respond in the absence of any of them, but with a very limited efficiency. The highest embryogenic response was obtained from the explants cultivated on medium supplemented with 20 µM α naphthaleneacetic acid (NAA) + 5 µM gibberellic acid (GA_3) under blue light. Contrary to expectations, paclobutrazol, an inhibitor of bioactive gibberellin biosynthesis, acted synergistically with NAA and GA_3 and further promoted somatic embryogenesis. In embryogenic explants, the expression levels of genes encoding the key enzymes involved in GA biosynthesis decreased, while those of genes involved in GA inactivation increased compared to non-embryogenic ones. Analysis of GA content using ultra-high performance liquid chromatography revealed enhanced GA metabolism in embryogenic explants during the first week of SE induction. During this period, the levels of endogenous bioactive GAs (GA_1, GA_3, GA_4 and GA_7) dramatically increased, confirming their important role in the acquisition of embryogenic potential.

Keywords: spinach, somatic embryogenesis, gibberellins, gene expression, paclobutrazol

Acknowledgment: Ministry of Education, Science and Technological Development of the Republic of Serbia supported this work through contract No. 451-03-68/2022-14/200007

Catmint (*Nepeta nuda* L.) Phylogenetics and Metabolic Responses in Variable Growth Conditions

IT4-3

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Nepeta nuda (catmint; Lamiaceae) is a perennial medicinal plant with a wide geographic distribution in Europe and Asia. This study firstly characterized the taxonomic position of *N. nuda* using DNA barcoding technology. Since medicinal plants are rich in secondary metabolites contributing to their adaptive immune response, we explored the *N. nuda* metabolic adjustment operating under variable environments. Through comparative analysis of wild-grown and *in vitro* cultivated plants, we assessed the change in phenolic and iridoid compounds, and the associated immune activities. The wild-grown plants from different Bulgarian locations contained variable amounts of phenolic compounds manifested by a general increase in flowers, as compared to leaves, while a strong reduction was observed in the *in vitro* plants. A similar trend was noted for the antioxidant activity of the extracts. The antimicrobial potential, however, was very similar, regardless the growth conditions. Analysis of the *N. nuda* extracts led to identification of 63 compounds including phenolic acids and derivatives, flavonoids, and iridoids. Quantification of the content of 21 target compounds indicated their general reduction in the extracts from *in vitro* plants, and only the ferulic acid (FA) was specifically increased. Thus, this study generated novel information on the regulation of *N. nuda* productivity using cultivation conditions, which could be exploited for biotechnological purposes.

Keywords: antibacterial, antioxidant, DNA barcoding, iridoids, phenolics

Acknowledgment: This work was financially supported by the Bulgarian National Science Fund (BNSF), Grant No. KP-06-N56/9/12.11.2021 and by the Ministry of Education and Science of Bulgaria, project BULCode No. Д01-271/02.10.2020, National Program "European Scientific Networks". Infrastructure support was provided by Grant BG05M2OP001-1.002-0012 "Sustainable utilization of bio-resources and waste of medicinal and aromatic plants for innovative bioactive products" co-financed by the European Union through the European Structural and Investment Funds, as well by the Bulgarian Ministry of Education and Science, through Operational Program Science and Education for Smart Growth 2014–2020.

Progress in disentangling the diversity of iridoids within the genus *Nepeta*: surprising biosynthetic and evolutionary insights

IT4-4

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Nepeta L. is the largest genus of the Lamiaceae family, and the exclusive representative of the subfamily Nepetoidae which produces iridoids. However, the genus *Nepeta* comprises both taxa producing iridoids (iridoid aglycones-IAs and/or iridoid glucosides-IGs), and those lacking iridoids. Combining omics-guided approach and functional genomics in elucidating the genetic background of the iridoid diversity within the genus *Nepeta*, we provide novel insights into molecular mechanisms driving the iridoid biosynthesis, and thus into chemical evolution of iridoids within the genus *Nepeta*. Multidisciplinary experimental approach is here explained on the example of iridoid synthases (IS), functionally characterized by *in vitro* enzymatic assays using recombinant proteins heterologously expressed in *Escherichia coli*. Functional ISs are expressed in both iridoid-producing *N. rtanjensis* (NrIS2) and *N. sibirica* L. (NsIS), as well as in iridoid-lacking *N. nervosa* L. (NnIS). *N. nervosa* possesses an iridoid biosynthetic platform, but inactive one, most likely due to the "switching off" or "silencing" of some gene(s) upstream in the pathway, which suggests that this species has lost the ability to produce iridoids during the evolution. Furthermore, comparative iridoid profiling and co-expression analysis of biosynthetic genes and transcription factors (TFs) during the exposure of chemodiverse *Nepeta* taxa to environmental stresses (e.g. dehydration, UV–B radiation, pathogens) or elicitors (MeJA), facilitated the explanation of regulatory mechanisms determining the productivity of plants, as well as of factors responsible for the presence/absence of iridoids or specific groups of iridoids (IAs and IGs), in these plants.

