Rooseveltov trg 6
HR-10 000 Zagreb
Tel. 01 460 62 67
Fax. 01 460 62 86
www.hdbb.hr

CROATIAN SOCIETY OF PLANT BIOLOGISTS

MINI-SYMPOSIUM OF THE CROATIAN SOCIETY OF PLANT BIOLOGISTS

Friday, 29th November 2019.

Lecture room -008, Department of Geology,
University of Zagreb, Faculty of Science
Horvatovac 102a, basement

Schedule:

-008 lecture room, Department of Geology

13:00-13:10 Greeting and award ceremony

13:10-14:00 Prof. Jutta Ludwig-Müller (Technische Universität Dresden, Institute for Botany, Dresden, Germany)
Control of the plant hormone auxin in different plant species and during the interaction of plants with their environment

14:10-16:55 Lectures

14:10-14:35 Ana Smolko, Ph.D. (Ruđer Bošković Institute, Zagreb)
How does disfunctionality of auxin-amidohydrolases in Arabidopsis reflect on auxin metabolome?

14:35-15:00 Lucija Markulin, Ph.D. (University of Zagreb, Faculty of Science, Department of Biology)
A novel role of Arabidopsis BPM1 protein in RNA-directed DNA methylation

15:00-15:25 Petra Peharec Štefanić, Assist, Prof. (University of Zagreb, Faculty of Science, Department of Biology)
Detection and localization of silver nanoparticles in plant roots

1st floor, seminar room of Division of Molecular Biolog, Department of Biology

15:25-15:45 Poster session and coffee break

-008 lecture room, Department of Geology

15:45-16:10 Mirta Tkalec, Assoc, Prof. (University of Zagreb, Faculty of Science, Department of Biology)
Impact of silver nanoparticles on photosynthesis in *Nicotiana tabacum* plants

16:10-16:35 Ivana Rešetnik, Ph.D. (University of Zagreb, Faculty of Science, Department of Biology)
Phylogeography of Amphi-Adriatic plant groups – ongoing research

16:35-17:00 Zrinka Ljubešić, Assoc. Prof. (University of Zagreb, Faculty of Science, Department of Biology)
Characterization of a photosynthetic marine pico green alga (Picochlorum, Trebouxiophyceae) isolated from the Adriatic Sea

1st floor, seminar room of Division of Molecular Biology, Department of Biology

17:00 - Banquet and poster session

Winner of the "Spiridion Brusina" award

Jutta Ludwig Muller, Prof.

Technische Universität Dresden, Institute for Botany, Dresden, Germany

Control of the plant hormone auxin in different plant species and during the interaction of plants with their environment

Plants need hormone substances to regulate a plethora of responses during their life cycle. One major hormone is called auxin which is involved in many developmental processes. Besides the major auxin indole-3-acetic acid, there are other auxin-like molecules present in some, but maybe not in all plants. Among these are the auxins with longer chains, indole-3-propionic acid and indole-3-butyric acid. The auxin-dependent growth response is dependent on the concentration of the compound. While lower concentrations are mainly growth promoting, high concentrations are actually inhibiting some developmental processes. Therefore, tight control of the auxin concentration is essential for proper growth and development. This can be achieved by altering the amount of active auxin via transport, biosynthesis, degradation or reversible conjugation to small molecules. In addition, plants use auxin during their interaction with the environment, for example during abiotic stresses such as salt, temperature or water stress to adapt the growth responses specifically. Furthermore, auxin is involved in the control of plant disease symptoms, such as tumor growth or aberrant tissue formation. However, together with other plant hormones such as salicylic acid auxin can also modulate disease progression or resistance in different plant – microbe combinations.

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Speakers

Ana Smolko, Ph.D.

Ruđer Bošković Institute, Zagreb

How does disfunctionality of auxin-amidohydrolases in *Arabidopsis* reflect on auxin metabolome?

Auxin-amidohydrolases are plant enzymes from the M20 metalopeptidases family, which specifically hydrolyse amide bond in the inactive auxin conjugates with amino acids thereby releasing free active auxin. Introducing small changes in the active site of these amidohydrolases results in different substrate preference, which is manifested in various monocot and dicot species, such as *Triticum aestivum*, *Brassica rapa*, *Medicago truncatula* as substrate specificity for longer side chain auxins such as amino acid conjugates with indole-3-butyric acid (IBA) and indole-3-propionic acid (IPA) over conjugates with the most common naturally occuring auxin indole-3-acetic acid (IAA). Interestingly, IPA and IBA are still not clearly enough defined as natural auxins in literature. In the work presented here, substrate specificity of ILL2 homologue enzyme from *Brassica rapa* towards long-chain auxin conjugates, as well as hydrolysis of these conjugates *in vivo*, has been confirmed using feeding experiments. Also, auxin metabolome of the *Arabidopsis thaliana* loss-of-function mutants, with dysfunctional amidohydrolases, was described in detail, showing that upon treatment with long-chain auxin conjugates the auxin distribution was expressed through enhanced auxin conjugation, and oxidation of surplus of auxin. New previously unknown substrate cleavage was

determined in *Arabidopsis*. Accumulation of auxin in the root of *Arabidopsis thaliana* reporter line *DR5rev::GFP* suggests an early plant response to auxins' treatment.

