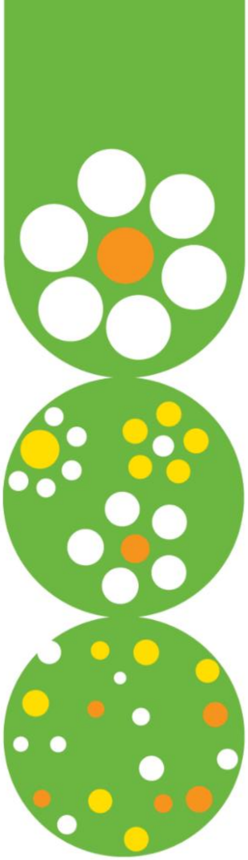

7th SLOVENIAN SYMPOSIUM ON PLANT BIOLOGY

with international participation

Biotechnical Faculty, University of Ljubljana

September 17 - 18, 2018, Ljubljana, Slovenia

Silva Slovenica
Ljubljana, 2018

Edited by: Špela Baebler
Marina Dermastia
Tine Grebenc
Špela Tomaž
Peter Železnik

Issued by: Slovenian Society of Plant Biology

Published by: *Silva Slovenica* publishing centre, Slovenian Forestry institute

Designed by: Jasna Dolenc Koce, Špela Tomaž

Edition: 1st electronic edition

Price: Free

Download: DOI 10.20315/SilvaSlovenica.0009

Kataložni zapis o publikaciji (CIP) pripravili v Narodni in univerzitetni knjižnici v Ljubljani
COBISS.SI-ID=296426496
ISBN 978-961-6993-44-9 (pdf)

Book of abstracts

Slovensko društvo
za biologijo rastlin

Slovenian Society
of Plant Biology

ORGANIZING COMMITTEE

Špela BAEBLER (Nacionalni inštitut za biologijo / National Institute of Biology) – Chair

Tine GREBENC (Gozdarski inštitut Slovenije / Slovenian Forestry Institute)

Peter ŽELEZNIK (Gozdarski inštitut Slovenije / Slovenian Forestry Institute)

Aleš KLADNIK (Biotehniška fakulteta, Univerza v Ljubljani / Biotechnical Faculty, University of Ljubljana)

Marina DERMASTIA (Nacionalni inštitut za biologijo / National Institute of Biology)

Matevž LIKAR (Biotehniška fakulteta, Univerza v Ljubljani / Biotechnical Faculty, University of Ljubljana)

Špela TOMAŽ (Nacionalni inštitut za biologijo / National Institute of Biology)

SPONSORS

The organization of the Symposium could not be done without the excellent sponsors whom we are grateful to:

mediline

bia
TRUSTED LAB PARTNER

METTLER TOLEDO

CHEMASS
MERILNI SISTEMI

vwr™
part of avantor

KEMOMED
BRINGING SOLUTIONS

Omega d.o.o.

Univerza *v Ljubljani*
Biotehniška fakulteta

NIB
NATIONAL INSTITUTE OF BIOLOGY

Silva
Slovenica

GENERAL INFORMATION

The symposium will start on Monday, September 17th 2018 at 9.00 a.m. and will close on Tuesday, September 18th 2018 in the afternoon.

The Symposium will be held in lecture hall B5 of the Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana. Coffee breaks will be served at the venue, while lunch will be provided in the cafeteria of the Faculty of Chemistry and Chemical Technology next door (see image). The symposium dinner will be held in the greenhouse next to the venue.

The Symposium reception desk will be open every day from 8.30 to 09.00.

Contributions will be presented as:

- invited lecture (IL)
- short lecture (SL)
- poster (P)

The speakers are kindly requested to deliver their presentation material in Microsoft PowerPoint (PPT) or PDF format on USB at least 15-20 minutes prior to the beginning of the session. We recommend using the Standard (3:4) slide size. The length of short oral presentations is 10-12 minutes, allowing 3-5 minutes for discussion.

Posters will be displayed in the main corridor of the Department of Biology, Biotechnical Faculty. Authors are kindly requested to mount their posters at the beginning of the Symposium and to dismount them after the lunch break on the second day in order to present their work throughout the period of the Symposium. At least one of the authors is requested to be available for discussion during the poster session that will take place on Monday before the Symposium diner.

SYMPOSIUM PROGRAM

Monday 17 September

08:30 - 09:00 Registration

Opening session (chair: Marina Dermastia)

9:00 Welcome address

9:15 **Opening lecture:** Johan Burger: Genome editing – a promising technology for Plant Pathology (IL1)

Session 1: Modern technologies (chair: Marina Dermastia)

9:50 Uroš Žibrat: Remote sensing applications for plant health and crop management (IL2)

10:20 Klemen Eler: Remote sensing of drought stressed maize in NE Slovenia (SL1)

10:35 - 11:05 Coffee break & posters

Session 2: Modern technologies & Plant phylogeny (chair: Johan Burger)

11:05 Marko Petek: Testing RNAi pesticide targets in Colorado potato beetle (SL2)

11:20 Jana Ambrožič-Dolinšek: Cryopreservation, an important tool for long-term preservation of endangered species (SL3)

11:35 Claudio Varotto: A transcriptomics view on the evolution of *Arundo donax*, a species with high potential for phytoremediation and bioenergy production (IL3)

12:05 Božo Frajman: Using an integrative approach to understand diversification of the *Heliosperma pusillum* group (Caryophyllaceae) (IL4)

12:35 Ales Lebeda: Research and exploitation of diversity of wild *Lactuca* germplasm in North America (SL4)

12:50 – 14:20 Lunch & posters

Session 3: Plant structure and function (chair: Andrea Schubert)

14:20 Primož Pelicon: Elemental and molecular imaging in plant biology (IL5)

14:50 Aleš Kladnik: Abscission in tomato leaves and flowers (SL5)

15:05 Pol Tijskens: Stability of variation: the case of tomato size in trusses. (SL6)

15:20 Joerg Fettke: Manipulation of the transitory starch granule number and morphology in *Arabidopsis thaliana* (SL7)

15:35 – 16:05 Coffee break & posters

Session 4: Plant structure and function (chair: Andrea Nardini)

16:05 Jožica Gričar: Plasticity of radial growth of trees from different environments (IL6)

16:35 Katarina Šoln: Ultrastructural changes in radish root tip after the exposure to rhizome extracts of *Fallopia japonica* and *F.* × *bohemica* (SL8)

16:50 Dominik Vodnik: Anatomical and physiological traits of *Quercus pubescens* Willd. from nearby sub-Mediterranean stands differing in soil and microclimate (SL9)

17:05 – 18:30 Poster session

18:30 Symposium dinner (greenhouse at the venue)

Tuesday 18 September

Session 5: Plant interactions with other organisms (chair: Dan Jacobson)

09:00 Maruša Pompe Novak: The influence of circadian rhythm on the potato response to PVY infection (IL9)

09:30 Tjaša Lukan: Spatiotemporal analysis of potato hypersensitive response-conferred resistance to potato virus Y: RBOHD is required for successful virus arrest (SL13)

09:45 Tjaša Stare: Unrevealing complex mechanisms of plant defense signalling using systems biology approach identifies StSAPK8 as novel regulator of potato immunity (SL14)

10:00 Anna Coll: The ethylene response factor StERF49 is a negative regulator of potato defence response against PVY (SL15)

10:15 Špela Tomaž: Potato immune signalization and potato virus Y proteins – towards analysis of 3D protein structures (SL16)

10:30 - 11:00 Coffee break & posters

Session 6: Plant interactions with environment (chair: Jasna Dolenc Koce)

11:00 Andrea Schubert: Do ABA and miR156 mediate the effects of strigolactones on drought stress tolerance and reproductive development? (IL7)

11:30 Andrea Nardini: Drought stress and xylem embolism: resistance and resilience of forest trees to climate change (IL8)

12:00 Angela Balzano: Effect of environmental factors on tree rings of Mediterranean species: investigation of xylogenesis and formation of irregularities in the wood (SL10)

12:15 Antonella Polzella: Effects of LED spectra in combination with biochar amendment on *Pisum sativum* L. and *Arabidopsis thaliana* L. growth (SL11)

12:30 Anja Kavčič: Selenate and selenite impact on mercury uptake and toxicity in plants (SL12)

12:45 – 14:15 Lunch & posters

Session 7: Plant interactions with other organisms (chair: Claudio Varotto)

14:15 Jasna Dolenc Koce: Biological activity of selected invasive alien plants (IL10)

14:45 Goran Kovačević: Interactions in green hydra symbiosis (IL11)

15:15 Jaka Razinger: Manipulating plant-microbe-insect interactions to enhance crop production and resistance to soil pests (SL17)

15:30 Karina Eva Hauer: *Colletotrichum coccodes* – how does the fungus operate in the shadows? (SL18)

15:45 Helena Volk: *Verticillium nonalfalfae* secretes a chitin binding protein to conceal itself from plant chitinases (SL19)

16:00 - 16:30 Coffee break

Closing session (chair: Špela Baebler)

16:30 **Closing lecture:** Dan Jacobson: Large Scale Systems Biology Approaches to Integrated Discovery: Explainable-AI and Supercomputing as the New Microscope for Complex Systems (IL12)

17:05 Concluding remarks

CONTENTS

Opening and closing lectures

Genome editing – a promising technology for Plant Pathology

Johan Burger* & Manuela Campa

Department of Genetics; Stellenbosch University; Private Bag X1; Matieland; 7602; South Africa
*corresponding author (jtb@sun.ac.za)

The challenges that modern agriculture faces to sustain food production are many and varied. One of the most important of these is the stabilisation of crop yield through the development of superior germplasm. The coevolution of plants and pathogens has resulted in a multifaceted, ongoing arms-race between the two, which seems to be slowly tipping towards the pathogens, especially considering the increasing numbers of infectious plant diseases caused by fungal, bacterial and viral pathogens. This situation necessitates the implementation of cutting edge technologies, like genome editing, for plant disease resistance breeding.

Genome editing comprises site-directed mutagenesis by the introduction of targeted double-strand breaks in DNA through the action of programmable nucleases, and the subsequent fixing of these breaks by one of two natural DNA repair mechanisms. CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/(CRISPR-associated protein9), the most widely used of these technologies, allows precise, targeted editing of commercial crop varieties, thus offering a viable, and much faster, alternative to traditional breeding methods. From a plant pathology perspective, additional advantages of this approach are that either key components of the host immunity system (*e.g.* susceptibility or resistance genes), or genome regions of the pathogen(s) (*e.g.* essential genes for replication) can be targeted, and that these modifications can be made in a non-transgenic manner.

CRISPR/Cas9 technology is based on an age-old adaptive immune system found in bacteria and archaea, aimed at protecting these organisms against virus infections. Scientific intervention has seen this system being adapted and optimised in a number of ways to allow for pin-point precision editing of the genome target sequences, potentially resulting in either knock-out or knock-in mutations. Here, we discuss the application of CRISPR/Cas9 technology in plant pathology. Aspects that are covered include experimental strategies for sgRNA design, choice of nuclease, vector construction, delivery systems, detection and analysis of *in vivo* mutagenesis, and analysis of off-target effects. Examples of CRISPR/Cas9 approaches to introduce resistance against fungal, bacterial and viral pathogens will be discussed and, finally, two case studies of current CRISPR/Cas9 research projects (grapevine and soybean) in our laboratory will be highlighted.

Large Scale Systems Biology Approaches to Integrated Discovery: Explainable-AI and Supercomputing as the New Microscope for Complex Systems

Daniel Jacobson*

Oak Ridge National Laboratory, 1 Bethel Valley Road, Oak Ridge, TN, USA
* corresponding author (jacobsonda@ornl.gov)

Integrated biological models need to capture the higher order complexity in the interactions that occur among cellular components. A full model of all of the higher order interactions of cellular and organismal components is one of the ultimate grand challenges of systems biology. The ability to build such comprehensive models will usher in a new era in biology. Success in the construction and application of computational algorithms will enable new insights into the molecular mechanisms responsible for complex biological systems and related emergent properties; using technologies not previously available on a scale not feasible before. A full systems biology model of all of the higher order interactions of cellular and organismal components would lead to breakthroughs, which would have profound effects on the field.

The cost of generating biological data is dropping exponentially, resulting in increased data that has far outstripped the predictive growth in computational power from Moore's Law. This flood of data has opened a new era of systems biology in which there are unprecedented opportunities to gain insights into complex biological systems. The dominant paradigm of high-throughput systems biology is the use of new technologies to generate massive amounts of data that can then be analysed computationally for new insights and hypothesis generation. Integrated biological models need to capture the higher order complexity of the interactions among cellular components. Solving such complex combinatorial problems will give us extraordinary levels of understanding of biological systems. Paradoxically, understanding higher order sets of relationships among biological objects leads to a combinatorial explosion in the search space of biological data. These exponentially increasing volumes of data, combined with the desire to model more and more sophisticated sets of relationships within a cell and across an organism (or in some cases even ecosystems), have led to a need for computational resources and sophisticated algorithms that can make use of such datasets.

We are using the bioenergy feedstock Populus trichocarpa (black cottonwood) and are currently using 10 million genome variants derived from the resequenced genomes of more than a thousand different genotypes and 160,000 phenotypes that have been measured across this population (including transcriptomics, metabolomics, microbiomics, and phenomics data). We are building increasingly comprehensive models of this species with the use of explainable-AI algorithms, network theory on peta- and exa-scale computing platforms at the Oak Ridge National Laboratory.

Modern technologies

Remote sensing applications for plant health and crop management

Uroš Žibrat*, Matej Knapič

Plant protection department, Agricultural Institute of Slovenia, Hacquetova 17, SI-1000 Ljubljana
* corresponding author (uros.zibrat@kis.si)

Monitoring pests and diseases is a fundamental tool in integrated pest management (IPM). With advances in satellite, on-ground, airborne and unmanned aerial vehicle (UAV) sensor technology, reflectance data are becoming more easily available for a wider audience. Spectral imaging allows reliable identification of plant species, and varieties, and their health status. In addition to plants, seeds of different varieties can also be distinguished, for example in tomatoes. Stress states can often be identified prior to the development of visible symptoms, enabling the development and use of reliable early-warning systems. For example, the presence of nematode infestations in tomatoes, including their severity, and drought stress can be detected in early stages of infection, as soon as approximately 2 weeks after infection. Furthermore, the presence and amount of certain chemical compounds (e.g. sugar in grapevine berries) can also be determined. Because these images also contain spatial information, we can generate heat maps of stress presence in entire fields. Heat maps of grapevine yellows infected grapevines and drought stress provide decision makers with precise spatial information on plant health and thus enable targeted management practices.

Remote sensing of drought stressed maize in NE Slovenia – A multilayer data integration

Klemen Eler[1], Pečan Urša[1], Marina Pintar[1], Jernej Šijanec[1], Matjaž Glavan[1], Damijana Kastelec[1], Žiga Kokalj[2], Dominik Vodnik[1]

[1] Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
[2] ZRC SAZU, Novi trg 2, SI-1000 Ljubljana, Slovenia
* corresponding author

Remote sensing (RS) systems are increasingly providing an important battery of technologies available for studying and managing agricultural systems. In last years RS has been widely used to study, monitor and predict drought - the single most devastating environmental stress factor, which decreases crop productivity more than any others. The advantages of RS include large scale (even global), near-real-time observations, consistent data records, and improved spatial resolution. The output of RS has to be related quantitatively to the values of the biophysical parameters being studied. Therefore, direct ground based measurements of soil (e.g. analyses of water availability) and plants (water status, nutritional status, growth - leaf area index, chlorophyll content, stress indicators (fluorescence, anti-stress compounds)) are needed to support multilayer data integration and modelling (calibration, validation).

In this study we present results from experiment in which drought on the maize fields in NE Slovenia (fields of Panvita Group, Murska Sobota) was remotely sensed at two spatial levels, namely from Sentinel 2 satellite (ESA Copernicus mission) and Unmanned Aerial Vehicle (UAV) by multispectral imaging. Irrigated and non-irrigated fields on two different soils (fine textured, coarse textured soil) were included into study. RS data from two seasons (2016, 2017) were integrated with ground based measurements (soil water content, leaf area index, relative water content of plants, chlorophyll content and fluorescence, etc.). Field study was additionally supported by controlled greenhouse experiment in which physiological response of maize to water deprivation was thoroughly studied. In the paper we discuss dynamics of drought – as revealed by integrated sensing approach- with respect to irrigation and soil texture.

Testing RNAi pesticide targets in Colorado potato beetle

Marko Petek*, Kristina Gruden

National Institute of Biology, Department of Biotechnology and Systems Biology, Večna pot 111, 1000 Ljubljana, Slovenia
* corresponding author (marko.petek@nib.si)

Colorado potato beetle (*Leptinotarsa decemlineata*; CPB) is a serious pest of Solanaceous crops such as potato, tomato and eggplant. The beetle's ability to rapidly evolve resistance to chemical pesticides and effective silencing of CPB genes trough feeding with long dsRNAs makes this beetle a good candidate for development of RNAi-based pest management treatments.

In order to identify new CPB pest management targets, we designed dsRNAs against three genes expressed in its gut and tested their insecticidal activity in feeding trials. For these trials, *in vitro* synthesized dsRNAs were sprayed onto potato leaves and used to feed the beetles. We followed beetle survival, weight gain and development and measured target gene knockdown by qPCR. We show that the efficiency of target knockdown differs between targets and how this correlates with dsRNA's insecticidal activity. Additionally, we have used RNA-seq to investigate the non-specific transcriptional response of CPB larval guts to dsRNA targeting efgp sequence. We have also used NGS to investigate the changes in gut metagenome composition induced by dsRNA feeding.

Cryopreservation, an important tool for long-term preservation of endangered species

Ambrožič-Dolinšek J.*[1,2], Ciringer T[1]

[1]University of Maribor, Faculty of Natural Sciences and Mathematics, Koroška cesta 160, 2000 Maribor, Slovenia
[2]University of Maribor, Faculty of Education, Koroška cesta 160, 2000 Maribor, Slovenia
* corresponding author (jana.ambrozic@um.si)

Cryopreservation is becoming an important tool for long-term storage of plant germplasm. Besides its agricultural importance, it also offers the potential for long-term preservation of endangered species like *Hladnikia pastinacifolia* Rchb. (Apiaceae), a monotypic endemic genus, with an extremely narrow distribution area, protected in situ in natural habitats and ex situ as seeds and whole plants. This plant species have short-lived seeds with complicated dimorphic dormancy, which make preservation in the form of seeds useless. Another difficulty is the plant's monocarpic life cycle: it flowers and produces seeds only once, usually several years after germination. Cryopreservation of tissue culture propagated plant material is an alternative conservation challenge used for the protection of "problematic" or/and protected species like *H. pastinacifolia*. Many cryopreservation techniques have been developed and successfully used with a range of cells, tissues and organs of plant species. We present and compare three relatively simple and widely used cryopreservation procedures, all tested on this species: encapsulation-dehydration (ED), encapsulation-vitrification (EV) and droplet vitrification (DV). All three include treatment in media containing high levels of sucrose and/or glycerol and first two encapsulation of plant material in calcium alginate beads. They have several technical advantages: the alginate beads with encapsulated explants are easy to manipulate and dehydration or vitrification are relatively easy to perform. Cryopreservation techniques, presented in our study are also inexpensive and they do not require special equipment.

Acknowledgements

The Slovene Ministry of Higher Education, Science and Technology supported this research within the program Research to Ensure Food Safety and Health within the Grant No. P1-0164, led by D. Škorjanc.

Metagenomic analysis and infectivity of plant viruses in wastewaters

Katarina Bačnik[1,2]*, Denis Kutnjak[1], Anja Pecman[1,2], Ion Gutierrez Aguirre[1], Nataša Mehle[1], Magda Tušek Žnidarič[1], Maja Ravnikar[1]

[1] National Institute of Biology, Department of Biotechnology and Systems Biology, Večna pot 111, Ljubljana, Slovenia
[2] Jožef Stefan International Postgraduate School, Jamova 39, Ljubljana, Slovenia
* corresponding author (katarina.bacnik@nib.si)

Aquatic environments represent a potential pathway for plant virus transmission, which is especially relevant in the case of recycled water use for irrigation purposes. New methods, such as high throughput sequencing (HTS), allow us to reveal a hidden diversity of viral species in aquatic environments. We analysed samples of the influent and effluent of wastewater treatment plant in Slovenia. Water samples were first concentrated using Convective Interaction Media (CIM) monolithic chromatography, a method that can efficiently concentrate viruses from high-volume water samples. Then, HTS was used to detect the presence of a wide array of viruses from different taxonomic groups. Using bioinformatics tools, we defined the viromes of water samples and compared their composition between influents and effluents. Such approach allowed us to characterise high diversity of plant viral species in the analysed samples. Moreover, specific virus detection for the most common plant viruses was done by quantitative PCR and their integrity was confirmed using transmission electron microscopy. Because the stability and mechanical transmission of several plant viruses found by HTS is known to be high, we aimed to confirm their infectivity. Using test plants mechanically inoculated with concentrated samples of influents and effluents, we were able to confirm the infectivity of some plant viruses, such as Pepper mild mottle virus (PMMoV), Tomato mosaic virus (ToMV) and Tobacco mild green mosaic virus (TMGMV) from Tobamovirus genus (family Virgaviridae). Together, these data demonstrate that plant viruses remain infective even after conventional wastewater treatment. Methods that are able to inactivate viruses in water environments, such as hydrodynamic cavitation, need to be further studied in the future. New knowledge and technologies should also bring more attention to plant health authorities to consider water monitoring for plant viruses and prevent plant diseases.