Keywords: *Nepeta*, dehydration, functional characterization of genes, iridoid synthase, nepetalactone

Acknowledgment: This work is financed by the Science Fund of the Republic of Serbia, Program IDEAS (project NEPETOME, No. 7749433), and is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/200007).

SELECTED TALKS

Distribution, host range, and genetic variability of the holoparasitic genus *Cuscuta* in Bulgaria

ST4-1

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Genus *Cuscuta* consists of approximately 200 species of holoparasitic flowering plants with a negative impact on plant productivity in agricultural areas and a significant ecological impact. Most of them are known as a generalist, e.g., parasitizing on numerous host species, and often simultaneously on several different ones. The genus is represented by up to 10 species in Bulgaria. During a four-year survey (2018 to 2021), we studied 54 specimens of *Cuscuta* spp., of which 35 belonged to the invasive, introduced species *C. campestris* and the rest belonged to *C. europaea* (4), *C. epithymum* (9) and *C. approximata* (6). A total of 114 host plant species, belonging to 33 families were identified, with *C. campestris* showing the widest host range with 52 host species from 19 families. All *Cuscuta* species were confirmed by ITS sequencing and sequences were submitted in GeneBank. Furthermore, the genetic diversity of all populations was studied by RAPD markers. In conclusion, our study showed that the introduced species *C. campestris* possesses significant genetic diversity and is dominant in comparison to the native species, thus representing a significant threat to both agriculture and natural plant communities.

Keywords: dodder, parasitic plants, RAPD markers

Acknowledgment: This research was funded by the National Science Fund of the Ministry of Education and Science, Bulgaria, grant number КП-06-Н31/10.

Variation in Hsp70 and Hsp101 levels in response to experimental warming in *Iris pumila* L.: an open-topped chamber experiment

ST4-2

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Global warming poses a serious threat to the survival of plant populations. To better understand plant responses to future temperature rise, two sun-exposed natural populations of *Iris pumila* were subjected to an open-top chamber (OTC) experiment over a two-year period. One half of all randomly selected clonal plants were experimentally warmed using clear-sided OTC, while the other half experienced ambient temperature conditions. In general, the air temperature within the OTCs was about 1-2°C higher than that outside of the OTCs. In spring and summer of 2018 and 2019, ramets of each clone growing inside and outside of OTC were analyzed for the amount of Hsp70 and Hsp101. The mean response profiles of both Hsps were parallel over time, but not flat. The amount of both Hsps tended to be greater in ramets growing inside the OTCs than in those growing outside of the OTCs, reaching its maximum in the summer. A repeated-measures profile analysis revealed statistically significant treatment, season and year effect exclusively for the Hsp101, and season effect for the Hsp70. Furthermore, profile analysis of the between-population effects showed that the mean response profiles, for both Hsps, differ between populations. Our results imply that small temperature increase can evoke changes in both the level and shape of the mean response profiles of Hsp70 and Hsp101 in *I. pumila* compared to that expressed at ambient air temperatures. This finding is especially important to forecast the fate of *Iris* populations under upcoming climate warming.

Keywords: Hsp70, Hsp101, *Iris pumila*, experimental warming, open-top chamber (OTC)

Acknowledgment: This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-68/2022-14/200007.

POSTER PRESENTATIONS

Orchid-soil System Relationship in the Serpentine, Silicate and Limestone bedrocks

PP4-1

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Anacamptis morio occurs on a wide range of habitats showing distinct tolerance to heterogeneous edaphic conditions. Assessment of Li, B, Ba, and Sr phytoavailability in the soils of western Serbia, and metal(oid) uptake by *A. morio* provided new information on less studied elements and revealed their relatively high mobility in the soil-orchid system. Although previous studies have found that *A. morio* prefers neutral and calcareous soils, our study shows that it also inhabits ultramafic and siliceous sites characterized by pronounced differences in soil chemical properties. BCR sequential extraction identified up to 60% of Li and more than 80% of Ba and Sr content as potentially phytoavailable. The total element analyses using ICP-OES showed that B, Ba and Sr predominantly accumulated in the roots, but did not exceed thresholds considered potentially phytotoxic. It revealed the exclusion strategy of *A. morio* which enables it to tolerate differences in elemental composition of contrasting bedrock types. The highest Li concentrations were detected in leaves, pointing to significant Li mobility within the plant. The contents of Li and Ca were highly positively correlated, which may result from Li physicochemical properties that are similar to those of alkali metals, allowing it to share the same transport carriers. Thus, Li could be easily transported to the aboveground plant parts and accumulated mainly in the organs with the highest Ca content, which was confirmed in this study. Bioconcentration factors >1 were detected only for B and Sr in all plants analyzed, irrespective of the bedrock type.