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Lucija Markulin, Ph.D.

University of Zagreb, Faculty of Science, Department of Biology

A novel role of Arabidopsis BPM1 protein in RNA-directed DNA methylation

Lucija Markulin, Mateja Jagić, Andreja Škiljaica, Karlo Miškec, Vedrana Vičić Bočkor, Nataša Bauer, Dunja Leljak-Levanić

Arabidopsis thaliana BPM1 protein belongs to six-membered MATH-BTB protein family. The best-known function of MATH-BTB proteins is their role as substrate-specific adaptors of cullin3-based E3 ligases in the ubiquitin-proteasome pathway. BTB domain enables assembly with the cullin while less conserved MATH domain serves as an adaptor and binds substrates destined for degradation *via* 26S proteasome. Targets of BPM1 include several transcription factors such as WRI1, HB6, DREB2A and MYB56. Through mediation of degradation of these transcription factors BPM1 is involved in the regulation of various plant processes including fatty acid metabolism, abscisic acid signalling regulation, flowering etc. Earlier results showed that BPM1 predominantly localizes in nucleus indicating cullin independent function of BPM1. Co-immunoprecipitation and mass spectrometry revealed interaction of BPM1 with DMS3 and RDM1, key components of RNA-directed DNA methylation (RdDM). However, exact role of BPM1 interaction with DMS3 and RDM1 and possible implications for RdDM pathway remain unknown. To elucidate putative role of BPM1 protein in RdDM yeast two hybrid and pull down assays were used to confirm direct interactions of BPM1 with DMS3 and RDM1. Additionally, role of MATH and BTB domain in the interaction with DMS3 and RDM1 was tested using truncated version of BPM1 protein with a single domain deletion. The results showed that BTB domain had higher affinity for interaction with RDM1, while both MATH and BTB domains appear to be equally important for interaction with DMS3. In addition, co-localization assay showed overlap of BPM1 and RdDM components while overexpression of BPM1 revealed change in DNA methylation pattern. These results indicate a novel role of BPM1 protein in RNA-directed DNA methylation pathway. Our next aim is to use ChIP-seq in order to identify DNA sites where transcription or RdDM methylation could be influenced by BPM1 protein.

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Petra Peharec Štefanić, Assist. Prof.

University of Zagreb, Faculty of Science, Department of Biology

Detection and localization of silver nanoparticles in plant roots

Petra Peharec Štefanić, Renata Biba, Bruno Komazec, Petra Cvjetko, David Vondrašek, Ilse Letofsky-Papst, Biljana Balen

Silver nanoparticles (AgNPs) are a very commonly used material in nanotechnology because of desirable antimicrobial and antifungal properties of the silver and due to their widespread use, there is growing concern about their safety and possible adverse environmental effects. As soon as AgNPs are released into the environment, their transformation begins which changes their properties and thus directly affects

the transport, fate and possible toxicity. Due to their large surface area, they are highly reactive and they are prone to aggregation to larger particles, oxidation of Ag^0 to Ag^+ and dissolution to Ag^+ species. Therefore, it is still unclear whether the toxicity of AgNPs originates from the NPs themselves or from dissociated Ag^+. As primary producers, plants are the vital part of a healthy ecological system, but they also play an important role in the transport and bioaccumulation of toxic substances in the food chain. Ag accumulation was analyzed in plants using the inductively coupled plasma mass spectrometry (ICP-MS) technique, but few studies have confirmed the accumulation and detected localization of AgNPs *per se* in the plant cell. In order to confirm that the particles found in the transmission electron microcopy (TEM) images are AgNPs, we combined analysis obtained by conventional TEM with energy-dispersive X-ray spectroscopy (TEM-EDX). Namely, some spherical NPs elicit a similar size to intracellular organelles (i.e. ribosomes) or some artefacts, which can interfere with detection of AgNPs within cells. Furthermore, we were interested to see the possible accumulation of AgNPs in the plant material and we used confocal microscope Leica SP8 with multiphoton excitation. This microscope enables to acquire images of materials by fluorescence and in reflected light with very high sensitivity. Furthermore, it has possibility of continuous excitation in the visible spectral range and the determination of the fluorescence lifetime in the samples (FLIM) enabling dynamic processes to be studied in living cells. As part of this talk I will give an overview of these two techniques which we used to visualize and detect accumulation of AgNs in root cells.