Alien Plant Species in the city – use of their wood for various products

Maks Merela[1], Denis Plavčak[1] , Aleš Straže[1], Željko Gorišek[1], Ervin Žveplan[1], Luka Krže[1], Jože Planinšič[1], Angela Balzano[1], Katarina Čufar[1*]

[1]Biotechnical Faculty, Department of Wood Science and Technology, University of Ljubljana, Ljubljana, Slovenia
* corresponding author (katarina.cufar@bf.uni-lj.si)

Invasive Alien Plant Species (IAPS) represent a big challenge in European ecosystems. They displace local vegetation, destroy agricultural land, and cause damage to economy. The City of Ljubljana recognized the problem of IAPS. Together with multidisciplinary partners it successfully applied for a project Applause, which is a project of UIA (Urban Innovative Actions) as Initiative of the European Union that provides urban areas throughout Europe with resources to test new and unproven solutions to address urban challenges. Three year project which started at the end of 2017 is financed by the European Regional Development Fund.

We will present the first results of the project which addresses unsolved questions regarding IAPS in terms of the zero-waste approach and circular economy. The project team from the Biotechnical Faculty, Department of Wood Science and Technology is focused to woody IAPS, which are currently mainly burned or composted. The team develops a pilot projects for processing their wood into various useful products, like paper and various wood products. To this purpose they intend to develop procedures for semi-industrial plants. In the first step the research is aimed to recognize the potential of IAPS, as well as to teach citizens to recognize and identify invasive non-native plant species, to properly remove, collect and prepared them to be processed into useful products. The further aim is to develop a circular economy model and find new use for all parts of the collected IAPS as well as upcycling the residual materials. The stakeholders involved are: kindergartens, schools, pupils, students, households, property owners, companies, tourists, professional organizations…

We will present the first results with the list of some of the most interesting IAPS appropriate for production of paper, wood and food products, dyes and home-made formulations against plant harmful organisms. We will present the development of a procedure to evaluate the raw material (wood) properties for production of the listed products. Examination of woody plant species involves evaluation of the anatomy, physical-mechanical properties, basic chemical composition, machinability, gluing, natural durability, resistance, and impregnability of the wood. We will also search for a solution for used of wood waste which now serves as an energy-source although it could be also processed into other useful products.

Establishment and characterization of biomass and astaxanthin content in the culture of alga *Haematococcus pluvialis*

Jana Ambrožič-Dolinšek[1,2], Zoltan Molnar[3], Veno Jaša Grujić[1,]*,

[1] University of Maribor, Faculty of Natural Sciences and Mathematics, Department of Biology, Koroška cesta 160, SI-2000 Maribor, Slovenia
[2] University of Maribor, Faculty of Education, Department of Elementary Teacher Education, Koroška cesta 160, SI-2000 Maribor, Slovenia
[3] Szechenyi Istvan University, Faculty of Agricultural and Food Sciences, Department of Plant Sciences, H-9200 Mosonmagyarovar, Hungary
*corresponding author (venojasa.grujic@student.um.si)

The purpose of the research was to establish the biomass and astaxanthin production in the culture of the alga *Haematococcus pluvialis*. The main goal is to present the design and establishment of the cultivation systems for microalgae and furthermore, compare some growth parameters of different strains. We screened biomass and astaxanthin production five microalgal strains obtained from CCALA (Culture collection of Autotrophic Organisms, http://ccala.butbn.cas.cz/en). The algal strains varied in the yields of biomass and the astaxanthin content. Among the five strains tested, the highest result was obtained from strains three and four, and the lowest from strains one and two. The strain five was contaminated and had been removed from the study. After the monitoring of biomass production and astaxanthin content, we selected the best and the most promising strains for further studies.

Keywords: algal culture, *Haematococcus pluvialis,* astaxanthin

A novel plasmid for synthetic biology of cyanobacteria, tested with cystatin as a reporter

Mojca Juteršek, Marko Dolinar*

Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, 1000 Ljubljana, Slovenia
* corresponding author (marko.dolinar@fkkt.uni-lj.si)

Photosynthetic organisms are gaining importance in biotechnology as sustainable factories for direct conversion of CO_2, H_2O and solar energy into valuable chemicals and biofuels. Amongst them, cyanobacteria provide genetically simple and fast-growing unicellular chassis employable for synthetic biology, but development of tools and biological parts is lagging behind in comparison with other organisms. We have decided to construct a new self-replicative shuttle plasmid for synthetic biology of model cyanobacteria *Synechocystis* sp. PCC 6803 with improved characteristics over widely used plasmid pPMQAK1. Moreover, we intended to evaluate cystatin as a potential new reporter protein for cyanobacteria. We have successfully assembled pMJc01 plasmid based on RSFmob-I replicon and cloning site along with selection marker from pSB3K3 plasmid and characterized it using GFP and cystatin reporter proteins. We demonstrated that pMJc01 can replicate both in *Escherichia coli* and *Synechocystis* sp. PCC 6803. Due to higher copy-number in stationary growth phase (25–30 in *Escherichia coli*) and efficient isolation by alkaline lysis compared to pPMQAK1 it enables faster cloning and effective transformation of *Synechocystis* sp. PCC 6803. Furthermore, we achieved high expression of GFP in *Synechocystis* sp. PCC 6803, indicating plasmid's potential not only for metabolic engineering but also for protein production in cyanobacteria. Its applicability for biotechnology is additionally improved due to the absence of mobilization regions and consequently increased biosafety. With replication machinery independent from host proteins, pMJc01 provides a platform for development of synthetic biology vectors for a wide range of organisms with a limited molecular tool-box. We have also successfully constructed a functioning cystatin expressing BioBrick and demonstrated its suitability as a reporter for quantitative analysis of regulatory genetic elements in both *Escherichia coli* and *Synechocystis* sp. PCC 6803 with some advantages over GFP.

Huang H.-H., Camsund D., Lindblad P. and Heidorn T. (2010). Nucleic Acids Research 38 (8), 2677-2593.

Strategy for development of immune response biosensors in potato

Valentina Levak*, Anna Coll, Tjaša Lukan, Maja Zagorščak and Kristina Gruden

Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia
*corresponding author (valentina.levak@gmail.com)

In biotic or abiotic stress, immune response is activated in infected plant cells. Information about the type of stress is shared between cells through hormones and other signal molecules synthesized in infected cells.

Immune response can be followed through the activity of promoters of the genes that are involved in the regulatory network of the immune response. With the selection of a gene promoter from a specific immune response signaling pathway, the spatiotemporal dynamics of the activation of chosen genes and consequently the expansion of the immune response across the tissue through time can be followed.

The goal of our work is development of a biosensor system, that will enable an insight into the spatiotemporal dynamics of immune response in potato tissue (*Solanum tuberosum*). Therefore, our work was directed towards the preparation of a vector with two sequences, coding beta-glucuronidase and fluorescent protein, that will, after fusion of promoters of selected genes with genes for reporter proteins, enable following of activity of selected promoters. The most suitable promoters were first selected by bioinformatic approach to reveal crucial cis-regulatory elements that are connected with plant immune response. According to *in silico* analysis results, the selected promoters will be fused with gene sequences coding for beta-glucuronidase and fluorescent protein and transiently transformed in tobacco (*Nicotiana benthamiana*) to follow their activity with beta-glucuronidase assay. Afterwards, the activity of the most promising promoters will be followed in potato stable transformants by means of fluorescent microscopy.

Optimisation of CRISPR/Cas9 technology for gene editing in potato

Lucija Luskar*, Barbara Dušak, Tjaša Lukan, Kristina Gruden

National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia
*corresponding author (lucija.luskar@gmail.com)

Site-directed mutagenesis offers an efficient way of studying gene function and fundamental biological processes. One of approaches to study role of a gene is by producing organisms with a specific gene knock-out. This can be a challenge, especially with tetraploids, such as potato (*Solanum tuberosum*). A way to edit multiallelic organisms is CRISPR-Cas9, one of the newest methods for genome editing.

In our research, we have optimised CRISPR-Cas9 for gene knockouts in potato. We chose the *StRbohD* gene, as the consequence of *StRbohD* silencing has already been determined by previous experiments at National Institute of Biology. *StRbohD* gene encodes an NADPH oxidase which plays a role in defence of potato against the potato virus Y (PVY). Silencing of this gene by short hairpin RNA causes loss of resistance of potato cultivar Rywal to PVY. In this case, the virus can spread to the upper non-inoculated leaves where necrotic lesions occur.

gRNA was designed to target a region, crucial for the function of StRbohD gene. We introduced guide RNA (gRNA), homologous to specific region in *StRbohD* gene into the plasmid pRGEB31, which also contained the sequence of Cas9 protein. We performed agrobacteria-mediated stable transformation of potato cultivar Rywal using the prepared construct. First shoots appeared 7 weeks after transformation. We collected 70 lines of transformed shoots. We randomly chose 36 lines and inoculate them with PVY N605-GFP to study the effect of StRbohD knock-out on virus resistance. Lesions appeared on upper non-inoculated leaves in 5 transgenic lines and we confirmed the presence of viral RNA with qPCR. Furthermore, we studied progress of lesion appearance in time in two transgenic lines. One of them outnumbered the other lines by the number of lesions which also had different phenotype in comparison to the lesions that were developed in cv. Rywal. Number of lesions was even higher than the number of lesions in NahG-Rywal plants that are sensitive to PVY. Optimisation of the method could provide faster and better understanding of genes in potato, one of the most important food crops nowadays.

Micropropagation of medical cannabis (*Cannabis sativa* L.)

Špela Mestinšek Mubi*[1], Marko Flajšman[1], Borut Bohanec[1], Jana Murovec[1]

[1]University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Slovenia
*corresponding author (spela.mmubi@gmail.com)

Plant tissue culture is an essential component of plant biotechnology. Since there are only a few reports about tissue culture of *Cannabis sativa* L. (Chaohua et al., 2016; Feeney and Punja, 2003; Lata et al., 2016), the development of an efficient micropropagation procedure may be of high commercial value for the medical cannabis industry. The study was performed as part of MGC Pharmaceuticals project "Breeding medical cannabis (*Cannabis sativa* L.)" in collaboration with the Biotechnical Faculty of the University of Ljubljana. Four experiments were done on their breeding lines MX-CBD-11 and MX-CBD-707 as follows: In the first experiment, sterilized nodes were inoculated on three different culture media. The results showed that 30 days after inoculation, in the breeding line MX-CBD-11 the basal MS medium supplemented with meta-topolin induced the higher number of axillary shoots and the longest shoots, while in the breeding line MX-CBD-707 the higher number of axillary shoots were induced on MS basal medium without hormones. The longest shoots were formed on the MS basal medium supplemented with meta-topolin. In the second experiment, we studied the effect of node position on initiation of shoot cultures. Nodes from five positions were inoculated on MS basal medium without hormones. For both breeding lines, nodes closer to the apical meristem produced less axillary shoots than those from distal ones. The results of the third experiment showed that the average number of shoots per node is increasing through the time for both breeding lines. The fourth experiment aimed to evaluate the effect of 10 different culture media, composed of basal MS medium supplemented with BAP and IAA at various concentrations, on *in vitro* culture initiation. The breeding line MX-CBD-11 produced a higher number and longer axillary shoots than the breeding line MX-CBD-707 on all tested media. With these experiments, we showed that different culture media are appropriate for growing *in vitro* culture of medical cannabis. Besides, shoot culture establishment is significantly influenced by genotype and nodal position.

Keywords: *Cannabis sativa* L., medical cannabis, micropropagation

Chaohua C., Gonggu Z., Lining Z., Chunsheng G., Qing T., Jianhua C., Xinbo G., Dingxiang P., Jianguang S. (2016) A rapid shoot regeneration protocol from the cotyledons of hemp (*Cannabis sativa* L.). Industrial crops and products 83: 61–65.

Feeney M., Punja Z. K. (2003) Tissue culture and agrobacterium-mediated transformation of hemp (*Cannabis sativa* L.). In Vitro Cellular & Developmental Biology-Plant 39: 578–585.

Lata H., Chandra S., Techena N., Khana I. A., ElSohlya M. A. (2016) *In vitro* mass propagation of *Cannabis sativa* L.: A protocol refinement using novel aromatic cytokinin meta-topolin and the assessment of eco-physiological, biochemical and genetic fidelity of micropropagated plants. Journal of applied research on medicinal and aromatic plants 3: 18–26.

Towards first F1 hybrids of hemp (*Cannabis sativa* l.) - haploid induction by pollination with irradiated pollen

Sinja Svetik, Marko Flajšman, Jana Murovec, Borut Bohanec*

University of Ljubljana, Biotechnical Faculty, Department of Agriculture, Slovenia
* corresponding author (Borut.Bohanec@bf.uni-lj.si)

F1 hybrids varieties of hemp (*Cannabis sativa* L.) do not exist so far. Although term “hybrid” is often used in cannabis literature, it does not represent true F1 hybrid (progeny produced with crossing of 100% homozygous plants, e.g .doubled haploids) but is rather used to describe other types of crossings (e.g. between varieties). Hemp varieties that now exist are either open pollinated or synthetic. Haploids of *Cannabis sativa* L. would be the first step toward the true F1 hybrids. This study aimed to develop a protocol for haploid induction from female gametes of Cannabis trough pollination with irradiated pollen. In this gynogenesis technique, embryo development is stimulated by pollen germination on the stigma. But pollen must be irradiated with optimal dose of rays, which allows it to grow the pollen tube within the style but is unable to fertilize the egg cell. So the development of embryos occurs but without the father's chromosome set.

In the experiments were included two cultivars ‘Finola’ as maternal line and ‘Tiborszallasi’ as pollen donor. To determine the percentage of germination of pollen *in vitro* several medium compositions were tested, of which two were most suitable. Pollen cells have to stay vital during and after irradiation treatment. The optimal procedure was to treat extracted pollen mixture in a petri dish with increased air humidity. Also, several regeneration mediums for immature embryos were tested, but there were shown no differences in growth of embryos. The optimal regeneration medium was MS medium with vitamins.

Irradiation treatment doses varied from 180 to 1326 Gy. Pollen germination decreased with increasing radiation dose. Even though that pollen germinated at high doses of radiation and embryos were formed in seeds in early stages, it was too damaging to obtain any regenerants. There were isolated 322 immature embryos in treatments with irradiation doses between 350 and 620 Gy of which 83 regenerants were formed. Preliminary results of ploidy test with flow cytometry showed the diploid status of all regenerants, except for two which were tetraploids and one mixoploid. They were also tested with SSR markers to determinate if there are any spontaneous doubled haploids. Results of testing with three SSR markers determined that three regenerants are homozygous. For more thorough analysis we are going to test them with more SSR markers.

Homozygous regenerants were treated with irradiation doses of 530 Gy and 443 Gy.

Digital PCR improves quantification of genetically modified organisms (GMO) in complex samples

Dejan Štebih[1,*], Alexandra Bogožalec Košir[1,2], Tina Demšar[1], Jana Žel[1], Mojca Milavec[1]

[1]Department of Biotechnology and Systems Biology, National Institute of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia.
[2]Jožef Stefan International Postgraduate School, Jamova 39, SI-1000 Ljubljana, Slovenia.
* corresponding author (dejan.stebih@nib.si)

Real-time quantitative polymerase chain reaction (qPCR) has been used for detection and quantification of GMOs for almost two decades. The quantification of GMOs with qPCR method is based on the use of standard calibration curve, prepared from reference materials. Especially for complex samples, quantification can be infeasible due to the sensitivity of qPCR to inhibitors from the sample. Digital PCR (dPCR) is a method where the reaction mixture is divided into many individual reactions called partitions, enabling absolute quantification without the need for a standard curve. Because of qualitative nature of PCR reaction in partitions, it is less sensitive to partial inhibition that could influence quantification.

In this study the potential of dPCR to overcome the difficulties of GMO quantification by qPCR in complex samples was investigated. Previous studies have shown that direct transfer of qPCR-validated methods to a dPCR system is possible, thus, we have transferred two simplex qPCR assays targeting the soybean species-specific gene and one of the most abundant soybean lines on the world market, commercially known as Roundup Ready® soybean, to dPCR system. We have also merged these two simplex assays in one duplex dPCR assay to enable more time and cost efficient analysis. The assays were assessed on certified reference materials and complex real-life feed samples using Bio-Rad's QX100™ and QX200™ Droplet Digital PCR Systems. Analysed DNA was extracted with two methods to investigate possible influence of DNA extraction.

Hyperspectral remote sensing of drought in vineyards

P10

Uroš Žibrat[1*], Matej Knapič[1], Monika Zovko[2]

[1] Plant protection department, Agricultural institute of Slovenia, Hacquetova ulica 17, Ljubljana, Slovenia
[2] Department of soil amelioration, Faculty of Agriculture, University of Zagreb, Svetošimunska 25, Zagreb, Croatia
* corresponding author (uros.zibrat@kis.si)

In the Mediterranean many parts of karst surfaces have been modified to make them suitable for agricultural use, mostly grapevine and olive production. In karst landscapes rain water percolates rapidly downward through the soil layers, making them highly fragile and vulnerable to anthropogenic as well as climatic stresses. Moreover, excessively dry topsoils can cause water run-off and significant evaporative loss of water, making lack of moisture an important risk indicator in vine production. Water measurements in karst soils are challenging, due to skeletal structure and great within-field variability. Under such conditions, irrigation scheduling should be mainly based on crop water status. Current standard procedures include measurements of individual plants, leading to extensive and time consuming field work, which is subject to measurement and sampling errors. We assessed the applicability of hyperspectral remote sensing for grapevine water status assessment. The research was performed in an experimental vineyard grown in artificially transformed kart terrain in Croatia, near Šibenik. The experimental design included four water treatments in three replicates: (1) fully watered (100% ETc), (2) 75% ETc, (3) 50% ETc, and (4) non-watered. Hyperspectral images were taken using two cameras (VNIR and SWIR), covering wavelengths fom 400 to 2500 nm. Data were analysed using Partial least squares discriminant analysis and Support vector machines. We achieved a classification success of 70 - 80% for determining watering status. The 75 and 100% groups could not be reliably distinguished between each other.

Plant phylogeny

A transcriptomics view on the evolution of *Arundo* donax, a species with high potential for phytoremediation and bioenergy production

Wuhe Jike[1,2], Enrico Barbaro[1], Giorgio Bertorelle[2], Mingai Li[1], Claudio Varotto[1,*]

[1]Research and Innovation Centre, Fondazione Edmund Mach, Via Mach, 1, 38010 San Michele all'Adige (TN), Italy
[2]Department of Life Sciences and Biotechnology, University of Ferrara, Via Luigi Borsari, 46, 44121 Ferrara, Italy
* corresponding author claudio.varotto@fmach.it

Arundo donax L. (also known as the giant reed) is a perennial C3 grass with fast growth. It is considered among the most promising bioenergy and phytoremediation crops in the Mediterranea area, due to its ability to produce large amounts of biomass from marginal soils not suitable for agricultural use. Despite its importance and value, many fundamental aspects of its biology and the precise phylogenetic relationships with respect to other species of the *Arundo* genus still remain to be fully elucidated.

We sequenced by 2x100 paired-end Illumina sequencing the leaf transcriptomes for all taxa of the *Arundo* genus and closely related outgroups. Assembly of leaf transcriptomes allowed the reconstruction of an average of 100000 transcripts per species, with an average length of nearly 1 Kbp. Analysis of gene onthology terms indicated that a uniform set of functional classes was obtained for all the species, suggesting that the dataset provided a comprehensive representation of both coding and non coding leaf transcriptome of the *Arundo* genus. Mining of putative microRNA primary transcripts with highly stringent *in silico* analyses identified 85 putative miRNAs belonging to 19 different families and a total of 197 high-confidence putative targets from the seven *Arundo* species analyzed. Gene Ontology functional annotation showed that miRNA targets are constituted mainly by transcription factors involved in multiple biological processes. Identification of a set of 150 of genes with exactely one ortholog per species allowed a robust phylogenomic reconstruction of the relationships among species, resulting in the precise positioning of *A. donax* and highlighting the mechanisms underlying evolution of chromosomal numbers within the *Arundo* genus.