Keywords: *Anacamptis morio,* metal exclusion, metal tolerance, BCR sequential extraction

Acknowledgment: This work was supported by the Serbian Ministry of Education, Science and Technological Development (Grants No. 451-03-68/2022-14/200178, 451-03-68/2022-14/200023, 451-03-68/2022-14/ 200007 and 451-03-68/2022-14/200168)

Changes in light intensity induce the developmental instability of *Iris variegata* flower parts

PP4-2

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The development sensitivities of flower parts of *Iris variegata* were studied in experimental conditions with changes in light intensity as stressor. As index of environmental stress and developmental instability we used indices of fluctuating asymmetry (FA8 as size-corrected index and FA17 as composite index of all traits measured on standard and fall flower parts). The 103 genotypes from three native habitats (open, shaded and semi-shaded) were examined in two light intensities (low and high). The measurements have been taken at five places along the longitudinal axis of the flower parts in relation to the width from the main nerve to the edge of the object. Larger values of the size-corrected index FA8 were found in the low light treatment for genotypes originating from the open, while for genotypes originating from the shaded habitat development instability was greater in the high light intensity treatment. Genotypes from semi-shaded habitat did not show significant responses in both fluctuating assymetry indices to changes in light intensity. The composite index FA17 showed the opposite pattern for genotypes from the shaded habitat with higher values in the low light treatment, while genotypes from open habitat showed no significant response. These results illustrated that light intensity may be an important factor contributing to bilateral fluctuating asymmetry of flower parts when environment of genotype origin is taken into consideration, and depending on the choosen index of developmental instability.

Keywords: flower developmental stability, fluctuating asimmetry, light intensity

Acknowledgment: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia contract number 451-03-68/2022-14/200007.

Flowering trends in *Iris pumila* in Deliblato Sand: Ten years after

PP4-3

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In 2012 we selected 35 experimental plots in *Iris pumila* L. (dwarf bearded iris) natural population in natural reserve Deliblato Sand, Serbia. Flowering was monitored in 2013, 2014 and 2015 on selected more than 4 000 square meters, and opening of every individual flower on *I. pumila* clones was recorded. Data revealed steady decline in number of flowering individual plants (ramets) through analyzed years. The same spots were revisited in 2021 and 2022 flowering seasons and occurence of all flowers was recorded by introducing drone digital photography in our long term study. Preliminary analysis on the subsample of monitored spots reveals that detected trend of decline in number of flowering ramets was not reversed after ten years. Detected long term trend has significant implications for studies of evolutionary relationship between vegetative and sexual modes of reproduction in this species, as well as possible conservation management strategies in the face of environmental change. Additional methods that can reveal evolutionary consequences of observed population dynamics on maintenance of within population genetic polymorphism (with special emphasis on flower color polymorphism and variability on DNA level) are discussed. We propose application of field spectography to huge flower color polymorphism observed in analyzed species coupled with pigment HPLC analysis on studied population level, as well as microsatellite genetic analyses of studied population composition as most suitable and efficient lines of further research of those topics in the studied populations.

Keywords: flowering phenology, sexual and vegetative reproduction, polymorphism maitenance

Acknowledgment: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia contract number 451-03-68/2022-14/200007.

Assessment of *Iris pumila* L. pollinator and florivore diversity in a common garden: a pan-trap experiment

PP4-4

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Nearly all botanists give pollinators credit for the remarkable diversity of flower color, shapes and sizes observed in nature. However, an increasing number of studies suggests that floral selection is a more pluralistic process, involving not only pollinators but also herbivores and factors of the plant's abiotic environment. To estimate *Iris pumila* pollinator and florivore diversity in a common garden of Institute for Biological Research in Belgrade, we used a pan trap approach. Since *I. pumila* displays a variety of flower color phenotypes ranging from white and yellow to various shades of purple and blue, we painted the traps in fluorescent purple, blue and yellow. A total of 391 insects were collected and identified to family or genus level. In general, insects were mostly attracted to yellow traps compared to blue and purple ones (69.3%, 19.7% and 11.0%, respectively). The most numerous potential *I. pumila* pollinators were bees (Anthophila) (89.8%) with predominantly genus *Halictus*, while sawflies (Tenthredinidae) were significantly less represented (10.2%) with only genus *Athalia*. As potential florivores, beetles *Tropinota hirta* (Scarabaeidae) and *Meligethes* sp. (Nitidulidae) were identified and all individuals were caught in yellow traps, while specimens of other species from Nitidulidae family were found only in the blue traps. Both pollinators and florivores showed pronounced affinity towards yellow traps, indicating that in the case of *I. pumila* insects have potential to be agents of floral selection. Nevertheless, it is necessary to evaluate pollinator and florivore diversity and their color preferences in *I. pumila* natural habitat.