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Mirta Tkalec. Assoc. Prof.

University of Zagreb, Faculty of Science, Department of Biology

Impact of silver nanoparticles on photosynthesis in *Nicotiana tabacum* plants

Mirta Tkalec, Petra Peharec Štefanić, Petra Cvjetko, Renata Biba, Sandra Šikić, Biljana Balen

Silver nanoparticles (AgNPs) are the dominating nanomaterial in various consumer products. They are often used in agriculture as vehicles for the delivery of fertilizers and pesticides. Increase in AgNPs production has raised many concerns about their possible toxicity, and many studies have already indicated that they are toxic to various organisms including plants. However, it is still not clear to which degree the toxicity of AgNPs results from AgNPs *per se* and how much toxicity is related to the released Ag^+. We investigated the effects of three types of laboratory-synthesized AgNPs with different surface coatings [citrate, polyvinylpyrrolidone (PVP) and cetyltrimethylammonium bromide (CTAB)] on photosynthesis in tobacco (*Nicotiana tabacum* L.). Adult plants were treated with 25, 50 and 100 μM of AgNPs as well as ionic form of silver ($AgNO_3$) for seven days. Treatments with all types of AgNPs and $AgNO_3$ resulted with the increased Ag uptake in the leaves. Treatments with $AgNO_3$ and AgNP-PVP, NPs with the low negative charge, decreased the majority of photosynthetic pigments, but mostly did not have effect on the chlorophyll fluorescence parameters. Positively charged AgNP-CTAB lowered most parameters of chlorophyll fluorescence and at higher concentrations decreased the concentration of photosynthetic pigments. Negatively charged AgNP-citrate exhibited the weakest impact by only increasing the non-photochemical quenching and concentration of lutein. Ultrastructural analyses performed on 100 μM AgNP-treated plants revealed that leaves of AgNP-PVP treated plants were characterized with thinner and longer chloroplasts with stacked thylakoids compared to control while the underdeveloped thylakoid chloroplast system were characteristic for

leaves of $AgNO_3$ treated plants. Chloroplasts in leaves of plants treated with AgNP-CTAB have a significantly damaged thylakoid system without proper organization and numerous large plastoglobules while chloroplasts of AgNP-citrate treated plants were swollen and ruptured but with well-developed thylakoids. Obtained results indicate that the phytotoxic effect of AgNPs is not simply due to the release of silver ions but can be correlated with their distinct surface coating and overall surface charge.

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Ivana Rešetnik, Ph.D.

University of Zagreb, Faculty of Science, Department of Biology

Phylogeography of Amphi-Adriatic plant groups – ongoing research

The Mediterranean peninsulas are recognized as major areas of biodiversity in Europe, where the interplay of geological, climatic and evolutionary processes shaped the distribution of species and the dynamics of speciation, dispersal, adaptation and extinction. Some of these factors have triggered common patterns on the phylogeographies of different species sharing similar habitats and areas. The Adriatic Sea presents a natural barrier between Balkan and Apennine peninsulas which are both recognized as important glacial refugia of temperate plant and animal species and a number of them exhibit amphi-Adriatic distributions. Such distribution patterns can be explained by land connections between the two peninsulas during the Messinian salinity crisis (Miocene/Pliocene) or Pleistocene climatic oscillations. However, a long-distance dispersal events, or migrations along the northern Adriatic coast in different periods cannot be excluded. The accumulation of molecular phylogenetic and phylogeographic data provides the baseline to infer shared spatio-temporal patterns and to define robust biogeographic hypotheses. Some examples of different amphi-Adriatic plant groups illustrating the most recent findings will be presented. *Campanula garganica* group includes 13 species whose diversification started in Miocene but some observed genetic variation within the group was also influenced by Pleistocene climatic fluctuations. Genus *Knautia* represents a prime example of Pliocene/Pleistocene rapid diversification and radiation and includes several transadriatic connections among closely related taxa. Genus *Aurinia* has the centre of origin and diversity in south-eastern Europe and three out of seven species exhibit species specific amphi-Adriatic distributions and different genetic patterns.

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Zrinka Ljubešić, Assoc. Prof.