These results pave the road to further elucidate the biology and evolution *Arundo donax* and other *Arundo* species. The dissection of the patterns of evolution in *Arundo* genus will support ongoing efforts to establish reverse genetics and functional genomics approaches in *Arundo donax*, thus contributing to provide promising candidate genes for the improvement of this biomass species.

Using an integrative approach to understand diversification of the *Heliosperma pusillum* group (Caryophyllaceae)

Božo Frajman[1*], Clara Bertel[1], Aglaia Szukala[2], Ovidiu Paun[2] and Peter Schönswetter[1]

[1]Department of Botany, University of Innsbruck, Sternwartestraße 15, A-6020 Innsbruck, Austria
[2]Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, A-1030 Vienna, Austria
*corresponding author (bozo.frajman@uibk.ac.at)

Variation in biotic and abiotic conditions in heterogeneous environments can lead to the formation of distinct populations adapted to their specific habitats. The *Heliosperma pusillum* group includes taxa morphologically and functionally adapted either to creeks and moist calcareous screes and rock crevices in the (sub)alpine belt, or to rock overhangs and shallow caves in the montane belt. Several of the low-elevation populations have been described as distinct species on the Balkan Peninsula and in the Eastern Alps, but recent analyses of genomic RADseq data rather suggest recurrent parallel origins of low-elevation populations treated as *H. veselskyi* in the Eastern Alps from widespread, high-elevation *H. pusillum*. We have used a broad set of anatomical, ecological, morphological, physiological and genomic (RADseq and RNAseq) analyses both in natural populations as well as in a common garden to disentangle the evolutionary patterns in this system. Phylogenomic patterns suggest that both "species" are merely ecotypes adapted to microclimatically divergent niches and that different populations of the montane ecotype ("*H. veselskyi*") evolved similar morphological and functional/physiological traits in parallel as result of adaptive evolution. RNAseq analyses show that drift and locally-relevant selection shape a major portion of expressed patterns, but also that natural selection shaped the expression patterns of a few ecologically relevant genes between both ecotypes. Last but not least, sequencing of ca. 300 nuclear DNA regions obtained by gene capture will answer whether similar evolutionary mechanisms are driving the diversification of this group also on the Balkan Peninsula, from where the majority of species has been described.

Research and exploitation of diversity of wild *Lactuca* germplasm in North America

A. Lebeda[1*], E. Křístková[1], I. Doležalová[2], M. Kitner[1], M.P. Widrlechner[3]

[1] Department of Botany, Faculty of Science, Palacký University in Olomouc, Czech Republic,
[2] Department of Genetic Resources for Vegetables, Medicinal and Special Plants of Crop Research Institute in Olomouc, Czech Republic,
[3] Departments of Horticulture and of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, Iowa, USA
[*] corresponding author (ales.lebeda@upol.cz)

Eleven wild and weedy species, either autochthonous (*L. biennis*, *L. canadensis*, *L. graminifolia*, *L. floridana, L. hirsuta*, *L. ludoviciana*, *L. terrae-novae*, *L. tatarica* subsp. *pulchella*) or allochthonous (*L. saligna, L. serriola, L. virosa*) represent the genus *Lactuca* (Asteraceae) in North America (Lebeda et al. 2007). Cultivation of lettuce (*L. sativa*) is highly important segment of vegetable production in the USA and Canada. Literature records on the history of the uses of cultivated lettuce and its wild North American relatives are presented. The ecogeography and diversity of wild *Lactuca* species, as well as recent trends in their exploitation in lettuce breeding, are reported. Literature data and the most important results of our observations and experiments provide background for a comprehensive characterization of the genus *Lactuca*: its taxonomy, biogeography, distribution, habitat ecology, and genepools, with an emphasis on its phenotypic variability, genetic diversity and disease resistance (Lebeda et al. 2011, 2012). The current status of *in situ* and *ex situ* conservation of wild and weedy *Lactuca* species in North America is reviewed; gaps and perspectives are analyzed and discussed. The protection, conservation and exploitation of autochthonous North American *Lactuca* species must be based on: i) knowledge of their diversity, phylogeny and taxonomy; ii) knowledge of their floristics, biogeography and ecology; iii) study of original accessions of wild *Lactuca* species from existing germplasm collections along with samples newly collected in North America; and iv) evaluations of their environmental adaptations, phenotypic and genetic variation, and resistance against diseases, pests and abiotic stresses, to determine their value for lettuce improvement (Lebeda et al. 2014).

Aleš Lebeda, Eva Křístková, and Miloslav Kitner were supported by internal grant IGA_Prf_2018_001 (Palacký University in Olomouc, Czech Republic), and by project MSM 6198959215 (Ministry of Education, Youth and Sports, Czech Republic). Ivana Doležalová was supported by grant LO1204 (Ministry of Education, Youth and Sports, Czech Republic).

Lebeda A., Doležalová I., Kitner M., Novotná A., Šmachová P. and Widrlechner M. P. (2011). Acta Hort 918, 475–482.
Lebeda A., Doležalová I. and Novotná A. (2012). Genet Resour Crop Evol 59, 1805–1822.
Lebeda A., Křístková E., Kitner M., Mieslerová B., Jemelková M. and Pink D. A. C. (2014). Eur J Plant Pathol 138, 597–640.
Lebeda A., Ryder E. J., Grube R., Doležalová I. and Křístková E. (2007). In: Singh R. J. (Ed.) Genetic resources, chromosome engineering, and crop improvement, Vol. 3, Vegetable crops. CRC Press, Boca Raton, FL: 377– 472.

Liverworts from genus *Conocephalum* in Slovenia

Tilen Miklavčič, Tinka Bačič, Simona Strgulc Krajšek*

University of Ljubljana, Biotechnical Faculty, Department of biology, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
* corresponding author (simona.strgulc@bf.uni-lj.si)

In Europe, there are two species of liverworts from the genus *Conocephalum*: *C. conicum* (L.) Dumort and *C. salebrosum* Szweyk., Buczkowska & Odrzykoski. The latter was described in 2005 by Szweykowski et al. as a separated species from *C. conicum*. The later floristic researches revealed that the new species is present in almost all European countries, as in *C. conicum* (Hodgetts 2015). It was expected to be more widespread in the Mediterranean area (Ros et al. 2007).

Until our research in 2018, there were only two localities of *C. salebrosum* known in Slovenia (Martinčič 2017). Our goal was to collect samples of *Conocephalum* in Slovenia to get more complete distribution patterns of both examined species. We have collected samples of *Conocephalum* liverworts from about 40 localities in Slovenia. The discriminative characters from the publication of original publication (Szweykowski et al. 2005) were examined on fresh material. All specimens were stored in Herbarium LJU. The results revealed that *C. salebrosum* is very common in Slovenia, as was already predicted by Martinčič (2017) and Ros et al. (2007). Among the collected specimens, *C. salebrosum* was even more frequent as *C. conicum*. On some localities both species grow together, *C. conicum* usually on wet and very humid ground on water banks and wet soil, and *C. salebrosum* on less moist sites, as shaded rocks, moist sand and similar habitats.

The revision of the old herbarium material from Herbarium LJU is planned. The collection contains about 150 herbarium sheets, all identified as *C. conicum*. We expect that many of them are in fact *C. salebrosum*. This data will supplement the knowledge about the distribution of both species in Slovenia, especially in the regions, where we haven’t sampled recently.

Hodgetts N.G. (2015), Checklist and country status of European bryophytes – towards a new Red List for Europe. Irish Wildlife Manuals, No. 84. National Parks and Wildlife Service, Department of Arts, Heritage and the Gaeltacht, Ireland: 125 pp.

Martinčič A. (2017), Hladnikia 40, 26-39.

Ros R. M, Mazimpaka V., Abou-Salama U., Alefi M., Blockeel Th. L., Brugués M., Cano M. J., Cros R. M., Dia M. G., Dirkse G. M., El-Saadawi, W., Erdağ, A., Ganeva, Gonzáles-Mancebo, J. M., Herrnstadt, I., Khalil, K., Kürschner, H., Lanfranco, E., Losada-Lima A., Refai M. C., Rodriguez-Nunes S., Sabovljević M., Sérgio C., Shabbara H. M., Sim-Sim M. and Söderström L. (2007), Cryptogamie, Bryologie. 28, 351–437.

Szweykowski J., Buczkowska K. and Odrzykoski I. J. (2005). Plant Systematics and Evolution 253, 133–158.

Plant structure and function

Elemental and molecular imaging in plant biology

Primož Pelicon[1,*] , Primož Vavpetič[1], Boštjan Jenčič[1], Mitja Kelemen[1,3], Katarina Vogel-Mikuš[1,2], Paula Pongrac[1], Esther Punzon Quijoirna[1], Marjana Regvar[2]

[1] Jožef Stefan Institute, Jamova 39, SI-1000, Ljubljana, Slovenia
[2] Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia
[3] Jožef Stefan International Postgraduate School, Jamova cesta 39, 1000 Ljubljana, Slovenia
* corresponding author (primoz.pelicon@ijs.si)

Probing the elemental and molecular distributions in the biological tissue has particular significance for the research in biology, medicine, food science, pharmacy and forensics.

The distribution of elements in the biological tissue can be acquired by a sequence of tissue cryo-sectioning, pre-processing and analysis by X-ray emission. In the case of micro-Particle Induced X-ray Emission (micro-PIXE), tissue slice is irradiated with 3 MeV focused proton beam, and quantitative elemental distributions of the elements spanning in the periodic table from Na to U are obtained in the form of two-dimensional elemental maps, resembling conventional microscopy images (*Vavpetič et al, 2015*). The detection limit is in the order of 1 ppm and the lateral resolution is 600 nm, which makes this method very effective in determining major and trace-element distributions in plant tissue, such as cereal grains (*Detterbeck et al, 2016*).

Imaging mass spectroscopy techniques offer insight into complex biochemical processes by detection of specific biomolecules and their distributions. To be able to determine the localization of large biomolecules in tissue and cells, it is important to extract significant fractions of non-fragmented molecular ions, as this allows for unambiguous identification of the molecular species. A novel technique of molecular imaging, MeV-Secondary Ion Mass Spectrometry (SIMS has been developed at Jožef Stefan Institute (*Jenčič et al, 2016*), which is able to detect the distribution of molecules with masses up to 2,000 Da directly in a form of non-fragmented molecular ions, with lateral resolution of 800 nm.

Vavpetič P., Vogel-Mikuš K., Jeromel L., Ogrinc Potočnik N., Pongrac P., Drobne D., Pipan Tkalec Ž., Novak S., Kos M., Koren Š., Regvar M., Pelicon P. (20015). Nucl. Instrum. Meth. B 348, 147-151.

Detterbeck A., Pongrac P., Rensch S., Reuscher S., Pečovnik M., Vavpetič P., Pelicon P., Holzheu S., Kraemer U., Clemens S. (2016). New Phytol. 211, 1241-1254.

Jenčič B., Jeromel L., Ogrinc Potočnik N., Vogel-Mikuš K., Kovačec E., Regvar M., Siketić Z., Vavpetič P., Rupnik Z., Bučar K., Kelemen M., Kovač J., Pelicon P. (2016). Nucl. Instr. Meth. B 371, 205-210.

Plasticity of radial growth of trees from different environments

Jožica Gričar[1*], Peter Prislan[1]

[1] Slovenian Forestry Institute, Večna pot 2, Ljubljana, Slovenia
* corresponding author (jozica.gricar@gozdis.si)

Tree growth is intimately linked to the environment, which directly influence physiological processes. Trees continuously adjust their structure to fit the changing environmental conditions. In the processes of radial growth, which encompasses xylogenesis and phloemogenesis, these structural adjustments can be achieved. Therefore, wood and phloem anatomy can represent a good indicator of tree success and performance. Xylem formation has already proven to be influenced by environmental factors, whereas drivers of phloem formation and structure have generally been less investigated. Unlike wood formation, annual formation of phloem is crucial for tree survival as it maintains the translocation pathways for photosynthates from leaves to the tissues where growth and storage occur. In the presentation, various intra-annual patterns of xylem and phloem development of trees from different environments will be shown. Cambial rhythm and xylem and phloem anatomy of trees from temperate and (sub)Mediterranean will be presented to demonstrate the plasticity of their radial growth, which enables them to respond and adapt to changing environmental conditions. Finally, we will discuss how radial growth studies can complement with ecophysiological and dendroecological observations. Understanding how the secondary growth of trees responds to changing environmental conditions contributes to the major scientific challenges related to future tree performance under global climate change.

Cuny H. E., Čufar K., Prislan P., Gričar J. and et al. (2015). Nature Plants, 1-6.
Gričar J., Prislan P., Gryc V., Vavrčík H., De Luis M. and Čufar K. (2014). Tree Physiology 34 (8), 869-881.
Gričar J., Prislan P., De Luis M., Gryc V., Hacurová J., Vavrčík H. and Čufar K. (2015). Frontiers in Plant Science 6, 730, 13 pp.
Gričar J., Lavrič M., Ferlan M., Vodnik D. and Eler K. (2017). European Journal of Forest Research 136 (4), 625-637.
Gričar J., Prislan P., De Luis M., Novak K., Longares L. A., Martinez del Castillo E. and Čufar K. (2016). IAWA J. 37 (2), 349-364.
Lavrič M., Eler K., Ferlan M., Vodnik D. and Gričar J. (2017). Frontiers in Plant Science 8, 314, 11 pp.
Martinez del Castillo E., Gričar J., Prislan P., Čufar K. and et al. (2016). Frontiers in Plant Science 7, 370, 20 pp.
Novak K., De Luis M., Gričar J., Prislan P., Merela M., Smith K. T. and Čufar K. (2016). IAWA Journal 37 (2), 260-274.
Prislan P, Gričar J, De Luis M, Smith K T. and Čufar K. (2013). Agricultural and Forest Meteorology 180, 142-151.
Prislan P., Gričar J., De Luis M., Novak K., Martinez del Castillo E., Schmitt U., Koch G., Štrus J., Mrak P., Tušek-Žnidarič M. and Čufar, K. (2016). Frontiers in Plant Science 7, 1923, 15 pp.
Prislan P., Čufar K., De Luis M. and Gričar J. (2018). Tree physiology 38(2), 186-197.
Rossi S., Anfodillo T., Čufar K., Cuny H. E., Gričar J., Prislan P and et al. (2016). Global Change Biology 22 (11), 3804-3813.
Sass-Klaassen U., Fonti P., Cherubini P., Gričar J, Robert, E. M. R., Steppe K., Bräuning A. (2016). Front Plant Sci 7, 1069, 6 pp.

Abscission in tomato leaves and flowers

Aleš Kladnik[1*], Marko Chersicola[2,3], Magda Tušek Žnidarič[4], Tanja Mrak[5], Katja Stare[4], Amnon Lers[6], Marina Dermastia[4]

[1]University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia, [2]GenePlanet, Ljubljana, Slovenia, [3]Jožef Stefan International Postgraduate School, Ljubljana, Slovenia, [4]National Institute of Biology, Ljubljana, Slovenia, [5]Slovenian Forestry Institute, Ljubljana, Slovenia, [6]Agricultural Research Organization, Volcani Center, Israel
* corresponding author (ales.kladnik@bf.uni-lj.si)

Abscission is a process in which various plant organs, including leaves, flowers and fruits separate from the plant in a precisely controlled manner. Regulation of abscission is important in plant production. Abscission is a natural stage in plant development and occurs specifically in the tissue of the abscission zone (AZ). AZ is located at the base of the organ to be shed. It is composed of a few layers of differentiated cells that separate after induction by dissolution of the middle lamella due to the action of hydrolytic enzymes, such as polygalacturonases and celulases. Molecular processes in differentiation of the AZ, induction and execution of abscission are being discovered only recently. Plant hormones, mainly ethylene and auxins, are crucial in regulation of abscission. The enzyme 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase or ACO) catalyses the last step in ethylene biosynthesis. In tomato, there are seven ACO genes. We have analysed the expression of two genes with contrasting patterns of expression, *ACO1* and *ACO4*. *ACO1* is induced with abscission, while *ACO4* expression is high before abscission and decreases following abscission induction. ACO1 protein was localized using immunolocalization on light microscopy and ultrastructural level, where it was found primarily in vascular tissue in the phloem companion cells of the flower pedicel. The ethylene produced by ACO in the vascular tissue may be related to the diffusible signal responsible for abscission induction. We have shown that programmed cell death (PCD) is involved in abscission of tomato leaves and flowers and demonstrated that different processes in AZ are asymmetrically distributed between the proximal and distal side of AZ. After induction of abscission we observed high metabolic activity and membrane transport in cells of the proximal AZ in leaves, including the process of endoreplication. Progression of PCD in distal AZ was evident by DNA fragmentation, changes in the ultrastructure of cells and expression of RNase *LX*, nuclease *TBN1* and NADPH oxidase *RBOH1*.

Chersicola M., Kladnik A., Tušek-Žnidarič M., Mrak T., Gruden K., Dermastia M. (2017). 1-aminocyclopropane-1-carboxylate oxidase induction in tomato flower pedicel phloem and abscission related processes are differentially sensitive to ethylene. Frontiers in plant science, doi: 10.3389/fpls.2017.00464.

Chersicola M., Kladnik A., Tušek Žnidarič M., Lers A., and Dermastia M. (2018). The pattern of 1-aminocyclopropane-1-carboxylate oxidase induction in the tomato leaf petiole abscission zone is independent of expression of the ribonuclease-LX-encoding *LeLX* gene. Plant Biology, doi:10.1111/plb.12730.

Stability of variation: the case of tomato size in trusses

Tijskens L. M. M.[1*], Schouten R. E.[1], Dreessen S.[2], van Mourik S.[2], Dieleman A.[3]

[1]Wageningen University Horticulture & Product Physiology, [2]Wageningen University Farm Technology, [3]Wageningen University Green House Technology
* corresponding author (pol.tijskens@wur.nl)

Tomatoes grow in trusses. The first tomato in a truss is always closest to the source of photoassimilates. It is therefore to be expected that the size of tomatoes in a truss decreases from the first one to the last in the truss. Based on the same line of reasoning, the time of flowering in a truss is expected to reflect that same pattern.

All this common knowledge leads to the questions

1. Can the size of fruit in a truss be related to its location in that truss?
2. Can the time of flowering in a truss be linked to the size?
3. If so, then the variation over tomatoes in a truss should be very stable and reproducible.

The result of an extended project on the size of tomatoes and its variation will be presented. Size of tomatoes in a truss is shown to be related to location and time of flowering. That would imply that, at least in the stable and well-defined circumstances in a greenhouse, the size is more or less predefined already at the moment of anthesis. Based on this, the same can be assumed for the availability of photoassimilates.

Manipulation of the transitory starch granule number and morphology in *Arabidopsis thaliana*

Prof Dr habil Joerg Fettke

University of Potsdam, Biopolymer Analytics, Karl Liebknechtstr. 24/25, 14476 Potsdam-Golm, Germany
* corresponding author (fettke@uni-potsdam.de)

Starch plays an important role in the plant life cycle. Furthermore, starch is an important source for industrial applications. However, the process of starch granule formation is so far obscure. Neither the physic-chemical mechanisms of starch granule formation nor all necessary proteins/enzymes have been identified. Our results clearly illustrate that starch synthase 4 (AtSS4) and the plastidial phosphorylase (AtPHS1) are differently involved in starch granule formation and do not act in series. Besides these two enzymes, also proteins related to starch granule degradation have an impact on the starch granule formation, especially on the granule surface properties and morphology. I discuss the generation of starch granules and the formation of specific starch granule morphologies, as the alteration of starch granules has a significant impact on applications.

Ultrastructural changes in radish root tip after the exposure to rhizome extracts of *Fallopia japonica* and *F.* × *bohemica*

Katarina Šoln, Nada Žnidaršič, Jasna Dolenc Koce*

University of Ljubljana, Biotechnical Faculty, Dept. of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia
* corresponding author (jasna.dolenc.koce@bf.uni-lj.si)

Secondary compounds of invasive alien plants have high allelopathic activity. According to novel weapon hypothesis allelopathy presents one of their main advantages (Hierro and Callaway, 2003). The main source of secondary compounds in invasive Japanese (*Fallopia japonica*) and Bohemian knotweed (*F.* ×*bohemica*) are their rhizomes. Knotweed extracts are known to inhibit seedlings growth. The main target of their action is a primary root (Dolenc Koce and Šoln, 2018). The aim of our study was to investigate the tissue and cell structure in seedlings roots treated with knotweed extracts. Radish as a fast-growing species was selected as a test plant. 10% (w/v) aqueous extracts were prepared from Japanese (FJ) and Bohemian knotweed (FB) rhizomes. Distilled water was used as the control treatment. After 7 days of exposure, morphological and ultrastructural changes in root cells were analysed with light and transmission electron microscope (TEM).