Keywords: pollinators, florivores, *Iris pumila*, pan traps, common garden

Acknowledgment: This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-68/2022-14/200007.

Plastic responses of *Iris pumila* leaf functional traits to experimental warming *in situ*

PP4-5

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The ongoing climate change creates critical challenges for plant population persistence in natural communities. To investigate plant responses to future climate warming conditions, we employed an *in situ* temperature manipulation experiment in two sun-exposed natural populations of *Iris pumila*. One half of each randomly chosen clonal plant was experimentally warmed (about 1-2 ºC) using a clear-sided open top chamber (OTC), while the other half faced the ambient temperature conditions. During the summer season, phenotypic values of key functional leaf traits - leaf dry mass per area (LMA), leaf succulence (SU) and stomatal density (SD) - were recorded simultaneously on the ramets from each clone growing inside and outside of the OTC. The phenotypic expression of all analyzed traits changed with temperature, being smaller in the ramets developing within the OTCs compared to those growing under ambient temperature conditions. Observed difference was found to be statistically significant for LMA and SU, while those related to SD could be described as tendency. In addition, the association between the pairs of leaf and abiotic variables was tested. The recorded soil temperature appeared to be significantly concordant with SD, but, as well as leaf and air temperature, discordant with SU. Our results suggest that *I. pumila* plants have the capacity to adjust to rising temperatures by plastic responses of leaf functional traits. This is of great importance to anticipate the future of *Iris* populations under upcoming warmer climate.

Keywords: *Iris pumila*, phenotypic plasticity, leaf functional traits, experimental warming, open-top chamber (OTC)

Acknowledgment: This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-68/2022-14/200007.

Iridoid profiles and expression patterns of iridoid-related biosynthetic genes in three *Nepeta nuda* L. accessions

PP4-6

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Nepeta nuda L. (family Lamiaceae) is a widespread species characterized by the prevalence of bioactive specialized metabolites from the groups of iridoids and phenolic. Analyzed plants were grown *in vitro* and in greenhouse from seeds collected from eastern Serbia ("Debeli Lug"), obtained from other research groups ("Rhodopes", Bulgaria), or commercially purchased ("Jellito", Germany). The chemical composition of *N. nuda* leaves was analyzed using the UHPLC/DAD/(±) HESI±MS^2 method, targeted towards dominant iridoid compounds. Both *in vitro* and greenhouse grown plants are rich in iridoid glycoside 1,5,9-epideoxyloganic acid (1,5,9– eDLA), and among analysed accessons *N. nuda* "Debeli Lug" was pointed out as the most productive one. When it comes to iridoid aglycones, it is noticeable that *N. nuda* "Jelitto" biosynthetizes *cis*, *trans*- isomer of nepetalactone (NL) while *N. nuda* "Rhodopes" and *N. nuda* "Debeli Lug" possess *cis*,*cis*-NL. Relative expression of early biosynthetic genes- EBGs (*NnGPPS*, *NnGES*, *NnG80*, *Nn8HGO*), in plants grown *in vitro*, is much higher in *N. nuda* "Debeli Lug" accession, compared to *N. nuda* "Rhodopes" and *N. nuda* "Jellito". In greenhouse grown plants, there is no particular regularity when it comes to the expression of EBGs in three *N. nuda* accessions. NEPS enzymes seem to be one of the key factors determining the metabolic flux and productivity of *N. nuda*. While plants grown *in vitro* and in greenhouse both express *NnNEPS2*, only plants grown *in vitro* express *NnNEPS1.2*. It is also noticeable that *N. nuda* "Debeli Lug" accession displays the highest *NnNEPS2* expression, and the lowest *NnNEPS1.2* expression.

Keywords: Nepeta nuda, chemical composition, iridoids, gene expression

Acknowledgment: This research was financed by the Science Fund of the Republic of Serbia (GRANT No. 7749433, "NEPETOME") and supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-68/2022-14/200007).