University of Zagreb, Faculty of Science, Department of Biology

Characterization of a photosynthetic marine pico green alga (Picochlorum, Trebouxiophyceae) isolated from the Adriatic Sea

Maja Mucko Judit Padisák, Marija Gligora Udovič, Tamás Pálmai, Tihana Novak, Nikola Medić, Blaženka Gašparović, Petra Peharec Štefanić, Sandi Orlić, Zrinka Ljubešić

Unknown photosynthetic picoeukaryote strains in diverse culture collections around the globe are emerging every day due to their possible role in biofuel production, larger carbon dioxide uptake, food and pharma industry. Still,

knowledge of these picoalgae is scarce. We cultivated marine pico green alga (strain PMFPPE4) from mixed net-phytoplankton sample taken in upper euphotic layer from the southern Adriatic Sea. Subsequent analyses were performed for its characterization: i) thorough morphological (light and electron microscopy), ii) phylogenetic (plastid 16S rRNA and nuclear 18S rRNA genes) and iii) physiological characterization (pigment and lipid composition screening and photosynthesis measurements). The strain PMFPPE4 proved to belong to the genus Picochlorum. The pigment composition of the strain was congruent with the class Trebouxiophyceae, having chlorophyll a and b, lutein, β-carotene, violaxanthin and neoxanthin. The lipid composition revealed dominant triacylglycerides and lipid remodeling from the exponential to stationary growth phase. The maximum photosynthetic activity was measured at 30°C, but the most rapid increase of photosynthetic activity over time was detected at lower temperatures (15-20°C). Additionally, this resilient strain exhibits no photoinhibiton until 40°C and it manages to survive for one month cultivation in complete dark. The PMFPPE4 resilience to low and no-light conditions, suggest a possible switch from autotrophy to mixotrophy under unfavorable growth conditions. This study contributes to physiological and phylogenetical information to be used in future biotechnological and taxonomical studies.

Poster presentations

1. Malenica N., Mlinarec J., Jurković A., Bohanec B., Grdiša M., Besendorfer V.
Manipulacija ploidijom u dalmatinskom buhaču (*Tanacetum cinerariifolium*)

2. Bruno Pavletić (Dunja Leljak-Levanić)
Detekcija virusa u hrvatskim autohtonim kultivarima vinove loze metodom RT-PCR prije i nakon ozdravljivanja

3. Renata Biba, Petra Cvjetko, Mirta Tkalec, Petra Peharec Štefanić, Ana-Marija Domijan, Sandra Šikić, Daniel Mark Lyons, Sanja Babić, Biljana Balen
Impact of silver nanoparticles on physiological parameters of tobacco seedlings

4. Karla Košpić, Renata Biba, Petra Cvjetko, Petra Peharec Štefanić, Biljana Balen
Oxidative stress assessment in tobacco plants exposed to silver nanoparticles and silver nitrate

5. Hrvoje Fulgosi, Lea Vojta, Anja Rac, Wolfgang Bilger, Sonja Veljović Jovanović, Marija Vidović
Fortifying plants with the FRAM (Free Radical Avoidance Mechanism)

6. Lea Vojta, Ana Tomašić Paić, Hrvoje Fulgosi
Studying dual localization of a protein that influences the fate of photosynthetic electrons

7. Klara Krmpotić, Iva Šutevski (Željka Vidaković Cifrek)
Učinak mehaničkog stresa na modelnu biljku *Arabidopsis thaliana* (L.) Heynh.

8. Vuković, Ana, Štolfa Čamagajevac, Ivna, Vuković, Rosemary, Matić, Magdalena, Velki, Mirna, Lončarić, Zdenko
Impact of different selenium forms on oxidative stress and antioxidative response in wheat seedlings (*Triticum aestivum* L.)

9. Begović Lidija, Mlinarić Selma, Cesar Vera
The effect of heat stress on photosynthetic efficiency in *Ginkgo biloba* L. and *Liriodendron tulipifera* L.

10. Selma Mlinarić, Lidija Begović, Vlatko Galić, Ivan Abičić, Alojzije Lalić
Exploring variations of chlorophyll a fluorescence induced by climate changes in selected genotypes of winter barley

11. Kekez Mario, Zanki Vladimir, Kekez Ivana, Matković-Čalogović Dubravka, Rokov Plavec, Jasmina
Plant seryl-tRNA synthetase as a link between translation and metabolism of brassinosteroid hormones

12. Ana Smolko, Nataša Bauer, Sarah Breitenbach, Jutta Ludwig-Müller, Branka Salopek-Sondi
Auxin homeostasis as a mechanism of abiotic stress adaptation

13. Ana Smolko, Nataša Bauer, Branka Salopek-Sondi
Short-term salt stress alters auxin distribution in Arabidopsis root

14. Ida Linić, Dunja Šamec, Valerija Vujčić, Sandra Radić Brkanac, Jiri Gruz, Branka Salopek-Sondi
Response of the Brassicaceae seedlings on short-term salt stress and role of specialized metabolites