Growth of treated radish roots was inhibited as the roots were up to 60% shorter compared to the control. Both extracts caused increase of the cell width and reduction of the cell length in cortex, resulting in increase of root diameter up to 38%. Also, number of cell columns in cortex was higher in knotweed-treated roots. Tissues were disorganized, especially the structure of the root cap and root meristem was altered so severely that it was difficult to delineate cell types. On the surface of many FJ-treated roots 5-6 layers of dead cells were observed. In the majority of treated roots, cells were irregularly shaped and cell membrane was detached from the cell wall. In the root cap cells nuclei were irregularly shaped, vacuoles were larger and more abundant, ring-shaped mitochondria were frequently observed and numerous dilated cisternae of granular endoplasmic reticulum were present. In some FJ-treated plants, root cap cells displayed ultrastructural characteristics indicating the programmed cell death. In most FJ-treated roots, the apical meristem was severely distorted and hard to define. In FB-treated roots, meristem was similar to control, except that cells contained many small vacuoles.

Hierro J. L. and Callaway R. M. (2003). Plant and soil 256, 29-39.
Dolenc Koce J. and Šoln K. (2018). Phyton 57 (1/2), 47-57.

Anatomical and physiological traits of *Quercus pubescens* Willd. from nearby sub-Mediterranean stands differing in soil and microclimate

Martina Lavrič[1], Dominik Vodnik[2]*, Mitja Ferlan[3], Klemen Eler[2], Jožica Gričar[1]

[1]Department of Yield and Silviculture, Slovenian Forestry Institute, Vecna pot 2, SI-1000 Ljubljana, Slovenia, [2]Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia, [3]Department of Forest Ecology, Slovenian Forestry Institute, Vecna pot 2, SI-1000 Ljubljana, Slovenia.
* corresponding author (dominik.vodnik@bf.uni-lj.si)

Pubescent oak (*Quercus pubescens* Willd.) typically grows in a Mediterranean-type climate region that among all European regions appears to be the most vulnerable to climate change. In Slovenia it is naturally present in SW, sub-Mediterranean part, on different soils: eutric cambisol on eocene flysch bedrock (deep, wet soil) and rendzic leptosol on paleogenic limestone bedrock (shallow, dry soil). Nearby oak stands with different soils and microclimate can be differently prone to drought and it is to expect that during the season trees there employ different mechanisms to optimise growth. In our research we were interested in water- and carbon-balance related anatomical and physiological responses of pubescent oak on different water availability on inter- and intra-annual level. For that purpose, we monitored leaf development, leaf gas exchange and leaf water potential, seasonal radial growth, xylem and phloem anatomy, non-structural carbohydrate (NSC) pools and xylem sap flow (SF) in two growing seasons, 2015 and 2016. We found that in both soils cambial activity started at the end of March and ended at the beginning of August; in the initial three weeks of radial growth phloem growth preceded that of xylem. About one month later first leaves appeared what coincided with the beginning of the initial earlywood vessel formation. In the period of intense radial growth and leaf development (i.e. April-May), highest values of free sugars in inner phloem (+cambium) were detected. On the other hand, highest amount of starch was observed in outer xylem at the end of growing season (in autumn). SF increased contemporarily with leaf development at the beginning of the growing season. During the growing season, SF was largely dependent on climatic factors, especially vapour pressure deficit and solar radiation and on the soil water content (SWC). On limestone bedrock SF rates exceeded ones measured on flysch bedrock, however, situation was reversed later in the summer when SWC was decreased and trees on limestone bedrock responded with stomatal closure and other drought stress coping mechanisms. Development of stress was delayed on flysch bedrock. Integrative structural-functional approach presented in this work revealed that soil properties substantially influenced secondary growth and xylem sap flow in the stem of *Q. pubescens*, whereas NSC amounts seemed to be less affected.

Vegetative reproduction of selected invasive alien plant species

Erazem Bahčič[1], Urban Čoko[1], Metka Škornik[1], Simona Strgulc Krajšek[2*]

[1] Gimnazija Bežigrad, Peričeva 4, SI-1000 Ljubljana, Slovenia
[2] University of Ljubljana, Biotechnical Faculty, Department of biology, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
* corresponding author (simona.strgulc@bf.uni-lj.si)

Invasive alien plant species are still present as ornamental or useful plants on many gardens. When gardeners decide to remove them, they throw the plant material into organic recycle bins or onto the compost heap and some even dispose the material in nature. Until recently there were neither collection centres for removed invasive plant species, nor is there a legislation that would lay down the procedure for their disposal.

We have decided to conduct research on how to handle the pieces of selected invasive plant species, with the aim of preventing their further dispersion. We have chosen eight species, which disperse either by growing adventitious roots from cut stem pieces (*Cornus sericea, Partenocissus quinquefolia* and *Buddleja davidii*), or with subterranean parts, such as rhizomes and tubers (*Solidago canadensis, S. gigantea, Rudbeckia laciniata, Helianthus tuberosus* and *Aster* sp.). We have subjected the biomass to three different storing conditions, namely drying, composting and storage in a black bin liner, all of which are easily created at home. Finally, we checked if this plant material planted in flower pots in controlled conditions developed new shoots or had died.

Drying and composting proved to be highly efficient, as the conditions killed the majority of the chosen plant species. Alternatively, storage in a bin liner proved to be highly inefficient because it neither killed nor stunted the growth of new roots in any of the chosen plant species.

Lately an increasing number of people have been using garden shredders to grind trees or shrub branches into smaller fragments for use of mulch. With this in mind, we conducted additional research on selected woody invasive plants (*Cornus sericea, Partenocissus quinquefolia, Buddleja davidii* and *Forsythia* × *intermedia*) to determine if their branch fragments could grow new roots and if the use of crushed branches could be one of the dispersal paths for woody invasive plant species. We compared the growth of the freshly ground fragments to the growth of the fragments that had been dried beforehand. We have observed that the dried fragments do not grow roots under any circumstances, while freshly ground fragments grow roots in some cases. From this we can infer that the use of mulch that has not been treated properly could foster dispersal of invasive plants.

Steps towards Revival of Moriculture in Slovenia – Biochemical Screening of Leaves of Centuries-Old Mulberry Varieties

M. Felicijan[1]*, P. Sušnik[1], T. Ugulin[2], A. Paušič[1], J. Rabensteiner[2], T.Bakonyi[3], R. Berčič[3], M. Mikulič Petkovšek[4], A. Urbanek Krajnc[1]

[1] Faculty of Agriculture and Life Sciences, University of Maribor, Slovenia
[2] Institute of Biology and Plant Sciences, University of Graz, Austria
[3] University of Veterinary Medicine Budapest, Department of Microbiology and Infectious Diseases, Hungary
[4] Biotehnical Faculty, University of Ljubljana, Slovenia
* corresponding author (mateja.felicijan@um.si)

Over centuries, in many European countries, including Slovenia, the white mulberry trees (*Morus alba* L., Moraceae) became an integral part of the cultural landscape. It was brought from Southeastern Asia in 12th century in order to establish a functioning European sericulture; since the leaves are the silk worm´s (*Bombyx mori* L.) preferred food. The cultivation of mulberries was introduced to Slovenia during the 16th century. It reached its peak in the mid-18th century, known as "The golden century of Slovenian sericulture". Despite the tradition of hundreds of years, the silk production in Europe decreasing and became irrelevant at the beginning of the 20th century because of the introduction of artificial silk [1-4]. Nevertheless, former sericultural regions retained a number of centuries old mulberry trees, which represent a still living cultural and natural heritage. The aim of the presented research was to collect data regarding geographical locations of historical mulberry trees in Slovenia and to screen the content of proteins and phenolics in leaves. During field excursions to different regions of Slovenia, we recorded exact GPS locations and collected leaf samples of more than 600 trees. The highest density of trees was recorded in submediterranean region, followed by subpannonean region and Southeast Slovenia. Wide variation in biochemical composition of mulberry leaves was observed between analysed genotypes. Mulberry leaves are shown to be rich in proteins (up to 16 g/ 100 g DW) containing threonine, asparagine, methionine, arginine, serine, glutamine, glycine and lysine as the most prominent amino acids. The total phenolic concentrations ranged from 0.7 to 2.3 g/ 100 g DW gallic acid equivalent. The main phenolic compounds were identified as caffeoylquininic acid derivatives, quercetin malonyl-hexoside, rutin, kaempferol acetyl-hexoside, quercetin-3-glucoside and *p*-coumaric acid derivatives. The statistical evaluation allowed us to present correlations between measured biochemical components with respects to pruning management and geographical distribution. The presented results will be the basic tools to defining superior high-yielding genotypes with optimum metabolic composition for silkworm feeding as well as for innovative usage in food processing and pharmaceutical industries.

[1] Deutsch H. Die Entwicklung der Seidenindustrie in Österreich 1660–1840, Wien Carl Konegen, 1909;16–17.
[2] Ipavec VM. Murve in kavalirji: Svilogojstvo na Goriškem. Ljubljana: Inštitut za slovensko narodopisje; 2008.
[3] Vijayan K, Jayarama Raju P, Tikader A, Saratchnadra B. Biotechnology of mulberry (*Morus* L.)–A review. Emir J Food Agric. 2014;26(6):472–496. http://dx.doi.org/10.9755/ejfa.v26i6.18019
[4] Žontar J. Svilogojstvo in svilarstvo na Slovenskem od 16. do 20. stoletja. Ljubljana: Inštitut za zgodovino SAZU; 1957.

Subcellular distribution of glutathione in wheat – a matter of light quantity and quality?

P13

Hauer Karina E.[1]*, Boldizsar Ákos[2], Möstl Stefan[1], Kocsy Gábor[2], Galiba Gábor[2], Zellnig Günther[1], Müller Maria[1]

[1] Institute of Biology, University of Graz, Austria;

[2] Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary

* corresponding author (karina.hauer@uni-graz.at)

Glutathione (γ-glutamyl-cysteinyl-glycine) plays as an antioxidant [1, 2] and a redox buffer [3] important protective roles under abiotic [4] and biotic stress conditions [5–7]. Glutathione biosynthesis is restricted to plastids and the cytosol and the product is distributed to the various organelles [8] by yet mainly unknown mechanisms. To determine the relative concentration of glutathione in different organelles immunogold cytohistochemistry based on anti-glutathione antisera and transmission electron microscopy (TEM) was used.

Leaves from *Triticum aestivum* plants cultivated under different light quantity [9] and quality as well as from *Arabidopsis thaliana* L. wild-type and mutant plants [10] grown under different light qualities [11] were investigated with a subsequent comparison of the results. Relative glutathione content and distribution differed between plants subjected to different light treatments (quality or quantity), between dicotyledonous (*A. thaliana*) and monocotyledonous (*T. aestivum*) and between selected thale cress mutants and wildtype plants.

Austrian FWF and the Hungarian National Research, Development Innovation Office (grants ANN 117949, TéT_15-AT-1-2016-0048) supported the work.

1 Anjum, N. A., Chan, M.-T. and Umar, S. (2010). Ascorbate-Glutathione Pathway and Stress Tolerance in Plants. Dordrecht: Springer Netherlands.
2 Hundal, T., Virgin, I., Styring, S. and Andersson, B. (1990). Biochim. Biophys. Acta (BBA) - Bioenergetics 1017 (3), 235–241.
3 Kocsy, G., Tari, I., Vanková, R., Zechmann, B., Gulyás, Z., Poór, P. and Galiba, G. (2013). Plant Sci. 211, 77–91.
4 Kocsy, G., Kobrehel, K., Szalai, G., Duviau, M.-P., Buzás, Z. and Galiba, G. (2004). Environ. Exp. Bot. 52 (2), 101–112.
5 Baltscheffsky, M. (Ed.) (2014). Current Research in Photosynthesis. Proceedings of the Viiith International Conference on Photosynthesis Stockholm, Sweden, Dordrecht: Springer Verlag.
6 Demmig-Adams, B. and Adams, W. W. (1992). Annu. Rev. Plant. Physiol. Plant. Mol. Biol 43 (1), 599–626.
7 Bela, K., Horváth, E., Gallé, Á., Szabados, L., Tari, I. and Csiszár, J. (2015). J. plant physiol. 176, 192–201.
8 Zechmann, B., Mauch, F., Sticher, L. and Müller, M. (2008). J. exp. Bot. 59 (14), 4017–4027.
9 Mishra, N. P., Fatma, T. and Singhal, G. S. (1995). Physiol. Plant. 95 (1), 77–82.
10 Heyneke, E., Luschin-Ebengreuth, N., Krajcer, I.; Wolkinger, V., Müller, M. and Zechmann, B. (2013). BMC plant biology 13, 104.
11 Rossel, J. B., Wilson, I. W. and Pogson, B. J. (2002). Plant physiol. 130 (3), 1109–1120.

The impact of mowing on the growth and blooming of common ragweed (*Ambrosia artemisiifolia*)

P14

Zala Krajšek[1], Špela Jakoš[1] Simona Strgulc Krajšek[2*]

[1] Gimnazija Šentvid, Prušnikova 98, SI-1210 Ljubljana–Šentvid, Slovenia
[2] University of Ljubljana, Biotechnical Faculty, Department of biology, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
* corresponding author (simona.strgulc@bf.uni-lj.si)

Common ragweed (*Ambrosia artemisiifolia*) is an invasive alien species originating in North America, that has spread widely in Europe in last decades. Its removal and prevention of its reproduction is mandatory for landowners in Slovenia, decreed by Slovene legislation. One of the removal methods is mowing, so we conducted a research project on the impact of mowing on the growth and propagation of the species.

We used the common ragweed plants that we collected in the spring at the road embankments and planted them in test pots. At the beginning of the summer, we cut the plants at three different heights, as if they were mowed at three different levels. A group of plants was left untreated and represented a control group for comparison with the test plants. We monitored the growth of plants until the end of September, by measuring the height of plants, the development of male inflorescences, and seed development.

We found out that the plants cut above the first pair of leaves were affected the most, while all the plants cut above the third pair, as well as plants of the control group, survived. Plants of the control group were the highest and had the highest dry mass at the end of the experiment as well. Contrary to expectations, differences in height and mass of test plants were negligible. Mowing has a major impact on the development of male inflorescences. Mowing above the 1st and 2nd pairs of leaves has reduced the overall length of male inflorescences to a third, while mowing over the 3rd pair almost halved the length of inflorescences. That resulted in a significant reduction in the amount of allergenic pollen. Altogether, most of the fruits (achenes) have grown on control plants. If we calculate the average number of fruits per plant, on average most fruits have developed on the plants, that were cut the lowest. A single mowing in a season, therefore, does not reduce the production of seeds, which is an important information for maintainers of public areas as well as landowners.

Monitoring of wood and phloem formation in beech and spruce

Peter Prislan[1*], Jožica Gričar[1], Maks Merela[2], Luka Krže[2], Angela Balzano[2], Jasna Štrus[3], Polona Mrak[3], Nada Žnidaršič[3], Martin de Luis[4], Magda Tušek Žnidarič[5], Katarina Čufar[2]

[1] Slovenian Forestry Institute, Večna pot 2, Ljubljana, Slovenia
[2] University of Ljubljana, Biotechnical Faculty, Department of Wood Science and Technology, Ljubljana, Slovenia
[3] University of Ljubljana, Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia
[4] University of Zaragoza, Department of Geography and Regional Planning, Zaragoza, Spain
[5] National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana, Slovenia
* corresponding author (peter.prislan@gozdis.si)

Cambial activity and radial growth was monitored over a 10-year period in European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) Karst.) at two contrasting sites in Slovenia; a lowland temperate Panška reka near Ljubljana (400 m a.s.l.) and an Alpine site on Menina planina (1200 m a.s.l.). Samples (micro cores) were collected from mature tree stems with a Trephor tool at weekly intervals during the growing seasons. Histological slides of cross-sections containing xylem, cambium and phloem were prepared according to standard procedure and observed with light microscope. The numbers of cells in the cambium, as well as current xylem and phloem increments, were determined. Phenological phases of xylem and phloem formation were recorded as day of the year (DOY), including: onset of cell production, onset of secondary cell wall formation and lignification, the occurrence of first mature cells, cessation of cell production, as well as cessation of lignification and transition from early to late phloem. Ultrastructural changes in cambial cells (between dormant and active state) and the development of xylem cell walls were observed with transmission electron microscope (TEM).

Weather conditions (temperature and precipitation) and xylem formation phenology were compared between sites and years to evaluate weather-growth relationships. Xylem formation seemed to be more affected by environmental factors than phloem formation. Average weather conditions before occurrence of observed phenological phases significantly differed between sites indicating high intra-specific plasticity of beech and spruce. Results also showed that precipitation is not a limiting factor for xylem growth in beech and Norway spruce at the two temperate study sites. At the ultrastructural level we observed changes in cell organelle size, distribution, number and shape. The changes could be observed one month before occurrence of newly formed xylem cells. This confirmed that classical methods of wood formation studies based on fixation with FAA and observations in the light microscope allow us to follow cambial cell production rather than real cambial activity. Thus, such information enables more precise interpretation of wood formation data. The obtained data provide a great potential for studying tree productivity and survival under climate change.

Scots pine (*Pinus sylvestris* L.) needles as a case study for element-specific tissue clustering

P16

Paula Pongrac[1,2,] , Edita Baltrenaite[3], Primož Vavpetič[1], Mitja Kelemen[1], Aleš Kladnik[2], Bojan Budič[4], Katarina Vogel-Mikuš[1,2], Marjana Regvar[2,]*, Pranas Baltrenas[3], Primož Pelicon[1]

[1]Jožef Stefan Institute, Jamova cesta 39, SI-1000 Ljubljana, Slovenia
[2]Biotechnical Faculty, University of Ljubljana, Jamnikarjeva ulica 101, SI-1000 Ljubljana, Slovenia
[3]Vilnius Gediminas Technical University, Vilnius, Lithuania
[4]National Institute of Chemistry, Hajdrihova ulica 19, SI-1000 Ljubljana, Slovenia Address
* corresponding author (marjana.regvar@bf.uni-lj.si)

Chemical diversity of plant tissues arises as a consequence of tissue differentiation being taken for granted. Yet, functional importance of diverse tissue biochemistry is still far from understood. The main aim of the present study was to reveal typical element profiles of tissues within the Scots pine needles and their qualitative and quantitative clustering with regard to their elemental biochemistry. Tissue-specific element profiles acquired using micro particle induced X-ray emission (μ-PIXE) available at the Microanalytical centre of the Jožef Stefan Institute. Spectral analysis and generation of numerical matrices (pixel-by-pixel concentration matrices) were generated by GeoPIXE II software package. These numerical matrices were used to extract element concentration profiles using ImageJ from which relative element distribution was calculated for each of the needle tissues (epidermis, mesophyll, endodermis, transfusion parenchyma, sclerenchyma, xylem, phloem, transfusion tracheids, Strasburger cells). Clustering analysis and hyperspectral image depicting tissues with distinctive element profiles were generated using Orange 3.13 software. Using this method, four tissue clusters were differentiated, based on Ca, K, Zn and Mg-S-Mn composition, indicating large differences in their functional significance for needle physiology.

Isoflavone content of selected Slovenian red clover accessions

Tanja Zadražnik[1], Špela Velikonja Bolta[2], Klavdija Poklukar[2], Lovro Sinkovič[2], Vladimir Meglič[2], Jelka Šuštar Vozlič[2]*

[1]University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Jamnikarjeva 101, SI-1000 Ljubljana
[2]Agricultural Institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana
* corresponding author (Jelka.vozlic@kis.si)

Red clover (*Trifolium pratense* L.) is an important forage crop for its crude protein and digestible fibre content. It also contains several phenolic compounds including isoflavones, natural phytoestrogens that are known for their beneficial effect in metabolic disorders. Over the past decade, isoflavones have been extensively studied in humans due to their estrogenic or anti-estrogenic effect and potential impact on human health. The aim of the study was to quantify selected isoflavones in 18 ecotypes of red clover, collected in different parts of Slovenia and stored in the gene bank of the Agricultural Institute of Slovenia. In addition, five commercial cultivars were included in the study. Methanolic extracts obtained by ultrasound-assisted extraction from above ground parts of the plants were used for the analysis by LC-MS/MS. The total isoflavone content in the analysed accessions varied considerably, from 177±89 µg/g dry weight (DW) to 1501±329 µg/g DW. The highest values were determined for ononin with its average content of 506 µg/g DW, while for formononetin and biochanin A concentrations vary greatly between and within the accessions. Genistin and prunetin were present in smaller contents, with the average content of 40 and 26 µg/g DW, respectively. The contents of daidzein and genistein were in many samples below the limits of detection. Glycitin was not detected by LC-MS/MS in our study. The data of total isoflavone content show significant differences between and within the accessions of red clover from the Slovene gene bank. Low levels of isoflavone compounds found in our study suggest that red clover may be a valuable source for applications in agronomy where low contents of isoflavones are desired.