Comparative metabolomics of two *Centaurium* species displaying variable flower coloration phenotypes

PP4-7

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Species of the genus *Centaurium* (family Gentianaceae), well-known medicinal plants, threatened in the nature, are a rich source of bioactive compounds that are of great interest for the pharmaceutical and biotech industry. While the phytochemical characterization was investigated in detail in roots and leaves, nobody has so far tackled the question about the specialized metabolites composition in the centaury flowers. In this work, using untargeted metabolomics approach, we examined phytochemical differences in flowers of two centaury species, *Centaurium pulchellum* (Sw.) Druce and *Centaurium tenuiflorum* (Hoffmanns. & Link) Fritsch, both displaying variability in flowers coloration, which grades from white to pink. UHPLC-Orbitrap MS characterization of methanol extracts in a negative ionization mode resulted in the detection of 82 compounds in total. The identified compounds represented six structurally distinct groups: phenolic acid aglycones and glycosides; iridoid glycosides and derivatives; flavonoid glycosides and aglycones; xantone glucosides and aglycones; hydorxycinnamic acid amides and other compounds. All compounds found were identified by exact mass search of their deprotonated molecule $[M-H]^-$, MS^2, MS^3 and MS^4 fragmentation behavior, as well as by comparison with the available literature. Flavonoid and xantone glycosides and aglycones are the main classes of metabolites identified, and their content was strongly associated with the coloration of the flowers. However, a clear insight into the molecular mechanism underlying the centaury flower coloration is still lacking. Herein, the ultimate goal of our research is to comparatevely profile the changes in metabolome and transcriptome between pink and white centaury flowers in order to pinpoint key compounds and genes controlling the flower color formation.

Keywords: *Centaurium*, chemical characterization, flowers, flavonoids, xantones

Acknowledgement: This work has been supported by the Serbian Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-9/2022-14/200007.

Ecological and chemical characteristics of stenoendemic species *Dalmatocytisus dalmaticum* (Vis.) Trinajstić

PP4-8

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Based on field research, an analysis of the ecology of the species *Dalmatocytisus dalmaticum* (Vis.) Trinajstić was conducted, and the collected plant material was subjected to phytochemical analysis. As a stenoendemic species, this plant is found only near the town of Sinj on the hills Poljakova greda, Šušnjevača and Medinjak. It grows in the composition of sub-Mediterranean dry grasslands of the class Festuco-Brometea Br.-Bl. & R.Tx. 1943, on which it forms carpet-like coverings. On Mount Greda it creates larger stands on shallow carbonate mud soils and it reproduces by creeping underground stems and to a lesser extent by seeds. Recently, the number of habitats of *D. dalmaticum* has been decreasing, due to afforestation with black pines and the succession from rocky grasslands to thickets. Isolation of essential oil from fresh plant material collected in 2021, was performed by water distillation in a modified Clevenger apparatus. Obtained oil sample was analyzed by GC/MS analysis. A total of 60 compounds were identified, accounting for 88.06% of the total oil. The major groups of compounds were monoterpene hydrocarbons (3.22%), oxygenated monoterpenes (5.12%), sesquiterpene hydrocarbons (4.54%), oxygenated sesquiterpenes (9.89%), diterpene alcohols (9.09%), carbonyl compounds (22.93%), and other compounds (29.84%). The most abundant compounds of each group in the extract are α-pinene, (E)-damascenone, (Z)-caryophyllene, hexahydrofarnesyl acetone, 4-vinylphenol, pentacosane, phytol, and palmitic acid. Some of the compounds mentioned are known to have cytotoxic, anticancer, antibacterial, and antiviral properties, suggesting the possibility of further research on this species.

Keywords: carbonyl compounds, essential oil, phytol, stenoendemic taxa, sub-Mediterranean grassland

Analysis of amplification of microsatellite loci in *Iris pumila*

PP4-9

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Utilization of microsatellite molecular markers was analyzed in *Iris pumila* and five other species belonging to the same genus: *I. humilis*, *I. sibirica*, *I. pseudacorus*, *I. spuria* and *I. variegata*, which inhabit the northern part of the Republic of Serbia. We selected 40 EST-SSR markers previously reported for the genus *Iris*. Sixteen markers that showed polymorphism were chosen for further research. To examine the potential of the selected microsatellite loci analysis in the detection of different *I. pumila* clones, we selected 18 individuals of this species that belonged to different genotypes. Optimization of amplification was performed for all 16 microsatellite loci: temperature program of the PCR protocol and number of amplification cycles were optimized as well as primer and DNA concentrations used in the reactions. Seven markers that were variable across the analyzed individuals were selected. The final analysis was performed using only 5 microsatellite loci since high-quality amplification for two loci was not possible. No greater deviations in the length of the fragments from the expected were observed, except for one primer. All microsatellite loci contained three-nucleotide repeats. *I. pumil*a is tetraploid species and in several plants the presence of four alleles were observed. The observed number of alleles per locus ranged from 3 to 9. In the results obtained across 18 *I. pumila* individuals using 5 microsatellite markers, 17 multilocus genotypes were observed.

Keywords: *Iris pumila*, EST-SSR markers, multilocus allelic phenotype

Acknowledgment: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia contract number 451-03-68/2022-14/200007.

Chloroplast *trnL - trnF* region variation in the genus *Centaurium* Hill as a molecular indicator of natural interspecific hybridization

PP4-10

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Species of the genus *Centaurium* are highly variable and clinal morphological variations caused by environmental conditions are very noticeable. Nevertheless, it is also well documented that species and subspecies of this genus hybridize readily in nature and it is considered that interspecific hybridization may be one of the leading causes of variability within the genus. Earlier studies indicate that the species *C. erythraea* Rafn and *C. tenuiflorum* (Hoffmanns. et Link) Fritsch in areas with a Mediterranean climate hybridize in nature. The taxonomic status of the hybridogenous taxon, which probably originated through the hybridization of the mentioned species, here called "*C. ×tenuiflorum*", has not been resolved. Since it has been proven to be a useful genetic marker in giving evidence of hybridization among several species of the genus *Centaurium*, the chloroplast *trnL - trnF* region was used for the study of hybridization between these particular species. The results of variation in the *trnL - trnF* region indicated that the taxon "*C. ×tenuiflorum*" shared a chloroplast haplogroup with the species *C. tenuiflorum*, which implies that *C. tenuiflorum* might be the donor of chloroplast DNA. In subsequent research, it is necessary to combine the data obtained from the analysis of the *trnL - trnF* region with the data that would be obtained from the analysis of other regions of genomic and/or chloroplast DNA to resolve the status of this potentially hybridogenic taxon.

Keywords: *Centaurium* Hill, interspecific hybridization, *trnL – trnF* cpDNA

Acknowledgment: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia contract number 451-03-68/2022-14/200007.

Metabolomic evaluation of three *Digitalis* species (fam. Plantaginaceae) using liquid chromatography with multistage mass spectrometry

PP4-11

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The aim of this research was to determine inter-species diversity in qualitative composition of methanol-soluble metabolites in leaves of three *Digitalis* species (*D. lanata*, *D. ferruginea*, and *D. ambigua*) from the central Balkans. Using the UHPLC-LTQ OrbiTrap MS technique, a total of 119 compounds were identified based on their monoisotopic masses, MS^n fragmentation, and previously reported MS data. All indetified compounds were clustered into seven groups: steroidal glycosides (43 compounds), steroid aglycones (12 compounds), phenylethanoid glycosides (15 compounds), flavonoid glycosides (18 compounds), flavonoid aglycones (9 compounds), phenolic acid derivatives (14 compounds), and 8 compounds belonging to other classes. In addition to the expected cardiac glycosides, steroidal saponins and pregnane and furostanol glycosides were also abundant in the extracts of analysed *Digitalis* species. A detailed LC/MS qualitative analysis revealed very similar profiles of steroidal glycosides in extracts of *D. lanata* and *D. ferruginea* leaves, which were characterized by significant amounts of pharmaceutically important digoxin, deslanatoside, and lanatosides A, B, and C. On the other hand, these compounds were not detected in leaves of *D. ambigua*. The UHPLC-LTQ OrbiTrap MS method was proved to be simple, rapid, and accurate strategy for unambiguous taxonomic determination and chemical differentiation of *Digitalis* species. It also alowed the selection of high-resolution chemical markers for revealing inter- and intra-population variability of *Digitalis* species adopting targeted metabolomic approach, which is the course of our further work.

Keywords: UHPLC-LTQ OrbiTrap MS, cardiac glycosides, steroids, phenylethanoids, flavonoids

Acknowledgment: This work is financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-68/2022-14/200007).

Essential oil composition of *Centaurea scabiosa* L. (Asteraceae)

PP4-12

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The essential oil composition of the greater knapweed (*Centaurea scabiosa* L., *Centaurea* sect. *Acrocentron*, Asteraceae) from Svrljig, Serbia, was analyzed. The volatile organic compounds (VOCs) were extracted from freshly frozen flowering heads (FH) and freshly frozen aerial parts (AP - leaves and twigs) by simultaneous distillation and extraction using Likens–Nickerson type apparatus and analyzed by GC-FID/GC-MS. In total 81 compounds were identified, representing 98.1% and 97.9% of the total VOCs composition of flowering heads and aerial parts, respectively. Aliphatic hydrocarbons were dominant class of compounds for both essential oils (FH 65.6%, AP 63.4%), followed by sesquiterpenes (FH 26.1%, AP 25.8%). Monoterpenes were the least represented (FH 2.6%, AP 6.3%). In the case of monoterpenes and sesquiterpenes, oxygenated derivatives were more abundant than hydrocarbons. The most abundant constituents were the same for both oils with hexanal being principal component (FH 14.5%, AP 13.3%), followed by (*E*)-2-Hexenal (FH 6.4%, AP 8.5%) and caryophyllene oxide (FH 5.2%, AP 6.2%). Results of this study can be potentially useful, based on chemophenetics of VOCs composition, for delimitation of *C. scabiosa* and other species from sect. *Acrocentron*.