Flow cytometric seed screen in diploid species of the genus *Onosma*

Dominika Vašková and Vladislav Kolarčik*

Univerzita P. J. Šafárika v Košiciach, Ústav biologických a ekologických vied, Katedra botaniky, Mánesova 23, 04154 Košice, Slovakia
* corresponding author (vladislav.kolarcik@upjs.sk)

In the double fertilization process, that has evolved in angiosperms, diploid embryo (2C) and triploid endosperm (nutritive tissue, 3C) have originated typically in sexual diploids. The flow cytometric seed screen (FCSS) was developed to analyse modes of reproduction in plants based on the endosperm to embryo DNA content ratio. A ratio of 1.5 represents a sexual mode of reproduction (3C:2C), whereas higher values of ≥ 2.0 indicate an asexual origin of the seed progeny, e.g., a ratio of 3.0 can be interpreted as a result of pseudogamous apomixis. In this study, 127 seeds of four diploid species of the genus *Onosma* (*O. thracica*, *O. stojanoffii*, *O. pavlovae* and *O. heterophylla*) distributed in the Balkan Peninsula were evaluated using FCSS. The ratio of 1.5 was determined in 31 seeds, the ratio of 3.0 in 75 seeds and the ratio of 5.0 was found in one seed. Finally, the endosperm peak was not detected in FCSS measurements of 20 seeds. These findings can be interpreted as a result of common endosperm endopolyploidisation in the early stages of its development rather than apomixis, which is present rarely in the plant kingdom at the diploid level. Both types of seeds with non-endopolyploid and endopolyploid (the ratio ≥ 3.0) endosperm were found among progeny of individual mother plants. At the population level, the frequency of seeds with endopolyploid endosperm was 39-93%.

Photosynthetic traits of two *Canabis sativa* breeding lines

Dominik Vodnik*, Jan Eržen, Damijana Kastelec, Marko Flajšman, Borut Bohanec, Jana Murovec

Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Slovenia
* corresponding author (dominik.vodnik@bf.uni-lj.si)

Despite its pharmacological importance and potential for bio-economy cannabis (*Cannabis sativa* L.) has been relatively poorly studied in terms of its physiology, response to environmental factors and linkage of physiology to biochemical traits. The exact scientific reports in peer-reviewed literature are very rare which holds true also for cannabis photosynthesis, a key process supporting growth and development, influencing also chemical composition of plants. Since photosynthetic characteristics vary between genotypes and clones of plants our study aimed to compare photosynthetic traits of two *C. sativa* breeding lines (MX-CBD-11 and MX-CBD-707) from a joint research project "Breeding medical cannabis (*Cannabis sativa* L.)" between MGC Pharmaceuticals and Agronomy Department of the Biotechnical Faculty, University of Ljubljana.

Measurements were performed in mid-January when plants were in the vegetative stage, additional measurements of instantaneous photosynthetic rate were made in March at flowering stage. We used Li6400xt (LiCor, USA) measuring system and analysed the following photosynthetic traits 1) photosynthetic rate under ambient, i.e. growth chamber conditions (A), 2) photosynthetic capacity (A_{max}), 3) photosynthetic light response (AQ_i) curves, curves, and 4) photosynthetic CO_2 response (AC_i) curves. Beside gas-exchange measurements, we assessed photochemical efficiency, analysed the content of photosynthetic pigments and measured some morphological parameters.

At vegetative stage, the photosynthetic rates of MX-CBD-11 and MX-CBD-707 were 9.76 ± 0.91 µmol CO_2 m^{-2} s^{-1} and 11.57 ± 0.91 µmol CO_2 m^{-2} s^{-1}., respectively. Similarly, higher photosynthetic rates in MX-CBD-707 compared to MX-CBD-11 were found at reproductive stage (10.69 ± 1.53 vs. 8.00 ± 0.91 µmol CO_2 m^{-2} s^{-1}). In both breeding lines, photosynthesis was saturated at PAR of ca. 600 µmol photons m^{-2} s^{-1}. Under saturating light and elevated CO_2 concentration (1000 ppm CO_2) photosynthesis (A_{max}) sometimes exceeded 35 µmol CO_2 m^{-2} s^{-1}, which is slightly higher than the values reported for some other *C. sativa* varieties in the literature. Average A_{max} was round 30 µmol CO_2 m^{-2} s^{-1}, and there were no significant differences between the breeding lines. This suggests that both breeding lines have similar photosynthetic capacity but differ in the photosynthetic activity when exposed to given chamber conditions. In the paper, we discuss Rubisco carboxylation capacity (V_{cmax}), triose phosphate utilization capacity (T_p) and day respiration (R_d), i.e. parameters derived from the AC_i models. Besides, other physiological traits (photochemical efficiency, transpiration, water use efficiency…) are presented and discussed with respect to the biochemical profiles of the plants.

Plant interactions with environment

Do ABA and miR156 mediate the effects of strigolactones on drought stress tolerance and reproductive development?

IL7

Schubert A[1*], Cardinale F[1], Korwin[1] P, Russo[1] G, Macchio M[2], Visentin I[1,2]

[1]University of Turin, Dept. Agricultural, Forestry, and Food Sciences, Grugliasco, Italy
[2] StrigoLab Srl, Turin, Italy
* corresponding author (andrea.schubert@unito.it)

Regulation of plant growth and reproduction must integrate with signal of environmental stress. Besides acting as ecological communicators with (micro)organisms in the rhizosphere, the recently discovered strigolactone (SLs) phytohormones induce primary and secondary growth of the main shoots, affect root morphology improving soil exploration, regulate reproductive development in certain species, and tune these effects to conditions of water or nutrient deprivation. SLs are predominantly synthesized in the roots and their concentration in the shoot is normally below the detection limit, thus their biological roles are mostly elucidated by the use of biosynthetic/signalling mutants or treatments with the artificial SL analogue GR24. The roles of SLs on drought stress tolerance and on reproductive development have been most studied in the Solanaceae, in particular tomato. Such effects of SLs are often linked with perturbations of the ABA message, involving both changes in ABA concentration but also modification of ABA transmembrane fluxes. Furthermore, SLs activate expression and increase the concentration of miR156, a pivotal hub of the control of reproductive development and tolerance to abiotic stress in plants. Not surprisingly, miR156 overexpressors mimic some SL- dependent phenotypes, in particular within the context of drought responses. The next challenge is to identify and better characterize mediators of these interactions in order to dissect their mechanistic integration.

Drought stress and xylem embolism: resistance and resilience of forest trees to climate change

Andrea Nardini

Department of Life Sciences, University of Trieste, Via L. Giorgieri 10, 34127 Trieste, Italia
* corresponding author (nardini@units.it)

The frequency and severity of drought and heat waves has increased in recent years over South Europe, with summer seasons 2012, 2015 and 2017 breaking new records in terms of temperature and precipitation anomalies over the last 200 years. These events produced severe impacts on forest trees, in terms of increased rates of dieback/mortality. Projected climate scenarios urge toward a better understanding of the physiological basis of plant resistance/resilience to drought.

Water is transported from roots to foliage under negative pressure (= tension), making the hydraulic system of trees vulnerable to cavitation and embolism. Xylem vulnerability to embolism and its anatomical and physiological correlates are key traits affecting species-specific drought resistance. In fact, hydraulic failure has emerged as the most ubiquitous cause of tree death under drought.

Even when non-lethal, drought-induced xylem embolism induces stomatal closure, leading to consumption of non-structural carbohydrates and potentially carbon starvation. In turn, non-structural carbohydrates might be important to restore the hydraulic functioning of trees after drought relief, but the underlying mechanism is controversial and currently debated.

Effect of environmental factors on tree rings of Mediterranean species: investigation of xylogenesis and formation of irregularities in the wood

Angela Balzano[1], Veronica de Micco[2], Giovanna Battipaglia[3], Maks Merela[1], Katarina Čufar[1*]

[1] Biotechnical Faculty, Department of Wood Science and Technology, University of Ljubljana, Ljubljana, Slovenia
[2]University of Naples Federico II, Department of Agricultural Sciences, Portici (Naples), Italy
[3] University of Campania Luigi Vanvitelli, Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Caserta, Italy
* corresponding author (katarina.cufar@bf.uni-lj.si)

In the Mediterranean increasing risk for drought stress and heat waves due to the ongoing climate change may impact wood formation in trees and forest productivity. Mediterranean woody species record environmental conditions in the wood (and its anatomical functional traits). This often results in changes in wood density within the tree ring, called Intra-annual Density Fluctuations (IADFs). The analysis of IADFs may provide accurate information on plants' growth responses to climate, encoding the anatomical intra-annual variability. To link IADFs with climate variables that trigger their formation, it is necessary to consider the various phases of xylogenesis (wood formation) occurring in different species in various environments. In this context, we monitored xylogenesis in *Pinus halepensis* Mill., *Pinus pinea* L. and *Arbutus unedo* L. at sites near Naples, Italy characterized by Mediterranean climate. The aims were to: identify the xylogenesis calendar; analyze the types of IADFs and the timing of their formation to evaluate which factors trigger their formation. To achieve these aims, microcores were collected weekly from eight trees of each species. They were prepared for light microscopy to observe the following cells and phases of cell development: cambial cells, post cambial cells, cells with developing secondary wall and mature cells with lignified secondary wall. Moreover, we applied traditional dendrochronological analysis for IADF identification and tree-ring dating. We found that all species formed the same type of IADFs (early wood-like cells within latewood) which were promoted by rainfall in autumn following a period of summer drought. Moreover in *P. pinea*, we found that successive tree rings were not always marked by a sharp boundary as in temperate trees. This is possibly due to the lack of a true cambial dormancy. In *A. unedo*, the formation of more than one IADF was detected within the tree ring formed in one year. In conclusion, the occurrence of IADFs in tree rings suggests high adaptation capability of the investigated species to frequent intra-seasonal variations of water availability, which is typical for trees growing on the Mediterranean sites. This can be considered a competitive advantage compared to those species, which are less prone to form IADFs. These findings assume considerable importance of wood anatomy to understand and forecast growth and adaptation capability of trees and thus forests suffering environmental changes.

Effects of LED spectra in combination with biochar amendment on *Pisum sativum* L. and *Arabidopsis thaliana* L. growth

Polzella Antonella[1*], Montagnoli Antonio[2], Terzaghi Mattia[2], Trupiano Dalila[1], Chiatante Donato[2], Scippa Gabriella Stefania[1]

[1]Department of Biosciences and Territory, University of Molise, Pesche, Italy
[2]Department of Biotechnology and Life Science, University of Insubria, Varese, Italy
[*] Corresponding author e-mail: a.polzella@studenti.unimol.it

Light and nutrients availability are the primary factors for plant growth and development. Artificial light sources can provide an enhancement or a decrease in crop yield and quality (Darko et al., 2013). Among artificial lighting sources, LED lighting represents an increasingly promising technology for a series of advantages (Wu et al., 2006). Besides a suitable light source, the plant also needs a soil type characterized by a good nutrient availability, which is not provided from synthetic fertilizers that have a negative impact on the environment. Biochar is a natural charcoal obtained by a controlled pyrolysis of organic materials, occurring at high temperatures and in an oxygen-deficient environment. The potential use of the biochar as a soil amendment can be considered as a mean for mitigating greenhouse gases emissions that increasingly contribute to the current global climate changes (D'Alessandro et al., 2010). Light-emitting diodes (LEDs) together with biochar soil amendment could be a potential combined strategy to improve indoor plant cultivation contributing to the mitigation of current climate changes. Therefore, in the present study we investigated the morphological changes at root and leaf level of Arabidopsis (*Arabidopsis thaliana,* Columbia - Col-0) and pea (*Pisum sativum* L.) plants grown in presence/absence of biochar and with either fluorescent light (Fluora T8, OSRAM) as reference (control) light, or one of three different LED light spectra (Valoya Oy; Helsinki, Finland): AP67, AP67-3L, and NS1. We also evaluated the plant photosynthetic machinery efficiency and stomata activity by analysis of chlorophyll fluorescence emission and stomatal conductance. Results clearly showed a species-specificity of both biochar and light spectra. Nevertheless, the AP67 light spectrum shows the best potentiality to improve plant growth and productivity, which in turn are improved with biochar addition.

D'Alessandro D. M., Smit B., Long J. R. (2010). Angewandte Chemie International Edition 49: 6058-6082.
Darko E., Heydarizadeh P., Schoefs B., Sabzalian M. R. (2013). Philosophical Transactions of the Royal Society B 369:20130243.
Wu M. C., HouC. Y., Jiang C. M., Wang Y. T., Wang C. Y., Chen H. H., Chang H. M. (2006). Food Chemistry 101:1753-1758.

Selenate and selenite impact on mercury uptake and toxicity in plants

SL12

Anja Kavčič[1*], Katarina Vogel-Mikuš[1,2]

[1]University of Ljubljana, Biotechnical faculty, Jamnikarjeva 101, SI-1000 Ljubljana
[2]Institute Jožef Stefan, Jamova 39, SI-1000 Ljubljana
* corresponding author (anja.kavcic@bf.uni-lj.si)

Mercury (Hg) is one of the most hazardous pollutants because of its high toxicity and persistent accumulation in the environment and consequently in the food webs. Plants can uptake Hg from water and soil via roots. Majority of Hg accumulates locally in the plant with low mobility, but small portions may be transported to the shoots. Nevertheless, a few studies have suggested that selenium (Se) can reduce Hg accumulation in plants. Furthermore, the studies indicated that both Se species, selenite ((Se(IV)) and selenate (Se(VI)), were equally effective in decreasing Hg accumulation by plants. However, there is still many questions opened, especially about Se dose, exposure time and Se tissue speciation after root or foliar application to the plants.

In this study we aimed to explore the potential effects of Se species (potassium selenite and potassium selenate) on Hg uptake into the roots and its translocation to the shoots under acute (one week) and chronic (four weeks) exposure to Hg.

Maize and sunflower plants were grown hydroponically in aerated Hoagland solution for one or four weeks. Solutions contained 2 μM Se(IV) or Se(VI) or a combination of 10 μM $HgCl_2$ and 2 μM Se(IV) or Se(VI). To evaluate Hg phytotoxicity at the end of acute/chronic exposure to Hg, physiological parameters such as chlorophyll content and lipid peroxidation were evaluated. Fresh and dry biomass of roots and shoots was measured.

Regardless of Hg treatment and time of exposure, plant chlorophyll contents were higher when Se(IV) was present in the Hoagland solution compared to the solutions without added Se. The lipid peroxidation of shoots and roots (with an exception of sunflower roots) was the lowest when no Se was added and the highest when Se(VI) was present in the solution. Biomass of roots and shoots was the lowest in Se(VI) treatments. Results also showed a retention of Hg in roots in both plant species. The retention of Hg in roots was higher if Se(IV) was added to solution. Consequently, in shoots both Hg and Se concentration were higher when plants were treated with Se(VI).

We conclude that addition of different Se species into nutrient solution affects Hg accumulation in plant roots and its transportation to the shoots. The addition of different Se species affects Hg uptake and toxicity in plants.

Salinity tolerance in a wheat mutant (Rht-B1c) with altered DELLA proteins

Maida Jusovic[1,2]*, Maya Y. Velitchkova[1], Svetlana P. Misheva[3], Anelia G. Dobrikova[1]

[1] Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 21, 1113 Sofia, Bulgaria
[2] University of Chemical Engineering and Metallurgy, 8 Kliment Ohridski Blvd., 1756 Sofia, Bulgaria
[3] Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 21, 1113 Sofia, Bulgaria
* corresponding author (maida.jusovic@gmail.com)

Soil salinity is one of the most severe factors that significantly affects plant growth and photosynthetic rate. As the wheat production in the world should be continuously increased, it is very important to improve the salt tolerance of wheat genotypes. In this study we investigated the impact of the salt stress, using two different concentrations of NaCl: 100 mM (equivalent to an electrical conductivity of 10.5 mS cm^{-1}) and 200 mM (equivalent to an electrical conductivity of 18.6 mS cm^{-1}), on the photosynthetic apparatus and the growth parameters of two near-isogenic wheat lines: *Rht-B1a* (wild-type) and *Rht-B1c* (dwarf mutant with modified DELLA proteins), grown on a half-strength Hoagland solution. Results revealed that salt toxicity was mitigated in the *Rht-B1c* mutant compared to the *Rht-B1a* wild-type, as demonstrated by the less-reduced growth parameters, relative water content, chlorophyll and carotenoid content as well as photochemical activity of photosystem II (PSII) and photosystem I (PSI) after 2, 7 and 9-day salt treatment of plants. For the first time, our data demonstrated that gibberellin (GA)-insensitive DELLA proteins increase the tolerance of wheat plants to salt stress at the early seedling stage as the mutant tolerance becomes more evident at higher salt concentrations and after long-term treatment for 9 days. In addition, compared to the wild-type wheat, the DELLA mutant plants showed a higher capacity for cyclic electron flow around PSI under salt treatment, thus preventing the photosynthetic apparatus from oxidative damage during exposure to different salt concentrations. Data suggested changes in thylakoid membrane proteins and/or their structural reorganization in the wheat DELLA mutant, which significantly contribute to the alleviation of salt-induced damage of the photosynthetic apparatus. Our previous work (Jusovic et al. 2018) showed that the long-term acclimation of mutant plants to higher salt concentrations (200 mM NaCl) included an increase of the PSI reaction center proteins (PsaB) and no changes in PSII reaction center proteins (D1), thus supporting the higher salt-resistance of photosynthetic activities of both photosystems. The observed better tolerance of the wheat DELLA mutant to higher salt concentrations can recommend it to be a suitable candidate for improving salt tolerance of wheat.

Endopolyploidy analysis in seedlings of four crop species growing under exogenous application of phytohormone gibberellic acid

Valéria Kocová, Vladislav Kolarčik*, Lukáš Koprivý, Viera Fraková and Pavol Mártonfi

Univerzita P. J. Šafárika v Košiciach, Ústav biologických a ekologických vied, Katedra botaniky, Mánesova 23, 04154 Košice, Slovakia
* corresponding author (vladislav.kolarcik@upjs.sk)

Endopolyploidy, repeated duplications of the nuclear genome within the cell without cytokinesis, is a common part of initial developmental stages in many plant species. The level of endopolyploidy (EL) is a result of interaction between several factors, e.g. systematic position, ploidy level, genome size and developmental stage of plant species, tissue and organ type, environmental conditions, biotic and abiotic stress. High EL is typical for cells of plant tissues with high metabolic activity (e.g. endosperm, cotyledons, tapetum), and for cells, which differentiation requires progressive cell growth (e.g. trichomes, cells of transport tissue, cells of pericarp). The character, level and extent of endopolyploidy at the tissue and organ level are rarely studied in comparative experiments. Phytohormones play a crucial role in the regulation of plant growth, metabolism and morphogenesis. They may have an impact on endopolyploidy level in plant organs. Generally, auxins and gibberellins (GA) enhance EL, while cytokinins have opposite effect. Contradictory results were obtained from experimental studies of GA effect on EL.

In the present study GA effect on EL was evaluated in seedlings of four crop species, *Trifolium pratense* cv. Dajana, *Solanum lycopersicum* cv. Stupické polní tyčkové rané, *Raphanus sativus* cv. Saxa and *Lepidium sativum*. Plants germinated and were cultivated in hydroponics with the addition of GA (0 M, 10^{-7} M a 10^{-5} M concentration). We used flow cytometry measurements of propidium iodide stained nuclei to evaluate EL in roots, hypocotyls and cotyledons of studied plants.

We did not record significant effect of GA on EL in organs of four studied plants with exception of root in *L. sativum*. In this case, application of 10^{-7} M GA led to increase of EL, while 10^{-5} M GA has no effect on EL compared to control. It suggests that GA did not effect EL significantly, but recorded increase of EL in roots of *L. sativum* may deserve further study. In general, the endopolyploidy level and its extent were similar in *R. sativus* and *L. sativum*, which probably reflects their phylogenetic relatedness. The highest EL was recorded in hypocotyls and cotyledons of *R. sativus*, followed by EL in hypocotyls and cotyledons of *L. sativum*. High EL in cells, which are not typically endopolyploid is common for representatives of the family Brassicaceae.

Elevational limits of *Lactuca serriola* L. and *L. virosa* L. in Central Chile

E. Křístková*, A. Lebeda

Palacký University in Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 27, 783 71 Olomouc – Holice, Czech Republic
* corresponding author (eva.kristkova@upol.cz)

The occurrence, distribution and character of populations of two spontaneous, Old World *Lactuca* species were monitored on 175 sites in Central Chile (southern part of region 4, and regions M, 5, 6, and 7) in 2016 and 2017. The occurrence of a single allochthonous species, *L. serriola,* was recorded on 144 sites, and another, *L. virosa*, was observed exclusively on 16 sites. Presence of both species was observed on 14 monitoring sites. *Lactuca serriola* was recorded equally in urban areas (in pavement, along local roads in suburbs, in areas with house construction, in ruderal sites) and along transport corridors (railway tracks, roads, highways) outside of cities. Population sizes varied from few (cca 5 to 10) plants to hundreds of individuals. *Lactuca virosa* was recorded most frequently along roads outside of cities, with several individuals to as many as dozens of plants at one site.