Keywords: *Acrocentron*, freshly frozen plant material, volatile organic compounds

Acknowledgment: Financial support was provided by the Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/200178).

Liquid chromatography with multistage mass spectrometry as a method for chemical differentiation of ten *Nepeta* species (fam. Lamiaceae)

PP4-13

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Nepeta species (fam. Lamiace, subfam. Nepetoideae, tribe Nepetae) are well known for their pharmacological properties, biopesticide potential and behavioral effects on cats. Different biological activities of *Nepeta* species are closely related to their secondary metabolites, among which iridoids predominate. Research objective of this study was to determine profiles of methanol-soluble iridoids in leaves of greenhouse-grown plants of 10 phylodiverse *Nepeta* species: *N. grandiflora* M. Bieb., *N. laevigata* (D. Don) Hand.-Mazz., *N. nervosa* Royle ex Benth., *N. stewartiana* Diels, *N. nuda* L., *N. parnassica* Heldr. & Sart., *N. rtanjensis* Diklić & Milojević, *N. ernesti-mayeri* Diklić & V. Nikolić, *N. cataria* L., and *N. sibirica* L. Untargeted metabolomics approach adopting UHPLC/LTQ Orbitrap MS^n analysis in a negative ionization mode revealed the presence of totally 15 iridoids in samples, of which 13 were glucosides and two were aglycons of iridoids. Iridoid compounds were identified based on their monoisotopic masses, MS^n fragmentation, and previously reported MS data. The results indicated that the iridoid profiles of the investigated species are very diverse both in terms of qualitative content of the compounds and their relative abundance in extracts. Among analysed *Nepeta* species, Balkan Peninsula endemics (*N. rtanjensis*, *N. parnassica*, and *N. ernesti-mayeri*) and *N. cataria*, are especially rich in isomers of 1,5,9-*epi*deoxyloganic acid. 5-Deoxylamiol, nepetariaside, nepetanudoside B, and ajugol were detected in majority of analyzed species. The UHPLC-LTQ OrbiTrap MS analysis, in combination with chemometric data processing, has proven to be an efficient tool for chemical differentiation between *Nepeta* taxa, and could aid the efforts to unravel the chemical evolution within the genus.

Keywords: *Nepeta* sp., iridoids, chemotaxonomic classification, chemical differentiation

Acknowledgment: This research was supported by the Science Fund of the Republic of Serbia, GRANT No. 7749433, "Omics-guided disentangling of the iridoid diversity in the genus Nepeta L. for in planta metabolic engineering towards sustainable exploitation- NEPETOME" (2022-2025), and is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-68/2022-14/200007).

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In memoriam

Dr Ana Simonović, Principal Research Fellow

(1969–2022)

Very unexpectedly, on June 17th 2022, Institute for Biological Research "Siniša Stanković", Univeristy of Belgrade, Serbian Society of Plant Physiology and Serbian Biological Society lost one of their most pioneering, gifted and respected colleagues, Dr Ana Simonović, Principal Research Fellow.

Dr Ana Simonović was born on December 26th 1969 in Belgrade, where she finished primary and secondary school. She enrolled in Molecular Biology and Physiology program at the Faculty of Biology, University of Belgrade, in 1989/90. After graduating in 1995, she continued her scientific education in Plant physiology and earned an MSc degree in 1998 at the University of Belgrade. Throughout her studies she was employed at the Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", first as the Research Trainee (1995-2000), then as a Research Assistant (2000). In November 2000, as an awarded scholarship holder (Presidential Doctoral Fellowship Award), she started her doctoral studies in the framework of the Cellular Molecular Biology program at the North Dakota State University (NDSU), Fargo, ND, USA. In the Department of Biological Sciences NDSU she was employed as a research assistant (2000-2005), then as a teaching assistant/lecturer (2005-2006). In March 2006, at the North Dakota State University, Dr Ana Simonović defended her PhD thesis, entitled *„Effect of low temperatures and light on glutamine synthetase isoforms in maize seedlings"*, under the supervision of Dr Marc Anderson. After returning to IBISS, Dr Simonović was elected a Research Associate in 2008 and a Senior Research Associate in 2013. She became a Principal Research Fellow in 2017.

The career of Dr Ana Simonović included numerous research directions. In her early works she studied physiology of seed germination. Later on she showed a keen interest in exploring the biosynthesis of pharmacologically important secondary metabolites from the group of sesquiterpene lactones and phenolic diterpenes. She also studied various aspects of *in vitro* morphogenesis of *Centaurium erythraea* and other *Centaurium* species, biotechnological solutions for the protection of horticultural species of the *Impatiens* genus from viral diseases and drought, and allelopathic effects of the weed species *Chenopodium murale*. The largest number of Dr Simonović bibliographic units refers to studies of the activity, regulation and structure of the key nitrogen assimilation enzyme in plants, glutamine synthetase (GS). The backbone of the GS test is an assay to detect the activity of GS isoforms, completed with a protocol for the extraction of different isoforms and their subsequent analysis and immune detection. Using these methods, cytosolic and chloroplast isoforms were successfully separated and analysed in numerous plant species. The most significant published result in this field by Dr Ana Simonović describes the quaternary structure of this enzyme in *A. thaliana*. Dr Simonović showed for the first time that the subunits of different GS isoforms can be freely combined into active heterodimers, which, bearing in mind the different catalytic properties of isoforms, indicates other possibilities of GS regulation.

Dr Ana Simonović also contributed to the research of sesquiterpene lactone (SLs) biosynthesis pathways in order to modify the level of their production in the species *Cichorium intybus* L ., while introducing new techniques of *Gateway* cloning, agroinfiltration and gene silencing using *amiRNA* into our laboratory practice.

In recent years, Dr Simonović focused on *Centaurium erythraea in vitro* morphogenesis, especially inducing somatic embryogenesis and shoot regeneration from leaf explants using plant growth regulators and wounding. This provided a basis for further studies of arabinogalactan proteins and peptides and their role in morphogenetic changes. Her work on centaury over the years culminated in her immense contribution to this species' molecular biology and bioinformatics, initiating and analysing two versions of *C. erythraea* transcriptome as well as a very recent genome sequence.

During her whole scientific career, including research in the fields of biochemistry and plant molecular biology, Dr. Simonović greatly valued bioinformatic analyses. In this area, fully supporting and accepting suggestions of her younger colleagues, she managed to reveal in more detail the structure of plant glycoproteins rich in hydroxyproline (HRGP), by establishing a new methodology for filtering and analysis of HRGP. The result of this investigation was a confirmation that the previously unknown domain DUF1070 is a conserved signal peptide that serves to add glucosylphosphatidylinositol to a large number of arabinogalactan peptides, which led to the renaming of the mentioned domain to "Arabinogalactan peptide" in the largest database of protein domains - PFAM.

Dr Simonović presented her results in numerous local and international conferences. She was a member of the Scientific board for the „2nd International Conference on Plant Biology and 21st Symposium of the Serbian Plant Physiology Society", member of the International program board of „Belgrade Bioinformatics Conference – BelBi 2016", member of the Editorial board of Journal of Plant Studies, as well as a member of a Work group for creating the *Glossary for biology*, formed by the Committee for education of Serbian Biological Society.

Dr Simonović has worked as a lecturer for the course "Biology 150L" at the North Dakota State University (2005), "Plant biochemistry and physiology" at the Faculty of Chemistry University of Belgrade (2011) and "New methods in plant biotechnology" in the PhD program of the Faculty of Biology University of Belgrade (2011). Same year Dr Simonović published a book titled *"Plant biotechnology and genetic engineering"* (NNK International, Belgrade) that reviewers, colleagues as well as students, deemed highly valuable.

From the beginning of her career, Dr Ana Simonović was a leading member of our team, altruistically giving all her knowledge and experience to anyone who needed it. Dr Simonović was involved in several PhD theses, and either directly supervised or helped plan and perform experimental tasks. She was also a valuable team member in international projects: FP7 (acronym TERPMED) - *Plant Terpenoids for Human Health: a Chemical and Genomic Approach to Identify and Produce Bioactive Compounds;* EU (2009-2013) and H2020-NMBP-BIOTEC-07-2017: *New Plant Breeding Techniques (NPBT) in molecular farming: Multipurpose crops for industrial bioproducts*.

With numerous collaborators Dr Simonović had over 100 scientific publications published in high ranking international journals. Her research findings have found their way into book chapters by internationally-recognised publishers, scientific journals and doctoral theses. According to the Science Citation Index she had over 700 citations, and an H-index of 16.

It is difficult for us, her colleagues and friends, to accept the fact that Ana is gone from our lives. She was a unique, unforgettable person, with relaxed and warm smile, always helpful to everyone, selflessly sharing the latest information and helping to overcome difficulties in scientific and experimental work. It was a privilege to work with her, and her enthusiasm and endless energy was contagious. She gifted us with memorable friendship and guidance for future generations of researchers.

We are sure that her influence will be felt among her associates for a long time and that her colleagues will maintain her fascination with biological dilemmas, as well as her spirit, zest and everlasting liveliness.

Dr Slađana Todorović, Principal Research Fellow
University of Belgrade, Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia

Dr Milica Bogdanović, Senior Research Associate
University of Belgrade, Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia

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ISBN-978-86-912591-6-7