The potential for species succession was observed along three roads with differing elevational gradients. Along R 60 (Los Andes – Portillo), *L. serriola* was recorded until an elevation of 2 235 m a.s.l.; at higher elevations of 2 365 - 2 707 m a.s.l., it was replaced by sparsely distributed plants of *L. virosa* along road. *Lactuca serriola* and *L. virosa* were both present along road G-27 (Cajón Maipú) from 903 m a.s.l. to 1 315 m a.s.l. (monitoring at higher elevations was technically impossible). Along road 115 (Lago Colbún – Lago del Maule), *L. serriola* was recorded alone at 1 057 m a.s.l., and then together with *L. virosa* on slopes above 1 139 m a.s.l., until at 1 361 m a.s.l., only *L. virosa* was recorded. From there, dense populations of *L. virosa* were observed continuously at higher elevations, up to 2 148 m a.s.l. in a gravel plateau over the Rio Maule, where relatively dense and stable populations of dozens of plants persisted and could regenerate. This phenomenon has not been observed in Europe under comparable eco-climatic conditions.

These observations documented that both allochthonous (Eurasian) wild *Lactuca* species (*L. serriola*, *L. virosa*) occur and regenerate in Central Chile, where *L. serriola* frequently forms dense populations in urbanized areas. Both species can expand along transport corridors to high elevations; resulting in the spread of *L. virosa* in dense populations even in extreme habitats above 2 000 m a.s.l. These species should be monitored (and treated as potentially invasive weeds) in urbanized and agricultural areas, either in unique local mountainous biotops.

This research was supported by the following grants: MSM 6198959215 (Ministry of Education, Youths and Sports, Czech Republic) and Internal Grant Agency of Palacký University in Olomouc (IGA_PrF_2018_001).

Response of the *Brassicaceae* seedlings on short-term salt stress and role of specialized metabolites

[1]Ida Linić*, [1]Dunja Šamec, [2]Valerija Vujčić, [2]Sandra Radić Brkanac, [3]Jiri Gruz, [1]Branka Salopek-Sondi

[1]Ruđer Bošković Institute, Department of Molecular Biology, Zagreb, Croatia
[2]University of Zagreb, Faculty of Science, Zagreb, Croatia
[3]Palacký University and Institute of Experimental Botany ASCR, Laboratory of Growth Regulators, Olomouc, Czech Republic
* corresponding author (ilinic@irb.hr)

Brassicaceae crops are among the oldest known cultivars and their use is widespread. Abiotic stress, such as increased salinity, and oxidative stress are serious threats to agriculture and the natural status of the environment. Increased salinity may cause nutrient deficiencies or imbalances, as well as reduced yield, growth and development of plants depending on their ability to overcome the effects of stress. The ability of plants to tolerate salt is determined by multiple biochemical pathways, those that lead to the synthesis of specialized metabolites and support scavenging of reactive oxygen species (ROS). For this reason, it is important to understand how *Brassicaceae* crops struggling against increased salinity. The aim of our research was to evaluate the short-term stress responses of three Brassica species/varieties (*Brassica rapa* spp. *pekinensis*, *B. oleracea* var. *capitata*, and *B.oleracea* var*. acephala*) exposed to saline stress. In order to determine the impact of the salt stress, one-day old seedlings were treated with NaCl (in range of conc. 50-200 mM), in comparison to corresponding controls, during 24h. The physiological effect of treatments was determined in vivo using the root growth assay. The amount of ROS (SO, H_2O_2) and GSH in seedlings is determined in vivo using a fluorescent microscope connected to the camera after the seedlings are stained with specific dies (dihydroethidium (DHE), dichlorodihydrofluorescein diacetate (DCFH-DA), and monochlorobimane (MCB)). Proline and specialized metabolites were determined using UV/VIS spectrophotometry, while phenolic acids profiles were determined by UHPLC-MS/MS. Ultimately, results indicate that stress response is species specific and depends on the salt concentration. The treatment of seedlings with different concentrations of NaCl cause gradual inhibition of biomass accumulation and root growth where *B. rapa* spp. *pekinensis* proved to be the most sensitive species while *B. oleracea* var. *acephala* the most tolerant one. Seedlings which are under stress conditions produce higher levels of ROS molecules (SO, H_2O_2) in comparison to the control, mostly in a dose-dependent manner. Stressful conditions also affect at the level of osmoprotectant proline, GSH as well as phenolic antioxidants. Profiles of phenolic acids were determined and their fluctuations upon salinity stress were discussed in comparison to seedlings growth inhibitions and stress tolerance of three *Brassica* varieties.

The influence of grafting on morphological properties of tomato

Marija Mandušić[1,*], Gvozden Dumićič[1], Branimir Urlić[1], Gabriela Vuletin Selak[1], Maja Jukić Špika[1], Dragan Znidarčič[2], Smiljana Goreta Ban[3], Katja Žanić[1]

[1]Institute for Adriatic Crops and Karst Reclamation, Put Duilova 11, Split, Croatia
[2]Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, Ljubljana, Slovenia
[3]Institute of Agriculture and Tourisam, Karla Huguesa 8, Poreč, Croatia
*corresponding author (marija.mandusic@krs.hr)

Grafting of tomato (*Lycopersicon esculentum* Mill.) seedlings onto selected rootstocks is an effective tool that may enable the susceptible scion to control biotic and abiotic stressors and increase yield. In these cases, the scion can modify its morphological properties. Grafting of tomatoes has been recently introduced in the Croatian greenhouse production. The aim of this study was to analyse the influence of commercial tomato rootstocks on the vegetative characteristic of tomato plants using the hydroponic growth system. Cultivars Clarabella and Estatio were grown as non-grafted, self-grafted or grafted onto rootstock cultivars Arnold, Buffon, Emperador and Maxifort. The experiment was set up in a greenhouse at the Institute of Adriatic Crops and Karst Reclamation during the spring summer season by random block design with four replications. During the nine measurements conducted over 61 days after planting (DAP), cv. Clarabella had more leaves compared to cv. Estatio at 32d. Grafted plants had a higher number of leaves compared to the non-grafted plants in the initial stages of growth (until 19 DAP). The height of the plants was affected by the scion during a period of 61 DAP. Cultivar Clarabella was higher than cv. Estatio for all measurements. The influence of the rootstock on plant height was expressed until 39 DAP after that the scion entered in to the generative phase. Clarabella cultivar had a higher plant diameter in comparison to the cv. Estatio, especially at the initial stages after planting. Also, there was a positive influence of the rootstock on the diameter of the plants. Plants grafted onto rootstocks Emperdor and Maxifort had a higher diameter compared to non-grafted or self-grafted plants. During all measurements, cv. Clarabella had a higher stem diameter compared to cv. Estatio. Rootstock failed to improve the diameter of stem in grafted cultivar Estatio. Rootstocks have improved the vegetative growth parameters of commercial tomatoes such as number of leaves, height, plant diameter and stem diameter. Improved morphological properties of grafted plants can enable to better withstand different stress conditions and achieve higher yields.

New Advances in the use of PGD biomonitoring for *Galanthus nivalis* L. cytogenome diversity assessment

Jasna Paradiž*[1], Jože Bavcon[2], Blanka Ravnjak[2]
[1]Dr. Jasna Paradiž, Private Researcher, Plant Cytogenetics and Biomonitoring, Hudovernikova 4, Slovenia
[2]University Botanic Gardens Ljubljana, Department of Biology, Biotechnical Faculty, Ižanska cesta 15, Ljubljana, Slovenia
*corresponding author (jasna.paradiz@gmail.com)

The aim of this study was to determine whether the variation responsible for intraspecific morphological recognition correlates with pollen grain deformation cytology, so that screening of deviating gametophytic stage could help preserve a diverse *ex situ* collection of *Galanthus nivalis* L. variants in University Botanic Gardens Ljubljana, Slovenia.

Pollen grain deformation (PGD) assay on 50 tall variant *Galanthus* transplants with wide leaves was performed for a six year (2011-2017) period at experimental Garden collection plots. Natural habitat localities and botanical survey details were previously described elsewhere (1). In the PGD analysis, pollen size and shape deformations (DEF) and nonviable sterile (STER) grains were scored. Preliminary interpretation of *ex situ* data in combination with *in situ* biomonitoring (from 1998 on) were used to identify cytogenome features and variations in species response to requirements of different environments. An assessment guideline was then developed based on PGD results covering phytogeographic regions where plants were present and pollen deformation variations linked with polyploidization processes were recorded.

The PGD *ex situ* bioassaying results revealed a considerable cytogenome DEF variation in 52% of *Galanthus* tall variants. Fluctuation peak frequency at DEF>STER was established in up to 2 years of cultivation, then for 1% higher average frequency of large pollen (L-DEF) was stated at low selection level STER<5-8,5% in 20% variants, but in 32% of them for 5% L-DEF higher frequency persisted at STER>8,5% up to 6 years. Considering the high STER/DEF ratio value, as a consequence of decreasing DEF dynamics in male gametic system, single balanced Dinaric and Submediterranean decorative biotype for horticulture use could be suggested. Some physiologic prematurity and reproduction mode disturbance within successive high STER levels could not be excluded as well.

However, mainly viable and fully nutritious pollen is of good enough quality even for apiculture, and a resource map based on plants' STER findings could be created for pollination operation. Local flora PGD scoring survey should be delivered to the international "Forum for environmental monitoring" for further protection of areas with identified or probable presence of valued species and habitats.

Reference

1. Bavcon J. (2008). Navadni mali zvonček (*Galanthus nivalis* L.) in njegova raznolikost v Sloveniji. Common snowdrop (*Galanthus nivalis* L.) and its diversity in Slovenia. Ljubljana: Biotehniška fakulteta, Oddelek za biologijo, 94 pp.

Uptake of heavy metals by the moss *Hypnum cupressiforme* from solid medium in controlled conditions

Jelena Stankovic[1], Katarina Vogel Mikuš[2,3], Mitja Kelemen[3], Primož Pelicon[3], Iztok Arčon[3,4,] Aneta D. Sabovljević[1], Marko S. Sabovljević[1]*

[1]Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia
[2]Department of biology, Biotechnical faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia
[3]Jozef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia
[4]University of Nova Gorica, Vipavska 13, 5000 Nova Gorica, Slovenia
* corresponding author (marko@bio.bg.ac.rs)

An ubiquitous moss species *Hypnum cupressiforme* is widely used in environmental studies e.g. heavy metal accumulation. However, its biological feature is rarely tested in controlled conditions. With this aim *H. cupressiforme* has been established in *in vitro* cultures and grown for five years in controlled conditions and minimal KNOP substrate to exclude synergistic or antagonistic effects of other factors.

This way obtained plants were used for test of toxic metal accumulation, namely Zn, Cu and Pb, which was added to solid media as potentially harmful acetate salts in concentration of 200 and 700 µM. The content of targeted metals and additional elements of (P, S, K, Ca, Fe, Sr) were measured by XRF analysis.

The results obtained clearly showed that *H. cupressiforme* accumulated Zn in both applied concentration and well survived. The uptake of copper was similar in both applied concentration, but survival was higher in 200 µM applied Cu-acetate. The viability of moss plants treated with copper was lower than those treated with zinc and this was also documented by the content of potassium that was significantly decreased in copper treatments. This was expected since copper is rather more toxic to plants compared to zinc. As concerned for lead, that was expected to be highly toxic, the survival was rather high and the detected content accumulated in *H. cupressiforme* was almost 20 times less compared to copper. Based on survival rate, it is assumed that lead did not enter the moss cells, either because of this moss unknown resistance mechanisms or because it was not available for uptake. Even though it is widely accepted that moss accumulate toxic metal mainly from the atmospheric deposits by whole surface, experiments with *H. cupressiforme* showed that substrate can play a significant role in loading mosses with heavy metals. Imaging of element distribution by proton-induced X-ray emission revealed cell to cell metal transport and metal compartmentation. Metal ligand environment was probed by synchrotron X-ray absorption spectroscopy, revealing for the first time metal ligand environment in moss species. Further studies, like the effects of the metal solubility in water solution, pH, the content of metals in water film wrapped the mosses, outside temperature, maturity of mosses, genotypes, hydratation of the moss plants or interaction with other compounds.

Uptake of selected metals by mosses is affected by solution pH in the rain simmulation controlled conditions

P28

Marko S. Sabovljević[1]*, Jelena Stanković[1], Marija Ćosić[1], Milorad Vujičić[1], Marieluise Weidinger[2], Ingeborg Lang[2], Aneta D. Sabovljević[1]

[1]Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia (*marko@bio.bg.ac.rs)
[2]Core Facility Cell Imaging and Ultrastructure Research, Faculty of Life Sciences, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Bryophytes are known to be good bioindicators, quickly indicating changes in the environment. Some bryophytes are known to cope with heavy metals, and other pollutants or tolerate them good. In the opposite, the other species are toxic element sensitive. Additionally, they receive the most nutrients by the trough fall and the specific binding process and the species differences remained rather unknown. With an aim to study species specific binding process and the influence of pH, we selected three different species of mosses: pleurocarp *Hypnum cupressiforme*, model moss in environmental studies, its counterpart *H. imponens* and acrocarp *Atrichum undulatum.* The axenic culture was established for each of the species and optimized production was achieved prior to use the lab-born plants in rain simulation experiments where rain was loaded with selected toxic metals, namely zinc, cadmium and copper. The time of exposure (raining) was varied as well as the pH of the rain. All the other biotic and abiotic variables were excluded and/or controlled. The growth parameters (multiplication index, secondary protonemal diameter), as well as semiquantitative analyses of metal content, were observed. The heavy metal content was measured by x ray energy dispersive spectroscopy (EDX). According the results obtained it can be said that binding pattern is not general, and it is rather species specific. The pH influence the binding of each metal tested, and the effect is different for different elements. In addition, the anion counterparts (cations) are also affecting the detection of target heavy metals within the moss bodies. Also, the gradient of heavy metal distribution along the moss stems or leaves can be observed in some cases depending on the treatment applied. It is clear that pH has an effect in heavy metals binding in mosses. However, this depends on other biotic factors as well. Further studies are needed to clear the process of heavy metal uptake in various moss species.

Gene expression and proteolytic activity of endopeptidases and aminopeptidases in common bean (*Phaseolus vulgaris* L.) exposed to drought

Aleš Sedlar[1,*], Marjetka Kidrič[2], Mateja Zupin[1], Jaka Razinger[1], Jelka Šuštar-Vozlič[1], Dominik Vodnik[3], Jerica Sabotič[2], Vladimir Meglič[1]

[1] Agricultural Institute of Slovenia, Ljubljana, Slovenia
[2] Jožef Stefan Institute, Ljubljana, Slovenia
[3] Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia
* corresponding author (ales.sedlar@kis.si)

Common bean is an important crop for human consumption and its yield is affected by exposure to drought. The response to drought is a complex process in which different classes of proteases have also been proposed to play a role [Budič et al., 2013, 2016]. To better understand the role of proteases in drought response, gene expression and protease activity of endopeptidases and aminopeptidases were determined in two European common bean cultivars differing in tolerance to drought and examined in correlation with plant physiology under different stages of drought and at rehydration. Gene expression of subtilisin endopeptidases (*PvSLP1*, *PvSLP2*), cysteine endopeptidases (*CysP1*, *CysP2*) and proline aminopeptidases (*PAP1*, *PAP2*) were determined by RT-qPCR and proteolytic activities for three substrates were determined using colourimetric assays. Gene expression of four examined proteases was predominantly up-regulated in drought-stressed plants with exception of subtilisin proteases. The up-regulation of *CysP1* during the severe drought was particularly pronounced. The gene expression of *PvSLP2* and *CysP1* during drought was cultivar specific, with greater up-regulation of these two proteases in drought less tolerant Starozagorski. After rehydration, gene expression returned to the level of control plants. Proteolytic activity at moderate drought was substrate dependent and at severe drought it declined for all substrates. Cultivar-specific increase was observed for Bz-Arg-pNA activity at rehydration. Under drought stress, the relative water content and water potential of leaves, as well as water use efficiency and several photosynthesis-associated parameters, were higher in Tiber than in Starozagorski plants. The differences in these physiological parameters indicate greater prevention of water loss in Tiber during drought. These results suggest that changes in the expression and activity of specific endopeptidases and aminopeptidases may be associated with the ability of plants to conserve water during drought stress.

Budič M., Sabotič J., Meglič V., Kos J., Kidrič M (2013). Plant Physiology and Biochemistry 62, 79-87.
Budič M., Cigić B., Šoštarič M., Sabotič J., Meglič V., Kos J., Kidrič M. (2016). Plant Physiology and Biochemistry, 109, 326-336
The study was supported by Slovenian Research Agency (project J4-4126, programme P4-0072 and Young researcher grant for Ms Zupin) and the FP7 Project CropSustaIn (FP7-REGPOT-CT2012-316205).

Influence on sex expression of *Cannabis sativa* L. and its determination using DNA markers

Miha Slapnik[1], Marko Flajšman[1], Sinja Svetik, Jana Murovec*[1]

[1]University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Slovenia
*corresponding author (jana.murovec@bf.uni-lj.si)

Cannabis sativa L. is a dioecious plant, which means that male inflorescence appear separately on male plants, and female inflorescence on female plants. There are also monoecious plants that are the result of breeding. Monoecious plants are particularly desirable in the production of hemp seeds and fibres (Divashuk et al., 2014). As with humans, sex of *Cannabis satvia* L. is determined by X and Y chromosomes. The expression of flower gender is controlled by genetic and epigenetic factors such as environmental factors, phytohormones, etc. (Tanurdzic and Banks, 2004). Studies have confirmed that endogenous hormones affect the expression of sex which is genetically determined and can be influenced by growth regulators, especially in polymorphic plants such as *Cannabis sativa* L. Gibberellin, auxins, ethylene and cytokinins affect sex expression in dioecious and monoecious plants. It was proven that chemical treatment can influence the ratio of endogenous hormones and hence the expression of sex. Gender changes occur only at the phenotypic level (Truta et al., 2007). In our research, the influence of growth regulators of gibberellic acid (GA3) and silver thiosulphate (STS) on the sex expression of dioecious female cannabis plants has been observed. We used six different treatments. The plants were sprayed one time with undiluted STS, thirty times diluted STS, gibberellic acid (GA3). Furthermore, application of 50 µg STS, 100 µg STS and 150 µg STS per growth peak was carried out by pipetted 10 µl of STS to the growth peak for five consecutive days. The most effective treatments turned out to be spraying with undiluted STS in which on average 331 male flowers per plant were observed and thirty times diluted STS where on average 297 male flowers per plan were observed. Treatments of 50 µg STS, 100 µg STS and 150 µg STS per peak followed. When treated with GA3, only few male inflorescences appeared (around eight male flowers per plant). In further research, the efficacy of three different DNA markers (SCAR119, SCAR323 and MADC2) for gender determination was tested. First we isolated DNA from the plant tissue and determined the effectiveness of the markers. We obtained proper results only with markers SCAR119 (amplicon length 119 bp) and MADC2 (amplicon length 390 bp), while marker SCAR323 was discarded as inaccurate. The most suitable annealing temperature was 52 °C. We also tested sex determination with direct PCR, for which it is not necessary to isolate DNA from plant tissues. We prepared and tested four different protocols for the preparation of the PCR solution and different annealing temperatures. We obtained the same results with molecular analysis of isolated DNA and with direct PCR.

Divashuk M.G., Alexandrov O.S., Razumova O.V., Kirov I.V., Karlov G.I. 2014. Molecular cytogenetic characterization of the dioecious Cannabis sativa with an XY chromosome sex determination system. PLoS ONE 9(1):e85118

Tanurdzic, M., Banks, J.A., 2004. Plant Cell 16(Suppl), 61-71

Truta E., Olteanu N., Surdu S., Zamfirache M.M., Oprica L. 2007. Some aspects of sex determinism in hemp. Analele Stiintificeale Universitatii BAlexandru Ioan Cuza, Sectiunea Genetica si Biologie Moleculara VIII: 31–39

Effects of low-temperature stress on specialized metabolites level in *Brassica oleracea* var. *acephala*

Šamec Dunja*, Stjepana Fistanić, Valentina Kruk

Ruđer Bošković Institute, Bijenička cesta 54, 10 000 Zagreb, Croatia
* corresponding author (dsamec@irb.hr)

Among the various abiotic stresses, low temperature is one of the major environmental constraints that limit the plant development and crop productivity. In the present work, we investigate the responses of kale (*Brassica oleracea* L. var. *acephala*), naturally cold-tolerant plants, to low temperatures because we expect that those plants have molecular mechanisms that enable them to effectively cope with low temperatures. Particularly, we were focused on specialized metabolites level because the role of specialized metabolites in low temperature stress tolerance is poorly understood. In a laboratory conditions plants at different growing stage- seedling, and 6-10 week old plants were exposed to chilling (8^0C) and freezing (-8^0C) temperature and changes in physiological, molecular and biochemical responses were evaluated in comparison with the control (21^0C). Among other parameters, the level of the main group of specialized metabolites- glucosinolates, polyphenols and carotenoids were evaluated by UV-VIS and/or HPLC-PDA. Low temperature stresses significantly reduce growth and development of the plants and affect specialized metabolites level.

Acknowledgement: This work has been fully supported by The Unity through Knowledge Fund (UKF) under the project "Metabolomic and transcriptomic response of kale to low temperature stress" (contract no 12/17).

Relation of long- and short-termed salt stress on pigment content in selected moss species

Milorad Vujičić[1,]*, Marko S. Sabovljević[1], Helena Šircelj[2], Aneta D. Sabovljević[1]

[1]Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia
[2]Agronomy department, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
* corresponding author (milorad@bio.bg.ac.rs)

Mosses are rarely recorded in salt environment. They inhabit almost all ecosystems on the Earth, but they are missing from the seas. Though, there are some representatives that are thriving exclusively in salt-harsh environment i.e. bryo-halophytes. With aim to study the effects of salts stress in mosses, three different moss species has been chosen, namely *Physcomitrella patens* (Hedw.) Bruch. & Schimp. (model moss), *Entosthodon hungaricus* (Boros) Loeske and *Hennediella heimii* (Hedw) RH Zander. The last two are known to tolerate salt and are competitive in salty habitat types. The *in vitro* culture of these species has been established and the growth was optimized in controlled condition. In the experimental approach, they were stressed with different concentrations of NaCl (0, 5, 10, 50, 100, 200, 300, 500 mM) that was added to the basal medium (BCD). The duration of salt stress lasts either 3 days (short term stress) or 3 weeks (long term stress). HPLC was used to detect the changes in pigment content (chlorophylls, carotenes and xanthophylls) both qualitatively and quantitatively. The results obtained clearly showed that in all tested moss species in short termed stress, low salt concentration does not harm the moss photosynthetic apparatus. In higher concentrations, the total chlorophyll content significantly decreased. In long termed stress the chlorophyll concentration decreased linearly. *E. hungaricus* has rather high total chlorophyll concentrations compared to the other two species. In all tested species, both in long and short-termed stress, β carotene was rather higher compared to α carotene. As referred to xanthophylls, the dominant forms were lutein and neoxanthin. Another detected xanthophylls were: violaxanthin, antheraxanthin and zeaxanthin. Nevertheless, the content of each of xanthophyll were stress duration, intensity and species dependent (i.e. specific). Zeaxanthin is rather absent in short-stressed *H. heimii,* but present in other tested species. In long-termed stress the three species express similar pattern: no zeaxanthin in lower concentration and presence in the highest concentration. Also, antheraxanthin in *H. heimii* tends to remain equal in all treatments in long-termed stress while in *E. hungaricus* it constantly decreased with salt concentration increase suggesting different strategies in salt stress survival of these two species.

Tomato grafting influences the ripening period and yield components

Gabriela Vuletin Selak[1,*], Gvozden Dumičić[1], Marija Mandušić[1], Branimir Urlić[1], Maja Jukić Špika[1], Dragan Znidarčič[2], Smiljana Goreta Ban[3], Katja Žanić[1]

[1]Institute for Adriatic Crops and Karst Reclamation, Put Duilova 11, Split, Croatia
[2]Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, Ljubljana, Slovenia
[3] Institute of Agriculture and Tourisam, Karla Huguesa 8, Poreč, Croatia
* corresponding author (gabriela.vuletin.selak@krs.hr)

Tomato (*Solanum lycopersicum* L.) grafting has received more attention in past years as a sustainable practice for mitigation of abiotic and biotic stresses. The aim of this experiment was to assess the length of the fruit development period, fruit characteristics at harvest and yield components in hydroponically grown grafted tomato. Cultivars Clarabella and Estatio were grown as nongrafted, self-grafted or grafted onto rootstock cultivars Arnold, Buffon, Emperador and Maxifort. The experiment was set up in a greenhouse during the spring-summer season by random block design with four replications. Cv. Estatio developed fruits 1.5-2 days earlier compared to cv. Clarabella. The rootstock influenced the ripening period at the first and the third flower of the fourth inflorescence. The weight of the fruits from the first four flowers was significantly influenced by the scion. Thus, the fruits of cv. Clarabella had 11-30% higher weight than those of cv. Estatio. Fruits of cv. Clarabella were significantly higher (5.69 cm), with wider columella (1.13 cm) but lower seed number (222.4 seeds/fruit) compared to cv. Estatio (5.51 cm; 0.91 cm and 295.3 seeds/fruit, respectively). Cv. Estatio showed the lowest fruit diameter at nongrafted (6.49 cm) and self-grafted (5.22 cm) plants, while the highest fruits (7.25 cm) were found for self-grafted plants. Self-grafted plants of cv. Estatio had the higher seed number per fruit (334.9) compared to nongrafted cv. Estatio (185.8) or plants grafted onto rootstocks Buffon (238.8) and Maxifort (259). The shortest length of collumela (1.03 cm) was found for fruits from plants grafted onto rootstock Arnold compared to self-grafted (1.80 cm) and nongrafted (1.47 cm) plants of cv. Estatio or self-grafted cv. Clarabella (1.60 cm). Fruits of cv. Estatio were harvested earlier compared to those of cv. Clarabella. The components of total yield (fruit weight and yield) were higher for scion cv. Clarabella. The total yield was improved with grafting onto rootstocks Buffon (12.6 t/0.1 ha), Emperador (12.3 t/ 0.1 ha) and Maxifort (12.3 t/ 0.1 ha) compared to nongrafted or self-grafted scion cultivars (10.1 to 11.2 t/ 0.1 ha). Higher fruit weight was found for scions grafted onto rootstocks Buffon (206.6 g) and Emperador (207.0 g) compared to nongrafted cv. Clarabella (191.1 g) or nongrafted (171.1 g) and self-grafted (185.8 g) cv. Estatio. According to results, the grafting of scion cultivars onto specific rootstock can modify the length of period from flowering to harvest, change the fruit qualities and increase the total yield.

Plant interactions with other organisms

The influence of circadian rhythm on the potato response to PVY infection

Maruša Pompe-Novak[1]*, Maja Švigelj[1], Timotej Čepin[1], Živa Ramšak[1], Maja Zagorščak[1], Juliane Pfeil[2], Andrej Blejec[1], Kristina Gruden[1]

[1]National Institute of Biology, Ljubljana, Slovenia
[2]Technical University of Applied Sciences in Wildau, Germany
* corresponding author (marusa.pompe.novak@nib.si)

The plant immune response is strictly controlled in order to effectively defend against pathogenic organisms and is regulated by abiotic factors such as light, humidity, circadian rhythm, temperature and others. Circadian rhythms are driven by biological clock of the organism and are a response to 24-hour changes in the physical environment. They are associated with day and night and also function when the plant is deprived of external time indications. Circadian clock is essential for the control of various physiological activities and gene expression (Greenham and McClung 2015). The core circadian oscillator generates rhythmic outputs, which in plants influence rhythmic expression of at least 30% of the genome. Consequently, circadian oscillations are observed also in plant metabolism, including photosynthesis, redox homeostasis, starch metabolism, nutrient assimilation and secondary metabolism (Barak et al. 2000).

We investigated the effect of inoculation with PVY on potato plants at different times during the day. In the case of infection in different parts of the day, the symptoms in certain times after inoculation were differently expressed and symptoms began to express at different times after inoculation. In general, PVY inoculation of potato plants at 2 p.m. and 8 p.m. in the early days after infection led to the appearance of symptoms on greater proportion of leaves than the infection at 8 a.m. During later course of disease, the proportion of leaves with disease symptoms in plants infected at 8 a.m., 2 p.m. and 8 p.m. equalized, 14 days after inoculation it was even higher in plants infected at 8 a.m. Very similar results were also obtained for the amount of viral RNA and viral protein in plant leaves.

Moreover, the expression of several potato genes involved in circadian clock, primary metabolism, generation of reactive oxygen species (ROS) and hormone metabolism was studied at different times of the day. The shortest paths between circadianially differentially expressed potato genes, the clock genes and PVY ORFs were extracted from a potato metabolic network model (Ramšak et al. 2018) and visualized by Cytoscape (Shannon et al. 2003).

Greenham K. and McClung C.R. (2015). Nature Reviews Genetics 16, 598–610

Barak S., Tobin E.M., Andronis C., Sugano S. and Greenet R.M. (2000). Trends in Plant Science 5, 517-522.

Ramšak Ž., Coll A., Stare T., Tzfadia O., Baebler Š., Van de Peer Y. and Gruden K. (2018). Plant Physiology, https://doi.org/10.1104/pp.18.00450.

Shannon P., Markiel A., Ozier O., Baliga N.S., Wang J.T., Ramage D., Amin N., Schwikowski B., Ideker T. (2003). Genome Research, 13(11), 2498-504.

Biological activity of selected invasive alien plants

Jasna Dolenc Koce*, Sabina Anžlovar, Jerneja Ambrožič Avguštin

University of Ljubljana, Biotechnical Faculty, Dept. of Biology, Večna pot 111, 1000 Ljubljana, Slovenia
* corresponding author (jasna.dolenc.koce@bf.uni-lj.si)

One of possible mechanisms that enable alien plants to become invasive in the newly colonised areas is allelopathy, the inhibitory effect on growth and development of neighbouring plants. The allelochemicals are released from roots or degrading plant material and affect the plants in interaction directly or indirectly via soil microbiota. The biomass of invasive alien plants (IAPs) is large and therefore these plants presents a potential source for the preparation of biologically active extracts.

Two invasive knotweeds (Japanese and Bohemian) and goldenrods (Canadian and gigantic) were selected to test their potential as a source for biologically active preparations. Different plant parts (rhizomes, leaves, flowers) were collected and extracts and/or essential oils were prepared in order to test their antifungal and antibacterial activity. The results show that essential oils (when applicable) have stronger inhibitory effects than extracts, and root extracts are more effective than shoots. Gram positive bacterial strains were more susceptible to IAPs than Gram negative. For antifungal activity, the growth of all species was reduced. Additionally, the IAPs were tested also as potential fungicides for wheat grain to prevent crop spoilage.

Knotweed extracts were also tested for allelopathic effects. Rhizome extracts caused oxidative stress and ultrastructural changes in exposed radish seedlings.

The observed inhibitory effects of selected IAPs present a basis for further studies in which their potential for preparations that control bacterial and fungal infections and also application as herbicides will be assessed.

Interactions in green hydra symbiosis

Goran Kovačević*

Department of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, HR-10000 Zagreb, Croatia
* corresponding author (goran.kovacevic@biol.pmf.hr)

Symbiotic associations are of a wide significance in evolution and biodiversity. Green hydra (*Hydra viridissima* Pallas, 1766) is a cosmopolitan freshwater cnidarian, a valuable model system for studying symbiotic interactions and it represents a typical example of endosymbiosis. In its gastrodermal myoepithelial cells it harbors individuals of *Chlorella*-like photoauotrophic algae. Up to 20 algae in one cell occupy 10% of the cellular volume. This symbiotic relationship is stable and well determined and it represents a biological advantage over the free-living brown hydra (Hydra oligactis Pallas, 1766). The bidirectional flow of metabolites between the host and the symbionts, and also the genetic flow through horizontal gene transfer might be the reason for the selective advantages. Endosymbiotic algae can be permanently isolated from its green hydra host showing that different genera and species of unicellular green algae could be present as symbionts in green hydra, but only one at a time, depending on the natural habitat of a particular strain of green hydra. All endosymbiotic algae isolated from green hydra with the characteristic of permanent stable lab growth belong to the *Chlorella zagrebiensis* group. The isolated zoochlorellae are also capable of modifying their morphology from coccoidal to cenobial, with transitional forms present. Newly described mechanisms of the hydra-alga symbiosis are widening of perialgal space, degradation and loss of symbiosome, fusion of symbiosomes and perialgal spaces, and their role in regular re-assembly of the symbiosis. Using the Chlorella bioassay huge differences are noticed in the growth rate of the endosymbiotic algae isolated from green hydra when compared to the growth rate of the free-living relatives (3.3% : 36.7%). These results indicate that isolated algae exhibit lower viability and are less adapted to unfavourable (micro-)environmental conditions than their free-living relatives. During millions of years of coevolution, preadaptations and interactions, the first phase of parasitic interactions secondarily became obligatory mutual, today being facultatively mutual. Endosymbiotic algae perform as the “stronger” symbiotic partner in green hydra symbiosis, pointing out also the evolutionary reversal. The results refer to the fact that symbiogenesis in green hydra has probably not been terminated yet and that hydra symbiosis biodiversity is much more complex than previously thought. Furthermore, if we consider the hydra as a holobiont that comprises three types of organisms (hydra host-algae-bacteria), we find ourselves in an exciting period of time for studying interactions in green hydra symbiosis.

Spatiotemporal analysis of potato hypersensitive response-conferred resistance to potato virus Y: RBOHD is required for successful virus arrest

Tjaša Lukan*, Maruša Pompe-Novak, Špela Baebler, Magda Tušek-Žnidarič, Aleš Kladnik, Andrej Blejec, Maja Zagorščak, Katja Stare, Tjaša Stare, Barbara Dušak, Anna Coll, Karolina Morgiewicz, Jacek Hennig, Kristina Gruden

National Institute of Biology, Ljubljana, Slovenia
* corresponding author (tjasa.lukan@nib.si)

Mechanisms of viral arrest in hypersensitive response (HR)-conferred resistance have remained elusive despite intensive ongoing research. We previously showed that in potato – potato virus Y HR interaction, as shown for some other plant-virus pathosystems, it is not the cell death or physical separation of the virus by callose deposition that prevents virus spread. Here we studied the molecular mechanisms of HR-conferred resistance in spatiotemporal manner. All analyses were performed also in SA-depleted transgenic plants, which cannot restrict viral spread. No particular ultrastructural features were detected distinguishing HR-conferred resistance and disrupted HR-conferred resistance. We further focused on spatiotemporal transcriptional responses. We selected 23 candidate genes for our analysis based on time stamped whole leaf transcriptomic data. These included genes involved in ethylene, jasmonic acid and salicylic acid signalling, metabolism of reactive oxygen species, response to redox potential changes and a set of immune signalling actuator genes. Analysis of small sections surrounding the site of viral infection has shown that responses for almost all selected genes are tightly spatiotemporally regulated. Interestingly, response of redox state related genes was showing spatiotemporal response that differed between both genotypes. In particular, responses of RBOHD gene are focused on the border region of the lesion and correlate with the expression of TRX-H gene known to be involved in regulation of SA signalling. Based on these results, we hypothesised that RBOHD is essential for signalling leading to successful arrest of the virus in HR-conferred resistance in potato. We have constructed transgenic lines with suppressed RBOHD gene activity. The virus can spread systemically in those plants, breaking the HR-conferred resistance, thus validating our hypothesis.

Unrevealing complex mechanisms of plant defense signalling using systems biology approach identifies StSAPK8 as novel regulator of potato immunity

Tjaša Stare*, Tjaša Lukan, Živa Ramšak, Maja Zagorščak, Anna Coll Rius, Kristina Gruden

Department of Biotechnology and Systems Biology, NIB, Ljubljana, Slovenia
* corresponding author (tjasa.stare@nib.si)

The interaction between plant and its pathogen initiates a complex signalling network, resulting in massive changes of the gene activity and extensive reprogramming of the cell metabolism. Using systems biology approach we were able to describe complex biological processes and dynamical mechanisms involved in potato response to viral infection and identify novel regulators of potato immunity.

Firstly, we constructed *Arabidopsis thaliana* signalling model describing the biosynthesis, hormone recognition and signal transduction leading to the activation of effector molecules of crucial phytohormones involved in plant defence: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) [1]. The primary model has been expanded with protein-protein interactors and translated to potato *(Solanum tuberosum*). In parallel with model construction, we have analysed dynamics of potato response to infection with Potato virus Y (PVY) [2, 3]. The dynamics of whole transcriptome changes of cultivar 'Désirée' and NahG-Désirée was analysed in inoculated and systemically infected leaves following 0, 1, 3, 4, 5, 7, 8, 9, and 11 days post inoculation (dpi). Proteome changes at the onset of viral multiplication were analysed and compared to transcriptional dynamics [4]. Dynamics of physiological changes were evaluated at the level of symptoms development, callose accumulation, measuring virus accumulation and its spread to uninfected tissue. In order to reveal hidden patterns of plant signalling dynamics, we have developed DiNAR, a user-friendly application with dynamic visualisation that integrates multiple condition high-throughput data and extensive biological prior knowledge. Integration of both, modelling and biological data, enabled us to identify novel regulators of plant defence against viral pathogens. The role of two kinases and two phosphatases has been evaluated with functional genomics. The results imply that novel players of virus induced-potato response gap the bridge between regulation of abiotic and biotic plant response signalling.

1. Miljkovic et al.: Signalling network construction for modelling plant defence response. PLoS One 2012
2. Stare T et al.: Bimodal dynamics of primary metabolism-related responses in tolerant potato-Potato virus Y interaction. BMC Genomics 2015
3. Baebler et al.: Dynamics of responses in compatible potato-Potato virus Y interaction are modulated by salicylic acid. PLoS One 2011
4. Stare et al.: Comparison between Proteome and Transcriptome Response in Potato (Solanum tuberosum L.) Leaves Following Potato Virus Y (PVY) Infection. Proteomes 2017

SL15

The ethylene response factor StERF49 is a negative regulator of potato defence response against PVY

Anna Coll[1]*, Tjaša Lukan[1], Barbara Dušak[1], Katja Stare[1], Marko Petek[1], Ingo Hein[2,3] and Kristina Gruden[1]

[1]Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia
[2] Division of Plant Sciences, University of Dundee (at JHI), Invergowrie, Dundee, United Kingdom
[3] Department of Cell and Molecular Science, James Hutton Institute (JHI), Invergowrie, Dundee, United Kingdom
* corresponding author (anna.coll@nib.si)

Potato (*Solanum tuberosum* L.) is the world's most widely grown tuber crop and potato virus Y (PVY) is one of the major potato pathogen causing severe crop loss in different areas worldwide. To better understand the potato-PVY interaction we analysed expression patterns of Ethylene Response Factors (ERFs) from group IX in potato cultivar exhibiting hypersensitive response (HR)-conferred resistance to PVY. Transcriptomics data previously obtained in our group suggested that StERF49 is a potential important player in Ny-mediated HR against the virus. Therefore, we selected the gene for further analyses. Localisation studies supported the importance of StERF49 as a signalling component in potato defence response since only after PVY infection the protein is strongly accumulated in cell nucleus. Using stable transgenic potato lines in which StERF49 was knockdown by short hairpin RNAs (shRNAs) we demonstrated that StERF49 has a positive role in PVY multiplication and symptom development. To further understand the underlying mechanisms of StERF49 function in PVY virus infection we performed RNAseq experiments on PVY infected and mock inoculated leaves of StERF49 silenced potato plants. We are also studying the possible regulation mechanism of this gene by means of in silico promoter analysis, Y1H, studies of promoter activity and gene expression analysis after hormonal treatments. Our results show the importance of StERF49 and its negative role in potato defence response to PVY. Therefore, the data will contribute to a better understanding of the complex network of plant defence signalling pathways.

Potato immune signalization and potato virus Y proteins – towards analysis of 3D protein structures

Špela Tomaž[1,2,4*], Ajda Taler-Verčič[2,3], Aleksandra Usenik[2], Anna Coll[1], Kristina Gruden[1], Dušan Turk[2,3]

[1] National Institute of Biology, Večna pot 111, 1000, Ljubljana, Slovenia
[2] Jožef Stefan Institute, Jamova 39, 1000, Ljubljana, Slovenia
[3] Centre of excellence for Integrated Approaches in Chemistry and Biology of Proteins, CIPKeBiP, Jamova 39, 1000, Ljubljana, Slovenia
[4] Jozef Stefan International Postgraduate School, Jamova cesta 39, 1000 Ljubljana, Slovenia
* corresponding author (spela.tomaz@nib.si)

Potato virus Y (PVY) is one of the most important potato crop pathogens. Different proteins respond to the infection of potato with PVY and cooperate in orchestration of the immune response. Studying protein interactions and their structural characteristics is crucial for understanding the processes, which occur during infection. In the scope of our study, we analysed known interactions between selected PVY proteins and potato immune signalization proteins and attempted to produce them in *Escherichia coli*. We were able to produce 13 of 38 selected proteins. We chose to produce potato protein TGA2.1 on a larger scale. We biochemically characterised TGA2.1 with determination of its oligomeric state, its solubility in different buffers and by N-terminal sequencing. TGA2.1 was crystallized in order to determine its structure with X-ray crystallography.

Manipulating plant-microbe-insect interactions to enhance crop production and resistance to soil pests

Jaka Razinger

Agricultural Institute of Slovenia, Hacquetova ulica 17, Ljubljana, SI-1000, Slovenia
* corresponding author (jaka.razinger@kis.si)

Microorganisms can have a beneficial effect on plants, for example, through their activities in rhizospheres. Such microorganisms (bacteria and fungi) can be enriched in rhizospheres of crops via bioaugmentation. The systems we are interested in are rhizosphere competent fungi that also have or could have an effect on pest insects (cabbage root fly (CRF) *Delia radicum* (Diptera: Anthomyiidae) on brassicas or *Agriotes* spp. wireworms (Coleoptera: Elateridae) on maize and wheat) because of their entomopathogenic activities. Presentation will focus on assessment of entomopathogenicity, rhizosphere competence, plant tissue colonization (endophytism) and potential plant growth stimulating effects of investigated fungi. Cauliflower and broccoli or maize and wheat were used with laboratory bred CRF or field-collected wireworms, respectively. The following fungal species were tested: *Trichoderma atroviride, T. koningiopsis, T. gamsii, Beauveria brongniartii, B. bassiana, Metarhizium robertsii, M. brunneum, Purpureocillium lilacinum* and *Clonostachys solani*. Several fungal isolates caused significant mortality to CRF and/or wireworms. The rhizosphere competence of the tested strains to cauliflower varied considerably, possibly due to the ecological preferences of the different fungal species. The fungi inoculated via drenching were successfully transferred via or grew with the elongating cauliflower roots. *M. brunneum* colonized the rhizoplane of broccoli plants but did not show endophytic characteristics. Moreover, the fungal inoculum recovered from the rhizoplane decreased over time. A significant increase of Mg and N concentrations was detected in *Metarhizium*-treated broccoli plants when grown in the greenhouse whereas no different nutritional values in plant tissue were observed in field-grown plants. Plants from the greenhouse also exhibited higher maximum quantum efficiency of PS2 photochemistry and electron transport rate as well as promoted growth when inoculated with *M. brunneum*. Regarding maize and wheat, all strains colonized the rhizoplane, however to varying extent. The selected taxa could not be identified as endophytes in maize and wheat. Some isolates stimulated growth of maize and wheat seedling, whereas others decreased root or shoot length or biomass. This effect was plant-fungus pair specific. On several occasions, contrasting results were observed between maize and wheat and the same fungal isolate. We conclude that the ecological preferences of the fungal species might influence their performance (i.e. virulence to insect pests) in different environments, method of bioaugmentation influences the plant-microbe interaction, and plant-microbe interactions can depend on the specific fungal strain and plant genotype.

Colletotrichum coccodes – how does the fungus operate in the shadows?

Hauer Karina[1], Schuhmann Clemens[1], Lienhart Wolf-Dieter[2], Stabentheiner Edith[1], Macheroux Peter[2], Müller Maria[1]

[1]Institute of Biology, Plant Sciences, University of Graz, Austria;
[2]Institute of Biochemistry, Technical University of Graz, Austria
* corresponding author (karina.hauer@edu.uni-graz.at)

Colletotrichum coccodes causes as primarily soil borne pathogen black dot disease at potatoes (*Solanum tuberosum*) and anthracnose symptoms on tomato (*S. lycopersicum*) crops [1–4]. Apart from black dot and tomato anthracnose *C. coccodes* is also able to infect many different host species from more than 13 families, mainly Solanaceae, Cucurbitaceae and Fabaceae [5, 6]. The harmful fungus influences plant health in many ways such as leaf and stem health, tuber development, crop yield and shelf life of harvested fruits and tubers [7–9].

The plant pathogen´s armoury ranges from morphological (e.g. appressoria) and propagation adaptations (e.g. conidia spores) to biochemical alignments (e.g. cell wall degrading enzymes) to facilitate effective infection and colonisation of host plants [10, 11]. The fungus´ morphological and physiological infrastructure and its dependence on abiotic factors [12] are investigated by microscopic (light microscopy, transmission and scanning electronic microscopy) and several biomolecular methods to achieve a better understanding of the fungus´ complex infection processes.

1 Nitzan N., Evans M. and Johnson D. A. (2006). Plant Disease 90(8), 999-1003.
2 Cruz-Hernández A., Tomasini-Campocosio A., Pérez-Flores L. J., Fernández-Perrino F. J. and Gutiérrez-Rojas M. (2013). Plant Soil 362 (1-2), 261–270.
3 Lees A. K. and Hilton A. J. (2003). Plant Pathology 52(1), 3-12.
4 Narayanasamy P. (2005). Postharvest Pathogens and Disease Management. – Hoboken, NJ, USA.
5 Photita W., Taylor P. W. J., Ford R., Hyde K. D. and Lumyong S. (2005). Fungal diversity (18), 117-133.
6 Chesters C. G. C. and Hornby D., (1965). Transactions of the British Mycological Society 48, 583–594.
7 Tsror L., Erlich O. and Hazanovsky M. (1999). Plant Disease 83(6), 561-565.
8 Gantotti B. V. and Davis M. J. (1993). Acta Horticulturae (341), 353–359.
9 Pasche J. S., Taylor R. J. and Gudmestad N. C. (2010). Plant Disease 94, 905-914.
10 Aqeel A. M., Pasche J. S. and Gudmestad N. C. (2008). Phytopathology 98 (8), 901–909.
11 Kim Young-Mi and Song Hong-Gyu (2009). J Microbiol. 47 (4), 420–424.
12 Sanogo S. and Pennypacker S. P. (1997). Plant Disease 81 (4), 333–336.

Verticillium nonalfalfae secretes a chitin binding protein to conceal itself from plant chitinases

Helena Volk[1], Kristina Marton[1], Marko Flajsman[1], Sebastjan Radisek[2], Ingo Hein[3], Crtomir Podlipnik[4], Branka Javornik[1], Sabina Berne[1]*

[1]Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
[2]Slovenian Institute of Hop Research and Brewing, Cesta Zalskega Tabora 2, SI-3310 Zalec, Slovenia
[3]The James Hutton Institute, Invergowrie, Dundee DD2 5DA, Scotland, United Kingdom
[4]Department of Chemistry and Biochemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Vecna pot 113, SI-1000 Ljubljana, Slovenia
* corresponding author (Sabina.Berne@bf.uni-lj.si)

Verticillium nonalfalfae (*Vna*) is a phytopathogenic fungus that causes Verticillium wilt on various crops, including hop. The plant immune system responds to fungal infections when the extracellular lysin motifs (LysM) of plant immune receptors recognize GlcNAc, the structural component of the fungal chitin cell wall. We report here that *Vna* outsmarts this recognition process by secreting a chitin binding protein (CBP) that disguises the fungus from the plant immune system.

Examination of the sequence variation of *Vna8.213*, the gene encoding for CBP, in different *Vna* and *V. alfalfae* (*Va*) isolates suggested that this gene is highly conserved and important for the fungal lifestyle. The highest expression of *Vna8.213* was detected in stems of susceptible hop at the latest stages of infection. Monitoring plant immune responses to recombinant CBP demonstrated that CBP is not recognised by the plant immune system. The nonspecific subcellular localisation of CBP::mRFP fusion protein transiently expressed in *Nicotiana benthamiana* additionally confirms these finding.

A carbohydrate sedimentation test showed specific binding of the recombinant CBP to chitin and the interaction was confirmed by surface plasmon resonance. 3D modelling of the interaction between $(GlcNAc)_7$ and CBP was performed in Yasara, to determine the interacting residues. In a cell protection assay, 3 μM recombinant CBP shielded *Trihoderma viride* from degradation by xylem sap.

This study revealed one of the secrets of the successful lifestyle of *Vna*. The secreted CBP binds to the chitin cell wall, interferes with the plant immune system by outcompeting plant LysM receptors and acts as a shield against xylem sap chitinases.

Identification of Avr effector of phytopathogenic fungus *Verticillium nonalfalfae*

P34

Klemen Dretnik[1], Kristina Marton[1], Helena Volk[1], Sebastjan Radišek[2], Branka Javornik[1], Sabina Berne[1,]*

[1]Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
[2]Slovenian Institute of Hop Research and Brewing, Cesta Žalskega tabora 2, 3310 Žalec, Slovenia
* corresponding author (sabina.berne@bf.uni-lj.si)

Avirulence (Avr) effectors are pathogen secreted molecules that are directly or indirectly recognized by cognate host resistance (R) proteins and trigger rapid and robust immune responses (ETI, effector triggered immunity), often associated with hypersensitive reaction. A recent *Verticillium nonalfalfae* secretome profiling study identified a candidate effector gene with high expression in roots of infected hop. *Vna7.443* knockout mutants did not exhibit any changes in fungal growth and sporulation, while the pathogenicity in susceptible hop was similar to the wild type. In this work, we combined subcellular localization studies with elicitor activity testing to decipher the molecular function of *Vna*7.443, which encodes putatively secreted 30.5 kDa neutral protein without cysteines. The predominantly unstructured protein with 16 aa signal peptide has two internal repeats (at 21-122 aa and at 161-262 aa) predicted by the SMART tool and, according to a Conserved Domains search at NCBI, belongs to the dnaA superfamily. Significant expression of *Vna7.443* in roots of resistant ('Wye Target') and susceptible ('Celeia') hop during early stages of disease was determined by quantitative PCR analysis of infected hop samples, while six-fold lower expression was observed in the stems. *Agrobacterium*-mediated transformation of *Vna7.443*-mRFP fusion construct, followed by imaging of *N. benthamiana* leaf sections under fluorescence microscope, showed transiently expressed mRFP-tagged *Vna*7.443 protein in the plant nuclei. Since a hypersensitive response was observed 5 dpi in agroinfiltrated leaves of *N. benthamiana*, elicitor activity testing of recombinant *Vna*7.443 in *Arabidopsis* and hop cell suspension cultures is underway. Yeast two-hybrid (Y2H) screening using *Vna7.443* as bait and the cDNA library of infected hop as prey, may reveal the cognate R protein responsible for recognition of this Avr effector, while the corresponding *R* gene would advance hop resistance breeding to verticillium wilt.

The role of *StRbohA* and *StRbohD* genes in defence of potato against PVY virus

Barbara Dušak*, Tjaša Lukan, Anna Coll-Rius, Kristina Gruden

Department of Biotechnology and Systems Biology, National institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia
* corresponding author (barbara.dusak@nib.si)

The gp91$_{\text{phox}}$ respiratory burst oxidase homologue family (RBOH) proteins are known to play an important role in the stress signalling of plants. Numerous studies have shown a connection between RBOH proteins and plant's defence against different types of pathogens but until now only a small number of studies has concentrated on defence against viral infections. Our aim was to study the role of potato genes *StRbohA* and *StRbohD* in response of potato against infection with potato virus Y. We prepared short hairpin RNA (shRNA) constructs to silence *StRbohA* and *StRbohD* genes in potato cultivars Rywal and Désirée. We successfully silenced the *StRbohD* gene in potato cultivars Rywal and Désirée. We inoculated potato cultivar Rywal with silenced *StRbohD* gene with potato virus Y N605-GFP and followed the symptoms. The number of lesions on inoculated leaves of potato cv. Rywal plants with silenced *StRbohD* gene was higher than on inoculated leaves of non-transformed plants. We were able to detect viral RNA of potato virus Y N605-GFP in inoculated leaves of potato cv. Rywal plants with silenced *StRbohD* gene 6 dpi but not in non-transformed plants. In inoculated potato cv. Rywal plants with silenced *StRbohD* gene, lesions appeared in upper (non-inoculated) leaves and we also detected viral RNA of potato virus Y N605-GFP in the upper leaves. We proved that silencing of the *StRbohD* gene disables the ability of potato cv. Rywal to stop potato virus Y from spreading.

Salicylic acid perturbs sRNA-gibberellin regulatory network in immune response of potato (*Solanum tuberosum* L.) to *Potato virus Y*^{NTN}

Maja Križnik[1,2]*, Marko Petek[1], David Dobnik[1], Živa Ramšak[1], Špela Baebler[1], Stephan Pollmann[3], Jan Kreuze[4], Jana Žel[1], Kristina Gruden[1]

[1]Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia
[2]Jožef Stefan International Postgraduate School, Ljubljana, Slovenia
[3]Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM) - Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Campus de Montegancedo UPM, Madrid, Spain.
[4]Global Program of Integrated Crop and Systems Research, International Potato Center (CIP), Lima, Peru
* corresponding author (maja.kriznik@nib.si)

Potato virus Y is the most economically important potato viral pathogen. We aimed at unraveling the roles of small RNAs (sRNAs) in the complex immune signaling network controlling the establishment of tolerant response of potato cv. Désirée to the virus. We constructed a sRNA regulatory network connecting sRNAs and their targets to link sRNA level responses to physiological processes. We discovered an interesting novel sRNAs-gibberellin regulatory circuit being activated as early as 3 days post inoculation (dpi) before viral multiplication can be detected. Two endogenous sRNAs, miR167 and phasiRNA931 were predicted to regulate gibberellin biosynthesis genes GA20-oxidase and GA3-oxidase. The increased expression of phasiRNA931 was also reflected in decreased levels of GA3-oxidase transcripts. Moreover, decreased concentration of gibberellin confirmed this regulation. The functional relation between lower activity of gibberellin signaling and reduced disease severity was previously confirmed in Arabidopsis-virus interaction using knockout mutants. We further showed that this regulation is salicylic acid-dependent as the response of sRNA network was attenuated in salicylic acid-depleted transgenic counterpart NahG-Désirée expressing severe disease symptoms. Besides downregulation of gibberellin signaling, regulation of immune receptor transcripts by miR6022 as well as upregulation of miR164, miR167, miR169, miR171, miR319, miR390, and miR393 in tolerant Désirée, revealed striking similarities to responses observed in mutualistic symbiotic interactions. The intertwining of different regulatory networks revealed shows how developmental signaling, disease symptom development, and stress signaling can be balanced.

Symptomatology, viral titer and virus populations' dynamics in experimental mixed infection of potato virus Y and pepino mosaic virus

P37

Denis Kutnjak*, Zala Kogej, Tjaša Pršin, Maja Ravnikar

National Institute of Biology, Department of Biotechnology and Systems Biology, Večna pot 111, Ljubljana, Slovenia
* corresponding author (denis.kutnjak@nib.si)

Plant viruses are often present in nature in mixed infections and, if pathogenic, the outcome of the disease in plant can be dependent on the type of the interaction between the viruses present in a mixed infection. This can range from synergistic (one or both viruses increase in their titer) to antagonistic (one or both viruses decrease in their titer). Moreover, the severity of disease symptoms these viruses are causing in plants can be increased or decreased, which is not necessarily connected with observed changes in viral titer. The most known reports on the synergistic interaction are between some potyviruses (family *Potyviridae*) and potexviruses (family *Alphaflexiviridae*). In the present research, we have established mixed infections of two economically important virus species with partially overlapping host ranges: *Potato virus Y* (genus *Potyvirus*) and *Pepino mosaic virus* (genus *Potexvirus*). Single infections, coinfections (infection with both viruses at the same time) and superinfections (first infection with one virus, later with the other) were established in tomato (*Solanum lycopersicum*) and *Nicotiana benthamiana*. Virus titers in plants were measured four weeks after the infection using quantitative PCR and compared between different modes of infection. A change in severity of disease symptoms was observed; most notably, *N. benthamiana* plants coinfected with both viruses showed much more severe symptoms than if infected only with one virus. Further, to elucidate the effect of mixed infection on the within-plant population structures and evolutionary dynamics of both viruses, we have performed an evolution experiment in which both viruses were serially passaged in *N. benthamina* plants in single and mixed infections. Viral titers were determined for selected passages and population structures of the viruses were characterized using small RNA deep sequencing.

Dark septate endophytic fungi affect the mineral nutrition and physiological state of goat willow (*Salix caprea*) in metal enriched soil

Potisek Mateja[1]*, Likar Matevž[1], Vogel-Mikuš Katarina[1,2], Regvar Marjana[1]

[1]University of Ljubljana, Biotechnical faculty, Jamnikarjeva ulica 101, 1000 Ljubljana
[2] Institut Jožef Stefan, Jamova cesta 39, 1000 Ljubljana;
*corresponding author (mateja.potisek@bf.uni-lj.si)

Plants roots are colonized by arbuscular (AM), ectomycorrhizal (EM) and dark septate endophytes (DSE) in areas contaminated with heavy metals (Likar and Regvar, 2009). Mycorrhizal fungi provide host plants with water and nutrients and thus improve their growth and survival under stress (Smith and Read, 2008). In contrast to mycorrhizal fungi, the effects of DSE on plant's fitness are poorly known, although they can extend from negative, neutral to positive and depend on DSE species and environmental/experimental conditions (Jumpponen, 2001; Mandyam and Jumpponen, 2005). DSE fungi may form associations with plants, functionally similar to mycorrhizal and also has an indirect impact on plant mineral nutrition through mineralization of the rhizosphere substrate (Mandyam and Jumpponen, 2005). The effects of DSE fungi on the host’s mineral nutrition and their physiological fitness in metal enriched areas are still poorly studied. Therefore we examined the effects of three DSE fungi with known plant protective functions against metal stress on mineral nutrition and physiological state of goat willow (*Salix caprea*) in an inoculation experiment in metal enriched soil. After one year, we determined the concentration of elements in leaves and roots (XRF), and physiological fitness of plants (photosynthetic pigments). Results show that DSE inoculation significantly affects the mineral concentrations in leaves and roots of the goat willows grow in metal enriched soil. Furthermore, inoculation affected the chlorophyll content which showed also correlation with leaf minerals. Nevertheless, the protective effect of the DSE isolates in our study was species specific, thus stressing the need to evaluate the individual fungus/plant combinations prior to any phytoremediation efforts.

Likar, M. and Regvar, M. (2009). Application of temporal temperature gradient gel electrophoresis for characterisation of fungal endophyte communities of Salix caprea L. in a heavy metal polluted soil. Science of the Total Environment, 407(24), 6179–6187.

Jumpponen, A. (2001). Dark septate endophytes - Are they mycorrhizal? Mycorrhiza, 11(4).

Mandyam, K. and Jumpponen, A. (2005). Seeking the elusive function of the root-colonising dark septate endophytic fungi. Studies in Mycology, 53, 173–189.

Smith, S. E., and Read, D. J. (2008). Mycorrhizal Symbiosis. Soil Science Society of America Journal (Vol. 137).

Silver fir (*Abies alba* Mill.) phenology and fungal community structure in Slovenia

Tina Unuk[1,*], Tijana Martinović[2], Domen Finžgar[1], Tine Grebenc[1], Hojka Kraigher[1]

[1]Slovenian Forestry Institute, Večna pot 2, SI-1000 Ljubljana, Slovenia
[2]Institute of Microbiology of the CAS, Benatska 2, CZ-128201, Prague, Czech Republic
* corresponding author (tina.unuk@gozdis.si)

Introduction: As higher plants are sessile organisms, they have to compete with other plants for resources, which require very efficient communication and co-ordination of resource allocation and signaling between above-and below-ground plant parts.

Materials and methods: We collected 24 soil samples with fine root from naturally regenerated silver fir plot at Lehen na Pohorju (Slovenia). Four different groups were selected based on phenological data of silver fir trees (project LIFEGENMON). For identification of root associated fungi, ITS2 region of fungal rRNA gene was amplified and sequenced on Illumina MiSeq platform. For diversity estimation, Shannon-Wiener and Simpson indexes were used. Differences in fungal community were tested using PERMANOVA on Bray-Curtis distances.

Results: Analyzed data showed statistically significant differences in fungal communities of silver fir between groups of early and late flushing saplings. Also, alpha diversity indexes differed between mentioned groups, as there was an indication on higher OTUs diversity in fungal community of silver fir of phenologically early young trees. Other groups have showed no statistically significant differences in fungal communities, although there were some differences in alpha diversity as well as in fungal composition between early flushing adult trees compared to late flushing adult trees.

Discussion: There is an increasing body of literature, showing that fungal communities impact on plant growth and vigor, as they influence the quality, direction and flow of energy and nutrients between plants and fungi. Moreover, many of the observed plant growth responses may be also regulated in part by alterations in fungi endogenous phytohormone levels.

Slovensko društvo
za biologijo rastlin

Slovenian Society
of Plant Biology